The colonisation of remains by the muscid flies *Muscina stabulans* (Fallén) and *Muscina prolapsa* (Harris) (Diptera: Muscidae)

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ABSTRACT

In the field, the muscid flies *Muscina stabulans* (Fallén) and *Muscina prolapsa* (Harris) only colonised buried baits in June, July and August. The two-species co-occurred on baits buried at 5cm but only *M. prolapsa* colonised baits buried at 10cm. Other species of insect were seldom recovered from buried baits regardless of the presence or absence of *Muscina* larvae.

In the laboratory, both *M. stabulans* and *M. prolapsa* preferentially colonised liver baits on the soil surface compared to those buried at 5cm. Baits buried in dry soil were not colonised by either species whilst waterlogged soil severely reduced colonisation but did not prevent it entirely. Dry liver presented on the soil surface was colonised and supported growth to adulthood but if there was no surrounding medium in which the larvae could burrow then they died within 24 hours.

M. stabulans showed a consistent preference for ovipositing on decaying liver rather than fresh liver, even when it had decayed for 41 days. The results for *M. prolapsa* were more variable but it was also capable of developing on both fresh and very decayed remains.

Blood-soaked soil and dead slugs and snails stimulated egg-laying by both species and supported larval growth to adulthood. Mushrooms, melon, and bananas also stimulated egg-laying although to a much lesser extent and very few larvae survived to adulthood. Horse faeces stimulated extensive egg-laying but the larvae invariably died during the first or second instar and none survived to pupariation.

This information could be useful when determining the forensic significance of *Muscina* larvae recovered from dead bodies.

1. Introduction

The muscid flies *Muscina stabulans* (Fallén) and *Muscina prolapsa* (Harris) (syn. *pabulorum*) are cosmopolitan species that are found as far north as Finland as well as tropical countries such as Malaysia. They are sometimes recovered from human remains [1, 2, 3] but their potential as indicators of the post-mortem interval has been exploited only occasionally. Nuorteva (1974) [4] describes a case in which *M. stabulans* larvae were reared from a blood-soaked shirt recovered from a rubbish bin. The adult flies ultimately emerged in two waves one of which corresponded with eggs laid on the date on which the person who was wearing the shirt was stabbed to death and the other date to eggs which were laid 13 days later when the shirt was disposed of in the bin. In the intervening period between the date of the murder and the date of disposal the shirt was stored in a plastic bag that excluded flies from accessing it. The larvae of *Muscina* species could be particularly valuable in the case of buried bodies because the adult flies lay their eggs on the soil surface above the remains and after hatching the larvae can crawl through loose soil to a depth of at least 40cm [5]. Even thin coverings of soil can prevent the colonisation of remains by the insect fauna that is more commonly used to determine the time since death. For example, Nuorteva (1977) [6] discusses a case from Finland in which the body of a young girl was disposed of in a forest and covered with moss and branches. Although blowfly larvae were recovered from the body, these were less developed than would have been expected from the state of decay of the body. By contrast, pupariae of *Muscina levida* (Fallén) (syn. *Muscina assimilis*) were found in the surrounding soil. It was thought likely that the blowflies were unable to colonise the body until some of the covering was eventually scrapped away by vertebrate scavengers whilst the development stage of the *M. levida* was a closer match to the date on which the girl was killed.

Recent papers have described means of distinguishing between species of *Muscina* larvae based on their morphology [7] and molecular identification techniques are now available [8, 9]. However, the value of *Muscina* species as forensic indicators is restricted by the limited published information on their biology much of which is anecdotal. For example, there are few surveys of when the adult flies are active and therefore likely to colonise remains. Similarly, there are differing accounts of the lifestyle of the larvae, their relative attractiveness to fresh and decomposed remains and their exploitation of alternative food sources. The latter is particularly important because it can compromise the minimum time since death calculation if there is a

probability that the larvae recovered from a body might have originated from elsewhere.

The larvae of both *M. stabulans* and *M. prolapsa* have reportedly been reared from the nests of the common wasp *Vespula vulgaris* (L.) [10] but it is not known whether this refers to active or dead nests or whether the fly larvae were feeding on living or dead wasp larvae or organic detritus in the hive. Skidmore (1985) [11] considered *Muscina* larvae to be predatory and even cannibalistic although he stated that *M. stabulans* larvae could develop on a wide range of foods whilst *prolapsa* required dead animal matter. However, some cases of pseudomyiasis (see below) are thought to have arisen through the consumption of fruit infested with *Muscina* larvae [12] and *M. levida* larvae are commonly found in fruit in the USA [13]. It is often stated that the larvae of *Muscina* cause myiasis in humans and domestic animals although most human infections relate to intestinal myiasis [14] and from the accounts these are more correctly considered as pseudomyiasis [15] in which the larvae are consumed unintentionally with food and then subsequently voided with the faeces. There are very few confirmed cases of wound myiasis due to *Muscina* larvae in humans and domestic animals [e.g. 16].

