Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
12(1): 220–237 (2022) ISSN 2229-2225
www.creamjournal.org **Article**
Doi 10.5943/cream/12/1/14

Dispersal distances of dung fungal spores: an *in vivo* experimental setup

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Van Asperen EN, Brennan J, Reid A 2022 – Dispersal distances of dung fungal spores: an *in vivo* experimental setup. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 12(1), 220–237, Doi 10.5943/cream/12/1/14

Abstract

Spores of coprophilous fungi from sedimentary sequences are increasingly used to study past herbivore presence and abundance. Dung fungal spores are regarded as very local indicators of herbivore dung due to their short dispersal distances. Previous studies of dispersal distances used highly artificial set-ups that can inform us about the dispersal potential of individual species, but do not reflect the field situation very well. We present an *in vivo* experimental setup using dung as a substrate, allowing the fungi to grow and compete as in the field situation, with a more natural light source, to assess dispersal distances.

Active dispersal can propel spores or spore clusters to great distances (up to 2.5 m in *Pilobolus*). However, most spores travelled no more than ~20 cm from the dung source. Apart from *Pilobolus* sporangia, most spores are not propelled very high (~10 cm). Spores are overwhelmingly dispersed towards the light. The aerodynamic features (mainly spore size and cluster size) of the spores may have less impact on dispersal distance than initial velocity, thermal convection generated by the decomposition of the dung and wind, although spores are only sporadically found in air samples. Dung fungal spores can thus be regarded as highly local indicators of the presence of animal dung.

Keywords – active discharge – coprophilous fungi – palaeoecology

Introduction

Fungi in different taxonomical groups have developed a range of adaptations for actively dispersing their spores using ballistic discharge (Ingold 1971). These mechanisms are particularly prevalent in coprophilous fungi, whose primary substrate is animal dung. Since grazing animals often avoid feeding in areas around dung patches (Ödberg & Francis-Smith 1977, Forbes & Hodgson 1985, Lütge et al. 1995), it is important that these fungi propel their spores as far as possible. In coprophilous ascomycetes, sexual spores are produced in asci, which often contain a full spore complement of 8 (or a multiple of 8) ascospores when mature. The asci have an apical pore through which the ascospores are forcibly discharged, generally using turgor pressure in the ascus (Ingold 1971, Trail 2007, Yafetto et al. 2008). Some coprophilous zygomycetes also disperse their spores, or more accurately, their spore-containing sporangia, using turgor pressure (Yafetto et al. 2008). In contrast, in coprophilous basidiomycetes a different discharge method is found using Buller's drop, which results in spores travelling only a very short distance (Zoberi 1969), with air

Submitted 25 June 2022, Accepted 30 August 2022, Published 15 September 2022
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movements being the primary means of transporting spores further away from the fruit body (Deering et al. 2001, Pringle et al. 2005, Stolze-Rybczynski et al. 2009). All these discharge methods are aimed at propelling the spores beyond the dung source and onto the surrounding vegetation (Deacon 2006, Yafetto et al. 2008). Many coprophilous spores have mucilaginous surfaces or appendages which cause them to stick to the vegetation (Krug et al. 2004). The spores can then be ingested by herbivores along with the vegetation, pass through the animal's digestive system, and complete the cycle by being voided with the dung (Deacon 2006). It is unclear whether the passage through the animal's gut plays any role in the germination of these spores (Janczewski 1871, Massee & Salmon 1902, Krug et al. 2004), but they are rarely found to be active on other substrates (Bell 2005, Doveri 2007, Kruys & Wedin 2009, Guarro et al. 2012, Newcombe et al. 2016).

Over the past few decades, spores of coprophilous fungi from sedimentary sequences have been increasingly used to study past herbivore presence and abundance (e.g. Davis 1987, Davis & Shafer 2006, Baker et al. 2013, Van Asperen et al. 2021). Dung fungal spores from such palaeoecological samples are regarded as very local indicators of herbivore dung (Graf & Chmura 2006) due to their short dispersal distances. However, previous studies of dispersal distances used highly artificial set-ups that can inform us about the dispersal potential of individual species, but do not reflect the field situation very well. Ingold & Hadland (1959) grew a culture of the coprophilous species *Sordaria fimicola* on filter-paper yeast-extract agar and placed this in a rectangular box which was blacked out except for a small light hole at one end of the box. The cultures were placed such that the light shone directly on the surface of the culture, so that the spores were shot out from the asci horizontally and then fell vertically on to a horizontal glass slide. Using the same method, Ingold (1961) repeated the experiment for a wider range of ascomycetes, including two further species of *Sordaria*, *S. destruens* and *S. macrospora*, and another coprophilous ascomycete, *Ascobolus leveilli*. Using a very similar experimental set-up to that of Ingold & Hadland (1959), Walkey & Harvey (1966) included the coprophilous ascomycetes *Ascobolus stercorarius, Podospora tetraspora, P. decipiens* (8-spored, 16-spored and 64-spored varieties), *P. curvula, P. coronifera, P. fimiseda* and *S. macrospora*. Similarly, Yafetto et al. (2008) studied *Ascobolus immersus* and *Podospora anserina* as well as the coprophilous zygomycete *Pilobolus kleinii* in their version of this experiment.

Whilst these studies revealed the remarkable dispersal distances some of these fungi can reach, they examined individual fungal species, which does not account for the effect of competition on fungal growth, maturation or sporulation that would occur in natural environments. The fungi were grown on dung agar rather than dung itself, which may be more variable in the distribution of nutritious compounds. The box allowed for only a small amount of light from one direction, whereas in a natural environment, variable amounts of light would be available from different directions at different times of the day. Finally, spore discharge was horizontal, whereas in *in vivo* situations zygomycete and ascomycete fruit bodies grow more or less vertically. Spores are thus shot vertically in an effort to break through the boundary layer of still air covering the first few millimetres above ground level and surrounding the fungus (Trail 2007) into turbulent air, where the spores become airborne and travel a longer arc.

Here we present dispersal data from a range of fungi growing spontaneously from spores present in freshly voided dung of free-ranging large mammals. Since dung was used as the substrate, the fungi experienced similar competitive circumstances as in field conditions. The experimental setup simulated natural light conditions, rainfall and dew. This provides a more naturalistic assessment of the dispersal distances of dung fungi that are commonly found in palaeoecological contexts.

Materials & Methods

Freshly voided dung of free-ranging feral cattle originating from a local breed of domestic cattle (*Bos taurus*) was collected in sterilised containers from Chillingham Wild Cattle Park (Chillingham, UK) on 10 October 2014 (sample 1), 2 December 2014 (sample 2) and 15 October

2015 (samples 3 and 4), from African elephant (*Loxodonta africana*) from Knowsley Safari Park (Prescot, UK) on 22 April 2015 (sample 5), and from domestic horse (*Equus caballus*) from Stevenage, UK on 12 May 2016 (sample 6). The samples were stored for 2d in the dark at 4 °C. 97-143.5g of each sample was placed on moist paper towels in a sterilised glass dish. The samples were placed in a sterilised transparent glass tank, L = 60 cm, W = 30 cm, H = 30cm in size, covered with a transparent lid and placed with one end facing a window and the other end a room where the lights were kept off, mimicking the natural daylight conditions at a field location that is partly in the sunlight and partly in shade. The samples were incubated for 30-66d at 20 °C (Krug 2004). The samples were kept moist by periodically wetting the paper towel with distilled water, simulating the effect of rainfall and dew. The experiment was executed in a laboratory environment rather than outside to limit the number of variables to be assessed. If the dung samples had been placed outside, further variables such as soil type and drainage, slope, aspect, and insect and invertebrate disturbance would complicate the analysis.

