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Myxomatosis: the introduction of the European rabbit flea *Spilopsyllus cuniculi* (Dale) into wild rabbit populations in Australia

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SUMMARY

1. The European rabbit flea *Spilopsyllus cuniculi* (Dale) bred successfully in wild rabbits on three properties in New South Wales and, within two breeding seasons, almost every rabbit shot within a quarter of a mile of a release site was infested.

2. It was demonstrated that the flea transmitted myxoma virus in the field.

3. In areas where more than 75% of the rabbits shot at the beginning of the breeding season were flea-infested and myxoma virus was present, populations failed to show the expected summer build-up.

INTRODUCTION

Myxomatosis is still of considerable economic importance in Australia, although the way it kills rabbit populations is less dramatic than it was when myxoma virus was first released in 1950. The transmission of myxomatosis has been mainly by mosquitoes (Fenner & Ratcliffe, 1965). Because they confer a selective advantage on attenuated 'field strains' of virus and because they are most prevalent in the hot months, during which rabbits have the best chance of recovery from myxomatosis (Mykytowycz, 1956; Marshall, 1959), mosquitoes are not ideal vectors of virus for rabbit control. Further, the presence of mosquitoes in numbers large enough to cause intense annual epizootics has a restricted geographical distribution. In many of the more elevated areas – on the northern tablelands of N.S.W., for example – mosquito-induced intense epizootics have been as infrequent as one year in four. There is evidence (R. T. Williams, personal communication) that the mite *Listrophorus gibbus* (Pagen) may be the vector responsible for winter epizootics in the Snowy Plains and Canberra areas. This mite is widespread throughout Australia and is thus probably part of the ecological background to any field studies of the flea.

The reintroduction of virulent virus into the field has been of limited value since it has not been able to maintain itself there (Fenner, Poole, Marshall & Dyce, 1957). To obtain the maximum usefulness from a reintroduction of virulent virus, it has been necessary to time the release of virus to coincide with mosquito activity and to release the virus on a wide scale (G. W. Douglas, personal communication). These procedures are likely to prove beyond the powers of the average landholder.

As early as 1956 the usefulness of inoculation campaigns to reintroduce virulent virus into the field came into question. Although it was conceded that, under favourable conditions, such campaigns could be useful, it was felt that it would be most profitable to allow myxomatosis to run its course and direct available resources into poisoning and other conventional methods of control (Fenner & Ratcliffe, 1965).

There seems every reason to believe that a better vector would improve the usefulness of myxomatosis in rabbit control, particularly if the vector were less limited by seasonal and geographical variation than the mosquito. In Britain the European rabbit flea *Spilopsyllus cuniculi* (Dale) is considered to be the principal vector for myxomatosis (Lockley, 1954; Armour & Thompson, 1955). Fleas are present on rabbits throughout the year (Allan, 1956), with the result that myxomatosis can be active throughout the year. In Kent 1964–7 at least 20% of each quarter year sample had virus (Vaughan & Vaughan, 1968). An understanding of the life-cycle of the flea by Mead-Briggs & Rudge (1960) enabled fleas to be bred in captivity. With the aid of this knowledge, the flea was introduced into Australia in 1966 (Sobey & Menzies, 1969) and released from quarantine restrictions in 1968.

Studies to determine how readily the flea would establish in the field were begun in June 1968. The present paper is an account of observations made during the 2 years following the release of the flea into wild rabbit populations.

MATERIALS AND METHODS

Experimental areas

Fleas were released on three properties in New South Wales:

(1) 'Millambri', a property of 3500 acres about 14 miles east of Canowindra in N.S.W., elevation about 2000 ft. One paddock of 500 acres was used on this property. Intense annual epizootics spread by mosquitoes occur on this property between December and March. When observations were begun in 1967 rabbit infestation was severe.

(2) 'Wing Vee', a property of 8500 acres about 45 miles south west of Mudgee in N.S.W. Several paddocks with a total area of 1700 acres were used; these are described more fully in the text. The elevation of the experimental area is about 2000 ft. Myxomatosis on this property has been sporadic and isolated and appears to have contributed little to rabbit control. During 1965–6 an extensive programme of poisoning by sodium fluoroacetate (1080) (Lazarus, 1956; Rowley, 1968) reduced the rabbits to very low numbers. By 1968, when experimental work was begun, rabbit numbers were increasing and during 1968 reached a high level.

(3) 'Longford', a property of 2500 acres about 25 miles west of Armidale. The elevation is 3400–3800 ft. Prior to experimental work myxomatosis was rarely reported on this property; there had been no explosive epizootic for the past 5 years.

Population density

On 'Millambri' a standard walk count (Myers, 1954; Rowley, 1968) was used as an estimate of population density. The same walk of about 1 mile, covered between 4.30 and 5.00 p.m., was made on each occasion. On 'Wing Vee' and 'Longford' counts were made by spotlight at night from a vehicle at a speed not exceeding 7 m.p.h. and expressed as rabbits per spotlight mile. Spotlight counts are much affected by the abundance of vegetation (B. Cooke, personal communication). Seasonal variation in vegetation cover can account for a two- or threefold variation in the number of rabbits counted.

