Investigations into the niche specialisation of the coprophilous mycoflora, with reference to herbivore faeces

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INVESTIGATIONS INTO THE NICHE SPECIALISATION OF THE COPROPHILOUS MYCOFLORA, WITH REFERENCE TO HERBIVORE FAECES

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TABLES

SUMMARY

1) Samples of herbivore dung and grass were collected from various pastures previously grazed by only one type of major herbivore. The grass was macerated, and incubated in the laboratory with the samples of dung.

Successions of fruiting bodies of coprophilous fungi occurred on all the samples. The occurrence of a succession of coprophilous species on macerated pasture grass has not hitherto been reported. The successions were characterised by the scarcity of Pyrenomycete fruiting bodies. An initial phycomycete phase occurred on the samples of macerated grass, this initial phase had previously only been known to occur on dung and wheat-straw compost.

Macerated grass from a lawn not grazed by large herbivores was also incubated, a coprophilous fungus Oedocephalum glomerulosum developed on this. This fungus has probably not been observed on this type of substrate before.

2) The energy content of the substrates before and after the succession of fungal fruiting bodies was determined by bomb calorimetry. The loss per gram initial dry weight was not found, but the energy value per gram of 'old' sheep dung was found to be very similar to that of fresh sheep dung, all the other values for fresh and 'old' samples of dung and macerated grass differed to quite a large extent.

3) Cultures of coprophilous fungi were inoculated onto autoclaved and non-autoclaved macerated grass, Very few

cultures produced fruiting bodies, but sporangiophores of Phycomyces blakesleeanus, Thamnidium elegans, and Coprinus cinereus developed on autoclaved grass.

4) Spores of Pilobolus kleinii, and spores still enclosed in their sporangia were incubated in water extracts of grass and cow dung. No germination was observed, probably because of self inhibition due to the high concentration of spores.

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INTRODUCTION

Herbivore dung is a good substrate on which to study microorganisms because it is easy to obtain, (except perhaps in the case of the horse, which is largely disappearing as a working animal), contains large amounts of nutrients, vitamins and minerals (Mason, 1976) and exhibits a wide array of microbial types. It has high levels of nitrogen, due to the activities of the gut microflora and microfauna, and contains quite large amounts of soluble carbohydrates not normally present in other substrates such as woody tissues.

The succession of coprophilous (dung-loving) fungi on herbivore dung under ideal conditions for fungal growth, such as under a bell-jar with adequate moisture in the laboratory, has been well described and documented by mycologists since early this century (Salmon & Massee, 1901, 1902).

For long considered a classical succession of fungi with time based on nutrition, Harper & Webster (1964) discredited the simple substrate-succession theory by experimental work. They showed that the observed succession might be simply one of the period of time taken for a fruiting body to be amassed; that is, larger fungal sporophores would appear later in time than smaller ones. They also showed the latent period before germination of the coprophilous fungal spores to be similar, so that at any one time all of the different fungal myceliums could be growing together in the dung mass.

Even so, the fungi that do appear on dung are each adapted to utilise a carbon source, or sources, within the

dung. The fruiting bodies that appear, do so, in fact, in an order in accordance with the schema of Garrett (1951) for the fungal colonisation of senescent tissues, which as a concept of ecological groups of soil fungi is also discussed by Burges (1939, 1958). Apart from the stage 1a of Garrett's which is made up of weak parasites, the other stages are the same. The primary saprophytic "sugar" fungi, typically exemplified by members of the Phycomycetes such as Mucor and Pilobolus, are the first fungi to appear. Amongst these fungi lignin decomposition is unknown and cellulose decomposition infrequent (Siu, 1951). They are limited to using the most simple carbon substrates such as simple sugars. But, despite this limitation, they have compensated by having a high growth rate and a rapid rate of spore germination - typical "r." strategists amongst the fungi. They are thus superior competitors for the soluble sugars, and can be compared with flowering plant colonisers of open habitats (Salisbury, 1942). These plants use up most of their food reserves to produce masses of small seeds.

Sporophores of cellulose decomposers are next to appear, members of the Ascomycotina, in particular, inoperculate Discomycetes such as Lasiobolus, Coprobia, and Cheilymenia. In the typical succession of fruiting bodies, Discomycetes are followed by Pyrenomycetes such as Sordaria and Podospora. This Pyrenomycete phase can often last for several weeks; Plectomycetes are often to be observed and Imperfect fungi develop along with all these groups.

The Ascomycete phase is followed by the appearance of basidiocarps of members of the Agaricales, such as Panaeolus and Coprinus species. These fungi make use of the most resistant, least readily decomposable component of the dung, the lignin, although they can also make use of cellulose. Much energy is it appears needed to produce specific lignin degrading enzymes, which increases the time before sporophores can be produced.

With the Basidiomycetes there is not a premium on fast germination and production of spores because of the more enduring nature of the lignin, so that they can be compared with the "K." strategists or plants that appear towards the climax of a succession.

Hudson (1968) has discussed how the mycoflora on dung is so unique when compared with the succession of fungi on other substrates above the soil surface. He considers that on the whole, most other substrates are less favourable for fungal growth. This is due to the specialisation of the coprophilous substrate, its improved aeration and higher moisture content which is subject to less marked fluctuations. These factors, combined with the comminution of the material may lead to a more rapid breakdown.

The only other succession that has previously been described which is at all similar is that occurring in wheat-straw compost. Chang and Hudson (1967) have demonstrated there to be an extremely brief phycomycete phase, followed by Ascomycetes, Fungi Imperfecti, and Basidiomycetes. Most of the fungi appearing in this succession are thermophilic,

unlike coprophilous species. But it is the initial phycomycete stage that is so unique to these two different substrates. This stage being initially absent from most other successions. Hudson attributes this similarity to the increased nitrogen supply, combined with the presence of readily available carbohydrates.

On other plant substrates successions of Ascomycetes and Fungi Imperfecti follow one another, and these may be followed by Basidiomycetes and other soil fungi. On lignin-rich substrates the colonisers are predominantly Basidiomycetes, especially Hymenomycetes (Mangenot, 1952). The sequence of occurrence of these fungi depends on the superficial humidity of the wood. The initial stage in the decomposition is attack by parasites such as Phellinus which enter via root systems and wounds. The second stage is equivalent to the phycomycete stage of dung and consists of the Phialophora phase. These fungi initially utilise simple carbohydrates, the members though are not phycomycetes and some are known to be cellulolytic.

Ingold (1971) has described some of the similar adaptations displayed by coprophilous genera concerned with spore dispersal. Young basidiocarps developing on herbivore dung initially show positively phototropic orientation so they grow towards the light; later this reaction begins to be masked as the stipe displays a negatively geotropic response. With Pilobolus kleinii, Dasyobolus immersus, and Podospora fimicola, the sporophores orientate themselves towards the light, the spore projectiles are all relatively large, and this increases the range according

to the equation; Distance = K x radius of projectile squared. This is achieved by the binding together of the small spores by mucilage in many other species, but in Dasyobolus immersus and Podospora fimicola the spores are also large. These spore projectiles are shot off onto the surrounding grass and vegetation, and it is significant that with many species the spores are darkly pigmented to protect them from the light whilst they are resting on the vegetation.

It is recorded that the spores are dispersed onto the vegetation where they may be grazed upon by herbivores, such as cows, sheep and horses. The spores will therefore traverse the length of the digestive system and be voided in the animals' faeces. Coprophilous genera are therefore 'caught-up' in the cyclical relationship; grass ——> animal gut ——> dung ——> grass, and are consequently termed entero-philous fungi, as dispersal occurs by means of the herbivore gut (Webster, 1970). An important point that Ingold emphasises is that by no means all of the dung fungi exhibit specialisation towards the dung habitat in terms of spore dispersal, and how spores of Mucor sp. and Pilaira sp. reach the vegetation can only be speculated upon at present.

Specialisation towards the dung habitat means specialisation towards the whole cyclical relationship described, that is - the dung niche, and this includes the passage through the herbivore gut. Buller (1931) showed that rolling a ball of horse dung in a heavy spore deposit of Coprinus sterquilinus did not result in colonisation and fruiting and he naturally

inferred that spores must be ingested for normal development to occur. Harper & Webster (1964) incubated spores in pancreatin for 5 hours at 37°C to simulate passage through the herbivore gut to a certain extent. (In cows, food is normally retained within the gut for a period of from 25 - 40 hours,(Webster, 1970)). They found that when coprophilous Mucorales spores were subjected to this treatment that spores of Pilaira anomala were encouraged to germinate, whilst the percentage germination of Pilobolus kleinii was decreased.

It is widely known that not all coprophilous fungal spores fail to germinate when inoculated onto fresh or sterilised dung. The work by Harper & Webster indicates variations amongst the fungi as to the necessity for enzymic breaking of dormancy. Roberts (unpublished results) has questioned the necessity for breaking of dormancy by factors in the herbivore gut and has found that species of Pilobolus, Coprobia, and Sordaria could be obtained by simply culturing macerates of grass. Little attention seems to have been drawn to the fact that coprophilous fungi have been observed growing on other substrates. Webster (1970) points out that there are relatively few coprophilous genera and goes on to say that many characteristically coprophilous species are to be found on decaying vegetation, seeds, etc., and that dung is only one of a range of habitats available.

The purpose of this study, therefore, is to investigate and to question the degree of niche specialisation of the coprophilous mycoflora. This is attempted by observing the

succession of fungal sporophores on macerated grass from herbivore grazed pastures and comparing it with the succession on herbivore faeces from the same pastures. In addition to this, the energetics of the two processes, the decomposition of the dung and grass is also investigated, and inoculation of cultures of coprophilic fungi onto fresh and autoclaved grass carried out to see if fruiting bodies are produced.

MATERIALS AND METHODS

Samples of herbivore faeces, and grass from herbivore-grazed pastures were gathered from several locations within County Durham on sunny dry days. It was important with regard to the experiment that the faeces to be collected were freshly voided from the animal. Older samples are subject to infestation by dung flies, dung beetles and other insects that utilise this substrate. Also, older dung may already have been utilised by some fungi; these fungi would be missed when the experiment was set up.

The theory behind the experiment depended upon the fact that only one type of major mammalian herbivore had been grazing in the pastures where the dung was to be collected. This 'mono-herbivore' grazing practice must also have been the condition for the immediately preceding period. It was important too that the animals were feeding only on grass and herbage in the fields, and not on hay brought in from other places.

Faeces samples were collected from three sites; cow faeces was obtained from enclosed pastures where dairy cattle were grazing, near to the village of Running Waters (Grid Reference NZ 324 408). Sheep dung was collected from pastures at Houghall Farm, Houghall, near to Durham (Grid Reference NZ 278 404), and horse dung from fields near to a housing estate in the centre of Coxhoe (Grid Reference NZ 317 363).

Vegetation that the herbivores were observed to be grazing upon included, *Lolium perenne, Poa pratensis,

*Nomenclature of vascular plants follows Clapham, Tutin & Warburg (1981).

Alopecurus pratensis, Anthoxanthum odoratum and Trifolium repens. Adequate samples of these plants were gathered, making sure that no roots were included. As far as possible, samples were collected from areas adjacent to old dried-up dung pats. As a control, grass was also gathered from a large lawn in the grounds of Durham University (Grid Reference NZ 275 416); this lawn is kept fairly closely mown throughout the year.

In the laboratory the samples of faeces and grass were kept separate according to their type. The faeces were fashioned into pats of the order of size of 2.5cm x 5cm, and placed into open plastic seed trays measuring 22cm x 37cm. The hands were washed each time before handling a new type of faeces. This was so that one type of faeces should not be contaminated with spores from another.

Two layers of tissue paper completely covered the tray bottoms; this paper was kept moist over the experimental period. It was important to moisten (but not flood) this paper with distilled water every day. The dung pats themselves were not sprayed with water. This could have altered the numbers of sporophores appearing because basidiomycetes are more susceptible to destruction by excess free water than ascomycete fruiting bodies (Richardson & Watling, 1968, 1969). Also, excess water encourages the proliferation of bacteria.

The trays were then covered with inverted transparent plastic jars. They were only partially covered so that there was free movement of air across the dung samples. Anoxic

conditions would tend to favour the development of mites and nematodes which break up the dung and alter the growth of fungi. The trays were placed next to a window so that they received an adequate amount of light. This light is needed for the formation of fruiting bodies of many of the fungi (Lilly & Barnett, 1951). Care was taken that the dung was not exposed to direct sunlight for long periods.

The vegetation taken from the sample sites was initially cut with scissors to a manageable size for the process of maceration. Separate samples were inserted into a macerator and distilled water was added. Maceration was carried out until the vegetation had a fibrous consistency resembling that of fresh horse dung. The macerates were then made into pats of a similar size to those of the dung samples, and were incubated in the same manner and under the same conditions of room temperature and daylength.

The dung samples and grass macerates were observed daily under a binocular microscope over a five week period for development of sporophores. The percentage cover of the fruiting bodies was very roughly assessed using a simple cover scale:

(+), up to 10% cover of the sample
(1), more than 10% and up to 20% cover
(2), more than 20% and up to 40% cover
(3), more than 40% and up to 60% cover
(4), more than 60% and up to 80% cover
(5), more than 80% cover

Each pat was individually assessed for percentage cover and a total average cover was calculated. There is evidently a bias here towards overestimation of percentage cover values

due to the fact that cover classes of less than the maximum value are still included under that class. This method, though, gives more information than a basic "presence or absence" technique that has been adopted by some workers.