To assess what proportion of spores were dispersed towards the lighter side of the tank and what proportion to the darker side of the tank, sample 1 was placed in the middle of the tank (Fig. 1a). Once per week, microscope slides made sticky with a thin layer of petroleum jelly were placed between the dish and the front of the tank, and between the dish and the back of the tank and left out to sample spores for 24 hours. The total length of the path sampled was 22.5 cm on each side of the dish. Since only a very small proportion of spores was dispersed towards the darker side of the tank (see Results), samples 2-6 were placed at the 'darker' end of the tank (Fig. 1b-c), with a total length of the sampling path of 43.0 cm. For samples 3 and 4, slides were also stuck on the 'dark-facing' wall of the tank to test heights to which spores were projected, up to 17.5 cm. These were left in place for the entire duration of the incubation. Slides were observed under a light microscope at 200-400x magnification. Spores were identified to genus, or, in some cases, to species based on spore morphology and the presence of fruitbodies of the same genus or species on the dung (see below) and their distance from the dung sources was noted down. It was assumed that groups of spores were expelled together from the asci, especially since these were often stuck together due to the presence of mucilaginous surfaces or appendages. The dung samples themselves were periodically examined using a stereomicroscope. Spore-producing fruit bodies growing on the dung were mounted in alcohol and lactophenol cotton blue, and identified under a light microscope. It was not possible to determine conclusively which dispersed spores belonged to which identified fruitbody, although spore and cluster size, spore ornamentation, and timing of growth and dispersal usually made it possible to correlate identified fruitbodies with particular types of spores. However, because of this residual level of uncertainty, some taxa are indicated as 'cf.' (Tables 1-2, Figs 2-7).

The distribution of dispersed sporangia, single spores and spore clusters was tested for normality with the Kolmogorov-Smirnov test where $n \geq 50$ and the Shapiro-Wilk test where $n < 50 \geq 10$. All tests were performed with the Statistical Package for the Social Sciences (SPSS) 26.

Results

The number of clusters analyzed per taxon for each sample is shown in Table 1, and mean, minimum and maximum overall dispersal distance and mean dispersal distance for each cluster size (where n>6) per taxon are shown in Table 2.

Dispersal distances of the sporangia, single spores and spore clusters were largely significantly not normally distributed, with a few exceptions: *Ascobolus immersus* clusters of 5, 6 or 7 spores on the light side, and *Podospora* cf. *intestinacea* clusters of 7 spores. For some taxa, the non-normal distribution is due to a diffuse distribution of spores across the entire length of the tank (*Pilobolus* for sample 1 light side, *Cheilymenia* cf. *granulata* for sample 1 dark side and sample 5, *Ascobolus albidus* and *Saccobolus depauperatus* for sample 5, *Ascobolus* cf. *michaudii* for sample 6). For other taxa, multiple peaks are present (*Cheilymenia* cf. *granulata* for sample 1 light side,

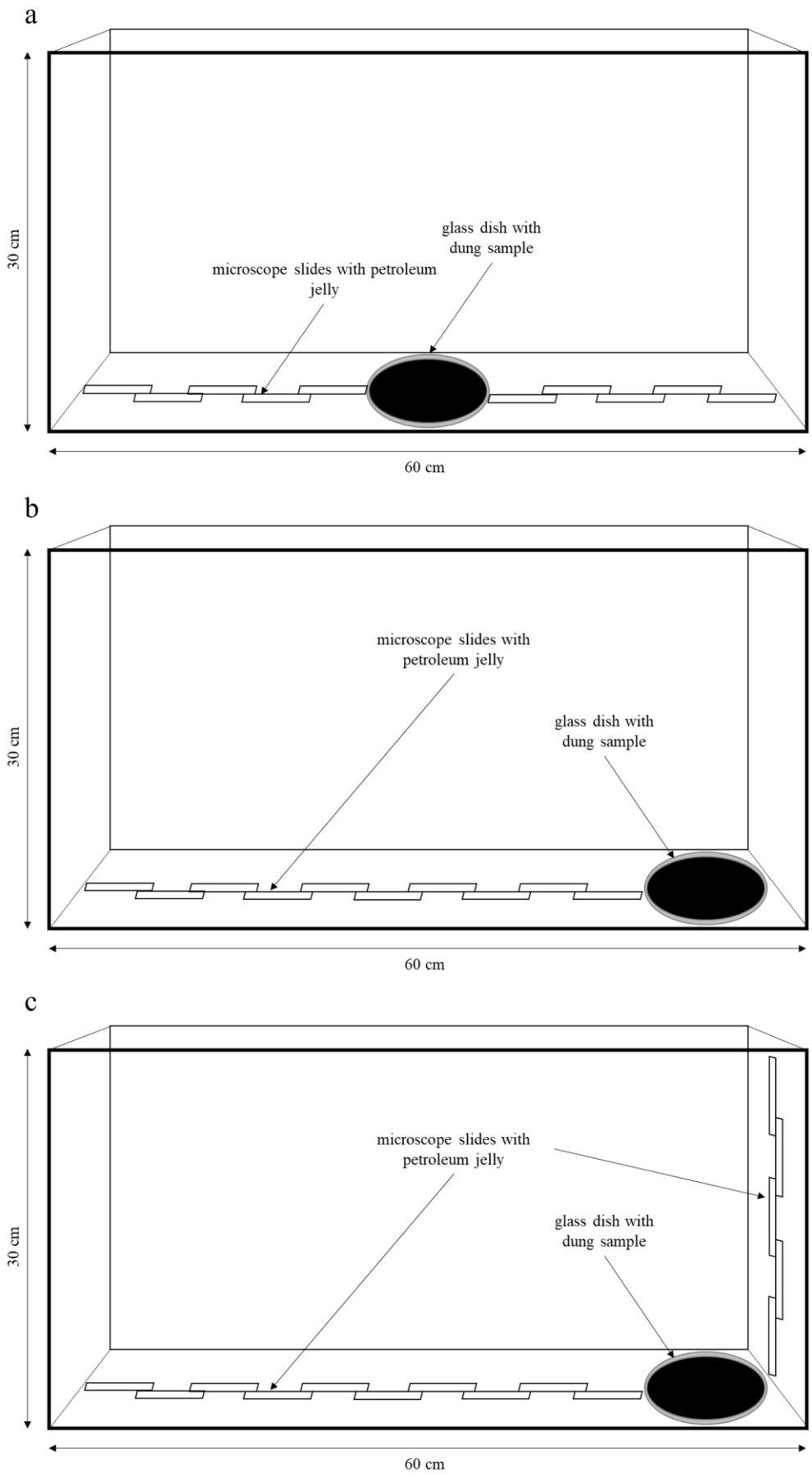

Fig. 1 – Experimental setup of glass tank with position of dung sample and microscope slides. a sample 1. b samples 2, 5 and 6. c samples 3 and 4.