The aim of the present study was to assess the environmental and biological factors that affect the colonisation of remains by *M. stabulans* and *M. prolapsa*.

2. Methods

2.1. Flies and Rearing Conditions

Culture conditions: Laboratory cultures of *M. stabulans* and *M. prolapsa* were established from buried baits placed in the wild and had been in culture for 12 months at the start of experiments. Flies were reared in gauze insect breeding cages (43cm x 45cm x 28cm; Watkin & Doncaster, Cranbrook, Kent, UK) in an indoor insectary maintained at 23 $\pm$ 1^{o}C and a 12h:12h light: dark regime. The flies were provided with an *ad lib* supply of water, sugar, and dried milk (as a protein source) and with fresh pig liver buried in loose soil at a depth of 5cm as an oviposition medium.

Soil: The soil used in all experiments was commercial John Innes number 2 potting compost. Soil moisture content was measured by drying the soil at 40^{o}C to constant

weight; soil organic matter was determined by heating dry soil in a muffle furnace and calculating the weight loss after ignition. The average soil moisture content was 0.38 $\pm$ 0.005 g g^{-1} soil wet weight, n = 7; the organic matter content was 0.17 $\pm$ 0.002 g g^{-1} soil wet weight and 0.27 $\pm$ 0.004 g g^{-1} soil dry weight, n = 7.

Baits: Unless otherwise stated, ~ 75 g fresh pig liver per jar was used as bait. All baits were presented in 1 litre glass jars (16cm deep x 9cm x 9cm) either on the surface of 7cm depth soil or 2cm soil depth soil was added to each jar, then the bait was added before being covered with a further 5cm (total volume = 480ml), or 10cm of soil (total volume = 820ml). In the laboratory work, the jars containing the experimental baits were not introduced into the culture cages until it was established that the flies were laying fertile eggs. All laboratory experiments were conducted with culture cages containing approximately 100 mixed sex adult flies. Jars containing baits were exposed for 6 hours unless otherwise stated. In those experiments in which a cage of flies was provided with a choice of two different baits then the jars containing them were both introduced at the same time and after the exposure period removed at the same time. After the jars were removed they were covered with nappy liner and maintained until all the adult flies had emerged and died - the number of flies in each jar was then counted. Once the experimental baits were removed the normal oviposition medium was replaced in the culture cages to confirm that the flies were capable of laying eggs both before and after the experimental period.

2.2. Annual Cycle of Activity

Jars containing fresh pig liver baits buried at depths of 5cm and 10cm were placed in a randomised array in an area of un-mown grassland close to the city centre of Liverpool between 28 September 2012 and 25 September 2014. The jars were covered with a metal mesh cage that allowed free access to insects but prevented disturbance by vertebrates. For each time interval there were 5 replicates of each depth (plus an un-baited control) and they were left exposed for 3 days at the beginning and end of each month after which they were collected and maintained in the insectary until all the adult flies had emerged – these were then identified and counted.

2.3. The relative attractiveness of exposed and buried remains

One jar of fresh pig liver bait on the soil surface and one jar of bait buried at 5cm were placed into each culture cage.

2.4. The influence of soil moisture on oviposition and larval development

Baits were presented in dry, normal, wet, and very wet (waterlogged) soil. Dry soil was prepared by drying soil to constant weight at 40^{o}C, normal soil was used unchanged from the sack it arrived in, wet soil was defined as 7cm depth soil (2cm base layer plus 5cm coverage of the bait) plus 50ml distilled water poured over the soil surface; very wet soil was defined as 7cm depth soil plus 100ml distilled water poured over the soil surface. Preliminary work indicated that 100ml was the most water that could be added without leaving a pool of free-standing water above the soil surface. Final moisture content was determined by allowing 60 minutes for the added water to be absorbed then drying subsamples of soil to constant weight at 40^{o}C. Average soil moisture content (g water g soil^{-1} $\pm$ se, n = 7) was as follows: dry soil: 0; normal soil 0.375 $\pm$ 0.0021; wet soil = 0.497 $\pm$ 0.0029; very wet = 0.611 $\pm$ 0.0065). The experiment was conducted in two parts, firstly baits buried in dry soil were placed in each cage (1 jar per cage) and secondly baits in either normal and wet or normal and very wet soil (1 jar of each soil type per cage).