Fungi were identified in the usual manner by mounting on a microscope slide in water and than staining with Lactophenol Cotton Blue. The keys to fungi on dung by Richardson and Watling (1968, 1969) were found to be most useful for the identification of some species. Naumov (1939) and Dennis (1960) were also consulted when identifying phycomycetes and ascomycetes.

Other fungi that could not be immediately identified were cultured onto agar plates. The method used consisted of brushing the fungus with a sterilised needle tip and streaking this onto the plates. The plates consisted of potato dextrose agar and separate plates of sheep, cow, and horse dung agar containing some antibiotic.

The potato-dextrose agar was made according to the method of Deverall (1969). 200 grammes of sliced, peeled potato tubers were boiled in water until soft. The mixture was then crushed and filtered through cheesecloth to give a volume of 500 ml, to which 20 grammes of dextrose was added. 20 grammes of agar was steamed and melted in 500 ml of water. The nutrients and agar were added together and dispensed into a flask and sterilised by autoclaving at 10lb pressure for ten minutes. After sterilisation the medium was poured into petri-dishes, about 15 mls per dish.

Dung agar was prepared by boiling 100 grammes of fresh dung in 1 dm^3 of distilled water for 30 minutes. To this, 15 grammes of agar and 0.025 grammes of chloramphenicol were added. The mixture was then sterilised by autoclaving at 10lb pressure for ten minutes. After cooling, this medium was poured into petri-dishes.

Two types of agar plate were prepared because it could be that some of the coprophilic fungi would not grow on the potato-dextrose agar and needed some extra nutrient supplement such as is present in dung.

After the succession of sporophores on each dung and macerate type had been completed (after about six weeks), then the samples were dried. Drying was carried out in an oven at about 50°C for a period of three days. It was important that the samples were not directly heated by flame to dry them because this might drive off volatile compounds. The purpose of drying is so that the energy content of the samples after fungal succession could be determined; excess direct heat could therefore cause a loss in energy content.

Also dried were samples of fresh grass and fresh dung from the same locations as where the previous samples for this study had been collected. Before drying, the grass samples were macerated as before, this was so that compressed pellets for use in the bomb calorimeter could be produced. Without maceration, the production of fully compressed pellets was found not to be possible. (If the grass is not compressed, it may burn too vigorously inside

the bomb calorimeter, and damage it.)

After drying the dung and grass macerates, samples were made into pellets of a suitable weight (from 0.3 - 0.4 grammes) and then weighted accurately. The pellets were then 'bombed' in a Gallenkamp Ballistic Bomb Calorimeter at an oxygen pressure of 25 to 26 atmospheres. The maximum deflection on the galvanometer associated with the calorimeter was read. Afterwards the oxygen inside the calorimeter was released and the machine was switched off. If there was ash present in the calorimeter crucible then this was accurately weighed.

A graph was produced of the galvanometer deflection caused by certain weights of benzoic acid; this had been previously determined using the calorimeter. The energy content of the benzoic acid was known, so that from the graph the energy contents of the samples (in kilojoules) could be calculated.

Plastic petri-dishes were set up, each containing a layer of moistened filter paper. Into these dishes were placed small 'pats' of autoclaved macerated grass. The grass selected did not contain much woody tissue and was therefore fairly soft. It was autoclaved as before, but was given slightly longer, i.e. a period of 30 minutes at 10lb pressure. The transfer of the grass was made swiftly to minimise contamination

The autoclaved grass was 'seeded' with various fungi obtained from the previous samples of dung and grass. These inoculations consisted of: (i) Pilobolus kleinii sporangia,

these had been ground against some fine sand-paper so that the spores were released. The sporangia were obtained by scraping them from the sides of the jars that had partially covered the dung and grass samples. (ii) Spores of Coprinus cinereus, the spores were collected by picking the auto-digesting caps of the fungus. Auto-digestion is a subject that has been discussed fully by Buller (1924). As the black spores are discharged from the basidia, then the edge of the gills becomes white, but the spore-free region is soon removed by self-digestion. A black fluid is thus produced which builds up and eventually flows down the inclined edges of the eroding gills and collects as an inky liquid around the margin of the cap. The cap therefore begins to decrease in size, many of the spores are shot off freely into the air, but some drift into the ink. (iii) Sclerotia of Coprinus cinereus, these sclerotia were obtained from beneath the pats of horse dung after the succession of fungi had terminated. They were washed before transference to the autoclaved grass. Some sclerotia were also transferred to pats of fresh macerated grass. (iv) A mixture of Coprinus cinereus and Pilobolus kleinii spores.

Inoculation was carried out by inserting the spores and sclerotia into the autoclaved grass using a sterilised needle. Spores were not left in direct contact with the air so that they could dry out, but were totally covered over by the macerated grass. The tops were then placed on the petri-dishes, and these were left in the laboratory to be observed; it was made certain that the filter paper was

kept moistened.

Information was also required as to whether other coprophilic genera would grow and produce fruiting bodies on fresh or autoclaved macerated grass. Fungi for this purpose were obtained from the Commonwealth Mycological Institute Culture Collection at Kew, Richmond, Surrey. Most of the samples, (Thamnidium elegans, Syncephalastrum racemosum, Phycomyces blakesleeanus, Sordaria fimicola, Podospora setosa, and Ctenomyces serratus) were supplied freeze-dried in ampoules. The cultures were revived by breaking the ampoules above the cotton-wool plug to release the vacuum. The plug was removed and enough sterile distilled water was added to cover the contents. The plug was then flamed and replaced in the ampoule. This was left for 15 minutes to absorb the water. Half of this mixture was used to streak suitable nutrient agar to keep the cultures going while the rest was added to the grass samples. These samples were set up in separate petri dishes as before. The culture mixtures were inoculated onto fresh and autoclaved grass.

from C.M.I.
Other cultures were obtained on malt and dung agar (Coprinus hexagonosporus, Ascophanus carneus, and Ryparobius polysporus), a portion of these colonies were inoculated onto separate grass macerates.

Experiments were also carried out using sporangia of Pilobolus kleinii. Sporangia were ground on some fine sandpaper, and the released spores added to petri-dishes lined with filter paper. This filter paper was soaked with the

water soluble extracts of dung and fresh grass. Whole sporangia were also added to separate petri-dishes containing these extracts. Every day, samples of the spores were taken, and observed under the high power of an optical microscope, to see which, if any, had germinated. The whole sporangia were also damaged so that the spores could be similarly observed.

PLATE 1. Sporangiophores of Pilobolus kleinii on cow dung ready to shoot their sporanigia.

PLATE 2. Apothecia of Coprobia granulata on cow dung.

PLATE 3. Immature basidiocarps of Coprinus heptemerus.

PLATE 4. Mature basidiocarp of Coprinus heptemerus.

PLATE 5. Basidiocarp of Coprinus cinereus at immature 'button'-stage..

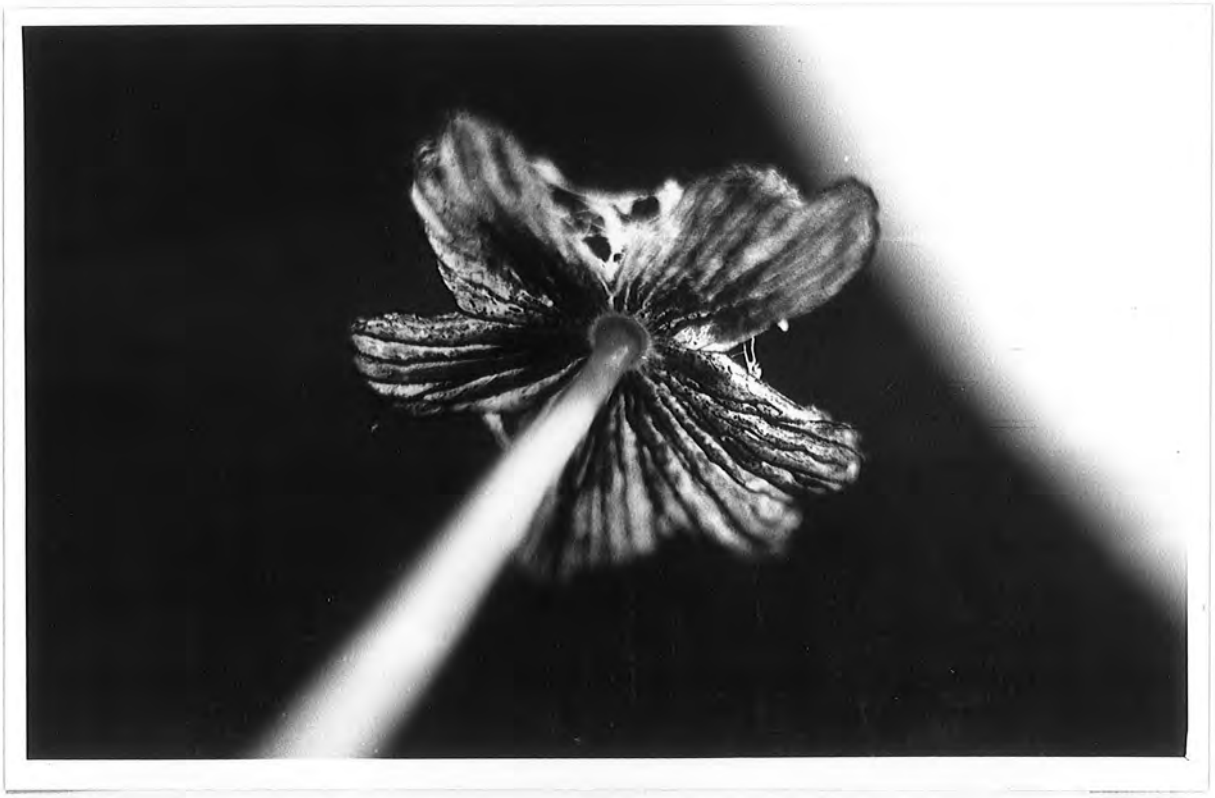


PLATE 6. Underside of pileus of Coprinus cinereus showing the gill arrangement for efficient dispersal of spores.

PLATE 7. A self-digesting pileus of Coprinus cinereus. (note bending of stipe due to the weight of the 'ink').

PLATE 8. A species of Fusarium forming a white covering over cow dung.

RESULTS

TABLE 1. The average percentage surface area of sheep dung pats covered by fungal fruiting bodies.

		Pilobolus kleinii	Pilaira moreaui	Basidiomycotina	Fungi Imperfecti	Mucor mucedo	Ascobolus furfuraceus	Cheilymenia theleboloides
May	28	0	0	0	0	0	0	0
	29	0	0	0	0	0	0	0
	30	65	14	0	0	0	0	0
	31	66	10	0	0	0	0	0
June	1	16	0	0	4	0	0	0
	2	25	1	0	5	0	0	0
	3	31	1	0	7	0	0	0
	4	12	0	0	6	1	0	0
	5	9	0	0	9	0	0	0
	6	9	1	0	6	0	0	0
	7	6	0	0	6	0	0	0
	8	5	0	0	7	0	0	0
	9	5	0	0	7	0	1	0
	10	10	0	0	7	0	2	0
	11	6	0	0	6	0	2	0
	12	4	0	0	3	0	1	1
	13	2	0	0	6	0	3	1
	14	1	0	0	8	0	5	1
	15	1	0	0	11	0	6	1
	16	3	0	0	14	0	9	1
	17	0	0	0	8	0	7	0
	18	1	0	0	9	0	5	0
	19	0	0	0	11	0	2	0
	20	0	0	0	10	0	1	0
	21	0	0	0	12	0	1	0
	22	0	0	0	12	0	0	0
	23	0	0	0	14	0	0	0
	24	0	0	0	14	0	0	0
	25	0	0	0	11	0	0	0
	26	0	0	0	14	0	0	0
	27	0	0	0	14	0	0	0
	28	0	0	0	14	0	0	0
	29	0	0	0	12	0	0	0
	30	0	0	0	11	0	0	0
July	1	0	0	0	14	0	0	0
	2	0	0	0	14	0	0	0
	3	0	0	0	10	0	0	0
	4	0	0	0	14	0	0	0

TABLE 2. The average percentage surface area of cow dung pats covered by fungal fruiting bodies

		Pilobolus Kleinii	Coprobia granulata	Coprinus ephemerus	Coprinus heptemerus	Coprinus stercoreus	Fusarium species	Ascobolus furfuraceus	Mucor mucedo
May	19	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0
	21	0	0	0	0	0	0	0	0
	22	10	0	0	0	0	0	0	0
	23	46	0	0	0	0	0	0	0
	24	36	0	0	0	0	0	0	0
	25	21	0	0	0	0	0	0	0
	26	10	2	0	0	0	0	0	0
	27	10	5	0	0	0	1	0	0
	28	12	13	0	0	0	5	0	0
	29	12	12	0	2	0	6	0	0
	30	5	16	0	2	0	10	0	0
	31	16	12	0	5	0	2	0	0
June	1	5	13	0	6	0	2	0	0
	2	6	9	0	7	0	2	0	0
	3	7	8	0	7	0	1	0	0
	4	2	3	0	7	0	1	0	0
	5	2	6	0	9	0	3	0	0
	6	2	3	0	8	0	1	0	0
	7	1	2	0	7	0	1	0	0
	8	0	4	1	7	0	1	0	0
	9	1	2	1	8	0	1	0	0
	10	2	2	1	5	0	1	1	0
	11	3	2	1	5	1	0	1	0
	12	1	2	2	2	1	0	2	0
	13	1	1	2	2	1	1	2	0
	14	1	0	2	2	1	1	1	0
	15	1	0	3	2	0	1	1	0
	16	0	1	3	2	0	1	1	0
	17	0	2	3	2	0	0	1	0
	18	2	2	4	2	0	0	2	1
	19	0	1	2	2	0	0	1	1
	20	0	0	0	0	0	0	0	0
	21	0	0	0	0	0	0	0	0