Table 1 Number of clusters analyzed per taxon.

Taxon	Total n clusters	Cluster size								Sample
		1	2	3	4	5	6	7	8	
Pilobolus sp.	214	na	na	na	na	na	na	na	na	1 light side
Ascobolus albidus	383	345	32	6	0	0	0	0	0	5
Ascobolus immersus	293	97	28	37	33	29	40	23	6	3 light side
Ascobolus immersus	60	31	7	4	6	6	2	2	2	3 dark side
Ascobolus cf. *michaudii*	463	438	15	3	0	1	0	0	6	6
Cheilymenia cf. *granulata*[a]	3099	-	-	-	-	-	-	-	-	1 light side
Cheilymenia cf. *granulata*	134	49	9	2	4	1	3	6	60	3 light side
Cheilymenia cf. *granulata*	73	4	3	0	0	2	9	5	50	4 light side
Cheilymenia cf. *granulata*	72	59	5	6	1	1	0	0	0	6
Cheilymenia cf. *granulata*[a]	1565	-	-	-	-	-	-	-	-	1 dark side
Cheilymenia cf. *granulata*	26	1	0	0	0	0	0	2	23	3 dark side
Cheilymenia cf. *granulata*	48	3	0	0	0	0	0	5	40	4 dark side
Podospora conica	60	55	0	0	0	2	1	2	0	6
Podospora intestinacea	250	209	5	1	6	4	9	16	0	6
Saccobolus depauperatus	133	0	0	0	0	0	0	0	133	6
Sporormiella sp.	26	20	2	1	0	0	1	0	2	2
Basidiomycetes cf. *Coprinus*	2501	2501	na	na	na	na	na	na	na	2
Basidiomycetes cf. *Coprinus*	455	455	na	na	na	na	na	na	na	6

[a] cluster size was not documented in this experiment

Table 2 Mean, minimum and maximum overall distance from dung source and mean distance from dung source for each cluster size per taxon; mean values were not calculated where sample size < 6.

Taxon	Overall distance			Mean distance per cluster size							Sample
	mean	min	max	1	2	3	4	5	6	7	8	
Pilobolus sp.	13.1	0.6	22.5	na	na	na	na	na	na	na	na	1 light side
Ascobolus albidus	22.3	0.2	42.6	22.5	22.9	12.4	-[b]	-	-	-	-	5
Ascobolus immersus	9.0	2.0	42.7	13.2	5.2	6.4	8.0	7.0	7.3	7.7	9.1	3 light side
Ascobolus immersus	6.0	2.0	17.5	4.8	4.7	-	7.4	8.6	-	-	-	3 dark side
Ascobolus cf. *michaudii*	15.1	0.0	38.1	15.5	10.9	-	-	-	-	-	0.5	6
Cheilymenia cf. *granulata*[a]	7.7	0.1	22.5	na	na	na	na	na	na	na	na	1 light side
Cheilymenia cf. *granulata*	7.2	2.0	41.9	9.9	13.4	-	-	-	-	10.5	3.6	3 light side
Cheilymenia cf. *granulata*	3.6	2.0	35.4	-	-	-	-	-	2.7	-	2.8	4 light side

Table 2 Continued.

Taxon	Overall distance			Mean distance per cluster size							Sample
	mean	min	max	1	2	3	4	5	6	7	8	
Cheilymenia cf. *granulata*	13.6	1.2	35.9	14.3	6.9	10.9	-	-	-	-	-	6
Cheilymenia cf. *granulata*[a]	11.0	0.1	22.5	na	na	na	na	na	na	na	na	1 dark side
Cheilymenia cf. *granulata*	3.0	2.0	12.6	-	-	-	-	-	-	-	2.6	3 dark side
Cheilymenia cf. *granulata*	3.3	2.0	24.7	-	-	-	-	-	-	-	2.6	4 dark side
Podospora conica	11.9	0.5	16.5	12.0	-	-	-	-	-	-	-	6
Podospora intestinacea	8.3	0.0	21.7	8.5	-	-	6.7	-	9.0	7.0	-	6
Saccobolus depauperatus	14.3	0.1	37.5	-	-	-	-	-	-	-	14.3	6
Sporormiella sp.	23.7	2.6	42.2	23.1	-	-	-	-	-	-	-	2
Basidiomycetes cf. *Coprinus*	11.6	1.4	42.1	11.6	na	na	na	na	na	na	na	2
Basidiomycetes cf. *Coprinus*	18.1	0.0	37.6	18.1	na	na	na	na	na	na	na	6

[a] cluster size was not documented in this experiment
[b] '-' indicates this cluster size was not encountered

Basidiomycetes cf. *Coprinus* sp. for sample 2, *Podospora* cf. *intestinacea* for sample 6) which could represent different cluster sizes or multiple taxa. Finally, many taxa show a clear peak with a long tail at one or either end of the distribution (*Ascobolus immersus* and *Cheilymenia* cf. *granulata* for the light and dark sides of samples 3 and 4 and *Podospora* cf. c*onica* and Basidiomycetes cf. *Coprinus* sp. for sample 6).

***Zygomycetes*: Pilobolus**

Whilst *Pilobolus* was also present on samples 2, 3 and 5, its sporangia were only measured for sample 1 due to the large number of sporangia produced. All sporangia were found on the light-facing side of the sample, with no sporangia found on the slides on the dark side of the sample. Sporangia were found across the entire length of the slide row (Fig. 2), without a clear peak distance. The glass of the side of the tank showed many sporangia in a diagonal line from about halfway its height to the top, and the tank lid was also covered in sporangia, suggesting maximum dispersal distances are significantly larger than could be measured within the tank.

***Ascomycetes*: Ascobolus albidus**

Spores of *Ascobolus albidus* (present on sample 5) largely dispersed individually, with a small number of clusters of 2 or 3 spores found. Spores are diffusely spread over the entire length of the tank, though there is a denser spread between 20 and 40 cm from the dung source, and spore numbers start to drop off beyond 40 cm (Fig. 3a).

***Ascomycetes*: Ascobolus immersus**

Ascobolus immersus was only observed on sample 3. Whilst clusters of 2-8 spores are relatively common, on the light side over 30% of spores

disperse as single spores. This is even more pronounced on the dark side, where over 50% of clusters consisted of a single spore. On the light side, dispersal distances peak around 2.2 cm, then fall off gradually (Fig. 3b). On the dark side, the peak falls between heights of 2.0-8.1 cm, with few spores above 8.1 cm (Fig. 4a). There is a slight difference in dispersal distances per cluster size. Most single spores disperse 0-5 cm, but there is a long tail in the distribution over the entire length of the tank, whereas the larger clusters rarely travel further than 20 cm.

