**Myxomatosis: some observations
on breeding the European rabbit flea *Spilopsyllus cuniculi*
(Dale) in an animal house**

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(Received 16 October 1973)

SUMMARY

Rabbit fleas for use in Myxomatosis investigations have been successfully bred on rabbits in an animal house. The timing of emergence appeared to be governed by a biological timing control interacting with different forms of disturbance. Yield was found to be related to litter size, the time the doe and her kittens were removed from the nest, the number of fleas put onto a doe before littering and the mean ambient temperature to which the doe was exposed in the week pre-partum. The survival rate of fleas in storage was affected by temperature, the degree of crowding, moisture content of the containers, whether fleas were fed or unfed and the source of fleas in terms of emergence times.

INTRODUCTION

The European rabbit flea, introduced into Australia in 1966 (Sobey & Menzies, 1969) has been studied in wild rabbit populations (Williams, 1971; Williams & Parer, 1971; Sobey & Conolly, 1971; Williams, Fullagar, Kogon & Davey, 1973) where it has readily established. The usefulness of the flea in terms of rabbit control is still under investigation. Should the flea be regarded as useful it could be required in large numbers for general dissemination, and information relating to the breeding of the flea would be of value. The key to breeding this flea is the dependence of its reproductive cycle on hormone(s) produced by the pregnant rabbit doe (Mead-Briggs & Rudge, 1960). This finding has been elaborated by Mead-Briggs (1964) and Rothschild & Ford (1964, 1966). The extraordinary dependence of this flea on the rabbit is exemplified by the requirement of the presence of kittens for copulation to take place, as was shown by Mead-Briggs & Vaughan (1969).

The present paper describes a method of breeding rabbit fleas in an animal house and some observations on the variables affecting flea production.

EXPERIMENTAL

Rabbits

Randomly bred white domestic rabbits, albino or vienna (vv) were used. A pelleted feed and water were supplied *ad lib*, supplemented twice a week by green feed in the form of freshly cut oats or millet.

Table 1. *The effect on yield of adding 0.5 in. of soil to the bottom of the breeding nest box*

Times	Treatment	Total yield	No. weaned	Yield/kitten
1	Soil	11,805	25	472
	No soil	16,530	26	636
2	Soil	33,120	105	315
	No soil	14,100	51	276
3	Soil	8,875	16	554
	No soil	9,025	17	531

Fleas

The fleas used were descendants of an importation from England in 1966 (Sobey & Menzies, 1969). To ensure genetic diversity and to avoid selection for our particular animal house conditions, the breeding fleas for seeding on to pregnant does were a mixture collected over the emergence period 17–45 days post-partum. To further ensure genetic diversity fleas were periodically collected from the field, screened to ensure they were virus free and added to the breeding fleas. Fleas put on to a doe before littering are referred to as 'seed' fleas.

Addition of soil to nest-boxes

When the flea breeding began it was standard practice to cover the nest-box tray with 0.5 in. of clean soil. Since it involved extra work the necessity for the addition of soil came into question and some comparative tests were made; these were conducted at three different seasonal times as it was thought that soil might become important at times when yields were low. The results given in Table 1 suggested that the addition of soil does not affect yield and its use was discontinued.

Assessment of flea numbers

Counting large numbers of fleas was found to be tedious and time consuming. Volumetric measurements were made and the relationship of these to count is illustrated in Fig. 1. These measurements were made with active, freshly combed fleas. Stored fleas, often more sluggish and with their stomachs depleted of blood, occupied less volume than active fleas. Active fleas allowed to stand for 15 min. and read undisturbed, showed a markedly reduced volumetric reading; however, if held in the hand for reading and thus disturbed slightly, the volume returned to that observed prestanding. In the present study, conversions from volume to numbers of fleas were made using Fig. 1.