Viruses

Two strains of virulent virus cloned in our laboratory were used: Glenfield (G.V.) strain no. 5 and Lausanne (Lu) strain no. 7: strain numbers according to Fenner & Marshall (1957). Virus samples collected from the field were passaged once and classified as 'virulent' or 'field strain' according to the type of lesion resulting from their subsequent intradermal inoculation into the shaved back of an unselected laboratory rabbit. Occasional checks on the classification of viruses as virulent, were made by noting survival time (S.T.); in no case did survival time contradict lesion-type assessment. Since virulent virus is seldom recovered from the field (Fenner & Chappel, 1965), any virulent virus recovered during the present study was regarded as having originated from releases made during the study.

After 1967 virus was disseminated on 'Millambri' and 'Wing Vee' by releasing infected fleas and on 'Longford' by inoculation via the eye of trapped rabbits that were then released (Sobey, Conolly & Adams, 1967). Fleas were infected by allowing them to probe skin, rich in virus, which had been prepared by scarifying as described by Rivers & Ward (1937). The skin was removed from the back of a rabbit 7 days after infection, stored at $-60°$ C. and thawed and warmed to 37° C. before feeding the fleas. Random samples from the infected fleas were tested for infectivity by feeding each flea on a marked area of the shaved back of an unselected domestic rabbit and noting the number which initiated lesions. Batch infectivity varied between 20% and 80% with a mean of 45%.

Rabbits were caught for infection via the eye by two methods:

(1) *Trapping*. Rabbits were trapped in gin-traps and the least damaged rabbits infected and released. Mature female rabbits were destroyed. On average, about 70% of the rabbits caught were inoculated.

(2) *Spotlight*. Rabbits caught in the beam of a spotlight mounted on the head of the operator could be immobilized by firing a shot from a 0·22 rifle an inch or two above the rabbit's head; the operator keeping the beam on the rabbit could then walk up to the rabbit and capture it.

Shot sample

Periodically, a sample, shot during the day or at night by spotlight, was taken from each property. A shot rabbit was collected immediately and combed for fleas. Only the head and ears were combed and only for 1 min. In Britain fleas were

found in numbers on the body for only about a fortnight during the whole year (Allan, 1956). Allan found most of the fleas on the ears (80 %) whereas in the present study fleas were found mainly on the head. A blood sample was taken from each rabbit (Sobey, Conolly & Adams, 1966). Sex was recorded and the abdominal cavity of all female rabbits was opened and the uterus exposed; where foetuses were present their age was estimated. Lactation (L) was also recorded. The testes of males were scored as exposed or withdrawn. Where present, virus was noted and sampled by taking an eyelid. Age was initially scored as adult or sub-adult on the basis of size. This classification was too coarse to give information about population age distribution and in the samples taken in the later part of the study age was determined by eye-lens weight (Lord, 1959; Myers & Gilbert, 1968).

Fleas

Fleas were bred in a small animal-house at a temperature of $72 \pm 5°$ F. with a minimum relative humidity of 55 %. One week before parturition a doe had 200 fleas placed on her head. She was supplied with a littering box with a removable bottom. Twelve to fourteen days after the litter was born, the litter and the doe were combed free of fleas and the nest in the cage bottom was removed and emptied into a large plastic bag which was hung from the ceiling of the animal house. From the time nests reached 20 days of age, measured from the time of parturition (i.e. 20 days after the litter was born), they were examined two to three times a week by being emptied into a large enamelled tray; any fleas were aspirated into 1 oz. McCartney bottles containing a strip of filter paper. Fleas were stored at $+2°$ C.

The introduction of the flea into the field in the early stages of the work was done by simply dropping fleas into an active warren opening in lots of 100 or 200. Infected fleas (IF) were spread similarly but in lots of 10–20 fleas.

RESULTS

Millambri

In each of the years 1966 and 1967, during the months October, November and December, about 500 rabbits were trapped across the whole property, inoculated via the eye with G.V. virus and released. Severe mosquito-borne epizootics occurred in December/January of both years, reducing the rabbit population in each case by an estimated 80–90 %. However, no G.V. virus was recovered during the course of either epizootic, thus confirming the findings of Fenner *et al.* (1957) that mosquitoes as vectors confer an overwhelming selective advantage on moderately attenuated strains of virus. In spite of the quite spectacular kills, sufficient survivors remained to ensure a resurgence of rabbits in the spring of 1967 and 1968.

Observations on fleas were made in a single paddock, 'Oak Hill', of about 500 acres which was inaccessible to vehicles and free from stock during the period of observation. No poisoning or other conventional forms of rabbit control were undertaken during the period of observation. The paddock is intersected by

numerous steep gullies and has a large, very steep, rocky outcrop at one end, from which it derives its name of 'Oak Hill'. From the base of the hill the paddock slopes up to a wooded rocky escarpment. The soil is shaley and not well grassed. There were numerous extensive rabbit warrens, particularly near the centre of the paddock in the region of two earth dams. The walk over which the count was made extended around the rocky outcrop to and from the dams.