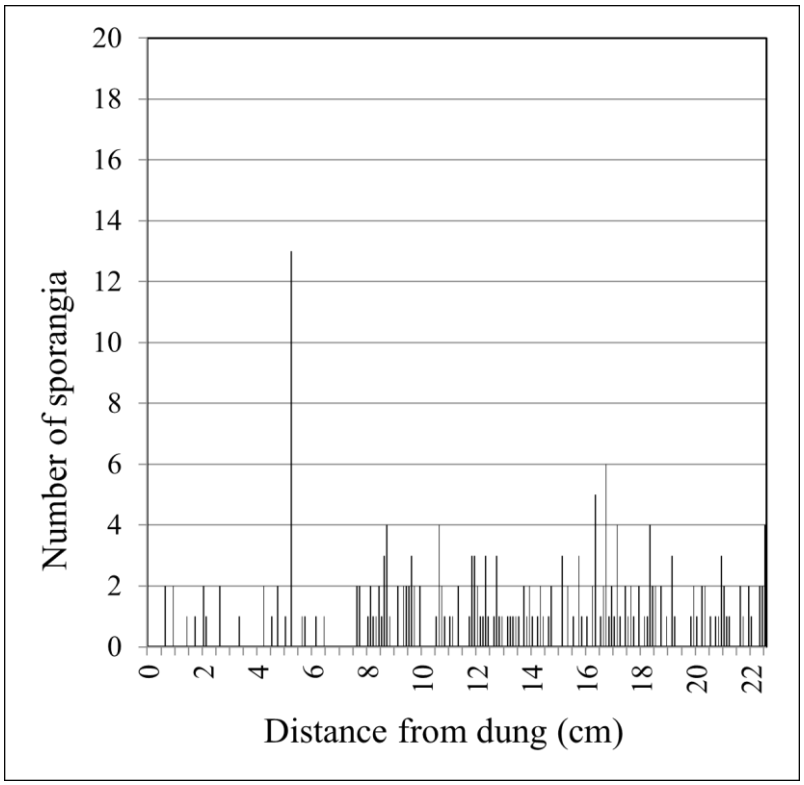

Fig. 2 – Dispersal distances of *Pilobolus* sporangia on the light side of the dung, sample 1.

***Ascomycetes*: Ascobolus cf. michaudii**

As in *Ascobolus* cf. *albidus*, the spores of *Ascobolus* cf. *michaudii* (present on sample 6) are largely dispersed individually, with a small number of clusters of 2 or 3 spores found, as well as a number of full spore complements (8 spores). Single spores on average show larger dispersal distances than larger groups of spores. Most spores disperse up to 15 cm from the dung source, with a slow drop in numbers beyond 15 cm (Fig. 3c).

***Ascomycetes*: Cheilymenia cf. granulata**

Whilst the number of spores were counted for sample 1, cluster size was not recorded for this sample. Counts from the other samples show that *Cheilymenia* spores are largely dispersed either as single spores or, more commonly, as full spore complements, with small numbers of groups of 2 to 7 spores also present. *Cheilymenia* cf. *granulata* spores from the light side of sample 1 show a multimodal distance distribution with peaks between 1.1 and 7.5 cm, at 12.5 cm and between 15.1 and 18.1 cm (Fig. 5a). On the dark side (horizontally), the distribution is diffuse with no clear peaks (Fig. 4b). For sample 3, a clear peak is present between 2.0 and 7.5 cm on the light side, with a long tail up to the end of the tank (Fig. 5b). On the dark side (vertically), the peak falls between 2.0 and 3.6 cm (Fig. 4c). giving an indication of the height to which these spores are shot. For sample 4, the peak on the light side falls between 2.0 and 4.0 cm (Fig. 5c), and on the dark side between 2.0 and 3.4 cm (Fig. 4d). Sample 6 shows a multimodal distribution, with peaks between 2.4 and 11.2 cm, at 15.0 cm and around 28.0-29.0 cm (Fig. 5d). The peaks are similar for single spores and for the full spore complements.

***Ascomycetes*: Podospora cf. conica**

Podospora cf. *conica* was found on sample 6. Virtually all spores disperse as single spores. Dispersal peaks between 15.4 and 16.5 cm, with a smattering of spores between 0.0 and 15.3 cm, and no spores beyond 16.5 cm (Fig. 6c).

***Ascomycetes*: Podospora cf. instestinacea**

Podospora cf. *intestinacea* was found on sample 6. Most clusters consist of single spores, but small numbers of clusters of 2-8 spores are also present. Dispersal is fairly continuous between 0.0 and 16.5 cm, with no spores beyond 21.7 cm (Fig. 6d).

***Ascomycetes*: Saccobolus depauperatus**

Saccobolus depauperatus spores were dispersed from sample 6 as full spore complements consisting of 8 spores. Most spore complements disperse up to 35 cm from the dung source, with a clear drop off beyond 35 cm (Fig. 3d).

***Ascomycetes*: Sporormiella sp.**

Sporormiella sp. was found on sample 2. Only a small number of clusters were found (n = 26). Most of these (n = 20) consisted of a single spore. Spores were scattered over the entire length of the tank (Fig. 6e).

***Basidiomycetes*: cf. Coprinus**

For sample 2, *Coprinus* spores were found over the entire length of the tank, with peaks around 6.0, 11.0 and 15.0 cm (Fig. 7a). For sample 6, there is a diffuse spread between 0.0 and 21.3 cm, followed by a peak between 21.8 and 27.9 cm, and a further smattering of spores up to 37.6 cm (Fig. 7b).

Discussion

Zygomycetes

Pilobolus is a common coprophilous zygomycete and occurred on all dung samples included in this study. The taxon is well-known for producing large sporangia containing a light-sensitive pigment on top of a pressurised vesicle. The sporangiophores orientate towards light. Unlike other coprophilous zygomycetes, which disperse their spores by sticking to passing animals or plant structures (e.g. *Pilaira*), growing to great lengths (e.g. *Phycomyces*), or passively, in *Pilobolus* the sporangium is propelled forwards by an explosive mechanism in which the vesicle ruptures (Deacon 2006).

Due to the strongly light-sensitive sporangia, all sporangia were dispersed towards the light and none were propelled to the dark side of the tank. The tank used in our experiments was clearly too small to measure the full range of dispersal distances for this taxon, which is borne out in other studies that found dispersal distances of up to 2.5 m (Yafetto et al. 2008, see Table 3). The fact that we found many spores on the tank's lid shows that the sporangia can be propelled to a relatively great height. However, not all sporangia travel this far, with some sporangia present at nearly every distance measured.

Ascomycetes

The asci in disc or cup fungi like *Ascobolus*, *Cheilymenia* and *Saccobolus* and the neck of the perithecia in flask fungi like *Podospora* and *Sporormiella* are generally phototropic (Ingold & Hudson 1993). It is therefore not surprising that in those experiments where spores were counted on the dark side of the tank as well as the light side, spores of the same taxon were 2-5 times more abundant on the light side of the tank.