TABLE 3. The average percentage surface area of horse dung pats covered by fungal fruiting bodies

		Pilaira moreaui	*Pilobolus kleinii*	*Mucor mucedo*	*Mucor haemalis*	*Ascobolus glaber*	*Ascobolus stercorarius*	*Coprinus cinereus*	*Sordaria fimicola*	Fungi Imperfecti
May	27	0	0	0	0	0	0	0	0	0
	28	21	0	0	0	0	0	0	0	0
	29	49	10	0	0	0	0	0	0	0
	30	54	19	0	0	0	0	0	0	0
	31	34	15	1	0	0	0	0	0	0
June	1	22	11	3	0	0	0	0	0	0
	2	11	14	2	0	0	0	0	0	0
	3	1	11	4	1	0	0	0	0	0
	4	0	21	6	6	0	0	0	0	0
	5	2	22	0	3	0	0	0	0	0
	6	3	6	1	1	28	0	0	0	0
	7	3	1	2	0	40	0	0	0	0
	8	2	0	0	1	39	0	0	0	0
	9	1	0	0	0	34	1	0	0	0
	10	0	0	0	0	16	1	0	0	0
	11	1	0	0	0	42	2	1	0	0
	12	0	0	0	0	11	2	2	0	0
	13	0	0	0	0	6	2	4	0	0
	14	0	0	0	0	4	2	7	0	0
	15	0	0	0	0	1	2	9	0	0
	16	1	0	0	0	0	1	9	0	0
	17	0	0	0	0	0	1	9	0	0
	18	0	0	0	0	0	1	8	0	0
	19	0	0	0	0	0	1	8	0	0
	20	0	0	0	0	0	0	8	0	0
	21	0	0	0	0	0	0	8	0	0
	22	0	0	0	0	0	0	6	0	0
	23	0	0	0	1	0	1	6	0	0
	24	0	0	1	0	0	0	5	0	0
	25	0	0	1	0	0	1	5	4	0
	26	0	0	2	0	0	0	6	5	0
	27	0	0	0	0	0	0	7	6	0
	28	0	0	0	0	0	0	7	6	0
	29	0	0	0	0	0	0	5	6	0
	30	0	0	1	0	0	0	6	6	0
July	1	0	0	2	0	0	0	7	6	1
	2	0	0	2	0	0	0	5	6	1
	3	0	0	3	0	0	0	4	6	1
	4	0	0	4	0	0	0	2	2	1
	5	0	0	3	0	0	0	4	4	1
	6	0	0	3	0	0	0	6	5	1
	7	0	0	3	0	0	0	4	4	1
	8	0	0	3	0	0	0	2	2	1
	9	0	0	3	0	0	0	1	1	1
	10	0	0	1	0	0	0	1	1	1
	11	0	0	1	0	0	0	1	0	1
	12	0	0	1	0	0	0	1	0	1
	13	0	0	0	0	0	0	0	0	1
	14	0	0	0	0	0	0	0	0	0

TABLE 4. The average percentage surface area of pat covered by fungal fruiting bodies developing on macerated grass pats from sheep grazed pastures.

		Pilaira moreaui	Pilobolus Kleinii	Mucor mucedo	Mucor haemalis	Fungi Imperfecti	Cheilymenia coprinaria	Ascobolus furfuraceus	Basidiomycotina (initials)
May	28	0	0	0	0	0	0	0	0
	29	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0
	31	17	17	0	4	0	0	0	0
June	1	46	10	0	0	0	0	0	0
	2	34	8	0	1	0	0	0	0
	3	25	0	0	1	0	0	0	0
	4	17	3	0	4	37	0	0	0
	5	11	3	0	1	41	5	0	0
	6	9	4	2	0	12	6	0	0
	7	6	4	5	0	3	8	0	0
	8	1	0	7	0	2	7	0	0
	9	0	0	18	0	9	7	1	0
	10	1	2	11	8	8	7	7	0
	11	3	0	16	0	5	6	7	0
	12	0	1	23	0	2	7	5	0
	13	2	1	19	0	3	5	5	0
	14	2	1	23	0	4	5	5	0
	15	2	1	21	0	5	4	4	0
	16	1	0	24	0	5	4	2	0
	17	2	0	21	0	1	3	1	0
	18	0	1	14	0	2	1	1	0
	19	0	0	14	0	2	1	0	0
	20	0	0	12	0	3	1	0	0
	21	0	0	12	0	2	1	0	0
	22	0	0	12	0	2	1	0	0
	23	0	0	12	0	4	0	0	0
	24	0	0	10	0	4	0	0	0
	25	0	0	10	0	5	1	0	1
	26	0	0	10	0	5	0	0	1
	27	0	0	10	0	5	0	0	1
	28	0	0	12	0	5	0	0	1
	29	0	0	12	0	5	0	0	1
	30	0	0	12	0	5	0	0	1
July	1	0	0	16	0	4	0	0	1
	2	0	0	19	0	5	0	0	1
	3	0	0	20	0	5	0	0	1
	4	0	0	9	0	4	0	0	1
	5	0	0	0	0	3	0	0	0
	6	0	0	0	0	1	0	0	0
	7	0	0	0	0	1	0	0	0

TABLE 5. The average percentage surface area of pat covered by fungal fruiting bodies developing on macerated grass pats from cow grazed pastures.

		Pilaira moreaui	*Mucor microsporus*	*Pilobolus kleinii*	*Mucor haemalis*	*Mucor mucedo*	Fungi Imperfecti	*Rhizopus* spp.	*Coprinus heptemerus*	*Ascobolus glaber*
May	19	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0
	21	0	0	0	0	0	0	0	0	0
	22	10	11	0	0	0	0	0	0	0
	23	14	12	5	0	0	0	0	0	0
	24	11	28	4	0	0	0	0	0	0
	25	8	18	6	0	0	0	0	0	0
	26	4	7	9	4	2	0	0	0	0
	27	1	0	15	10	6	1	6	0	0
	28	3	0	13	2	2	46	0	0	0
	29	19	0	5	1	0	37	0	0	0
	30	6	0	1	0	0	25	0	0	0
	31	3	0	1	0	0	34	0	0	0
June	1	1	0	0	0	0	53	0	0	0
	2	1	0	0	0	0	28	0	0	0
	3	2	0	1	0	0	25	0	0	0
	4	1	1	1	0	0	44	0	0	0
	5	0	0	0	0	0	42	0	0	0
	6	0	0	0	0	0	28	0	0	0
	7	1	0	0	0	0	15	0	0	0
	8	0	0	1	0	0	33	0	0	0
	9	0	0	0	0	2	36	0	0	0
	10	0	0	0	0	1	36	0	0	0
	11	0	0	0	0	0	42	0	0	0
	12	0	0	0	0	0	33	0	0	0
	13	0	0	0	0	0	34	0	0	1
	14	0	0	0	0	0	37	0	1	1
	15	0	0	0	0	0	41	0	1	1
	16	0	0	0	0	0	41	0	1	1
	17	0	0	0	0	6	6	0	1	1
	18	0	0	0	0	0	42	0	0	1
	19	0	0	0	0	0	28	0	0	1
	20	0	0	0	0	0	5	0	0	0
	21	0	0	0	0	0	0	0	0	0
	22	0	0	0	0	0	0	0	0	0

TABLE 6. The average percentage surface area of pat covered by fungal fruiting bodies developing on macerated grass pats from horse grazed pastures.

		Pilaira moreaui	Mucor mucedo	Pilobolus kleinni	Fungi Imperfecti	Mucor haemalis	Piptocephalis repens	Basidiomycotina
May	27	0	0	0	0	0	0	0
	28	0	0	0	0	0	0	0
	29	0	0	0	0	0	0	0
	30	28	0	1	1	0	0	0
	31	45	1	2	0	0	0	0
June	1	47	0	1	0	0	0	0
	2	47	0	1	0	0	0	0
	3	49	0	0	0	0	0	0
	4	11	1	0	1	4	1	0
	5	1	9	0	61	0	2	0
	6	0	0	0	54	0	0	0
	7	0	0	0	47	0	0	0
	8	1	0	0	46	2	1	1
	9	1	1	0	19	1	1	4
	10	1	0	0	11	1	1	1
	11	0	0	0	9	0	0	1
	12	0	1	1	8	0	1	1
	13	0	1	1	9	0	1	1
	14	0	1	0	11	0	1	1
	15	1	1	0	12	0	1	1
	16	0	4	0	13	0	1	2
	17	0	4	0	9	0	1	2
	18	0	3	0	6	0	0	1
	19	0	4	0	6	0	0	1
	20	0	3	0	8	0	0	2
	21	0	2	0	9	0	0	1
	22	0	2	0	10	0	0	1
	23	0	1	0	9	0	0	2
	24	0	1	0	9	0	0	1
	25	0	1	0	11	0	0	2
	26	0	1	0	6	0	0	2
	27	0	0	0	6	0	0	2
	28	0	0	0	7	0	0	2
	29	0	0	0	8	0	0	1
	30	0	0	0	9	0	0	2
July	1	0	0	0	10	0	0	1
	2	0	0	0	12	0	0	1
	3	0	1	0	10	0	0	1
	4	0	1	0	11	0	0	1
	5	0	0	0	10	0	0	1
	6	0	0	0	8	0	0	1
	7	0	0	0	6	0	0	0
	8	0	0	0	6	0	0	0
	9	0	0	0	9	0	0	0
	10	0	0	0	4	0	0	0
	11	0	0	0	4	0	0	0
	12	0	0	0	1	0	0	0
	13	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0

TABLE 7. The average percentage surface area of pat covered by fungal fruiting bodies developing on macerated grass pats from a lawn in the grounds of Durham University.

	Mucor mucedo	*Oedocephalum glomerulosum*	*Coprinus* spp.
August 4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	40	0	0
9	40	0	0
10	0	10	0
11	0	25	0
12	0	40	0
13	0	80	0
14	0	100	0
15	0	100	0
16	0	90	0
17	0	80	0
18	0	75	0
19	0	75	0
20	0	75	0
21	0	65	0
22	0	60	0
23	0	55	0
24	0	55	0
25	0	45	0
26	0	40	0
27	0	35	0
28	0	25	0
29	0	20	0
30	0	20	0
31	0	20	0
September 1	0	10	2
2	0	10	2
3	0	10	2
4	0	10	0
5	0	10	0
6	0	10	0
7	0	10	0
8	0	7	0
9	0	7	0
10	0	0	0

FIG. 1.

Conidiophores of the imperfect fungus Oedocephalum glomerulosum (not to scale), drawn from those occurring on samples of macerated grass from a lawn in the grounds of Durham University. The fungus was initially pure white but later changed to a pale salmon colour. The enlarged apex and free conidia are shown. This species is characterised by its large conidia (22 - 28 x 13 - 18 μ.)

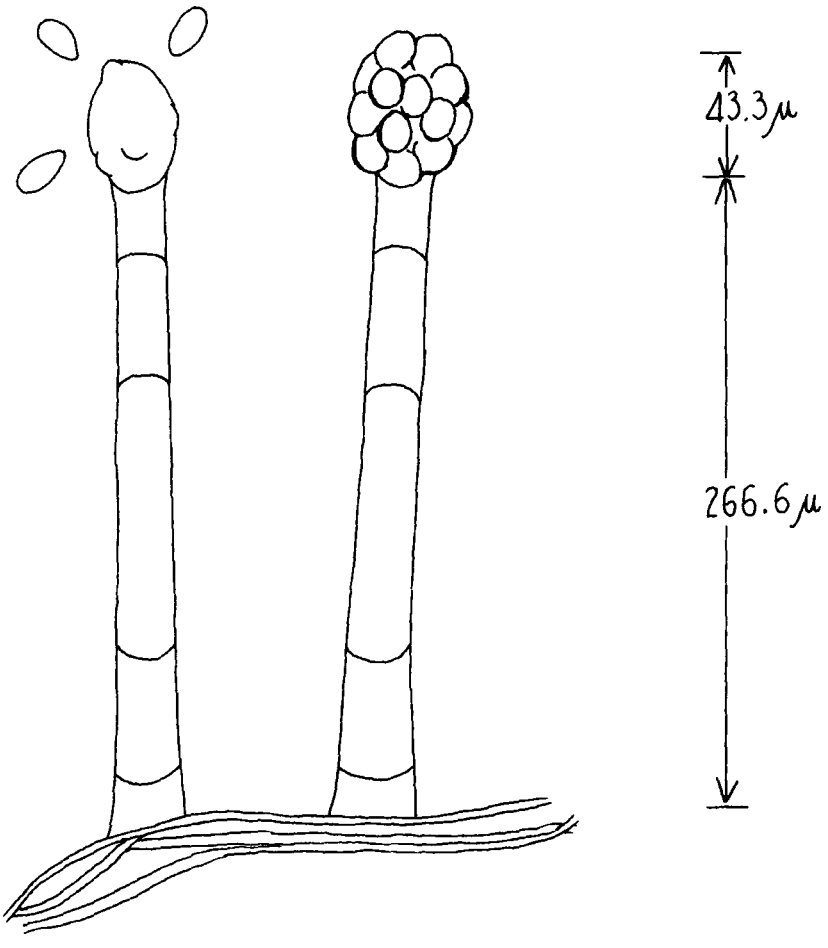
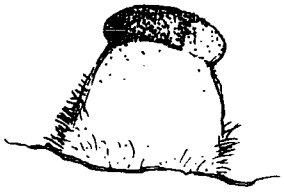

43.3μ
266.6μ

FIG. 2.