2.5. The influence of bait moisture content on oviposition and larval development

Samples of fresh pig liver (77.04 $\pm$ 0.514g, n = 22) were dried at 40^{o}C in an oven to constant weight (23.27 $\pm$ 0.238g, n = 22) and then presented either on the surface of 7cm depth soil or within an empty glass jar. After drying, pig liver still had a strong smell and if flexed it would first bend and then fracture. Each cage was presented with either one jar of dry bait on the soil surface or one jar containing only dry liver.

2.6. The influence of bait decomposition on oviposition and larval development

Fresh pig liver was placed in clear 3.4 litre plastic discard containers (~1kg per container) that were covered with a loose muslin cloth to allow access to bacterial and fungal spores but exclude flies and other invertebrates. The containers were left on a laboratory bench at ambient temperature (~22^{o}C) for 48 hours after which a plastic lid

was loosely attached to prevent excessive evaporation but allow some air access and then placed in a fume cupboard until required. A separate container was used for each decomposition period and the baits were weighed after they had decomposed. The baits (~75g per jar) were buried at 5cm as described previously. In the laboratory experiments, each culture cage contained 1 jar of fresh liver and 1 jar of either 7, 14, 21, 28, or 41, day-old liver. Field experiments were conducted in June 2015 (fresh and 49 day-old liver) and repeated in August 2015 (fresh and 43 day-old liver).

2.7: The ability of alternative baits to stimulate oviposition and support larval development

A range of animal and plant baits were presented on the surface of 7cm depth soil. One jar containing bait was presented per cage and during the time of exposure it was the only bait available to the flies.

Fresh pig blood: 100ml fresh pig blood was poured over the surface the soil and the baits exposed for 6 hours.

Dead slugs: *Arion hortensis* (Férussac) and *Deroceras reticulatum* (Müller) were killed by freezing and after defrosting 19-25g of slugs were placed on the surface of the soil. The baits were exposed for 6 hours. Additional dead slugs were added on subsequent days as necessary to ensure that the larvae did not run out of food.

Dead snails: *Cornu aspersum* (Müller) (syn. *Helix aspersa*) and *Cepaea nemoralis* (L.) were killed by freezing and after defrosting placed on the surface of the soil. The snails were presented in their shells and exposed for 6 hours. After the larvae had pupariated the snail shells were re-weighed after removing any remaining organic matter in order to calculate the original weight of the snail flesh presented to the flies.

Horse faeces: Fresh horse faeces (107.02 $\pm$ 0.683g, n = 14) was presented on the surface of 7cm depth soil and exposed for 24 hours. The horse had not been treated with anthelmintics or insecticides for at least 4 weeks before passing the faeces.

Fruit and fungi: Bananas (*Musa* spp.), melon (*Cucumis melo* L.), and commercially grown flat cap mushrooms, *Agaricus campestris* (L.) were purchased from a local supermarket. The bananas were cut into two (76.24 $\pm$ 0.755g, n = 10) and part peeled to allow access to the inner flesh but limit water loss. The melon was cut into slices (87.67 $\pm$ 0.955g, n=11) and the rind and seeds retained, whilst the mushrooms were cut into two and one half of each mushroom (50.11 $\pm$ 1.941g, n=14) was placed in

each jar. All baits were placed on the surface of 7cm depth soil and exposed for 24 hours during which time they were the only bait in the cage.

2.8. Statistical analysis

Descriptive statistics and Mann Whitney analysis were performed using SPSS (version 21).

3. Results

3.1. Annual Cycle of Activity

A total of 166 jars containing baits at both 5cm and 10cm were deployed between 28 September 2012 and 28 September 2014. No baits were colonised in the months between October and May; the earliest colonisation of baits by *M. stabulans* occurred on 13th June and the latest on 30th August whilst for *M. prolapsa* colonisation occurred between 13th June and 17th September. Between June and September 2013 and June and September 2014 a total of 84 baits buried at 5cm were deployed out of which 11 (13%) were colonised by *M. stabulans* and 16 (19%) were colonised by *M. prolapsa*; *M. stabulans* was only found in association with *M. prolapsa* (Table 1). More *M. prolapsa* than *M. stabulans* emerged in 8 out of the 11 jars in which the two species occurred together (73%; Chi squared = 569.3, $P < 0.001$). Only *M. prolapsa* colonised baits buried at 10cm but just 5 of 84 baits deployed (6%) were colonised. Fewer *M. prolapsa* were recovered from baits buried at 10cm than at 5cm (median 77.7% lower, $U_{16,13} = 49.0$, $P<0.015$). Other species of insect recovered from buried baits included *Calliphora vicina* (Rob-Desvoidy), *Alysia manducator* (Panzer), *Nasonia vitripennis* (Ashmead), *Fannia canicularis* (L.), *Fannia scalaris* (Fabricius), *Hydrotaea ignava* (Harris), *Lasiomma picipes* (Meigen), and *Leptocera fontinalis* (Fallen). However, all of these species only occurred in 1-3 jars over the whole survey period.