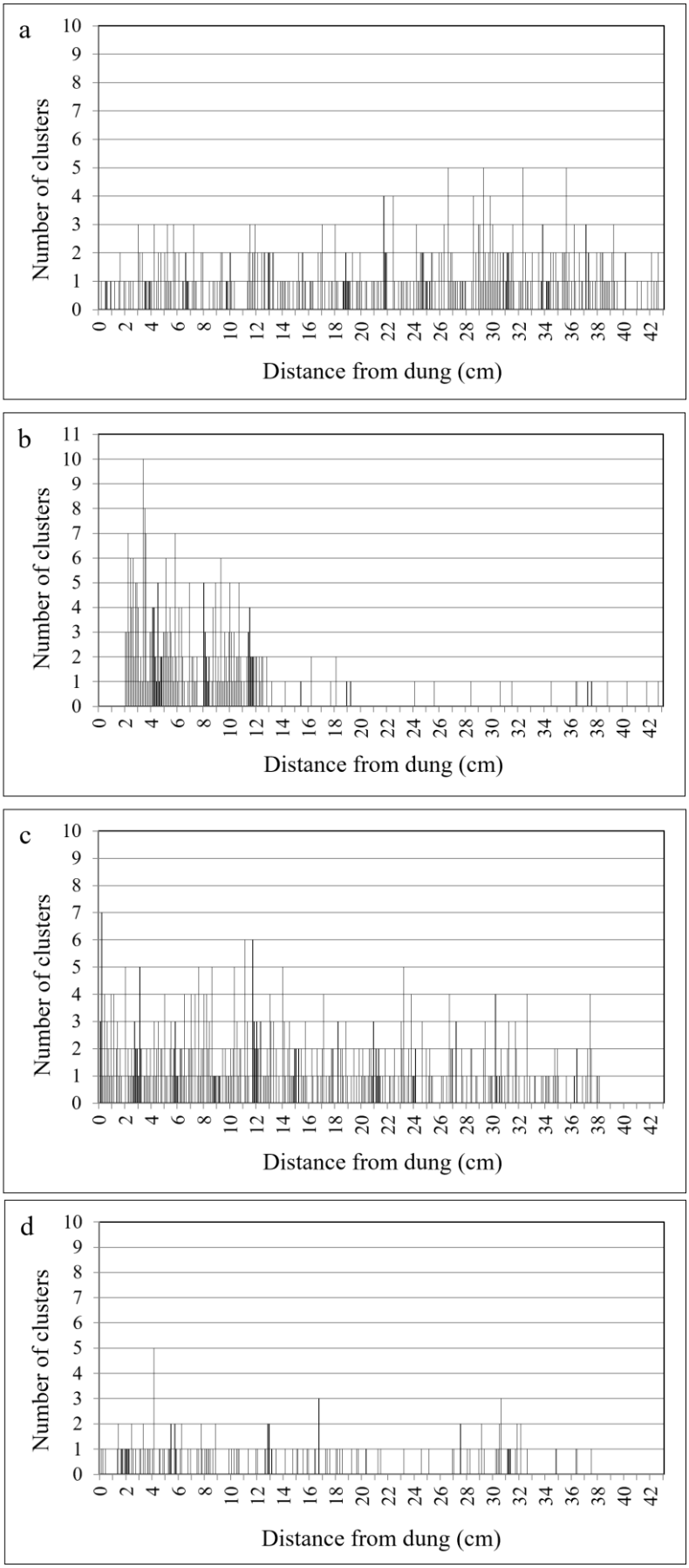

Fig. 3 – Dispersal distances of spores on the light side of the dung. a sample 5, *Ascobolus albidus*; b sample 3, *Ascobolus immersus*. c sample 6, *Ascobolus* cf. *michaudii*. d sample 6, *Saccobolus* cf. *depauperatus.*

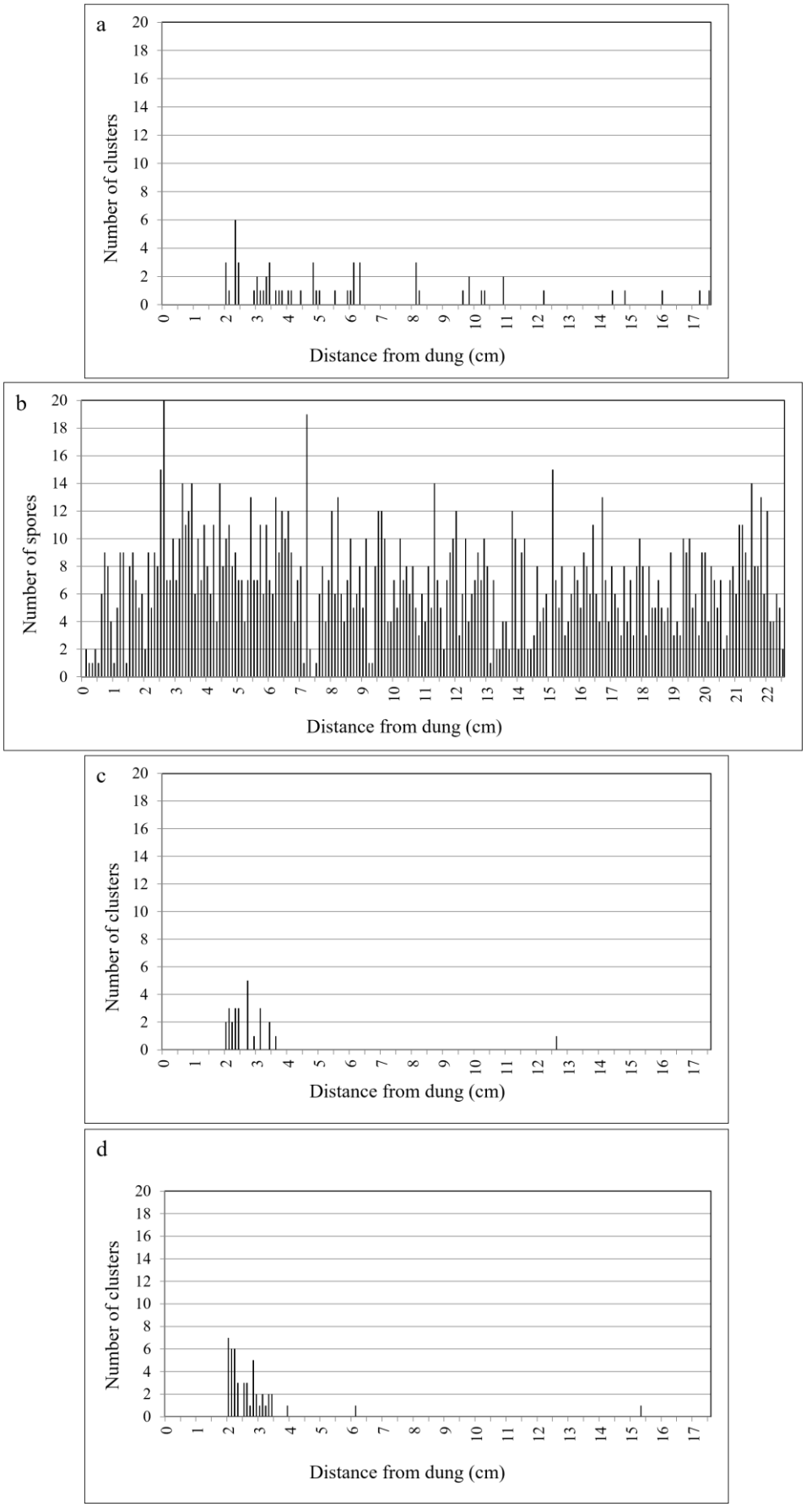

Fig. 4 – Dispersal distances of spores on the dark side of the dung. a sample 3, *Ascobolus immersus*. b sample 1, *Cheilymenia* cf. *granulata*. c sample 3, *Cheilymenia* cf. *granulata*. d sample 4, *Cheilymenia* cf. *granulata.*