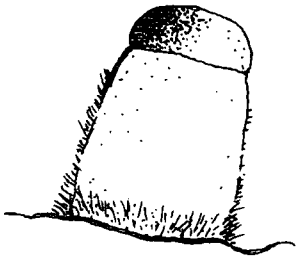
Initial stages of basidiocarp development of *Coprinus heptemerus*, observed on cow dung samples. (x 30)

(a)

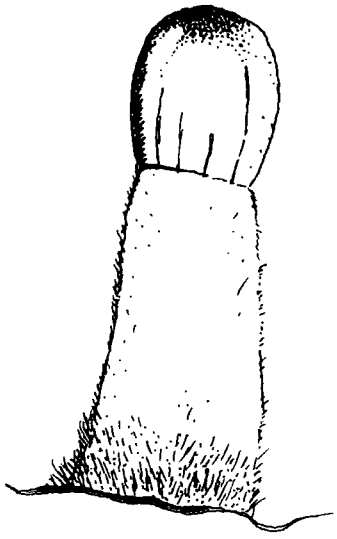
(b)



(c)



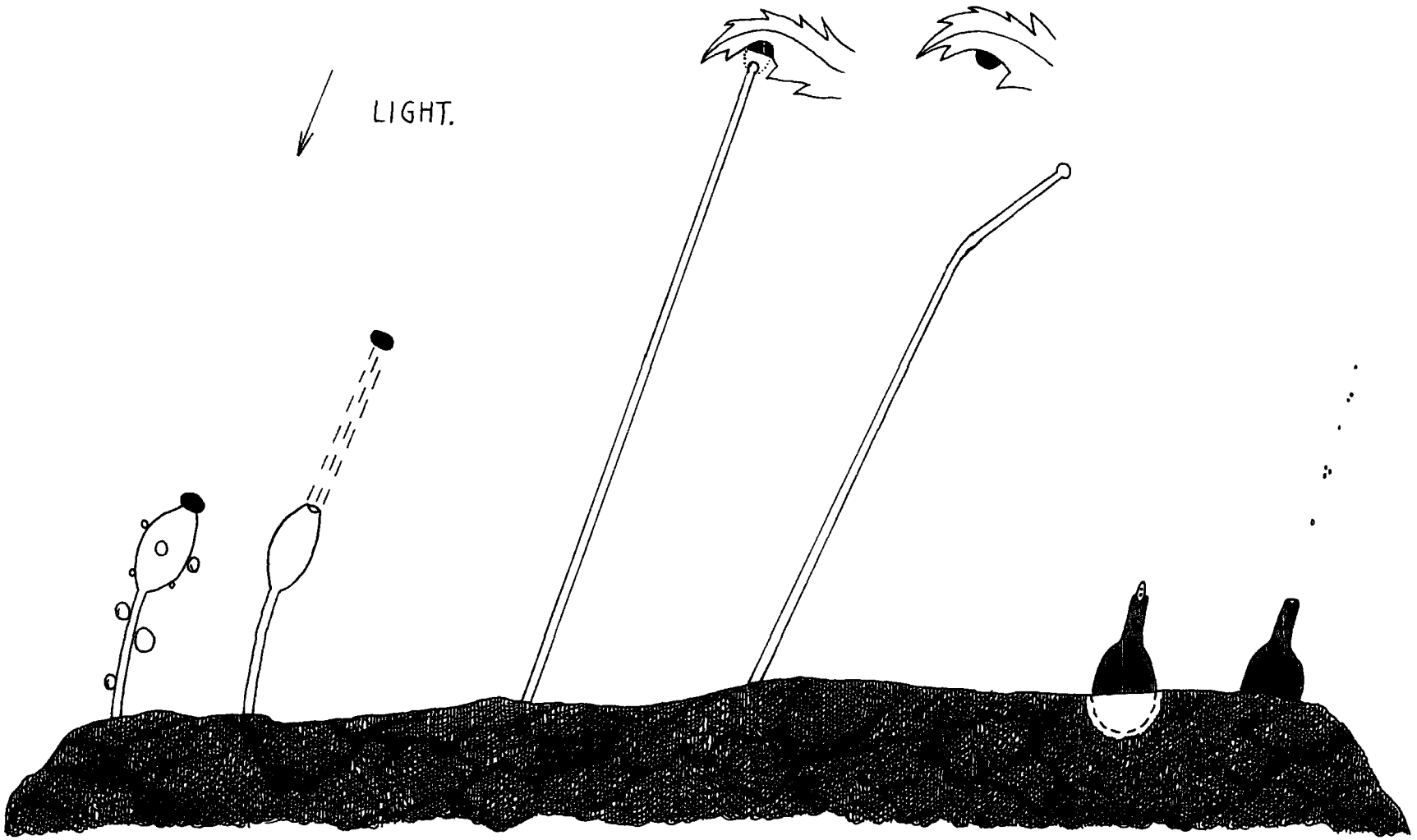
(d)



(e)

FIG. 3

Methods of spore dispersal in three coprophilous fungi; (a) Pilobolus kleinii, shooting its heavily pigmented sporangia. (b) Pilaira moreaui, in this figure the sporangia attaches to some vegetation. (c) Sordaria fimicola.



(a). (b). (c).

TABLE 8. Calorimeter results. Calculation of the energy content of sheep dung before fungal succession

Sample Weight (Grams) A.	Ash Weight (Grams) B.	Ash free dry wt. (Grams) A - B	Corrected Galvanometer Reading (x - 2)	Wt. Benzoic Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as that produced by 1 Gram of dung	Wt. Benzoic Acid giving equal reading as 1 Gram of Ash free dung	Energy Content of sample KJ/g	Energy Content of Ash Free sample KJ/g
0.36649	0.0305	0.3360	16.8	0.232	0.6330	0.6904	16.74	18.26
0.37444	0.03263	0.3418	18.3	0.252	0.6730	0.7372	17.80	19.49
0.39246	0.04736	0.3451	18.6	0.257	0.6548	0.7447	17.31	19.69
0.39148	0.03975	0.3517	17.0	0.233	0.5952	0.6625	15.74	17.52
0.39752	0.04514	0.3524	18.0	0.245	0.6163	0.6952	16.30	18.39
0.39839	0.04213	0.3563	18.9	0.264	0.6626	0.7409	17.52	19.59

mean $\bar{x}$ = 16.90 ± 0.32 95% confidence = 16.90 ± 0.62

mean $\bar{x}$ = 18.82 ± 0.36 95% confidence = 18.82 ± 0.714

TABLE 9. Calorimeter results. Calculation of the energy content of sheep dung after fungal succession

Sample Weight (Grams) A.	Ash Weight (Grams) B.	Ash Free Dry Wt. (Grams) A - B	Corrected Galvanometer Reading (x - 2)	Wt. Benzoic Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as that produced by 1 Gram of dung	Wt. Benzoic Acid giving equal reading as 1 Gram of Ash Free Dung	Energy Content of Sample KJ/g	Energy Content of Ash Free Sample KJ/g
0.33434	0.04676	0.287	15.1	0.202	0.604	0.704	15.97	18.62
0.37781	0.5578	0.322	17.0	0.232	0.614	0.720	16.24	19.04
0.35242	0.04608	0.306	15.9	0.218	0.618	0.712	16.35	18.83
0.35501	0.04300	0.312	17.0	0.232	0.653	0.743	17.27	19.65
0.37205	0.05579	0.316	16.3	0.222	0.597	0.702	15.79	18.57
0.39518	0.05153	0.344	17.8	0.245	0.619	0.712	16.37	18.83

mean $\bar{x}$ = 16.33 ± 0.21
95% confidence = 16.33 ± 0.41

mean $\bar{x}$ = 18.92 ± 0.16
95% confidence = 18.92 ± 0.31

TABLE 10. Calorimeter results. Calculation of the energy content of cow dung before fungal succession.

Sample weight (Grams) A.	Ash Weight (Grams) B.	Ash Free Dry Wt. (Grams) A - B	Corrected Galvanometer reading (x - 2)	Wt. Benzoic Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as 1 gram of dung	Wt. Benzoic Acid giving equal reading as 1 gram of Ash Free Dung	Energy Content of Sample KJ/g	Energy Content of Ash-free Sample KJ/g
0.34675	0.06483	0.2819	15.3	0.205	0.5912	0.727	15.64	19.23
0.37593	0.05670	0.3192	16.8	0.232	0.6171	0.727	16.32	19.23
0.39997	0.06530	0.3347	19.5	0.270	0.575	0.806	17.85	21.32
0.39809	0.05618	0.3419	18.3	0.252	0.633	0.737	16.74	19.49
0.30669	0.05470	0.2519	14.2	0.190	0.619	0.754	16.37	19.94
0.36425	0.07840	0.2858	15.4	0.208	0.571	0.727	15.10	19.23

mean ($\bar{x}$) = 16.33 ± 0.38
95% confidence = 16.33 ± 0.75

mean ($\bar{x}$) = 19.74 ± 0.34
95% confidence = 19.74 ± 0.66

TABLE 11. Calorimeter results. Calculation of the energy content of cow dung after fungal succession.

Sample weight (Grams) A.	Ash Weight (Grams) B.	Ash Free Dry Wt. (Grams) A - B	Corrected Galvanometer reading (x - 2)	Wt. Benzoic Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as that produced by 1 gram of dung	Wt. Benzoic Acid giving equal reading as 1 gram of Ash free dung	Energy Content of Sample KJ/g	Energy Content of Ash free Sample KJ/g
0.33637	0.09378	0.2426	11.5	0.148	0.4399	0.6100	11.63	16.13
0.35282	0.09881	0.2540	12.5	0.163	0.4619	0.6417	12.22	16.97
0.35340	0.09130	0.2621	12.5	0.163	0.4612	0.6219	12.19	16.45
0.35393	0.0874	0.2665	12.0	0.157	0.4436	0.5891	11.73	15.58
0.39383	0.09762	0.29621	14.6	0.192	0.4875	0.6481	12.89	17.14
0.35550	0.08845	0.2670	13.8	0.167	0.4697	0.6254	12.42	16.54

mean ($\bar{x}$) = 12.18 ± 0.19
95% confidence = 12.18 ± 0.36

mean ($\bar{x}$) = 16.47 ± 0.23
95% confidence = 16.47 ± 0.45

TABLE 12. Calorimeter results. Calculation of the energy content of horse dung before fungal succession

Sample weight (Grams) A.	Ash weight (Grams) B.	Ash free dry wt. (Grams) A - B	Corrected Galvanometer reading (x - 2)	Wt. Benzioc Acid to give same deflection of Galvanometer (Grams)	Wt. Benzioc Acid giving equal reading as that produced by 1 gram of dung	Wt. Benzoic Acid giving equal reading as 1 gram of ash-free dung	Energy Content of Sample KJ/g	Energy Content of ash-free sample KJ/g
0.32549	0.03239	0.2931	15	0.204	0.6267	0.6960	16.57	18.41
0.34988	0.04105	0.3088	15.3	0.205	0.5859	0.6638	15.49	17.56
0.33998	0.04658	0.2934	16	0.219	0.6440	0.7464	17.03	19.74
0.33973	0.04428	0.2954	16.5	0.228	0.6711	0.7718	17.75	20.41
0.34516	0.02896	0.3162	15.3	0.205	0.5939	0.6483	15.71	17.15
0.37233	0.04543	0.3269	17.2	0.237	0.6365	0.7249	16.83	19.17

mean ($\bar{x}$) = 16.56 ± 0.35
95% confidence = 16.56 ± 0.67

mean ($\bar{x}$) = 18.74 ± 0.52
95% confidence = 18.74 ± 1.01

TABLE 13. Calorimeter results. Calculation of the energy content of horse dung after fungal succession.