A general method used for breeding fleas

The following method was planned to accommodate the dependence of the breeding cycle of the flea on that of the rabbit. Does were palpated to confirm pregnancy and introduced into a breeding cage at approximately 9 days pre-partum at which time they were seeded with fleas. The number of fleas put onto a doe varied in general between 100 and 500. The breeding cage was supplied with a sheet metal nest-box, 20 in. long by 12 in. wide by 12 in. high. The nest-box had

Fig. 1. The relationship of volume to number for freshly combed 1st and 2nd emergence fleas.

a detachable floor tray with a 3 in. high surround. The floor of the breeding cage and the nest-box tray were covered with straw to a depth of about 3 in. Litters were not counted until 5 days post-partum; the number then found was referred to as the number born. The doe and her kittens were removed from the breeding cage between 12 and 20 days post-partum, combed free of fleas and moved to a fresh breeding cage. The nest-box tray was then detached and either emptied into a large plastic bag or placed in a floor-pen containing a 'sweep' rabbit, usually an adult doe. Sweep rabbits were counted three times a week and the number of fleas was counted or measured volumetrically and recorded. The large plastic bags, 'bagged fleas', were emptied every 2 or 3 days into a large enamelled tray with 8 in. sides and the emerged fleas aspirated, counted and recorded.

The observations to be described cover two periods, the first when all nests were bagged and the second when fleas were collected via a sweep rabbit. The reason for changing from bags to sweep rabbits was to reduce the labour involved in flea collection. Variation in the rate of multiplication, in terms of fleas reaped divided by fleas seeded, over the total period of observation is illustrated in Fig. 2. During the period when nests were bagged as a routine, there was only limited temperature and humidity control in the animal house. Multiplication rate varied widely according to the time of the year, with the highest rates occurring between June and December and the worst, including many complete failures, during January to May. After 1971 with better temperature and humidity control, seasonal variation disappeared.

Fig. 2. Mean monthly multiplication rates, number of fleas seeded and reaped, and number of does seeded annually during 1969 to 1973.

Fig. 3. Some examples of emergence patterns. (A) The nest in its tray was removed from the doe and her litter on the 17th day post-partum and placed in an undisturbed cabinet with a collecting bottle at the base. At 44 days the nest was scraped out of the nest-box tray into a floor pen housing a sweep rabbit. (B) The nest in its tray was removed to the laboratory at 23 days post-partum and suspended at room temperature in a polythene bag with a small opening into a collecting bottle. It was disturbed at least once daily by gentle movement of the straw from above and a few sharp blows underneath. At 55 days it was emptied into a floor pen with a sweep. Counts of emerging fleas were made daily. (C) The nest in its tray was placed in a floor pen with a sweep rabbit at 18 days post-partum and the sweep combed every 2-3 days. At 44 days the nest was scraped from its tray and returned to the sweep. The mean temperature for the week pre-partum was 19.5° C. (D) The nest in its tray was placed in a floor pen with a sweep rabbit at 16 days post-partum and the sweep combed every 2-3 days. At 36 days the nest was scraped from its tray and returned to the sweep. The mean temperature for the week pre-partum was 22° C.

Flea emergence

In general an early and a late emergence was found for both bagged fleas and fleas collected via a sweep. Some examples of the types of emergence patterns observed are illustrated in Fig. 3. In A the nest-box was removed from the doe and her litter on the 17th day post-partum. The tray of the nest-box was placed in a dark cabinet, with a collecting bottle in the base, and left undisturbed until 44 days post-partum when the nest was scraped out into a floor pen containing a sweep rabbit. Few fleas emerged until the nest was vigorously disturbed. In B the nest in its tray was removed to the laboratory at 12 days post-partum and suspended in a polythene bag with a small opening leading into a collecting bottle. The nest was disturbed at least once daily by gentle movement of the straw from above and a few sharp blows from below. At 55 days post-partum the nest was scraped into a floor pen containing a sweep rabbit. Counts of emerging fleas were made daily. With regular disturbances an emergence pattern with two main peaks was observed. The violent disturbances of scraping the nest out at 55 days post-partum resulted in further emergence of fleas. In C and D the nests in their trays were placed in two floor pens, each with a sweep rabbit. C was removed from the

Table 2. *Correlations between the time post-partum at which emergence peaks occurred and the mean ambient temperature to which does were exposed pre- and post-partum within the range of mean temperature 18.9 to 23.3° C. (min. 15, max. 27). N refers to the number of nests on which the correlation was based*