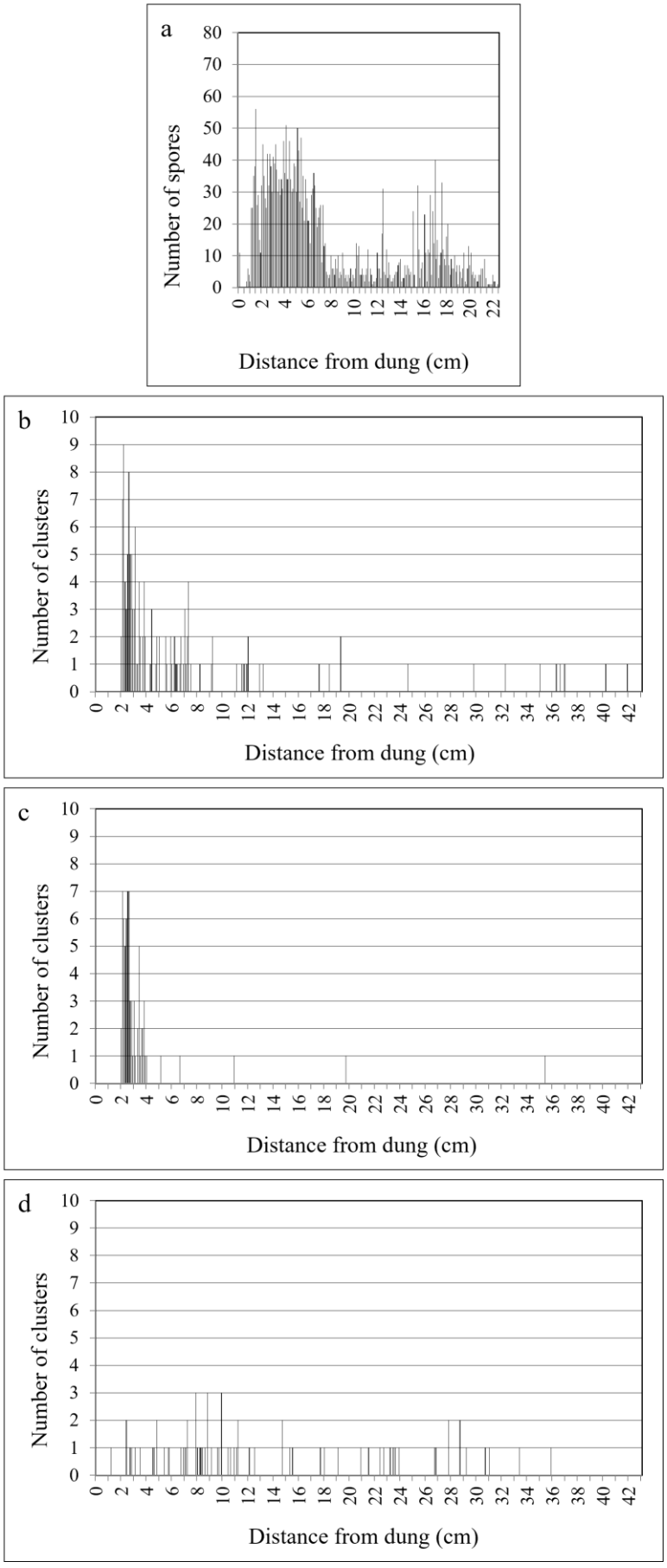

Fig. 5 – Dispersal distances of *Cheilymenia* cf. *granulata* spores on the light side of the dung. a sample 1. b sample 3. c sample 4. d sample 6.

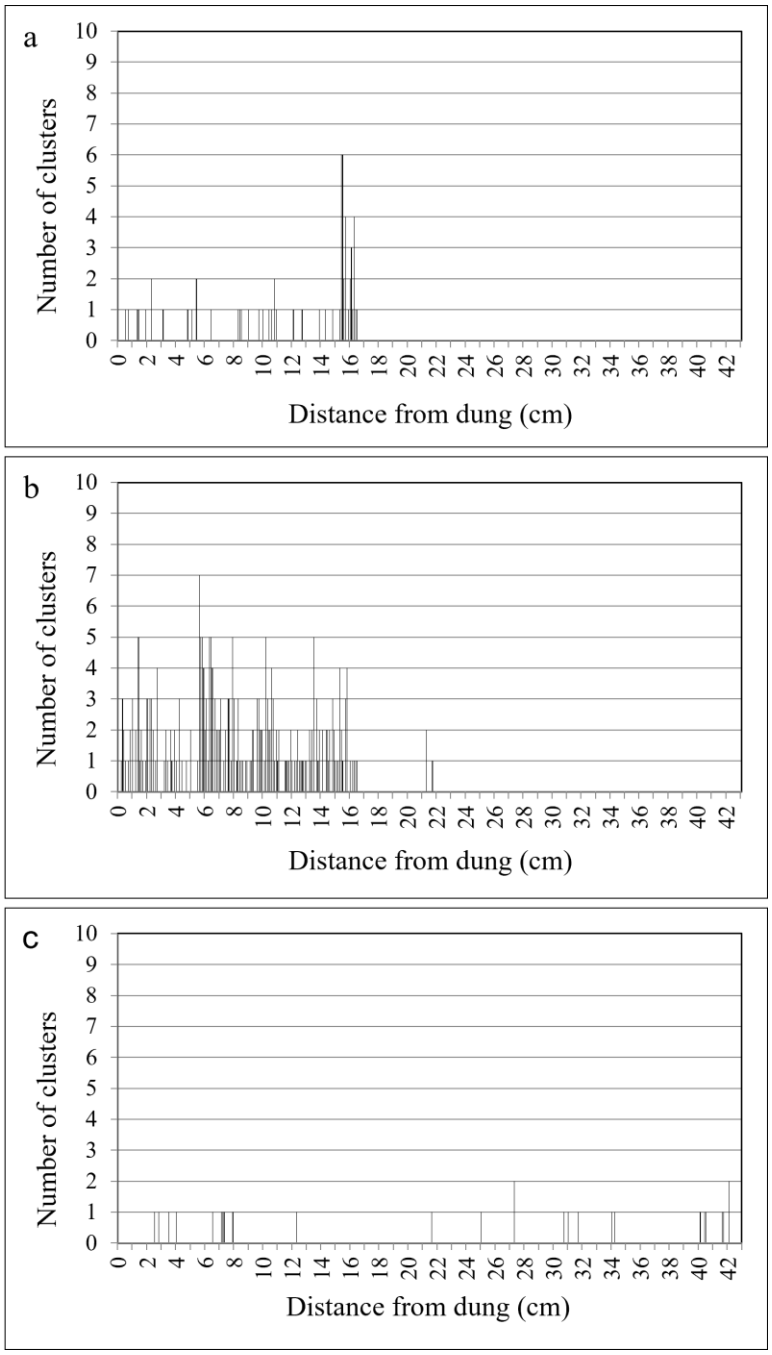

Fig. 6 – Dispersal distances of spores on the light side of the dung. a sample 6, *Podospora* cf. *conica*. b sample 6, *Podospora* cf. *intestinacea*. c sample 2, *Sporormiella* sp.