Table 1

Field data demonstrating the the number of instances in which *Muscina stabulans* and *Muscina prolapsa* produced single species infestations and co-infestations of buried remains.

Species	*M. stabulans*	*M. prolapsa*	*M. prolapsa*	*M. prolapsa*
	Co-occurring with *M. prolapsa*	Co-occurring with *M. stabulans*	Sole *Muscina* species present	Sole *Muscina* species present
Bait depth	5cm	5cm	5cm	10cm
Total number of jars analysed	84	84	84	84
Number of jars colonised by *Muscina* spp.	11	11	5	14
Median number of adult flies emerging	26	74	135	16.5
Interquartile range	66	74	153.5	33.8

3.2. The relative attractiveness of exposed and buried remains.

In every replicate, both *M. stabulans* and M. *prolapsa* laid more eggs on exposed baits than on those that were buried (Table 2).

Table 2

The relative attractiveness of exposed and buried baits to *Muscina stabulans* and *Muscina prolapsa* under laboratory conditions.

	Muscina stabulans		*Muscina prolapsa*	
	exposed	buried	exposed	buried
n	9	9	8	8
Median % adult flies emerging	98.3	1.7	82.4	17.6
Interquartile range	43.6		27.6	
Min %	54.6	0	70.8	0.3
Max %	100	45.4	99.7	35.4
Chi squared	1352.6		1114.6	
P	<0.001		<0.001	

3.3. The influence of soil moisture on oviposition and larval development

Although flies probed the soil surface above baits buried in dry soil, no larvae or adults were reared from any of the replicates. For both *M. stabulans* and *M. prolapsa*, more adults were reared from baits buried in wet soil in 4/6 replicates but baits buried in very wet soil were either never colonised or produced few adults (Table 3).

Table 3

The effect of soil moisture content on the colonisation of buried baits by (a) *Muscina stabulans* and (b) *Muscina prolapsa*.

a.

	Muscina stabulans		*Muscina stabulans*	
Water content g water g soil^{-1}	Control 0.375 ± 0.0021	Wet 0.497 ± 0.0029	Control 0.375 ± 0.0021	Very wet 0.611 ± 0.0065
n	6	6	6	6
Median % adult flies emerging	37.7	62.3	100	0
Interquartile range	62.5		0.3	
Min %	0	5.4	99.3	0
Max %	94.6	100	100	0.69
Chi squared	62.08		1229.04	
P	<0.001		<0.001	

b.

	Muscina prolapsa		*Muscina prolapsa*	
Water content g water g soil^{-1}	Control 0.375 ± 0.0021l	Wet 0.497 ± 0.0029	Control 0.375 ± 0.0021	Very wet 0.611 ± 0.0065
n	6	6	5	5
Median % pupariae	46.7	53.3	99.8	0.2
Interquartile range	61.8		5.82	
Min %	5.3	0	88.9	0
Max %	100	94.7	100	11.1
Chi squared	188.47		478.3	
P	<0.001		<0.001	

3.4. The influence of bait moisture on oviposition and larval development

Dry liver presented in an empty jar attracted adult *M. stabulans* and *M. prolapsa* to feed but they laid either no or few eggs. Those eggs that were laid probably died before hatching as no larvae were observed in any of the dry liver replicates. By contrast, if dry liver was presented on the surface of soil, egg-laying was stimulated on and around the liver and some larvae developed to adulthood in all the *M. stabulans* replicates and most of the *M. prolapsa* replicates (Table 4).

Table 4

The ability of *Muscina stabulans* and *Muscina prolapsa* to colonise and develop upon dry liver

	Muscina stabulans		*Muscina prolapsa*	
	Dry liver in empty jar	Dry liver on soil surface	Dry liver in empty jar	Dry liver on soil surface
Number of replicates	5	6	5	6
Number yielding adult flies	0	6	0	5
Median number of flies (excluding zero value)		113.0		65.0 (68.0)
Minimum		15		0 (24)
Maximum		287		306
Interquartile range (excluding zero value)		233.0		225.8 (221.5)

3.5. *The influence of bait decomposition on oviposition and larval development*

In almost all replicates, egg-laying occurred on both fresh and decayed liver when exposed under laboratory conditions. However, *Muscina stabulans* showed a consistent preference for ovipositing on decaying liver, even when it was heavily decomposed, whilst the results for *Muscina prolapsa* were more variable (Table 5).