Emergence	1 week pre-	1 week post-	2 weeks post-	3 weeks post-
1st peak	-0.59	-0.48	-0.13	-0.20
N	40	43	36	34
2nd peak	-0.42	-0.18	-0.43	-0.28
N	38	41	33	31

doe and her kitten 18 days and D 16 days post-partum and the sweeps were combed every 2-3 days. Clearly the two main emergence peaks occur in the presence of continued rabbit disturbances. The difference between C and D in the timing of the emergence is possibly a result of the mean ambient temperature to which the does were exposed in the week pre-partum, C 19.4° C. and D 22.2° C. respectively. On the basis of the above data nests put to a floor pen and sweep rabbit were removed from the doe and her kittens at or about 17 days post-partum. Occasionally a nest was fouled by the doe with urine which raised the humidity, particularly in bagged nests. This resulted in a low yield at 2nd emergence and infestation of the fleas by different species of mites (*Acarus siro* Linnaeus, *Proctolaelaps* sp. Berlese, *Cheyletus malaccensis* Oudemans, and *Macrocheles* sp. Latreille). Fouling of the nest-tray by a sweep rabbit resulted in a low yield particularly among the late hatch fleas.

Temperature

Decreased mean ambient temperature increased the time post-partum that the emergence peaks occurred. Correlations between the time post-partum that the emergence peaks occurred and mean ambient temperatures to which does were exposed pre- and post-partum are shown in Table 2. The effect of temperature on the first emergence peak was greatest during the week pre- and the week post-partum, with the emergence peaks occurring later as the mean temperature decreased. Second peaks also occurred later at lower temperatures. An example of this peak shift with temperature is shown in Fig. 2C, D. The mean temperature to which the doe was exposed during the week before she littered was negatively correlated with yield ($r = -0.31$ est. s.e. 0.1) within the range of mean temperature 18.9 to 23.3° C. (min 15°, max 27° C.).

Litter size

The correlation between yield and the number of kittens born was $r = 0.33$ (est. s.e. 0.16) and between yield and the number of kittens weaned was $r = 0.32$ (est. s.e. 0.16). The data are limited because when nests were first exposed to sweep rabbits for flea collection more than one nest was put to one sweep. Later when more floor pens became available single nests were given individual sweeps and the above data are based on single nests. Nests where all the kittens died generally failed to produce fleas.

Fig. 4. Changes in mean yield for different mean numbers of kittens weaned for different mean ambient temperatures to which the does were exposed in the week pre-partum. ●, 19-21° C.; ×, 21-22° C.; ○, 22-23° C.

Table 3. *A comparison of the yield from nests in which no kittens were lost with those in which one was lost in the first week post-partum*

No. kittens born	No. lost in first week	No. of nests	$\bar{x}$ flea yield $\times (5 \times 10^2)$
9, 10, 11	0	7	9.3
9, 10, 11	1	8	6.6
8	0	8	6.7
8	1	10	6.4
7	0	21	10.1
7	1	7	9.1
6	0	12	9.8
6	1	7	7.2
5	0	13	7.3
5	1	6	5.3
4	0	11	6.4
4	1	4	5.9

Only single nests were included during a time when yields were good.

The relationship of yield with the number of kittens weaned and the temperature to which the doe was exposed in the week before littering is illustrated in Fig. 4. Yield increased with increased number of kittens weaned and at each mean the yield was highest where the temperature to which the doe was exposed in the week pre-partum was lowest. There was a suggestion that at temperatures above 21° C. litters greater than six weaned have no advantage over those with six weaned.

The loss of a kitten in the first week post-partum was found to depress flea yield, as shown in Table 3. The loss of yield was consistent over the range of litter

Table 4. *The yield of fleas in relation to the number of fleas seeded onto does 9 days pre-partum*

Seeding rate, fleas/doe					
500		1,000		1,500	
No. weaned	Yield	No. weaned	Yield	No. weaned	Yield
5	798	4	1,410	8	2,380
8	4,910	5	237	5	825
6	4,150	4	3,698	8	2,305
2	25	5	570		
6	735	6	3,315		
2	1,280				
8	6,295				
3	4,090				
40	22,283	24	9,230	21	5,510
Yield/kitten	557		385		262
Yield/doe	2,785		1,846		1,836

sizes examined. Further, in comparisons of litters where no kittens were lost with litters which were reduced to the same size by the loss of a kitten, the litters without loss yielded more fleas on the average. In litters where all the kittens were lost between 5 and 10 days post-partum fleas were recovered from only a few nests. This observation must be qualified to the extent that many of these nests were discarded early, and might, had they been late yielding nests, have produced some fleas.