There is some variation in dispersal distances both within and between genera, but most spores land around 10 cm from the edge of the dung source, with only *Ascobolus albidus* and *Sporormiella* averaging more than 20 cm. It must be kept in mind that the dung patches themselves were around 20-30 cm in diameter, so that a fruitbody in the centre of the dung patch would have to disperse its spores 10-15 cm further than a fruitbody on the edge of the dung patch to reach the surrounding vegetation. However, these distances are similar to distances recorded in previous studies (Ingold 1961, Walkey & Harvey 1966, Ingold & Oso 1968, Yafetto et al. 2008, see Table 3).

For small projectiles, drag is a significant factor in travel through air. This increases in smaller objects with a larger surface to volume ratio, as well as with the presence of appendages (Trail 2007). Larger spores can therefore be expected to travel further. However, our data do not bear this out, with spore clusters of different sizes within the same taxon showing largely similar

dispersal profiles. Walkey & Harvey (1966) found that full spore complements tended to travel further than single spores or smaller clusters of spores, but single spores travelled further than small clusters. In contrast, Ingold & Hadland (1959) found that in *Sordaria fimicola*, the mean dispersal distance increased with cluster size. Ingold (1961) also observed that fungi with a larger full spore complement had larger mean dispersal distances. However, the primary taxon included in these two studies was *Sordaria* spp., which we did not find in our experiments, so these patterns may differ between genera. Furthermore, size, shape and surface features of spores primarily affect aerodynamics, whereas dispersal distance is mainly dependent on-air mass movement, turbulence and thermal convection (Lacey & West 2006), as well initial velocity of the expelled spores.

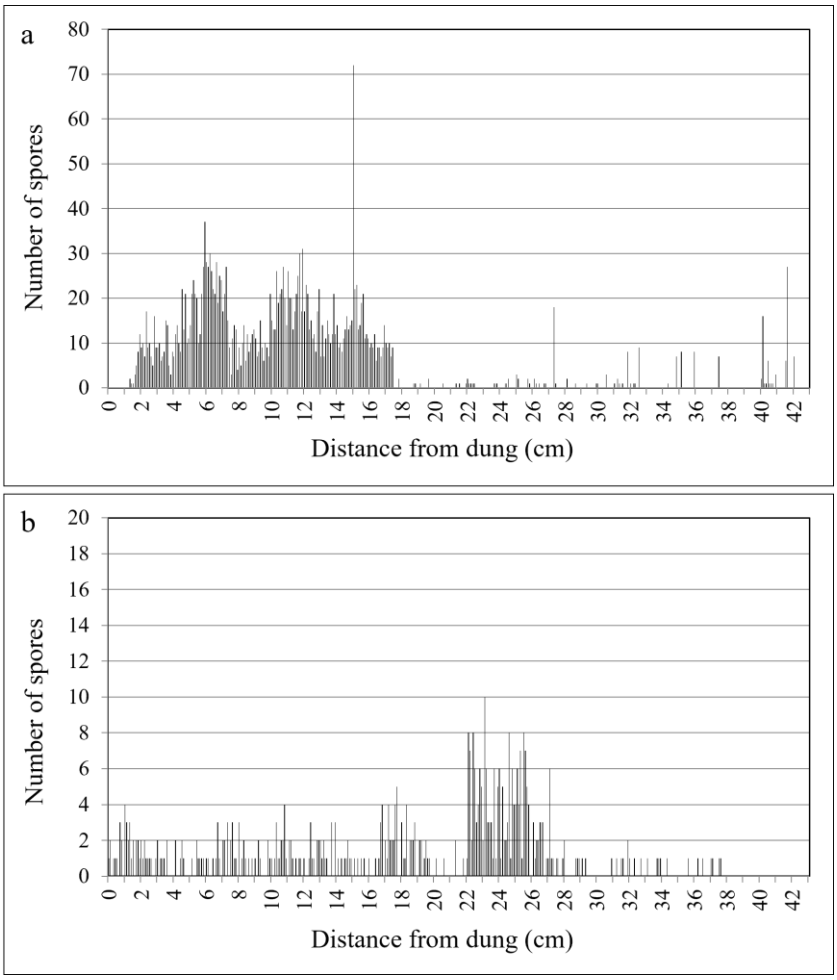

Fig. 7 – Dispersal distances of Basidiomycete spores (cf. *Coprinus*) on the light side of the dung. a sample 2. b sample 6.

Table 3 Dispersal distances for coprophilous fungal species from previous studies.

Taxon	Mean discharge distance (cm)	Maximum discharge distance (cm)	Reference
Pilobolus kleinii		250.0	Yafetto et al. (2008)
Ascobolus immersus		30.0	Yafetto et al. (2008)
Ascobolus leveillei	11.5 (8 spores)		Ingold (1961)
Ascobolus stercorarius	5.7[a]	19.5	Walkey & Harvey (1966)
Ascobolus viridulus	~1.0 (background discharge)		Ingold & Oso (1968)
Ascobolus viridulus	~5.0 (puffed spores)		Ingold & Oso (1968)
Podospora anserina		20.0	Yafetto et al. (2008)
Podospora coronifera	19.9[a]	36.5	Walkey & Harvey (1966)

Table 3 Continued.