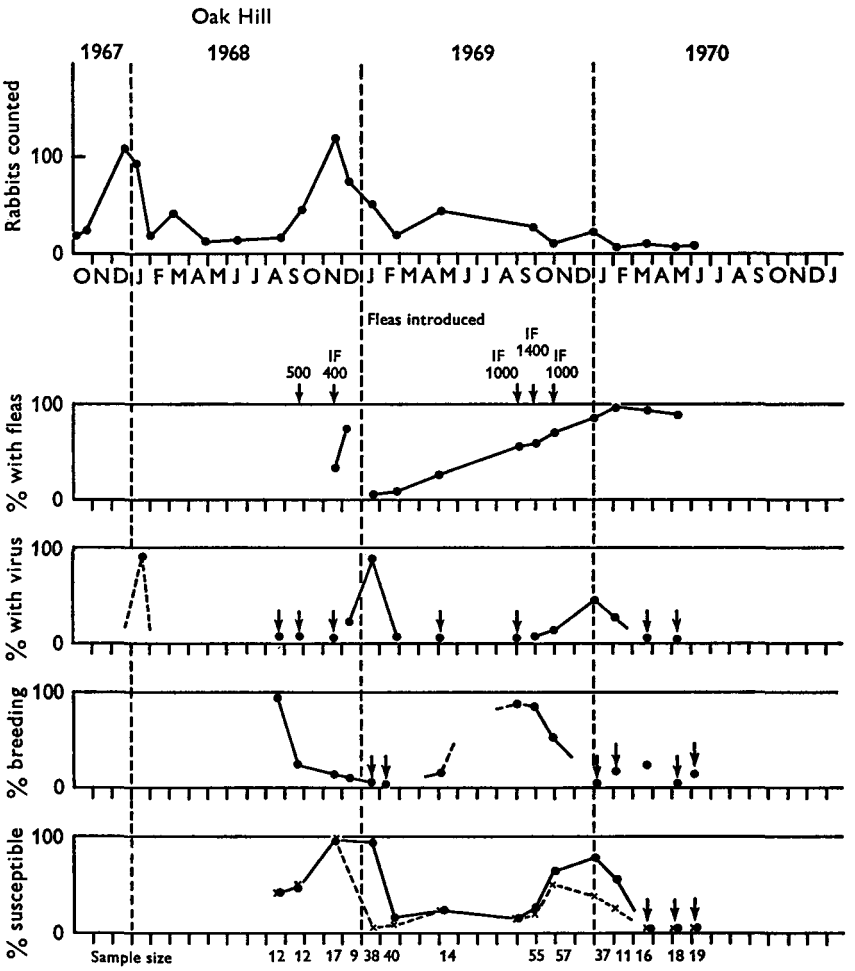

Fig. 1. Walk counts made in the paddock Oak Hill and the number of fleas introduced, together with data from shot samples showing the percentage of animals with fleas, with virus, breeding and susceptible. [× – – – ×, including those with active virus; ●—●, excluding those with active virus, of the % susceptible.]

Fleas were introduced into Oak Hill in September 1968 towards the end of the breeding season, as shown in Fig. 1. One hundred fleas were introduced into each of five major warrens in and around the area of the dams near the centre of the paddock. Fleas were found on rabbits from a shot sample in November, at which time Lu virus was released by the introduction of 400 infected fleas. Of nine rabbits shot in December seven had fleas and two of those with fleas were infected with Lu virus.

During January 1969 there was an epizootic probably involving mosquitoes from which only attenuated field strains were recovered and the proportion of rabbits with fleas fell to two out of thirty-eight sampled. In February 1969 the proportion of rabbits with fleas was still low, but subsequently, and coinciding with the 1969 breeding season, the proportion increased linearly with time until February 1970, when almost every rabbit shot had fleas. Infective fleas were distributed in Oak Hill in September and October 1969. The eight virus samples collected in September and October were all Lu virus. In January 1970, at the height of the epizootic, Lu virus was recovered from some of the infected animals, but in February all the virus recovered was attenuated field-strain.

Throughout the 1969 breeding season the shot samples contained fewer susceptible animals than from the previous year and this could be attributed to the fleas transmitting the virus to the young animals before they entered the count. In October 1968 58% of the shot sample was adult and in October 1969 84% was adult.

The salient features of the data presented in Fig. 1 are (1) the linear increase in the population of rabbits with fleas from about 5% to about 100% during the 1969 breeding season, (2) the absence of a population build-up in the spring of 1969 when compared with 1967 and 1968 as judged by the walk count, (3) the extended and less intense 1969 epizootic compared with the shorter and more dramatic 1968 epizootic, and (4) a reduced build-up of susceptible animals in the population of 1969/70 when compared with that of 1968/69.