Seeding rate

Early experiments suggested that although lower seeding rates (100/doe) gave a higher rate of multiplication than higher rates (500/doe), the higher seeding rate produced more total fleas per doe and fewer complete failures. As fleas became more plentiful, seeding rates in excess of 500/doe were examined as a possible means of increasing flea production but they proved disappointing. The comparative yields from seeding rates of 500, 1000 and 1500 fleas per doe, are shown in Table 4. These data suggest that seeding rates in excess of 500 may confer some disadvantage to flea production as compared to 500 or less. This is further illustrated in Fig. 5 where the data suggest that the rate of multiplication decreases with increased seeding rate: further, yield per doe was highest and fewer nests failed to produce more than the number of fleas seeded, where 500 fleas were seeded. These data must be interpreted with some caution since the different seeding rates were not examined concurrently and all nests were not reaped in the same manner.

Storage of fleas

Allan (1956) was able to store fed and unfed fleas for nine months at -6.7°C . Chapple & Lewis (1965) had 20% survive at 20 days and none at 42 days in a refrigerator at 4°C . In bottles stored underground they observed a 5% survival after 74 days. In the present work a temperature of 2°C . was selected as it was felt that temperatures between 2° and 4°C . would be readily available to workers

Fig. 5. Multiplication rates (fleas reaped/fleas seeded) observed for different numbers of fleas seeded.

producing fleas, whereas temperatures from 0 to -10°C . might be difficult to obtain even if they proved better for flea storage.

It soon became apparent that the storage survival rate was very variable and affected by such things as size and type of container, degree of crowding, moisture content of containers, whether fleas were fed or unfed and the source of fleas in terms of emergence times.

In most of the work reported here, fleas were stored in 3 ml. flat-bottomed auto-analyser cups (plastic bottles) made of clear polystyrene and sealed with polyethylene caps. These tubes were initially selected because they were cheap and suitable as disposable containers for fleas being disseminated in the field. Their being plastic did not appear to confer any disadvantage. When compared with screw capped glass 1 oz. McCartney bottles with roughly equal numbers of fleas per volume, the 50 % survival time for the McCartney bottles was approximately 25 % lower than that for the plastic bottles. It was found that 100 fleas/3 ml. of container volume approached the 'overcrowding' limit for storage. With 150 or more/3 ml., fleas were, when removed from the cold storage, mostly dead, but on occasions anaesthetized, giving the appearance of being dead. We have observed apparently dead fleas show 100 % recovery after 2–3 hr. exposure to fresh air. Fleas from overcrowded bottles, whether or not they survived after storage, were observed to have their legs drawn up close to their bodies in contrast to fleas which

Table 5. *The percentage survival of fleas kept at room temperature and at 2° C. with and without the addition of moistened filter paper*

Treatment	% Survival 9 days at 22–24° C.		56 days at 2° C.
	In light	In dark	
No filter paper	26	60	52
With filter paper added	25	33	30
With moist filter paper added	47	80	64

Fleas were stored in 3 ml. auto-analyser bottles at 20 fleas/bottle. Filter paper added was 1 cm.² and where moistened approximately 10 % by weight water added.

Table 6. *The half-life in days of fleas fed or unfed for different emergence times, stored at 2° C. in auto-analyser bottles at 100 fleas/bottle with no moisture or filter paper added*

Time of emergence	Half-life in days	
	Fed	Unfed
At or near 1st peak	55–65	60–70
Between 1st and 2nd peaks	50	—
At or near 2nd peak	70–80	90–120

Table 7. *The percentage survival of fleas from the peaks of 1st and 2nd emergence stored for 80 days at –1° C. or at 2–3° C.*

Origin of fleas	Treatment (° C.)	Sex	% Survival at 80 days	No. of fleas
1st Peak of Emergence	–1	♂♂	9	347
		♀♀	13	503
	2–3	♂♂	13	339
		♀♀	22	510
2nd Peak of Emergence	–1	♂♂	33	263
		♀♀	34	303
	2–3	♂♂	55	290
		♀♀	60	299

died from other causes where the legs were usually extended. The role of CO₂ or other toxic gases in this intoxication has not been determined.