Taxon	Mean discharge distance (cm)	Maximum discharge distance (cm)	Reference
Podospora curvula	14.0[a]	23.0	Walkey & Harvey (1966)
Podospora decipiens (8-spored variety)	18.4[a]	28.0	Walkey & Harvey (1966)
Podospora decipiens (16-spored variety)	8.2[a]	23.0	Walkey & Harvey (1966)
Podospora decipiens (64-spored variety)	36.6[a]	51.0	Walkey & Harvey (1966)
Podospora fimiseda	33.4[a]	50.5	Walkey & Harvey (1966)
Podospora tetraspora	6.7[a]	12.0	Walkey & Harvey (1966)

[a] Mean dispersal distance varied with the number of spores in the projectile (1 to 8)

Another important factor in dispersal distances is the height to which the spores are projected, which is related to the propulsive force with which the spores are expelled and the orientation of the asci. The data from the dark side of the tank gives some indication of this. Most spores of *Cheilymenia* were found below a height of 5 cm, indicating these spores were not projected much vertically, although a smattering of spores was found to the top of the 22.5 cm tall sample path. Similarly, *Ascobolus* spores were largely found below 10 cm, with a small number of spores higher up the side of the tank. To successfully disperse their spores, the fungi must break through the laminar boundary layer, the layer of still air above the earth's surface that is between 1 mm and 10 cm thick (Lacey & West 2006, Trail 2007). Most of the taxa studied here seem to provide just enough propulsive force to accomplish this. Once the spores break through the boundary layer, the amount of turbulence and thermal convection will determine whether spores are carried further away or whether the spores precipitate under the force of gravity. The activity of decomposing organisms can produce higher temperatures in dung pats than that of the surrounding environment (Webster 1970, Lundqvist 1972), creating thermal convection. The air movement caused by the placement and retrieval of the microscope slides, in combination with the thermal convection caused by decomposing organisms, and the spread of the fruitbodies across the diameter of the dung patches could account for the fact that the spores of some genera show a diffuse distribution over the entirety of the tank, or a long tail away from the primary peak.

Dung fungal spores have been sporadically encountered in air samples (e.g. Gonianakis et al. 2005, Hernández Trejo et al. 2012, El Haskouri et al. 2016), implying that at least some spores are dispersed more widely. However, whilst wind could increase the dispersal distance in field situations, the spores are not shot very high so this is not expected to affect the spores on a large scale, with most spores being deposited relatively locally.

There is variation in cluster size both within and between taxa. Although for most taxa all cluster sizes were encountered, some taxa disperse mainly as individual spores (*Ascobolus* cf. *albidus*, *Ascobolus* cf. *michaudii*, *Podospora* cf. *conica*, *Podospora* cf. *intestinacea*, *Sporormiella* sp.), whereas others mainly disperse as full spore complements (*Saccobolus depauperatus*). In *Cheilymenia* cf. *granulata*, individual spores and full spore complements were significantly more common than other cluster sizes. In *Ascobolus immersus* all cluster sizes were relatively common. Walkey & Harvey (1966) found that in the *Podospora* species they studied, clusters nearly always consisted of the full spore complement, whereas in *Ascobolus stercorarius*, most spores dispersed individually. Our samples contained other species of *Podospora* and *Ascobolus* that had different dispersal patterns, showing that there is variation within these genera. In some species of *Ascobolus*, the asci of a single fruitbody release all their spores in one 'puff'. This produces clouds of single spores that behave as one larger body, reducing drag and increasing dispersal distance (Ingold & Oso 1968, Trail 2007, Roper et al. 2010). Whilst we did not observe this directly, this could explain the abundance of single spore clusters in *Ascobolus* cf. *albidus* and *Ascobolus* cf. *michaudii*. In *Podospora*, the tip of the ascus is discharged together with the spores, which often

stick together by mucilaginous sheaths, intertwined appendages, and ascus fluid (Ingold 1971, Yafetto et al. 2008). Although both *Podospora conica* and *P. intestinacea* have significant appendages, and although their spores are quite different in size, with the spores of *P. intestinacea* being about twice the size of those of *P. conica*, in our study both species dispersed individual spores.

Basidiomycetes

Coprophilous basidiomycetes use a more passive method of discharge using Buller's drop. We would therefore have expected that spores would travel only a very short distance. However, in our experiments, cf. *Coprinus* spores travelled a similar distance to most ascomycete spores. As discussed above, the main sources of air movement within the tank were thermal convection from the warm dung patch and movement caused by the placement and removal of the microscope slides. From the distribution of the small-sized cf. *Coprinus* spores, it is clear this can increase dispersal distance significantly.

Conclusion

Since grazing animals often avoid feeding in areas around dung patches (Ödberg & Francis-Smith 1977, Forbes & Hodgson 1985, Lütge et al. 1995), coprophilous fungi must actively disperse their spores away from the dung substrate that they grow on to complete their lifecycle. Active dispersal can propel spores to great distances (up to 2.5 m in *Pilobolus*; Yafetto et al. 2008). However, in our experiments most spores travelled no more than ~20 cm from the dung source. Apart from *Pilobolus* sporangia, most spores are not propelled to great heights (~10 cm). Thermal convection generating by the heat produced by the decomposition of the dung may play some role in dispersing the spores beyond the reach of their propulsive distance, and when there are wind dispersal distances may be even larger. Furthermore, these spores can lay dormant for significant amounts of time, and as the dung disintegrates and grazing animals begin to use the area near the dung patch again, these spores can be ingested and reactivated.

Due to phototropic parts of the sporangium or fruitbody spores overwhelmingly dispersed towards the light. This may ensure that spores are propelled towards the most productive parts of the herbaceous vegetation that are therefore most attractive to herbivores, away from areas of shade under canopies or rocks.

In contrast to some earlier studies, we found no clear relationships between overall spore size, cluster size or spore complement size and dispersal distance. Most spores dispersed individually or as a full ascus complement. The aerodynamic features of the spores may therefore have less impact on dispersal distance than other factors, such as initial velocity, thermal convection and wind.

Our experiments used a more naturalistic setup than previous studies. Our light source was more similar to the natural environment, with the incidence of the light changing with the sun's movement throughout the day. This made the direction the spores were dispersed in less predictable. Furthermore, we used dung rather than dung agar as a substrate, and multiple species of dung fungi competed for the same resource. This produced a more naturalistic orientation of the fruitbodies, which were not positioned horizontally but instead used the phototropic parts of the sporangium or fruitbody to orientate themselves to the light. Since the dung patches themselves were around 20-30 cm in diameter, the dispersal curves of fruitbodies at different locations on the dung patches were overlaid to create a more diffuse overall spore distribution. However, our study shows that under these circumstances, mean dispersal distances are similar to those found in earlier, less naturalistic studies, indicating that such studies provide a good approximation of dispersal distances in field situations.

Although our experimental setup did not include an analysis of the impact of wind on dung fungal spore dispersal, our study confirms that dung fungal dispersal is most likely largely local. In field situations, dispersal distances may be somewhat larger due to wind, but as the spores are propelled only just beyond the boundary layer, and spores are only sporadically encountered in air samples (e.g. Gonianakis et al. 2005, Hernández Trejo et al. 2012, El Haskouri et al. 2016), this is

likely of limited impact. The effect of water transport on the spores once they adhere to surrounding vegetation also needs more study (Etienne et al. 2013, Ahlborn et al. 2015, Baker et al. 2016, Van Asperen et al. 2020). Our study should also be repeated in a fully naturalistic setting to address the effect of variables such as different soil types and drainage, slope, aspect, and insect and invertebrate disturbance. However, it seems justifiable to assume that most dung fungal spores present in palaeoecological samples reflect a highly local presence of animal dung.

Acknowledgements

This work was supported by a Leverhulme Trust Early Career Fellowship (ECF-2013-517, Van Asperen) and the Quaternary Research Fund of the Quaternary Research Association (Van Asperen). Knowsley Safari Park, Chillingham Wild Cattle Association and Alice Smith contributed greatly to this study by facilitating our work.

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