Wing Vee

The counts on this property were made by the two owners of the property, one driving and one counting. This arrangement enabled counts to be made by a single operator using the same spotlight and vehicle throughout. The same fixed transect was driven on each occasion and care was taken to select dark nights, avoiding rainy or windy conditions. Counts using a tallycounter were made while driving at speeds not exceeding 7 m.p.h.

Fleas were first released in June 1968 into a paddock of 350 acres called Cattle Station. Nine hundred fleas were released into four openings in a major heavily populated warren. The first flea to be recovered from the field was found on a rabbit shot in this paddock in August of 1968. By the end of October 1968, 70% of the shot sample had fleas, these rabbits being shot over the whole paddock and not just in the vicinity of the release site. As shown in Fig. 2, the percentage of rabbits with fleas fell to just below 50% during March and April 1969 but then steadily rose during the breeding season to 100% in July 1969. The percentage of rabbits with fleas remained high thereafter, with a fall to 80% during March/April and a subsequent rise to 100% in May 1970.

There was an increase in the rabbit count during 1968/9 but, as the percentage of rabbits with fleas rose above 75%, the count fell and remained low without any summer build-up in 1969/70 in spite of almost continuous breeding. Counts recorded in Braziers, a paddock of 435 acres adjoining Cattle Station, are given in Fig. 2. In contrast with the population in Cattle Station, the population in Braziers rose steeply in the breeding season of 1968/9 when there were few fleas

in Braziers. There were two transects through Braziers, No. 1 running the length of the western boundary and No. 2 circuiting the area adjoining Cattle Station. Although the shot sample from Braziers was not separated in relation to these two transects, it was observed that the percentage of rabbits with fleas increased more rapidly in the area of Route 2 than Route 1. Running parallel to a non-rabbit

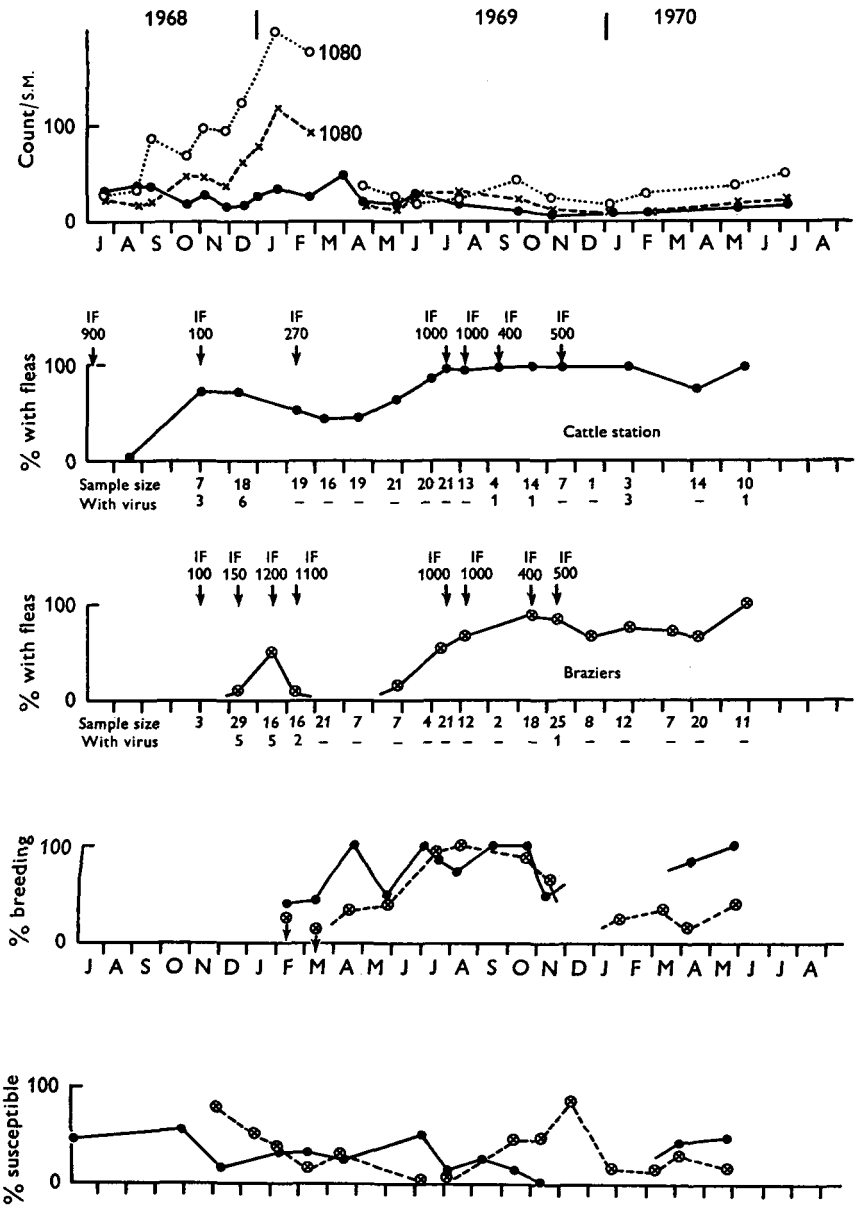

Fig. 2. Spotlight counts in two routes in Braziers prior to and after 1080-poisoning and in Cattle Station. Fleas added to and the build-up of fleas in these two paddocks is shown together with virus found, percentage breeding and percentage susceptible. ● — ●, Cattle Station; ○·····○, Braziers route 1; × – – – ×, Braziers route 2; ⊗ – – – ⊗ or ⊗———⊗, Brazier's route (1 and 2).