To examine the effects of moisture on storage, 1 cm.² pieces of filter paper with and without the addition of 10 % water by weight, were added to the auto-analyser cups. The percentage survival for different treatments is shown in Table 5. Fleas were stored at the rate of 20 fleas/bottle. Dry filter paper reduced the percentage survival at both temperatures and the addition of water increased the survival. At room temperature (22–24° C.) fleas survived half as long again when kept in the dark than when kept in the light.

Unfed fleas, collected from bags, stored better than fed fleas combed from

sweeps, as shown in Table 6. Second emergence fleas stored better than 1st emergence fleas and fleas collected at times other than the main emergence times stored badly. The effect of emergence times is further illustrated in Table 7 where considerably more 2nd emergence fleas survived to 80 days than 1st emergence fleas. These data also suggest that storing fleas below 0° C. did not confer a storage advantage. Other data illustrating the storage advantage of 2nd emergence fleas are presented in Fig. 6. Fleas reaped in the emergence trough between 1st and 2nd emergence stored badly.

Occasionally fleas were left at 2° C. for long periods. One batch of 2nd emergence unfed fleas after 7 months had 11 % (20/177) survivors. With another batch of 2nd emergence unfed fleas, put on a rabbit after 10 months storage, 2 out of 1600 were recovered.

DISCUSSION

It is clear that fleas can be successfully bred in an animal house under quite wide ranges of temperature and humidity although control of these factors provides increased breeding efficiency. Our observations suggest that flea yield is greatly depressed by high humidity and maximum ambient temperatures in excess of 24° C. In the absence of adequate temperature and humidity control, seasonal variation in total yield was observed. It is interesting that the time of maximum flea yield, June to December, corresponded with the breeding season in the field suggesting a parallel response, possibly hormonal, of wild and domestic rabbits to the same seasonal effect to which the fleas respond. A clear seasonal effect on litter size and conception rate both negatively correlated with ambient temperature was reported by Sittmann, Rollins, Sittmann & Casady (1964). The effect of increased flea yield with decreased ambient temperature to which a doe is exposed in the week pre-partum, seems likely to be physiologically related to the apparent temperature effect on rabbit breeding efficiency.

The observations on emergence suggest that while fleas emerge in response to a disturbance of the nest there is a biological timing mechanism present which allows only part of the yield to respond early, 15–30 days post-partum, and the rest to respond after 30 days post-partum. This timing of emergence could be a survival mechanism related to the breeding behaviour of the rabbit. The fleas emerging before 30 days could leave the nest with the doe and the departing kittens whereas the later emerging fleas would be available for the following litter whether it followed immediately or after the break of the non-breeding season. The observation that late emerging fleas are better able to survive storage than early emerging fleas supports this idea. Second emerging fleas occupy a greater volume per flea than first emerging fleas (Fig. 1) either because they are bigger, or more active, or both, suggesting a physiological difference which could be related to their storage capabilities.

It seems likely that the correlation between the number of kittens in a litter and the yield of fleas may represent a causal relation in that the larger the litter, the greater the probability of high levels of sex hormones available to the fleas via both the doe and the kittens, ensuring a maximal ovarian maturation (Rothschild

& Ford, 1966). Further, the 'factor(s)' needed to bring the male rabbit flea to the correct 'physiological state' for mating (Mead-Briggs & Vaughan, 1969) and to ensure the adequate transfer of sperm (Rothschild, Ford & Hughes, 1970) is (are) probably quantitatively correlated with litter size.

Although the best total yield was achieved with a seeding rate of 500 fleas (Fig. 5) the maximum multiplication rate at this level of seeding, 17, was well below the maximum of 26 where the seeding rate was between 100 and 200 fleas. At seeding rates of 500 or more the maximum multiplication rate was only 5. The cause of this decreased multiplication rate with increased seeding is unknown.

We are indebted to Professor David Lee of the School of Public Health and Tropical Medicine, University of Sydney and Dr R. Domrow of the Queensland Institute of Medical Research for their help in identifying the mites found on our fleas.

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