Table 5

The effect of decomposition on the colonisation of buried liver baits in the laboratory by: (a) *Muscina stabulans* and (b) *Muscina prolapsa*.

a)

Muscina stabulans										
Length of decay (days)	0	7	0	14	0	21	0	28	0	41
Number of replicates	7	7	7	7	7	7	6	6	7	7
Number of jars with over 50% of flies	2	5	2	5	0	7	0	6	1	6
Median % adults	47.4	52.6	39.3	60.7	28.6	71.4	22.5	77.5	23.3	76.7
Interquartile range	43.6		51.4		15.7		23.6		45.4	
Min %	22.5	22.6	1.8	8.9	15.5	61.2	9.1	58.5	0	15.7
Max %	77.4	77.5	91.1	98.2	38.8	84.5	41.5	90.9	84	100
Chi squared	276.6		458.9		370.6		539.9		498.1	
P	<0.001		<0.001		<0.001		<0.001		<0.001	

b)

Muscina prolapsa										
Length of decay (days)	0	7	0	14	0	21	0	28	0	41
Number of replicates	8	8	7	7	6	6	6	6	5	5
Number of jars with over 50% of flies	1	7	5	2	0	6	2	4	5	0
Median % adults	26.7	73.3	66.6	32.4	26.1	73.9	34.6	65.4	84.4	15.6
Interquartile range	34.2		49.1		24.0		49.2		19.0	
Min %	9.8	22.8	0	1.3	22.1	52.0	5.0	0.7	68.7	3.4
Max %	77.2	90.2	98.7	100	48.0	77.9	99.3	95.0	96.6	31.3
Chi squared	611.4		397.4		268.3		274.3		344.6	
P	<0.001		<0.001		<0.001		<0.001		<0.001	

Under field conditions, *M. prolapsa* colonised both fresh and heavily decomposed liver but *M. stabulans* was never recorded (Table 6). There was no significant difference in the number of *M. prolapsa* emerging from fresh and 43- or 49 day-old liver (Mann Whitney $U_{7,7} = 21.0$, $P > 0.05$; $U_{8,8} = 27.0$, $P > 0.05$). The only other insect recovered was the muscid fly *Hydrotaea ignava* (Harris) which colonised 2 jars of fresh liver and 4 jars of 43 day-old decayed liver but was only present when *M. prolapsa* was absent. Only one of the fresh liver baits and two decayed liver baits (49 day-old) were not colonised by insects.

Table 6

The effect of decomposition on the colonisation of buried liver baits under field conditions.

Length of decay (days)	0	43	0	49
Date out	6 August 2015	6 August 2015	25 June 2015	25 June 2015
Days exposed	5 days	5 days	4 days	4 days
Number of jars	7	7	8	8
Number colonised by *M. stabulans*	0	0	0	0
Number colonised by *M. prolapsa*	6	3	5	6
Median number *M. prolapsa* ± lower/upper CI (excluding zero values)	79.0 ± 28.3/98.0 (79.5 ± 44.4/102.9)	0 ± -11.6/118.4 (127 ± 28.9/220.4)	18.5 ± -5.3/143.1 (122.0 ± -1.45/221.9)	12.0 ± -7.6/55.6 (19.5 ±-11.2/75.2)
Number colonised by *Hydrotaea ignava*	0	4	3	0
Median number *Hydrotaea ignava* ± lower/ upper CI (excluding zero values)	0	65.0 ± -6.26/134.8 (88.5 ± 6.90/ 218.1)	0 ± -18.5/159.0 (215.0 ± -14.2/ 388.8	0

3.6. Influence of alternative baits on oviposition and larval development

Fresh pig blood attracted adult flies to feed and lay eggs within minutes of being introduced into the culture cages and it supported larval growth to adulthood (Table 7). Dead slugs and snails also rapidly attracted adult flies to feed and lay eggs of

them. On the slugs, eggs were scattered over the body surface and on the nearby soil whilst on the snails the eggs were laid on the shell surface, on any exposed flesh, and on the surrounding soil. Even large slugs (~5g) with thick skins could be exploited by first instar larvae. Both slugs and snails supported larval growth to adulthood.

Fresh horse dung attracted adult flies to feed within seconds of being introduced into the culture cages. Although flies were stimulated to lay eggs in large numbers (estimated >100 per jar), the majority of larvae died during the first instar and none of the replicates yielded pupariae or adult flies. In none of the replicates did the soil or baits totally dry out and in none of them was the bait fully consumed. The death of the larvae was therefore not a consequence of starvation or desiccation.