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netting fence, Route 1 was more prone to changes due to the movement of rabbits from untreated areas. Infected fleas were introduced into Braziers towards the end of 1968 and fleas were recovered in January 1969. During February, March and April 1969, when rabbit numbers were very high, fleas were not recovered. Subsequent to 1080-poisoning in April 1969, fleas were recovered in a progressively increasing proportion of rabbits during the 1969 breeding season, reaching 90% of rabbits in October of that year. The proportion of rabbits with fleas remained high but did not approach 100% until June 1970. There was no marked build-up in rabbit numbers after the 1968/9 breeding season.

Fleas were released in a number of paddocks along the transect route late in the 1968 breeding season, and before the middle of the 1969 breeding season almost every rabbit shot in these areas had fleas. In three areas, Davis A, Davis B and Hoppin' Charlie, which had a different history of flea release, the build-up of fleas was slower and it was not until the end of the 1969 breeding season that flea infestation reached very high levels. Changes in the rabbit spotlight count, expressed as a fraction of the June count in relation to the build-up in rabbits infested with fleas, are illustrated in Table 1.

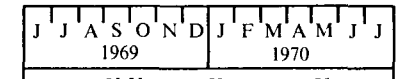
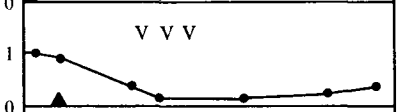
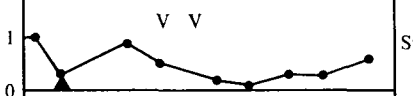
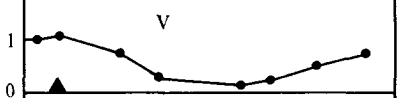
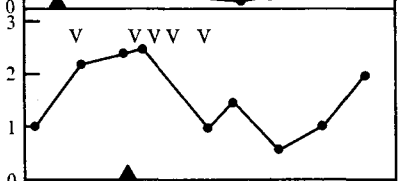
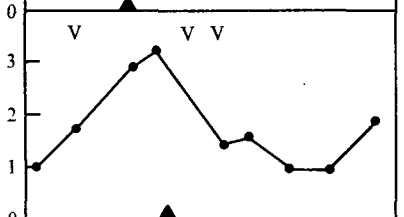
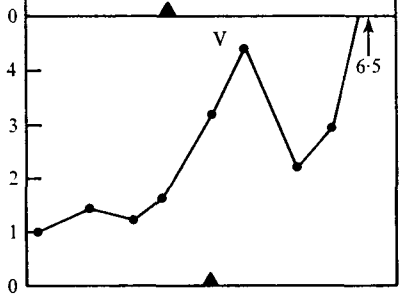
Cattle Station, Plain Station, Sugarloaf and Braziers all reached a high percentage of rabbits with fleas (75% or more) by July 1969 and each showed a decline in the rabbit population by July 1970, with an absence of any summer build-up in rabbit numbers in spite of a normal breeding season. By October 1969 none of the shot samples from these areas contained more than 20% young animals. No fleas were introduced into Davis A and Davis B during 1968. An introduction of 1500 fleas was made into each of Davis A and Davis B in June 1969 and Davis A was subsequently treated with infected fleas, whereas no further fleas were introduced into Davis B. The build-up of fleas in Davis A was advanced by a month when compared to Davis B, but any difference this might have made to changes in population went unrecorded owing to the absence of counts during November and December 1969. In both areas a build-up of rabbits occurred between June and October 1969 (60% young in the October shot samples), at about which time a high level of flea infestation was reached, and in both areas the count fell steeply. By July 1970 the count was double the June 1969 number in both Davis A and B.

Three hundred fleas were introduced into Hoppin' Charlie in November 1968, i.e. at the end of the breeding season. This paddock was 1080-poisoned in April of 1969 and the build-up of flea infestation was slow, not reaching a high level until January 1970. No virulent virus was introduced into Hoppin' Charlie. By February 1970 a 4·5 times count increase had been observed. There was a fall in the count during March and April 1970, following myxomatosis, but by July a 6·5 times increase was observed.