Sample weight (Grams) A.	Ash weight (Grams) B.	Ash Free dry weight (Grams) A - B	Corrected Galvanometer reading (x - 2)	Wt. Benzioc Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as that produced by 1 gram of dung	Wt. Benzoic Acid giving equal reading as 1 gram of ash-free dung	Energy Content of Sample KJ/g	Energy Content of ash-free Sample KJ/g
0.37583	0.06652	0.30931	16.2	0.220	0.5854	0.7112	15.48	18.81
0.37501	0.07017	0.30484	16.0	0.219	0.5839	0.7184	15.44	19.00
0.38273	0.08184	0.30089	16.1	0.218	0.5695	0.7245	15.06	19.16
0.36752	0.07852	0.2890	14.8	0.200	0.5442	0.6920	14.39	18.30
0.38501	0.07839	0.30662	15.7	0.212	0.5506	0.6914	14.56	18.29
0.34462	0.06517	0.27945	14.5	0.193	0.5600	0.6906	14.81	18.26

mean ($\bar{x}$) = 14.95 ± 0.18 95% confidence = 14.95 ± 0.36

mean ($\bar{x}$) = 18.63 ± 0.16 95% confidence = 18.63 ± 0.32

TABLE 14. Calorimeter results. Calculation of the energy content of macerated grass from sheep grazed pastures before fungal succession.

Sample weight (Grams) A.	Ash weight (Grams) B.	Ash-free Dry weight A - B	Corrected Galvanometer reading (x - 2)	WtBenzoic Acid to give the same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as 1 gram of macerate	Wt. Benzoic Acid giving equal reading as 1 gram ash-free macerate	Energy Content of Sample KJ/g	Energy Content of ash-free Sample KJ/g
0.3218	0.0088	0.313	16.2	0.212	0.6588	0.6773	17.42	17.91
0.31491	0.0050	0.30991	15.00	0.204	0.6480	0.6582	17.14	17.41
0.32908	0.00488	0.3242	17.8	0.243	0.7384	0.7495	19.53	19.82
0.35078	0.00749	0.34329	20.00	0.278	0.7925	0.8098	20.96	21.42
0.35105	0.00610	0.34495	18.00	0.248	0.7064	0.7189	18.68	19.01
0.35168	0.0052	0.34648	18.20	0.251	0.7137	0.7244	18.88	19.16

mean value ($\bar{x}$) = 18.76 ± 0.57
95% confidence = 18.76 ± 1.11

mean value ($\bar{x}$) = 19.12 ± 0.58
95% confidence = 19.12 ± 1.13

TABLE 15. Calorimeter results. Calculation of the energy content of macerated grass from sheep grazed pastures after fungal succession.

Sample weight (Grams) A.	Ash weight (Grams) B.	Ash Free Dry weight (Grams) A - B	Corrected Galvanometer reading (x - 2)	Wt. Benzoic Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as 1 gram of macerate	Wt. Benzoic Acid giving equal reading as 1 gram of ash-free macerate	Energy Content of Sample KJ/g	Energy Content of ash-free Sample
0.37967	0.03841	0.34126	15.3	0.205	0.5399	0.6007	14.28	15.89
0.38569	0.02277	0.36292	12.0	0.157	0.4071	0.4326	10.77	11.44
0.37755	0.02263	0.35492	17.0	0.233	0.6172	0.6565	16.32	17.36
0.36414	0.03671	0.32743	15.7	0.214	0.5877	0.6535	15.54	17.28
0.32442	0.03009	0.29433	14.0	0.185	0.5702	0.6285	15.08	16.62
0.37086	0.02678	0.34408	16.8	0.232	0.6256	0.6743	16.55	17.83

mean ($\bar{x}$) = 14.76 ± 0.86
95% confidence = 14.76 ± 1.7

mean ($\bar{x}$) = 16.07 ± 0.96
95% confidence = 16.07 ± 1.8

TABLE 16. Calorimeter results. Calculation of the energy content of macerated grass from cow grazed pastures before fungal succession.

Sample weight (Grams) A.	Ash weight (Grams) B.	Ash Free dry weight (Grams) A - B	Corrected Galvanometer reading (x - 2)	Wt. Benzoic Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as 1 gram of macerate	Wt. Benzoic Acid giving equal reading as 1 gram of ash-free macerate	Energy Content of Sample KJ/g	Energy Content of ash-free Sample KJ/g
0.36019	0.0244	0.3367	17.7	0.243	0.6746	0.7217	17.84	19.08
0.3060	0.01198	0.2940	14.0	0.185	0.6046	0.6292	15.99	16.64
0.3196	0.0100	0.3096	15.5	0.210	0.6571	0.6783	17.38	17.94
0.3639	0.01607	0.3478	17.9	0.246	0.6760	0.7073	17.88	18.71
0.3501	0.01499	0.3351	17.00	0.233	0.6655	0.6953	17.60	18.39
0.3374	0.01232	0.3251	16.9	0.232	0.6876	0.7136	18.19	18.87

mean ($\bar{x}$) = 17.48 ± 0.32
95% confidence = 17.48 ± 0.63

mean ($\bar{x}$) = 18.27 ± 0.37
95% confidence = 18.27 ± 0.72

TABLE 17. Calorimeter results. Calculation of the energy content of macerated grass from cow grazed pastures after fungal succession.

Sample weight (Grams) A.	Ash weight (Grams) B.	Ash free dry weight (Grams) A - B	Corrected Galvanometer reading (x - 2)	Wt. Benzoic Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as 1 gram of macerate	Wt. Benzoic Acid giving equal reading as 1 gram of ash-free macerate	Energy Content of Sample KJ/g	Energy Content of ash-free Sample KJ/g
0.31521	0.031	0.28421	14.5	0.193	0.6123	0.6791	16.19	17.19
0.2990	0.02691	0.27209	12.0	0.157	0.5251	0.5770	13.88	15.26
0.30917	0.04172	0.26745	12.5	0.163	0.5272	0.6094	13.94	16.12
0.2900	0.03652	0.25348	10.5	0.128	0.4414	0.5049	11.67	13.35
0.31854	0.05268	0.26586	12.0	0.157	0.4929	0.5905	13.04	15.62
0.3273	0.03812	0.28918	13.20	0.171	0.5224	0.5913	13.79	15.64

mean ($\bar{x}$) = 13.75 ± 0.6
95% confidence = 13.75 ± 1.18

mean ($\bar{x}$) = 15.66 ± 0.61
95% confidence = 15.66 ± 1.19

TABLE 18. Calorimeter results. Calculation of the energy content of macerated grass from horse grazed pastures before fungal succession.

Sample weight (Grams) A.	Ash weight (Grams) B.	Ash-free dry weight (Grams) A - B	Corrected Galvanometer reading (x - 2)	Wt. Benzoic Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as 1 gram of macerate	Wt. Benzoic Acid giving equal reading as 1 gram of ash-free macerate	Energy Content of Sample KJ/g	Energy Content of ash-free Sample KJ/g
0.36142	0.01312	0.3483	18.8	0.260	0.7194	0.7465	19.03	19.74
0.30895	0.01174	0.29721	15.3	0.205	0.6635	0.6897	17.55	18.24
0.35212	0.01397	0.33815	16.9	0.233	0.6617	0.6890	17.50	18.22
0.32856	0.0110	0.31756	15.5	0.209	0.6361	0.6581	16.82	17.41
0.34336	0.0140	0.32936	17.00	0.233	0.6786	0.7074	17.95	18.71
0.36062	0.01362	0.34699	17.5	0.241	0.6683	0.6945	17.68	18.37

mean ($\bar{x}$) = 17.75 ± 0.30
95% confidence = 17.75 ± 0.58

mean ($\bar{x}$) = 18.45 ± 0.31
95% confidence = 18.45 ± 0.60

TABLE 19. Calorimeter results. Calculation of the energy content of macerated grass from horse grazed pastures after fungal succession.

Sample weight (Grams) A.	Ash weight (Grams) B.	Ash free dry weight (Grams) A - B	Corrected Galvanometer reading (x - 2)	Wt. Benzoic Acid to give same deflection of Galvanometer (Grams)	Wt. Benzoic Acid giving equal reading as 1 gram of macerate	Wt. Benzoic Acid giving equal reading as 1 gram ash-free macerate	Energy Content of Sample KJ/g	Energy Content of ash-free Sample KJ/g
0.34812	0.05393	0.29419	12.8	0.168	0.4826	0.5711	12.76	15.11
0.39448	0.06128	0.3332	14.8	0.200	0.5070	0.6002	13.41	15.87
0.35900	0.05142	0.30758	13.3	0.174	0.4847	0.5657	12.82	14.96
0.36749	0.05510	0.31239	13.4	0.175	0.4762	0.5602	12.59	14.82
0.36204	0.05569	0.30635	14.5	0.193	0.5331	0.6300	14.10	16.66
0.35960	0.04251	0.31709	12.9	0.169	0.4699	0.5330	12.43	14.10

mean ($\bar{x}$) = 13.02 ± 0.26
95% confidence = 13.02 ± 0.51

mean ($\bar{x}$) = 15.25 ± 0.36
95% confidence = 15.25 ± 0.70

TABLE 20. The results of inoculating fungi on fresh and autoclaved macerated grass for the purpose of determining fruiting body formation. (Experiment undertaken for a period of 30 days).

	Whether fruiting bodies were produced after inclubation on the substrate	
Fungal inoculum	Macerated grass	Autoclaved macerated grass
Pilobolus kleinii spores	X	X
Coprinus cinereus spores	X	✓
Coprinus cinereus sclerotia	✓	✓
Coprinus cinereus spores - *Pilobolus kleinii* spores	X	X
Phycomyces blakesleeanus spores	X	✓
Thamnidium elegans spores	X	✓
Syncephalastrum racemosum spores	X	X
Podospora setosa spores	X	X
Ascophanus carneus mycelium	X	X
Ryparobius polysporus mycelium	X	X
Sordaria fimicola spores	X	X
Ctenomyces serratus spores	X	X
Coprinus hexagonosporus mycelium	X	X

X = indicates no fruiting bodies observed.

TABLES 21 & 22 Percentage Germination* of Pilobolus kleinii spores incubated on filter paper moistened with water soluble extracts of (i) Dung, and (ii) Macerated Grass

(i)

	Percentage of Pilobolus kleinii spores, observed under the microscope, which have germinated	
Period of Observation	Sporangia ground before incubation	Sporangia not ground before incubation
Up to 7 days	0	0
Up to 14 days	0	0

(ii)

	Percentage of Pilobolus kleinii spores, observed under the microscope, which have germinated	
Period of Observation	Sporangia ground before incubation	Sporangia not ground before incubation
Up to 7 days	0	0
Up to 14 days	0	0

* Percentage germination is expressed as a percentage of 100 spores observed under the microscope using a x 40 magnification

The different mycofloras that developed on the dung and grass macerate samples can be compared by calculating similarity coefficients. The data does not really lend itself to rigorous analysis, but Adansonian Similarity Coefficients can be easily calculated by directly comparing the different fungal species. Adansonian Similarity Coefficients were devised by Michel Adanson for use in the numerical taxonomy of bacteria (Ainsworth & Sneath, 1962). The assumption made is that each phenotypic character is given equal weighting so that it is possible to express numerically the taxonomic distances between organisms, in terms of the number of characters they share, relative to the total number of characters examined. The phenotypic characters used here can be substituted for the fungal species observed according to the formula:

$$\text{Similarity Coefficient } (S_J) = \frac{a}{a + b + c}$$

Where, a = Number of species positive to both samples

b = Number of species positive to sample 1, negative to sample 2

c = Number of species positive to sample 2, negative to sample 1

It is difficult to compare the samples in terms of the numbers of fruiting bodies developing on the dung or macerated grass. Percentage cover of sporophores on one day may be exactly the same 'individuals' as seen on the previous day, or

the fruiting bodies from the previous day may have disappeared and others have been produced in their place. A simple approach though, is to calculate the percentage of similarity. This is based on a comparison of the make-up of the two samples in terms of individuals of the various species; because of this it places the emphasis on the dominant species (Raabe, 1952).

Percentage similarities in this study were calculated by using the highest percentage cover value for a particular species on a certain sample. This is not strictly accurate but does give a value that has some relevance to the interpretation of the results. Raabes' value of percentage similarity for a pair of samples is given by the summation of the smaller values of the percentage of the total individuals, i.e.

$$\% \text{ Similarity} = \sum \min (a, b, \ldots . x).$$

Tables 23 and 24 show the calculated similarity coefficients, in these tables sheep, cow and horse macerate refers to macerated grass from pastures grazed by these animals.

Data concerning the period of time required for the production of fruiting bodies was collected for species of fungi occurring on both samples of dung, and also macerated grass from the herbivore grazed pastures.

The experimental design of this study was not produced with the comparison of the time required for fruiting in mind. The dung and macerated grass samples were set up in different separate trays, this was so that fungi occurring on one type

TABLE 23. Percentage Similarities for dung and macerated grass.

Cow Dung.						
Cow Macerate.	27					
Horse Dung.	33	48				
Horse Macerate.	17	85	71			
Sheep Dung.	63	45	44	34		
Sheep Macerate.	31	86	75	98	57	
	Cow Dung.	Cow Macerate.	Horse Dung.	Horse Macerate.	Sheep Dung.	Sheep Macerate.

TABLE 24. Adanson Similarity Coefficients for dung and macerated grass.