Melon, banana, and fungi all attracted adults to feed and eggs were laid on both the bait and on the surrounding soil. Melon was particularly attractive and every replicate resulted in large numbers of first instar larvae feeding on the fruit. However, none of diets supported the development of more than a few insects to adulthood and most larvae died during the first or early second instar. In none of the replicates did the soil or baits totally dry out and in none of them was the bait fully consumed. The death of the larvae was therefore not a consequence of starvation or desiccation.

Table 7: The ability of pig blood, dead molluscs, horse faeces, mushrooms, banana, and melon to support the growth of *Muscina stabulans* and *Muscina prolapsa* to adulthood.

Muscina species	bait	Number of replicates	Number of replicates yielding adults	min	max	median	Interquartile range
stabulans	pig blood	6	6	75	555	250	378.0
prolapsa	pig blood	4	4	40	93	67	44.8
stabulans	dead slugs	4	4	101	238	160.5	107
prolapsa	dead slugs	4	4	34	138	119.5	86.3
stabulans	dead *Cornu aspersum*	2	2	50	251		
prolapsa	dead *Cornu aspersum*	1	1	214	214		
stabulans	dead *Cepaea nemoralis*	1	1	52	52		
prolapsa	dead *Cepaea nemoralis*	1	1	37	37		
stabulans	horse faeces	7	0				
prolapsa	horse faeces	7	0				
stabulans	mushrooms	6	2	0	6	0	3.0
prolapsa	mushrooms	8	4	0	3	0.5	1.75
stabulans	banana	5	3	0	2	1	1.5
prolapsa	banana	5	0				
stabulans	melon	6	6	1	5	2.5	4.0
prolapsa	melon	5	3	0	2	1	1.5

4. Discussion

Although both *M. stabulans* and *M. prolapsa* breed throughout the year under laboratory conditions, in the field the adults are found most commonly during the warmest months in those countries with temperate climates. For example, in Central Spain, adult *M. stabulans* and *M. prolapsa*, are virtually absent during the winter, most abundant during the summer months and found to a lesser extent in spring and autumn [17]. A similar distribution pattern has been described in Lisbon in Portugal although somewhat fewer *M. stabulans* were recorded in summer than in the spring and autumn [18]. Skidmore (1985) [11] stated that in the UK, adult *M. stabulans* and *M. prolapsa* are active from April to June and from August to early November

although I recorded a much more restricted period of adult activity that included July. In Germany, Bernhardt et al (2016) [19] found that adult *M. prolapsa* were active between early March and late October/ early November but with evidence two peaks of activity in June and in late July/ early August. Similarly, also in Germany, Schroeder et al (2003) [20] noted two peaks of colonisation of human corpses in spring and late summer by *M. stabulans*. The adult flies have been recorded as hibernating overwinter in houses and cellars [21] although some authors state that state that they usually overwinter as pupae [22].

My field experiments demonstrate that *M. prolapsa* and *M. stabulans* larvae are able to co-exist and the absence of *Muscina* larvae was not associated with an increase in the diversity and abundance of other insect larvae. It is therefore unlikely that *Muscina* larvae were limiting the colonisation of remains by other Diptera as has been observed with highly predacious species such as *Chrysomya albiceps* (Wiedemann) [23]. None of the *Muscina* species reared from field-collected insects produced insect parasitoids. However, because the baits were exposed for only a few days before being transferred to the laboratory, this would exclude species that infect 3rd instar larvae and pupariae. It is therefore uncertain to what extent living in the soil profile protects *Muscina* from attack by insect parasitoids.

Under laboratory conditions, jars containing exposed remains yielded more adult flies than those in which the baits were buried. Presumably, this was a consequence of the greater odour released by the exposed remains attracting flies and stimulating oviposition. However, in most reports of field experiments with fresh exposed remains the vast majority of insects recovered are almost invariably larvae of blowflies such as *Calliphora vicina* (Rob-Desvoidy), *Lucilia sericata* (Meigen) and *Chrysomya albiceps* [24, 25]. The relative paucity of recordings of *Muscina* species may therefore be a consequence of them being outcompeted and/or tending to feed underneath the remains where they are less likely to be sampled rather than the adults avoiding laying on fresh exposed remains. However, if the density of *Muscina* larvae is very high (hundreds of larvae) then some of them move above ground and form a larval mass around the food source in a similar manner to *Lucilia sericata* larvae but without exhibiting the frothing that is a characterises this species (pers. obs.).