Spotlight counts can be affected by the density of the pasture; when pasture is dense counts can be low because rabbits are not seen, and when pasture is flattened counts can be high because all rabbits are easily seen. An increase in pasture density from June to December 1969 and thereafter a decrease in the pasture density from March to July 1970 could have affected the data in Table 1; ageing rabbits by the weight of the eye lens suggests that very few (9/146) new rabbits entered the popu-

lation between March and July 1970 and yet the count doubled in most areas. Factors other than pasture cover must also have affected counts. Rabbits counted in June 1969 were clearly born before this date and 80 % of the shot sample were immune and thus unlikely to be affected by myxomatosis. By January 1970 the

Table 1. *Counts, expressed as a fraction of the June* 1969 *count, in different paddocks where the build-up of fleas reached* 75 % *or more of the rabbits at different times*

Increase in count/spotlight mile from June 1969	Paddock	Count details: No./s.m.* in June 1969	Route mileage	Max. increase	Increase by July 1970	Month when no. of rabbits with fleas exceeded 75%	Virulent virus introduction
	Cattle Station	29	2·0	0·6	0·6	May 1969	July–Nov.
	Plain Station	85	0·8	0·9	0·4	July 1969	July–Nov.
	Sugarloaf	52	1·0	0·9	0·6	July 1969	July–Nov.
	Braziers Route II	26	0·8	1·1	0·8	July 1969	July–Nov.
	Davis A	31	1·8	2·7	1·9	October 1969	Sept.–Dec.
	Davis B	22	1·7	3·2	2·0	November 1969	None, except within 5–10 chains of ⌓1/4 of the count route
	Hoppin' Charlie	9	1·2	6·5	6·5	January 1970	None, within 1/4 mile of the route

* Number per spotlight mile. ▲ Rabbits with fleas exceed 75%. V Virus recovered.

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counts had fallen, and age estimates of shot samples by eye-lens weight indicated that only 10 % were born before May 1969. However, by July 1970 the counts had risen and 30–50 % of the shot sample were found to have been born before May

Table 2. *The number of infected fleas per* 10 *acres introduced into different paddocks and the virus recovered* (*only virulent virus was introduced*)

	1969						1970					
Paddock	J	A	S	O	N	D	J	F	M	A	M	J
Cattle Station												
Virus added*	30	30	10	—	15	—	—	—	—	—	—	—
Virus found†	—	—	V	V	—	—	AA	—	—	—	A	—
Plain Station												
Virus added	55	55	—	20	30	—	—	—	—	—	—	—
Virus found	—	—	—	V	V	V	—	—	—	—	—	—
Sugarloaf												
Virus added	25	25	—	10	—	—	—	—	—	—	—	—
Virus found	—	A	—	—	V	VA	—	—	—	—	—	—
Braziers												
Virus added	25	25	—	10	10	—	—	—	—	—	—	—
Virus found	—	—	—	—	V	—	—	—	—	—	—	—
Davis A												
Virus added	—	—	400	150	100	100	—	—	—	—	—	—
Virus found	—	A	—	V	VVA	VVA	VAA	—	A	—	—	—
Davis B												
Virus added	—	—	—	—	—	—	—	—	—	—	—	—
Virus found	—	A	—	—	—	AA	VAA	—	—	—	—	—
Hoppin' Charlie												
Virus added	—	—	—	—	—	—	—	—	—	—	—	—
Virus found	—	—	—	—	—	—	AAAA	—	—	—	—	—

* Expressed in terms of infected fleas per 10 acres.

† V, Virulent virus; A, attenuated virus. The number of A's or V's indicates a quantitative estimate of virus found.

Table 3. *Illustrating the greater proportion of old immune rabbits* (*born before March* 1969) *and the higher proportion of immunity in current season's rabbits* (*born March–December* 1969) *on Wing Vee where fleas were active than on adjoining properties where there were no fleas*

	Wing Vee Cattle Station and Plain Station	Edge Hill and Gundowda	χ^2 (1)	*P*
No. in sample*	50	60	—	—
Rabbits born before March 1969	13	7	38	< 0·001
No. immune	13	6	—	—
Rabbits born March–Dec. 1969	37	53	—	—
No. immune	17	11	6·5	0·002–0·01

* Rabbits shot March–May 1970

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1969. In spite of these general trends, the four paddocks which had a high percentage of rabbits with fleas early completed the year with a decreased count. In the three paddocks where high flea infestation was late the counts increased rapidly at a time when pasture density would have been depressing the count. All three showed a marked fall in count when the percentage of rabbits with fleas became high.

Where virulent virus was introduced into the field via infected fleas it was recovered from infected rabbits shot a month later, as shown in Table 2: thus, the virulent virus was transmitted at least once from rabbits infected in the field. Attenuated strains were active before, during and after the time when virulent virus was recovered.

During March, April and May 1970 a shot sample of rabbits was taken (by spotlight) from two properties each 4–5 miles from Wing Vee. A comparison of these rabbits with rabbits shot in Cattle Station and Plain Station on Wing Vee during the same period is given in Table 3. The Wing Vee shot sample contained a smaller proportion of animals born in the 1969/70 breeding season and of these more were immune than those from the adjoining properties.