Cow Dung.						
Cow Macerate.	0.31					
Horse Dung.	0.21	0.5				
Horse Macerate.	0.25	0.45	0.45			
Sheep Dung.	0.34	0.33	0.33	0.4		
Sheep Macerate.	0.25	0.42	0.42	0.66	0.66	
	Cow Dung.	Cow Macerate.	Horse Dung.	Horse Macerate.	Sheep Dung.	Sheep Macerate.

of sample could not shoot their spores onto the other type of sample. These spores could possibly germinate on the other sample and produce erroneous results. If the time required for fruiting was to be compared between dung and grass samples then the samples should have been set up with regard to a randomised block design. That is, the dung and macerated grass samples should have been arranged together in one tray during incubation. This is done because of the physical heterogeneity of the environment. If samples were in separate trays then sunlight, for instance, could fall for a different time on one tray compared with the other, even though the trays are only a short distance away. On analysis of the results, a statistician would say that if there was a difference in the time required for fruiting between the dung and grass samples, then this could be due to the different periods of sunlight falling on them, and not to the physical and chemical conditions within the samples. When considering the results present, the fact that the samples were not 'randomised' must be borne in mind.

The Median Test was used to see whether fungi occurring on the two types of sample had the same median number of days before production of fruiting bodies. The null hypothesis (H_o) is that the two samples, dung and grass, have the same median. The alternative hypothesis may be that the median of the population of fungi occurring on macerated grass is different to that of fungi on dung (a two-tailed test.).

To perform the median test, the median number of days before the production of fruiting bodies for the combined

group is determined (i.e. the median for all scores in both samples). Both sets of scores are then dichotomised at that combined median and these data are cast in a 2 x 2 contingency table.

To test the null hypothesis the Fisher Exact Probability Test is used. The exact probability of observing a particular set of frequencies in a 2 x 2 contingency table, when the marginal totals are

	number of samples equal to or below combined median (days)	number of samples above combined median (days)	Totals
Grass	A.	B.	A + B
Dung	C.	D.	C + D
Totals	A + C	B + D	

2 x 2 contingency table

regarded as fixed is;

$$p = \frac{(A + B)!\ (C + D)!\ (A + C)!\ (B + D)!}{N!\ A!\ B!\ C!\ D!}$$

The exact probability of the observed occurrence is found by taking the ratio of the product of the factorials of the four marginaltotals to the product of the cell frequencies multiplied by N factorial. For a two tailed test this probability value is then doubled. In the results, sheep, horse and cow macerate refer to macerated grass from pastures grazed by these animals.

(i) Latent period before production of sporangiophores of Pilaira moreaui

	2	3	no. of days ⟶
Number of samples of sheep dung	16		
Number of samples of sheep macerate		15	median = 2 days

$p = 0.33 \times 10^{-8}$ (1 tailed)

$p = 0.66 \times 10^{-8}$ (2 tailed)

This value is significant so reject the null hypothesis.

	1	2	no. of days ⟶
Number of samples of horse dung	16		
Number of samples of horse macerate		15	median = 1 day

$p = 0.33 \times 10^{-8}$ (1 tailed)

$p = 0.66 \times 10^{-8}$ (2 tailed)

This value is significant, so reject the null hypothesis.

(ii) Latent period before production of sporangiophores of Pilobolus kleinii

	2	3	4	⟶ no. of days
Number of samples of sheep dung	16			
Number of samples of sheep macerate		13	2	median = 2 days

$p = 0.33 \times 10^{-8}$ (1 tailed)

$p = 0.66 \times 10^{-8}$ (2 tailed)

This value is significant, so reject the null hypothesis.

	2	3	4	——› no.of days
Number of samples of horse dung	13	2		
Number of samples of horse macerate		1	3	median = 2 days

p = 0.0039 (1 tailed)

p = 0.0078 (2 tailed)

This value is significant, so reject the null hypothesis.

	3	4	5	6	7 ——› no. of days
Number of samples of cow dung	12				
Number of samples of cow macerate		7	1	5	4 median = 4 days

p = 0.00097 (1 tailed)

p = 0.0019 (2 tailed)

This value is significant, so reject the null hypothesis.

(iii) <u>Latent period before production of sporangiophores of Mucor mucedo</u>

no.of days ——›	5	6	7	8	9	10	11	12	13	14	15	16
Number of samples of horse dung	2			3		3						
Number of samples of horse macerate				3	10				1			1

median = 9 days

p = 0.172 (1 tailed)

p = 0.34 (2 tailed)

This value is not significant, so do not reject the null hypothesis.

(iv) Latent period before production of sporangiophores of Mucor haemalis

	7	8	9	no.of days
Number of samples of horse dung	2	5	1	
Number of samples of horse macerate		5		

median = 8 days

p = 0.615 (1 tailed)

p = 1.23 (2 tailed)

This value is not significant, so do not reject the null hypothesis.

(v) Latent period before production of apothecia of Ascobolus furfuraceus

no. of days ⟶	12	13	14	15	16	17	18	19
Number of samples of sheep dung	1	2	2	1			4	3
Number of samples of sheep macerate	9							

median = 13 days

p = 0.0004 (1 tailed)

p = 0.0008 (2 tailed)

This value is significant, so reject the null hypothesis.

(vi) Latent period before production of fruiting bodies of Fungi Imperfecti

No. of days ⟶	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Number of samples of sheep dung	3		1	2	1	1									1	1
Number of samples of sheep macerate				14												

median = 7 days

p = 0.0019 (1 tailed)

p = 0.0038 (2 tailed)

This value is significant so reject the null hypothesis.

	8	9	10	11 ——› no.of days
Number of samples of cow dung	1	3		4
Number of samples of cow macerate	1	8	1	2

median = 9 days

p = 0.199 (1 tailed)

p = 0.398 (2 tailed)

This value is not significant, so do not reject the null hypothesis.

DISCUSSION

The amount of fungus present on the samples of dung and macerated grass was estimated by observing the percentage cover of the sample surface area by the fungal fruiting body. This value may not relate to the amount of biomass of fungal mycelium within the sample itself. Although it may be that the fungus that has amassed more reserves, and produced more mycelium will thus produce more fruiting bodies, but this is a gross oversimplification.

Mycelial growth in the dung can be estimated by direct observation, such as the method of Dickinson and Underhay (1977). They took cores from cow dung, and homogenised samples from these in a waring blender, diluted them with water and made a dung agar mixture. The well of a haemocytometer slide was then filled with this mixture. When this was set, the film was floated off in water, stained with Phenolic aniline blue, cleared in Euparol essence and mounted in Euparol for observation under the microscope. Although the amount of mycelium can be calculated, it is not possible to identify it to genus level, only to say whether it is septate or non-septate etc. Thus when looking at fungal mycelium 'one sees what one cannot identify', whereas with fruiting bodies 'one identifies what one cannot see' (i.e. the mycelium).

It may be difficult to relate the percentage cover values of fungi such as members of the Mucorales to those of the Agaricales. The mucorales tend to have thin fruiting bodies, and when a value is given, then it means that most of the area of dung surface is covered by these fruiting bodies. A similar

figure for the Agaricales means only that the pileus covers this surface area of the dung. The stipe itself covers a much lower percentage surface area.

Although the method of cover analysis is 'borrowed' from plant ecological studies, it therefore does not have so much relevance for this sort of study. In microbial ecology in general, the description of communities seems to present many problems due to the differing scale and the degree of separation in space. Dimensions are extremely important, habitats can be of as little as a micrometre, or as large as a millimetre (Campbell, 1977). Also the dung communities cannot be classified according to the Braun-Blanquet system for higher plants (Braun-Blanquet, 1927). There are many reasons for this; the Braun-Blanquet classification describes various plant successional seres. But dung pats are a transient phenomenon, localised pockets of energy, that do not ultimately become more complex and stable in terms of their communities. A stable community only occurs when there is a continuous influx of energy and an efficient recycling of nutrients. Therefore different dung pats cannot be compared to see which has the most number of species and so the highest diversity and stability (Elton, 1958). The relationship between increased diversity and stability is very dubious anyway and certainly is not applicable here.

Successions of fungal fruiting bodies occurred on all the samples of dung and macerated grass, indicating that the laboratory conditions were acceptable for fungal growth and development. Examples of classic coprophilic fungal successions

occurred on both the macerated grass and dung samples, confirming initial suspicions that coprophilic fungi could grow on macerated decaying grass. The statistical tests used in determining whether there was a difference in the time before fruiting bodies were produced on dung and macerated grass proved that in many cases there was a significant difference. This is to be expected because dung and macerated grass are quite different in terms of their physical and chemical properties.

None of the fungi that appeared on the samples were exceptionally uncommon, although it is doubtful whether they could be observed on decaying dung in the field at the same time because of weather conditions causing drying of pats, and thus unsuitable conditions. The species that were observed in this study could be called fairly typical coprophilic representatives. On incubation of the macerated grass used as the control, i.e. the sample collected where no large herbivores were known to graze, the developing mycoflora was dominated by the imperfect fungus Oedocephalum glomerulosum. The work of Massee and Salmon (1902) mentions records of this fungus found growing on horse dung. Included in this work is a reference to Oedocephalum glomerulosum found growing on the branches of trees, but this observation is reputed to be a false one. It is stated that in this country the fungus has been met with only on dung. More up to date literature on the habitat relations of this species has not been found.

This information is interesting because it means that coprophilous fungi are even appearing on the macerated grass

used as the control. This highlights the suitability of macerated grass for the growth of coprophilous fungi and provides more evidence to cast doubt upon the degree of niche specialisation of many coprophilous species.

The Adansonian Similarity Coefficients calculated were quite useful in that they indicated that the mycofloras of the different dung types were most unlike each other in terms of the different fungal species occurring. This indicates that different fungal species may be suited to growth within certain dung types. This has been highlighted by Richardson (1972) who analysed the occurrence of ascomycetes on 137 samples of sheep, horse, cow, roe deer, rabbit and hare dung. He concluded that many dung fungi were cosmopolitan, while others such as Cheilymenia spp, Coprobia granulata, Ascobolus immersus, A.furfuraceus, Ascophanus microsporus, Lasiobolus ciliatus, and Podospora curvula were confined to ruminant dung. Sporormia bipartis, Coniochaeta spp, Thelebolus stercoreus and Podospora appendiculata were found to be mainly confined to lagomorph dung. Observations such as these cannot be made from this study because too few samples were taken and observed. Richardson considered that the factors responsible for the occurrence of these specialised groups were: the physical nature of the dung; its consistency, and the other organisms which develop on and in it.

The discomycete Coprobia granulata was found only on cow dung in this study, it did not develop on macerated grass from cow grazed pastures. This is the type of dung with which it is most commonly associated. Indeed, it is well known that

if there are a mixture of different herbivores grazing in a field, including cows, sheep and horses, then it is more than likely that Coprobia granulata will be found only on cow dung. This leads to the question as to whether there is a specific nutritional factor found to an adequate concentration only in cow dung, that this fungus requires.

Richardson found also that Ascobolus furfuraceus occurred most frequently on cow dung. In this study it occurred on cow dung but seemed to occur in most profusion on the macerated grass from the sheep grazed pastures. This result would seem to lend some doubt towards the suggestion that A.furfuraceus is specialised towards occurrence on ruminant dung. Richardson's study also indicated that Cheilymenia coprinaria was found most commonly on sheep dung; it was not observed on sheep dung in this study but was found on the macerated grass from sheep grazed pastures. This result may have occurred just by chance, that is the grass the sheep ate did not have associated with it any C.coprinaria spores, so that none were voided in the faeces. There is also the possibility that the ingested spores were inviable. Other sheep dung collected from the same pasture would perhaps have developed colonies of C.coprinaria upon incubation. For the fungus to have developed on the macerated grass means that it was probably growing at some time on sheep dung in the same pasture; it is doubtful if spores had blown onto the grass from long distances away by the wind.

Pilobolus kleinii germinated and produced sporangiophores on all the various samples of dung and macerated grass. This data therefore confirmed the observations made by Roberts that

Pilobolus will grow on decomposing vegetation, a phenomenon that has not hitherto been recorded in mycological papers in Britain before.

Hesseltine, Whitehill et al. (1953) found that Pilobolus species required a growth factor present in dung, which they called Coprogen. Their observations showed that Pilobolus could not be cultured without dung extract. It was found that the Coprogen was being produced by bacteria, possibly as a waste metabolite.

This suggests that either (a) the same bacteria are growing in the decaying grass macerates and producing Coprogen, so that Pilobolus trophocyst formation can be initiated, or (b) that when growing on grass macerate Pilobolus does not require Coprogen. There is also the possibility that decomposition products of the grass can substitute for Coprogen.

The question may be posed 'why does Pilobolus not appear on decaying vegetation in the field?' This question may simply be answered by examining the gross physiology of the sporangium. Observation of a mature sporangiophore shows that the sporangium is attached to the top of the ovoid bulb; below the sporangium is a ring of mucilage which is revealed when the top of the sporangiophore wall ruptures.

When the sporangial projectile is liberated, then it travels in a drop of cell sap, attached to the ring of mucilage. When this drop strikes an object such as a leaf of some vegetation, the sporangia rises to the top of the sap because it is hydrophobic, being studded with minute project-

ing crystals of calcium oxalate. When the sap evaporates then the sporangium becomes tightly attached to the leaf surface by means of the mucilage which has dried. Thus the sporangium resembles a cap tightly enclosing thousands of spores. Buller (1931) has demonstrated how the sporangia are effectively attached by their mucilaginous bases to the herbage, so that in dry weather they cannot be detached from the herbage by vigorous winds and in wet weather heavy rain will not successfully dislodge them. The enclosed spores cannot be released to germinate until the sporangial wall has been removed or ruptured.