M. prolapsa larvae have been said to be predominantly associated with large dead bodies, such as those of deer [11]. However, I found that in both the field and the laboratory baits weighing approximately 75g will stimulate oviposition by both *M. stabulans* and *M. prolapsa* and support growth of their larvae to adulthood. Both species are therefore potential exploiters of both whole corpses and body parts. It is also clear that they will colonise blood-soaked soil and therefore may be present at the site where a body was left before it was moved elsewhere. Similarly, from the case described by Nuorteva (1974) [4] they will also infest blood-soaked clothing and probably also cloth furnishings and bedding and the chances of them completing their development will be enhanced if it does not subsequently dry out.

Soil moisture has a marked effect on the normal soil invertebrate fauna [26, 27, 28] and is an important factor in the rate of decomposition of buried remains [29]. My results indicate that even remains buried in waterlogged soil can be colonised but it substantially reduces the likelihood of infestation and the number of larvae that survive to adulthood. Although more adults were reared from wet soil than 'normal' soil, the differences were slight and suggest that the soil must be extremely damp before the colonisation of buried remains is compromised. Dry soil prevented the colonisation of buried remains: presumably, any larvae died of desiccation before or shortly after hatching. This could be a major factor during dry spells in the summer when the surface layers of soil become baked dry. However, extremely dry soil starts to crack and adult *Muscina* will then followcrevices in the soil to get as close as possible to buried bait before laying their eggs (pers. obs.). By contrast, Szpila *et al* (2010) [30] found that the first instar larvae of the sarcophagids *Eumacronychia persolla* (Reinhard) and *Phylloteles pictipennis* (Loew) are able to locate baits buried in dry sand so perhaps they have adaptations that reduce excessive water loss. They also found that in field experiments in Poland that *M. prolapsa* and *P. pictipennis* would occasionally co-infest rat carcasses buried at ~45cm.

The low moisture content and tough consistency of dry and mummified remains presents a serious challenge to insects that are sensitive to water loss and those lacking robust mouthparts. As a consequence they are colonised by a specialist invertebrate fauna composed predominantly of Coleoptera such as dermestid beetles [24, 25]. It is therefore not surprising that liver baits that were completely dry were

not colonised by *Muscina* larvae if these were presented in an empty dry environment. Dry baits were, however, exploited if they were presented on the surface of soil within which the larvae could shelter and avoid desiccation. In the latter circumstance, the flies tended to lay their eggs on the soil around the dry liver and the larvae remained in the soil for most of the time. The dry liver absorbed some moisture from the soil over time but it remained tough and leathery throughout the period the larvae were developing. The larvae mostly fed on the under-surface of the dry liver where it was in contact with the soil but the presence of holes in the liver indicated that they fed on it rather than microbes growing in the soil or the surface of the liver. The results indicate that *M. stabulans* and *M. prolapsa* cannot exploit mummified remains if these are in an exposed dry environment but if they are placed on the surface of moist soil then colonisation is possible. Similarly, the larvae may be able to exploit remains at the later stages of decay when these are starting to dry out provided that the remains are on the surface of moist soil. The dry liver was extremely attractive to *Dermestes maculatus* (De Geer) adults and larvae. When exposed to the beetles in an otherwise empty plastic container the dry liver became covered in actively feeding individuals within minutes. The dry liver therefore did not present a physically difficult substance to insects that are adapted for this sort of material.

The invertebrate fauna associated with a corpse changes as the body decays and can be used as an indicator of the time since death [24, 25]. Nevertheless, although there are many field studies on the colonisation of human and animal remains [31, 32, 33] the species recorded are strongly determined by what is present in the locality at the time. One is therefore uncertain whether a species that reportedly did not arrive until the later stages of decay did so because it was not present earlier was previously being outcompeted by other species, or fresh remains did not stimulate oviposition. For example, the stratiomyid fly *Hermetia illucens* (L.) is usually considered to colonise remains that are starting to dry out but its larvae have been found colonising the body of a pig that had been dead for less than 7 days [34]. There are few experimental studies on the relative attractiveness of fresh and decomposed remains when both are presented simultaneously although female burying beetles *Nicrophorus vespilloides* (Herbst) are more strongly attracted by the odours emitted by pigs at the later stages of decay than those that are freshly dead [35].