Longford

This property is run by C.S.I.R.O. as an experimental sheep-breeding station. Counts begun in March 1968 on Longford were made by the same two C.S.I.R.O. employees stationed on the property, using a fixed transect of 3·8 miles throughout. From October 1967 until fleas were first released in July 1968, between five and fifteen rabbits were caught once or twice a week. The mature females, as judged by appearances, were destroyed and the rest were infected with virulent G.V. virus via the eye. Subsequent to January 1969 rabbits were caught only by spotlight. The monthly totals of rabbits infected are shown in Fig. 3, together with other relevant data.

Virus was recovered every month between October 1967 and July 1968. The susceptibility remained between 50 and 75%, and as no counts were made until March no assessment of population fluctuations can be made. All of the nine virus samples tested during this period were attenuated strains. The prolonged virus activity suggests the presence of some vector other than mosquito or the flea or of contact transmission.

The population was reduced to a very low level in June 1968 by poisoning. Fleas were introduced in July, September and November of 1968 and, by July 1969, were found on every rabbit shot or captured. From June 1968 to May 1969 the count had increased by a factor of 5 or 6 and was significantly lowered by further poisoning. During the following year there was very little change in the count in spite of continuous breeding; flea infestation remained high. When virulent Lu virus was introduced into the population in January and February 1969, the rabbits were largely susceptible and there was a rapid spread of virus with a consequent fall in number of susceptible animals. Of ten virus samples collected during March, April and May 1969, five were virulent Lu and five attenuated field-strain. With the fall in the number of susceptible animals the reintroduction of

virulent virus became increasingly ineffectual, as may be seen in Fig. 3, from the number of susceptible animals that were infected – an average of only two per month. Virus was recovered during most months of the year and two of those sampled in June 1970 were attenuated field-strains. The rise in the number of susceptible rabbits during December, January 1969/70 and the subsequent fall, without any apparent change in size of the total population, suggests some continuing change in the age structure of the population during this period.

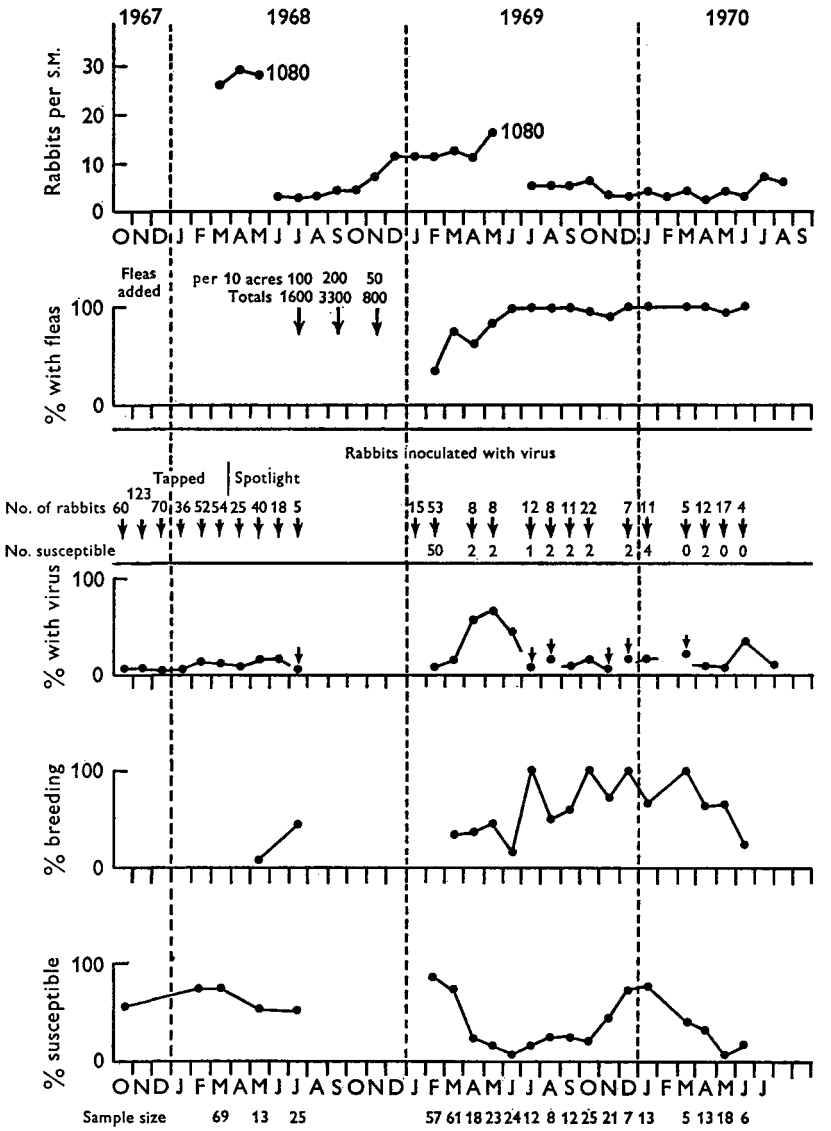

Fig. 3. Spotlight counts on Longford, together with data on fleas added and percentage fleas, virus, breeding and susceptible, in shot samples. Not all rabbits inoculated with virus were susceptible: where blood samples were taken the number of susceptible rabbits is shown.