In this study the sporangial wall was removed when the grass samples were macerated. Maceration also served to distribute the spores fairly evenly amongst the grass. When a herbivore ingests vegetation then the sporangial wall may be ruptured by the teeth of the animal during chewing. The sporangial wall may also be affected by chemical conditions within the animal's gut. Digestion will remove the vegetation that the sporangium is attached to so that spores will be released in this way. Travel through the herbivore gut may therefore 'stimulate' germination in two ways, by way of removing and rupturing the sporangium and also by chemical factors removing dormancy. This latter point has still not been disproved and must be borne in mind.

Perhaps then, in the field, if the sporangial wall was ruptured and spores were released, then these could germinate and grow on dead or decaying vegetation providing that conditions of say, humidity were correct. Thus if these actually occured on senescent vegetation, they would produce sporangia which would be shot off onto herbage, so that the inoculum of Pilobolus spores ingested by the herbivore could

be from two different sources. This has not been observed in nature so far. Pilobolus may have sacrificied quick propagation on senescent vegetation in favour of prolonged viability of sporangium-protected spores.

Apart from the macerated grass from cow grazed pastures, the other grass macerates resembled their respective dung types in terms of fungal species to quite a high degree. This is the result to be expected if coprophilic fungi can also grow on decaying vegetation. High similarity coefficients were also found when comparing the mycofloras of the different grass macerates together, showing that maybe they have certain species in common that are well adapted for growth on decaying grass.

Inthe analysis of fungal populations colonising herbivore faeces, similarity indices have also been used by Angel and Wicklow (1975). They used a different index of similarity, one which was described by Sorensen (1948). Their studies indicated that the highest index of similarity was associated with cattle and pronghorn faeces and concluded, as described previously, that this was because both animals are ruminants, therefore the fungi were associated with ruminant faeces.

The values for percentage similarities that were calculated are probably not so useful a concept for this study as the similarity coefficients. Percentage similarities only indicate which samples had the same maximum numbers of certain species at a particular time. These values only really show which samples had equal maximum numbers of mucoraceous and imperfect fungi, the other fungal types counting for very little as far as percentage cover of dung pat or grass macerate is concerned.

It is significant though that the highest values seem to be found when comparing the grass macerates. Other values when compared seem to be very varied and so are not revealing.

It is interesting and possibly significant that on each sample type examined, the number of species recorded only ranged from between 7 and 9. It could be that the sample can only really support this number of species, so that these most effectively decompose the substrate. More than 7-9 species may possibly create severe antagonism between species. Ikediugwu and Webster (1970) have demonstrated the effects of antagonism between Coprinus heptemerus and other coprophilous fungi. When studies on rabbit pellets and agar plates were carried out these workers found that the suppression of fruiting of Pilobolus crystallinus and Ascobolus crenulatus by C.heptemerus was by hyphal interference. This hyphal interaction caused intracellular changes in the other fungus such as loss of hydrostatic pressure, vacuolation and granulation of the cytoplasm. A preliminary investigation was made on the nutritional relationships between C. heptemerus and A.crenulatus and no evidence was obtained that the basidiomycete derives nutrients from the affected cells of Ascobolus. It was also shown that apothecia of A. crenulatus produced in the presence of C. heptemerus were smaller in size, ill-developed and lost activity earlier than those developed in its absence. It was however found that the Coprinus antagonism does not seem to affect the life span of those apothecia which succeeded in developing in its presence. These results confirmed earlier findings of Harper & Webster (1964) and suggested that the limitation of duration of fruiting of coprophilous fungi which

happens in the presence of C. heptemerus is a natural phenomenon.

Burns first noted that there were sporangiophores of Pilobolus which collapsed before discharging their sporangia. This affect was caused by hyphae of Coprinus, although germ tubes of Coprinus can have the same affect. No affected Pilobolus sporangiophores were observed during this study. This may mean that there was not enough mycelium in the vicinity of the Pilobolus. But Ikedingwu and Webster have also shown that direct contact with the hyphae of the antagonist is not strictly necessary for antagonism to occur. An interference factor has been shown to diffuse from the basidiomycete mycelium, so that other fungal mycelium some distance away may be affected. It would be interesting to find out the rate of diffusion of this interference factor in dung.

The data presented here do not lend themselves to analysis for antagonistic effects. It is difficult to relate the duration of certain phases, such as the mucoraceous phase to the percentage cover of Coprinus and again, the number of basidiomycete fruiting bodies may not relate to the amount of mycelium within the dung. On the grass macerate samples where only basidiomycete initials were formed, the mucoraceous primary sugar fungus phase seems to be of no greater period of duration than on other samples where a fairly high number of basidiomycete sporophores were formed. It is notable too that none of the ascomycete fruiting bodies formed during this study were ill-developed, indicating that there is little

evidence for severe antagonistic effects on samples in this study.

Dickinson and Underhay (1977) observed there to be an infrequent and erratic appearance of fungi on samples of cow dung in the field, and they concluded that their data only partially supported the thesis that there is a regular sequence of fungi fruiting on dung. Similarly, Wicklow and Moore (1974) also noted that there was an absence of certain groups of fungi from decomposing rabbit pellets. Data from this study shows there to be a paucity of Pyrenomycete perithecia observed on the dung and macerated grass samples. In the classic succession of fruiting bodies on dung the pyrenomycetes usually follow the discomycetes.

Sordaria fimicola was the only pyrenomycete observed and this only occurred on samples of the horse dung. Sordaria fruiting bodies appeared at a very late stage in the fungal decomposition of dung in this study, they appeared after the basidiomycete stage. Harper and Webster (1964) quote that 9 days had elapsed before fruiting of Sordaria fimicola on incubated dung. In this study 29 days had elapsed before fruiting finally occurred. This could be because of many reasons; (i) the antagonistic effects of Coprinus cinereus present in the horse dung, although it has been found that antagonistic effects apparently have little effect on the actual time of appearance of fruit bodies, but are only important in limiting the duration and intensity of fruiting, (ii) Nutritional Factors - it has been shown that Sordaria fimicola is deficient for biotin and requires an exogenous

supply of this vitamin for sporulation (Lilly and Barnett, 1951). This is shown if Aspergillus rugulosus and Sordaria fimicola are placed on an agar plate containing only trace amounts of biotin. Sordaria, being a faster grower, produces a sparse mycelium, which surrounds the slower growing colony of the Aspergillus. The mycelium of the Sordaria directly next to the Aspergillus colony soon shows stimulated growth, which is followed by the formation of many perithecia in this area. This stimulating effect has been attributed to the extra biotin, produced by Aspergillus rugulosus, diffusing into the medium. In the horse dung in this study it may be that there were only small amounts of biotin, and that associated coprophilic fungi that could synthesise biotin were not present.

Spores of Sordaria fimicola and the other pyrenomycete obtained, Podospora setosa, did not germinate and produce any perithecia within the period of 30 days when inoculated onto non-autoclaved and autoclaved macerated grass. This does not prove a lot, as many inoculation experiments ought to be carried out with a single species, but it may be that these species are not adapted for growth on macerated grass. Alternatively it could be that fruiting bodies may be produced after a much longer period of time than 30 days.

Of the cultures obtained from culture collections, the only fungi to produce fruiting bodies on the grass samples were Phycomyces blakesleeanus and Thamnidium elegans; the sporangiophores of these fungi occurred only on the autoclaved macerated grass. This failure with the other species may mean that microbial antagonism or competition could be preventing the germination of the spores and formation of fruiting bodies by Phycomyces and Thamnidium on unsterilised macerated grass.

The process of autoclaving may also be destroying chemical inhibitors of germination present in the grass. The failure with the other cultures was not further investigated.

Sclerotia of Coprinus cinereus germinated and produced fruiting bodies on both non-autoclaved and autoclaved macerated grass. These sclerotia were collected from the horse dung samples after fungal succession, the fact that they germinated shows that their period of constitutive dormancy was over. Linderman and Gilbert (1969), found that volatile chemicals from the initial stages of decomposition of plant remains appeared to act as stimulators for the germination of sclerotia of Sclerotium rolfsii. The same type of volatile chemicals may similarly be acting as stimulators for the germination of sclerotia of Coprinus cinereus. These sclerotia germinated on the non-autoclaved macerated grass; sclerotia are notverysusceptible to microbial antagonism because they possess a thick, resistant, pigmented rind on the outside. They are also not so dependent on nutrients within the grass samples because they contain quite large amounts of food reserves.

Fruiting bodies were produced on autoclaved macerated grass inoculated with spores of Coprinus cinereus. In a great many species basidiospores are hard to germinate, but under certain conditions the spores of some species, such as Coprinus cinereus germinate freely, (Ingold, 1979). Fruiting bodies were not, however, produced on the non-autoclaved macerated grass, this again could be due to microbial antagonism, or the process of autoclaving may be removing some chemical inhibitor present in the grass.

It may be useful to compare the fungi developing on macerated grass with those on decaying intact grass. Several studies on the latter subject have been documented. Webster (1956, 1957) observed the succession of fungi occurring on decaying Cocksfoot culms (Dactylis glomerata) during a two-year period following flowering.

Initial development on the culms follows attack by parasitic fungi, which enter the dying plant by one of three methods: (i) through stomatal openings, (ii) by direct penetration of herbaceous tissues. This involves entrance of the fungus through layers of wax, cutin, pectin, and a network of cellulose fibrils impregnated with other wall polymers before making contact with the living protoplasm (Dickinson and Lucas, 1977). Many fungal pathogens are known to be able to enter their hosts by these methods, and non-specific pathogens such as Botrytis can enter directly through the epidermal layer. The final method is; (iii) through wounds caused by other fungi, animals, or by direct damage caused by the wind, etc.

Succession of entry is dependent upon many factors, significant ones being the amount of food reserve present in the spore and also environmental conditions. High humidity is knownto greatly facilitate entry.

The primary flora following this parasitic attack consists chiefly of Hyphomycetes such as Cladosporium herbarum, Epicoccum purpurascens, Alternaria tenuis, Botrytis, and Pyrenomycetes such as Leptosphaeria microscopica and Pleospora vagans. The upper internodes of the grass culm are more suit-

able for rapid colonisation by this microflora and this could be due to the more complete lignification of the medullary parenchyma at the lower levels of the culm, this provides a substratum for slow growing fungi.

In contrast, an initial phycomycete phase occurred on all of the macerated grass samples. This was probably because the process of maceration released the simple soluble sugars present in the grass. These fungi would not be able to utilise these sugars from intact grass. This phase was of the same duration and intensity as that occurring on the samples of dung. These results indicate that macerated grass can therefore be added to Hudson's (1968) list of substrates which possess this unique initial phase. Why is it that the fungal succession that develops on intact grass does not occurr on the macerated grass? Spores of the typical primary hyphomycete microflora will almost certainly be present attached to the grass blades (phylloplane) and stems before they undergo maceration. This can be stated with confidence due to the results of air spora samples.

Many surveys have been undertaken to determine the fungal spore content of the air. Hirst (1953) showed that Cladosporium and Alternaria were part of the dry-air spora, and that they had afternoon maxima. While Richards (1956) exposed Petri-dishes at nine localities in Great Britain during April - October 1952. He recorded about 70 species of fungi, of which Cladosporium was the commonest. Cladosporium, Epicoccum, Botrytis, Alternaria, and Pullularia were relatively more important in rural areas than in cities, and Cladosporium

made up 69.6% of the catch while the second dominant in terms of numbers was Pullularia at 5.5%.

These spores will be deposited on most of the above-ground herbage by several ways: by sedimentation at terminal velocity under gravity; scrubbing of the atmosphere by rain; and by impactation, being blown by the wind against a projecting surface. The rules for deposition on leaves and stems, which are the same as those for deposition efficiency on bluff trapping surfaces, show that impactation is greatest on smaller leaf or stem surfaces facing the wind (Gregory, 1976). The stems and leaves of grasses, because they are so thin, provide a relatively small obstruction so that they will trap many spores.

Pugh and Buckley (1971) determined the percentage isolation of phylloplane fungi on monthly samples of 50 washed leaf squares. They found that during May, June, July and August, the period during which this present study was carried out, the percentage occurrence of Aureobasidium pullulans, Cladosporium herbarum, and Epicoccum nigrum were at its greatest, while maxima for Alternaria tenuis occurred during June, July and August. This is good evidence then that these spores will be present in the macerated grass.

Although fruiting bodies of these hyphomycetes were not observed, it could be possible that they were actively growing in the samples of macerated grass. Webster (1957) found that the primary hyphymycete phase only exhibited continued sporulation on the lower internodes. He explained this by saying

that the fungi might demand for growth some substance present in the decaying grass, which under the high humidity regime of the lower internodes is more rapidly depleted. Hudson and Webster (1958) modified this hypothesis slightly, and restated it in terms of substances necessary for sporulation rather than growth. This hypothesis might well apply to the macerated grass samples, but as yet it seems that workers have found little evidence in support of this hypothesis.

If hyphomycetes are not developing within the macerated grass, then this could be because of many reasons. The primary phycomycete phase of fungi, by utilising the simple sugars, may be restricting the germination of hyphomycete spores. The simplest reason though is that these fungi are not adapted for growth on this type of substrate.