My studies demonstrate that adults of both *M. stabulans* and *M. prolapsa* are attracted to liver that has undergone extensive microbial decomposition both to feed and to lay their eggs. Even liver that was over 40 days-old and its black decomposition fluids were instantly attractive to the adult flies and supported larval development to adulthood. It is therefore clear that both species are able to colonise remains that are fresh and those that are in the later stages of decomposition. Indeed, these results suggest that decomposed remains can be even more attractive than those that are fresh. This would accord with the statement of Thomson (1937) [36] that *M. stabulans* and *M. pabulorum* (= *prolapsa*) are more likely to be associated with decomposed animal matter that has already been exploited by blowflies. In field experiments, *M. prolapsa* has been found to colonise unburied pigs in the second year of exposure but not the third [37]. Consequently, if *M. stabulans* and *M. prolapsa* are to be used to determine the minimum time since death one must be careful to consider also the state of decay since the eggs could have been laid at any point from immediately before death on soiled clothing to over a year after the person died. Although heavily decomposed remains may attract insects and stimulate egg-laying they may not provide the optimum nutrition for the larvae because of the changes in the biochemical composition of remains. For example, as little as 7 days decomposition of pig's liver can reduce the development rate of *Calliphora vicina* larvae considerably [38].

The ability of both *M. stabulans* and *M. prolapsa* to develop on dead slugs and snails indicates that they are not restricted to mammalian corpses and they will exploit even small dead invertebrates. *M. stabulans* has been reared from dead snails [39] and *M. levida* (syn. *assimilis*) has been recovered from dead *C. nemoralis* on several occasions [40]. Therefore, the consumption of dead invertebrates is not likely to have arisen as a laboratory artefact as a consequence of the absence of alternative larval food. I have observed *M. stabulans* to lay eggs on dead final instar *Mamestra brassicae* (L.) and *Galleria mellonella* (L.) caterpillars and the larvae subsequently develop to adulthood (pers. obs.). There are several accounts of *M. stabulans* being reared from other insects although it is not always easy to determine from some of them whether these are instances of predation, parasitism, or scavenging [41, 42, 43]. Nevertheless, it is clear that the larvae are general detritivores but both species require a high protein diet that may be of either vertebrate or invertebrate origin. The larvae

do, however, migrate away from any food source if the larval density is high and/or the food starts to run out (pers. obs.). Consequently, if either species is recovered from a dead human it would be important to check the vicinity for not only vertebrate corpses but other decaying animal matter.

Smith [44] states that *Muscina* larvae are more commonly associated with faeces than dead bodies but my data indicates that whilst horse faeces attracts adult flies and stimulates oviposition it does not offer an effective larval rearing medium. Within 24 hours of being in the culture cages over 100 first instar larvae were visible within each jar but most of these subsequently died either before or shortly after reaching their second instar. The dead larvae were visible on the sides of the jars and the surface of the soil and horse faeces and therefore mass mortality was not a consequence of cannibalism. Portchinsky (1913) [45] considered the larvae of *M. stabulans* to be active predators but stated that he had never observed cannibalistic behaviour. It is possible that omnivore and carnivore faeces may be a more effective larval diet since it contains more protein than horse faeces. There are several reports of *M. stabulans* larvae being found in poultry manure [46, 47] and Hafez (1941) [48] states that *M. stabulans* can breed in the faeces of humans, horses and cattle. The attraction of *Muscina* to human faeces means that it can be a useful indicator in cases of neglect in which diapers remain unchanged for prolonged periods. For example, Benecke & Lessig (2001) [49] describe a case in which the presence of third instar *M. stabulans* larvae in the diapers of a dead child indicated that he had been neglected for several days before he had died.

There are reports of adults of various species of *Muscina* visiting the fruiting bodies of fungi but only *Muscina angustifrons* appears to utilise fungi as a major larval food source [50]. Our results indicate that although both *M. stabulans* and *M. prolapsa* can complete larval development on *Agaricus campestris* it does not stimulate intensive egg-laying and most larvae die before pupariating so it is unlikely to be a major food source in the wild.

According to Catts & Mullen (2002) [22] adult female *Muscina* will lay their eggs on the surface of rotting fruit and the presence of *Muscina* larvae in fruit has been given as the explanation for some cases of pseudomyiasis. I can confirm that

melon, and to a lesser extent bananas will stimulate oviposition but the vast majority of larvae died during the first instar and hardly any developed to adult emergence. This would emphasise the need of both *M. stabulans* and *M. prolapsa* larvae for a high protein diet to complete their development.

5. Conclusion

The data indicates that *Muscina stabulans* and *Muscina prolapsa* share a very similar biology. Both species are active primarily during the summer months and both fresh remains and those that have undergone extensive decomposition are attractive to adult flies and support larval development to adulthood. Dry soil prevents the colonisation of buried remains and dry remains cannot support larval development unless there is a moist medium within which the larvae can retire. Waterlogged soil reduces the colonisation of buried remains but does not prevent it entirely. Although horse faeces and vegetable matter stimulate oviposition they do not provide a complete larval diet and the majority of larvae die before they reach the third instar.

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