It is interesting to note that hyphomycetes such as Pullularia pullulans, which is part of the initial flora on dying grass stems, breaks down plant tissues effectively itself by producing pectolytic enzymes and possibly lethal factors to kill the cells. The enzymes separate cells by degrading the pectic substances in the middle lamella between cells, thereby facilitating the colonisation of host tissues (Smit and Wieringa, 1953), the lethal factor will then kill the separated cells.

Later in the succession of fungi on Dactylis stems, when they have collapsed due to the action of the primary flora, they are colonised by other species. This flora is very varied and includes various Pyrenomycetes, Discomycetes, Hyphomycetes and Coelomycetes, with Helminthosporium hyalospermum, Tetraploa aristata, and Menispora ciliata being the dominant species. It is significant that very few basidiomycete species were recorded in the later phases during the two year study, although the distribution of mycelium within the tissues has suggested a capacity for lignin decomposition. On all of the samples of macerated grass in this study the final fungal phase included basidiomycetes, but only one species was identified.

Basidiomycete rudiments appeared on the samples of macerated grass from the sheep grazed pastures but they developed no further than this. The reason for this is probably not due to conditions within the grass pat, but due to external conditions. It is documented that light inhibits the growth of some rudimentary fruit bodies. Buller(1931) observed that numerous fruit-body rudiments of Coprinus sterquilinus came into existence all over the free surface of dung balls. He found that the rudiments which escaped abortion and ultimately developed into mature fruit-bodies always had their origins at or near the bases of the balls. Those rudiments that arise in well lighted positions are inhibited by the light from further growth. However, once the fruiting bodies have grown sufficiently large to push upwards into light, owing to an internal change they become immune to the sun's inhibitory action. The fruiting bodies

on the macerated grass samples may have been exposed to an inhibitory concentration of light so that their growth was checked. Another possibility could have been that the samples used were of a size too small to provide necessary reserves for sporophore production.

Incubation of spores of Pilobolus kleinii in water extracts of cow dung and macerated grass was carried out over a period of two weeks. The experiment was set up to see if free spores, and spores still enclosed by their sporangia could germinate in these extracts.

Percentage germination was assessed over this period. Germination is the act of protrusion of the germ tube from the spore wall. The exact moment when this occurs is probably impossible to determine, although a close approximation can be made by using the electron microscope. The arbritary criterion of germination used in this study was that used by Manners (1949) who defined a spore as germinated when the germ tube is as long as it is broad.

Initial growth of Pilobolus kleinii should be favoured by short latent periods of germination and high rates of germ tube extension. Pilobolus is a phycomycete and must make quick use of the ephemeral soluble sugars present in the substrate. However, none of the spores incubated in the various extracts were observed to have germinated. This result is the opposite to that of Harper and Webster (1964) who found that there was a 90.5 percentage germination of spores of Pilobolus kleinii treated in water and incubated on potato dextrose agar after 20 hours. It would be expected that after incubation in the extracts the spores would have germinated, because sporangiophores of Pilobolus kleinii

appeared on many of the samples of dung and macerated grass. Data from the incubation of macerated grass lends support to the theory that passage through the herbivore gut may not be necessary to break spore dormancy. The results from the germination experiment though tend to contradict this assumption. How can this problem be solved? If it is assumed that the spores collected were viable, then reasons why these spores did not germinate can be speculated upon.

Dormancy in fungus spores can be of two kinds; exogenous and constitutive (Sussman, 1965). Exogenous dormancy occurs when development is delayed because of unfavourable chemical or physical conditions of the environment. It may be that certain nutritional factors required by Pilobolus spores to germinate were not provided in the water extract, or were not extracted in sufficient quantity. Constitutive dormancy is a condition wherein development is delayed because of an inbuilt characteristic of the dormant stage. This could be a metabolic block, a barrier to nutrients, or the production of a self inhibition. The spores used in the germination experiment were incubated in the grass and dung extracts about two weeks after they had been shot from the sporangiophores. The spores germinating on the macerated grass collected from the field may have been lying attached to vegetation for a long period of time, probably for several months, so that there would no longer be a constitutive period of dormancy.

The most likely reason for no germination is probably self-inhibition. This phenomenon is most apparent when high concentrations of spores fail to germinate. An early observa-

tion of this was made by Edgerton (1910) who noted that when 12 to 15 conidia of Colletotrichum lindemuthianum were included in 1 cubic millimetre of medium the percentage of germination was reduced. The chemical nature of the inhibitors(s) is not known for most spores, but some suspected inhibitors have been isolated from urediospores of Puccinia graminis tritici.

The concentration of free spores incubated in the extracts in this experiment was not determined, but was very high, there was little free distance between neighbouring spores. This experiment should have been performed using different concentrations of spores in the extracts. Self inhibition is probably, if not certainly, the reason why spores would not germinate within the sporangia of Pilobolus.

ENERGY DETERMINATIONS.

Initial Energy Content Of Samples.

When compared with each other, the different types of dung had approximately similar values for initial energy content per gram of dry weight. This was not the case however for the various samples of macerated grass from different sites.

Dickinson,Underhay and Ross (1981) found that cow-pats used in their experiments had an energy content of about 11-12 kJ per gram of dry weight. Cow-pats used in this study had an initial energy content of about 16 kJ per gram dry weight. That this figure differs substantially from that determined by Dickinson et al (1981) may be for some of many reasons. Cows, and indeed all of the herbivores considered here, may differ as regards the

physiological working of their individual digestive systems. Also, different types of grass are found in different pastures, and these species probably differ somewhat in their energy content. An important consideration is the season at which the experimental work was carried out, because plants differ in their energy content according to the time of year.

The Use Of Energy By The Mycoflora.

Both Odum (1962) and Macfayden (1963) have emphasised that in many instances detritus chains may be important in that they can support substantially greater amounts of secondary production and thus account for a greater portion of the energy flow through a system.

The information obtained from the energy determinations of the samples before and after fungal succession is interesting, but unfortunately, by itself, is not revealing about the energy flow, and the use of energy by the mycoflora.

Conclusions cannot be drawn about the energy obtained by the fungi, without taking into account of what has happened to a given mass of sample. The important value to find (and the one that was not determined during this study) is the amount of weight loss of a sample over the time period of the fungal succession. The total amount of energy used by the fungi cannot be determined because the fungal mycelium that is produced is oxidised in the calorimeter, but this weight loss is mainly the proportion of organic matter respired by the mycoflora and bacteria.

Although it could be calculated that the dung and grass samples lost xkJ of energy over the time period, this

would only be an average value and means little. From these results, what can be said is that the energy content of 'old' sheep dung is very similar to that of fresh sheep dung, while all the other values for fresh and 'old' dung samples differed to quite a large extent. The values for all the samples of fresh and 'old' macerated grass also differed.

Some of the individual readings for the energy content of the dung and grass samples after the fungal successions on them are higher than those of the fresh samples. The reason for this might be explained by assuming a relatively greater consumption of less energy rich materials in the sample. For example, take a hypothetical situation:- If the fresh dung were composed of 90% carbohydrate, and 10% fat, and if the fungus that grew on it digested most of the carbohydrate, forming a little fungal tissue, carbon-dioxide, and water, and leaving most of the 10% of fat undigested. Then, if this sample were burnt in the calorimeter, then it would be found that the energy value per gram of dung plus mycelium increased. A similar situation would arise if carbohydrate were utilised and largely converted to fat in the mycelium.

Even the simplest studies in microbial ecology are demanding and time consuming and this study was no exception. Sufficient time was not available for studies on the energetic processes in more depth, and the question of energy flow and the use of energy by the mycoflora had to be left unanswered.

In this discussion what can be done is to suggest what further experimentation would have been carried out if sufficient time had been available.

A substantial set of samples of dung and macerated grass

would be taken from the grazed field under study. These aliquot samples would all be of similar determined fresh weight. Some of the samples would be taken and dried in the oven under standard conditions to constant weight. Then the energy content per gram dry weight would be found using the bomb calorimeter. The other samples placed on separate aluminium dishes of known weight would be covered by simple net tents to protect them from the effects of flies and other insects. The samples would be kept moist by adding sufficient water to prevent drying up. The fungi appearing on the samples would be identified in the usual way, subculturing them if necessary. Then, every four days, one of the samples would be removed. The fresh and dry weight would be determined and the energy content per gram of dry weight found using the bomb calorimeter. This procedure would be carried out until the succession of fruiting bodies had finished, that is, right past the basidiomycete stage. Because a given mass of sample is being used, and the weight loss is known, then calculations will be able to show the amount of energy that has been lost over the period of incubation. It would be necessary to avoid loss of substantial quantities of spores or falling fruit bodies.

To take a hypothetical example; suppose the initial dry sample weighed 10 grams and that the initial energy content per gram dry weight was 12 kJ, so that the initial total energy content of the sample would be 120 kJ. Then, suppose that the reduction in dry weight over a time period of four weeks may have been 2 grams, and that the energy content of the sample at the end of this period had been determined to be 13 kJ per gram of dry

weight. Then the total energy content of the material at the end of the period would therefore be 104 kJ ((10-2)x 13), so that 16 kJ (120-104) of energy would have been consumed as metabolic energy in respiration and 104 kJ left as the energy content of the residue. To quote an example of work already carried out, Dickinson et al (1981) found that about 1.82 kJ of energy were lost per gram of dung after eighty days. Their experiments were carried out in an outside enviroment as compared with these laboratory experiments. Thus, decomposition of dung under fairly strict conditions of moisture regime and temperature can proceed much more efficiently and quickly in the laboratory, even without the physical effects of earth worms and dung beetles, which would be present in an external enviroment. An important point concerning cow-dung is that it dries out very quickly and develops a tough resistant external crust. This crust can markedly affect the rate of disappearance of cow-dung in the field (Holter,1979). During the summer months there are often no fungal fruiting bodies seen on the dung in the field and the persistence of cow-pats is often of great trouble to farmers and landowners.

The results obtained from a study on the energy loss of the samples with time could be coupled with the study of the species of fungi observed on the samples. The fruiting bodies of a species may be seen on a sample at a certain time and the energy content of the dung at that time could then be determined. It would not however, be valid to assume that this fungus had made use of a large part of the energy lost from the dung because other fungi are growing and respiring in the mass of the sample even though their fruiting bodies may not be visible.

Even though the loss of energy from the dung and grass samples during the experimental period might be found, this cannot be solely attributed to fungi. Bacteria are also present, and probably in vast numbers, so that loss of carbon and energy occurs by bacterial metabolism. 1 gram of cow faeces has been estimated to contain 63 to 80 million bacteria. Using the plate method, manure of stall-fed cattle was found to contain between 1 and 120 million of bacteria per gram, while that of cattle on pasture contained only 1 to 4 million (Gruber, 1909). These figures are underestimates of the true numbers of bacteria present, numbers found will vary according to the method used for their estimation. The bacterial content of straw has also been found to vary greatly, 74 thousand to 11,540,000 bacteria were found per gram of straw (Hoffman,1908). Numbers per gram of hay may vary between 10 and 400 millions per gram (Duggeli,1906). Myxomycetes and actinomycetes may also be present on the samples of dung and grass although they were not directly observed by fruiting body presence. Fungi are efficient decomposers of the dung because they can ramify throughout the whole substrate. Cellulolytic fungi have the advantage over cellulolytic bacteria which can only accomplish breakdown by enzymic action at a free surface (Hudson,1972). However, the actual rate of contributions of the different organisms has not yet been assessed.

Invertebrates may also be responsible for some energy loss; it was observed that some mites and also large numbers of nematodes were present on most of the samples of faeces. Although the dung was collected freshly voided from animals, flies were found to have emerged from pupal cases on the surface of the samples.

Leaching of nutrients may be occuring from some of the faeces during watering. In the field, soil beneath dung pats soon becomes enriched after the dung is deposited. MacDonald and Watkin (1972) have found that levels of nitrogen, phosphorus and potassium in a 0 to 1 cm layer of the soil immediately beneath a dung pat increased markedly over the first ten days after the pats were deposited. Dickinson, et al, (1981) determined that more substantial falls in dung mineral content were observed in those treatments where the dry weight losses were also large. Thus, high levels of leaching are associated with high levels of dung breakdown.

The export of fungal spores accounts for a proportion of the loss of energy and nutrients, although this is fairly small. Pilobolus kleinii, Mucor species, and Pilaira moreaui produce many sporangia. In the case of Pilobolus these are fired from the sporangiophores by an efficient mechanism (Page, 1964). The turgid sporangiophore bursts along a predetermined circular line of weakness just below the sporangium, and then the stretched wall contracts squirting out liquid contents with the sporangium on top, up to a distance of about two metres (Buller, 1931., Ingold, 1971). The average weight of a single sporangium of Pilobolus kleinii was found to be about 0.14×10^{-3} grams. Assuming that on one of the substrate pats about a thousand sporangiophores could be produced, this means an export of sporangia around somewhere in the order of only 0.14 grams from about 10 grams of sample.

An insight into the fate of the organic matter of the dung or macerated grass would be provided if studies on the chemical composition of the samples could be performed

after successive intervals of time. This would involve determining: (a) the dry weight of the soluble fraction (cold and then hot water); (b) the dry weight of extractable fat (ether soluble); and (c) the dry weight of residual matter (cellulose, hemicellulose, lignin). Studies could also be made on the amount of insoluble and soluble organic nitrogen present in the samples at successive stages.

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