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Fleas and virus transmission

During the period fleas were building up in the field, before all rabbits had fleas, it was possible to compare the proportion of rabbits, with and without fleas, from the same area, that were infected with myxomatosis. Shot samples taken during September, October and November 1969, in areas where Lu virus was being actively disseminated, are shown in Table 4. All of the infected rabbits had fleas. Not all the diseased rabbits were infected with Lu, some were infected with attenuated field strains, suggesting that the fleas were the main vectors and were transmitting whatever virus they came in contact with.

Table 4. *To illustrate the association of virus and fleas in rabbit populations where the proportion of rabbits with fleas was increasing* (*Sept.-Nov.* 1969)

	Rabbits with fleas			Rabbits without fleas		
	No. in sample	No. with virus	% with virus	No. in sample	No. with virus	% with virus
Wing Vee	128	25	20	43	0	< 2
Millambri	35	9	26	14	0	< 7
Total	163	34	21	57	0	< 2

Flea infestation rates

When it became clear that most rabbits in the experimental areas were carrying fleas, an attempt was made to estimate the numbers of fleas on each rabbit. From March 1970 each shot rabbit was scored by inspection on the following scale: +, 1–4; ++, 5–20; +++, 21–100; ++++, 101–500; and +++++, > 500 fleas. The data collected from March to September 1970 on Wing Vee are summarized in Table 5, where the scale has been converted back to flea numbers on the basis + = 3, ++ = 15, +++ = 50, ++++ = 200 and +++++ = 700 fleas. It is probable that the numbers arrived at are underestimates of the actual numbers. Scoring was done by artificial light at night on wild agouti rabbits and the time allowed for scoring was restricted to 1 min. From the September sample, pairs of ears from seven shot rabbits were put into plastic bags, brought back to the laboratory and the fleas in each counted. A +++++ gave 765 fleas and six ++++'s gave 246, 378, 727, 241, 123 and 196 fleas. The complete rabbits would undoubtedly have had additional fleas in each case.

In general, the breeding animals carried more fleas than the non-breeding animals. Females that were lactating only or lactating and less than 10 days pregnant carried fewer fleas than females pregnant and not lactating, or lactating and pregnant more than 10 days. This is simply explained in that most of the fleas these animals were carrying prior to parturition would have been in the nest. The 1970 breeding season commenced about April and as it proceeded to September the mean number of fleas per rabbit increased. Flea infestation in excess of 100 per rabbit was generally restricted to pregnant does or does lactating and pregnant more than 10 days. However, an occasional breeding buck (testes exposed) was

found with more than 100 fleas. The very high numbers, greater than 700 fleas per rabbit, are in excess of the highest numbers, 450 fleas per rabbit, reported by Allan (1956) from rabbits captured in Scotland.

Table 5. *Estimates of the mean number of fleas found on different classes of rabbits at different times between March and September* 1970

	Males		Females				
Date	Testes up	Testes down	Non-breeding	P only	L and P < 10 days	L and P > 10 days	Total
10.iii.70	2 (37)	5 (12)	3 (29)	—	3 (1)	—	3 (79)
6.iv.70	3 (12)	18 (35)	7 (33)	10 (2)	6 (7)	—	11 (89)
26.v.70	19 (3)	13 (43)	8 (10)	25 (3)	18 (7)	—	13 (66)
4.viii.70	8 (5)	8 (44)	22 (8)	70 (13)	11 (24)	50 (11)	22 (105)
15.ix.70	*5 (24)	12 (34)	*5 (9)	125 (2)	25 (19)	140 (16)	34 (104)

P, Pregnant; L, lactating.

The numbers in parenthesis are the numbers of animals on which the estimates are based.

* Kittens present in sample.

DISCUSSION

Within two breeding seasons the fleas released on each of three properties in New South Wales multiplied to a point where almost every rabbit shot within at least a quarter of a mile radius of a release site was infested with fleas. When, within a given area, the number of rabbits infested with fleas reached a high level, it remained high. The number of fleas per rabbit was highest during the breeding season, particularly on the ears and heads of pregnant does. The highest numbers per rabbit counted were in excess of those reported by Allan (1956) in Scotland. During the hot summer months fleas were particularly active and often deserted a shot rabbit within minutes.

There is no doubt that the flea transmits virus in the field. Virulent Lu virus was recovered from the field in areas where it had been introduced only via infected fleas and during the build-up of flea populations there was a very strong association between fleas and the presence of virus of all kinds, no virus being found in 57 rabbits that had no fleas, whereas out of 163 rabbits with fleas 34 had virus. The data presented suggest the long-term usefulness of the flea as an aid in rabbit control. It is interesting that in the areas on all three of the properties where the flea has been established, by the beginning of the breeding season, the rabbit population showed no increase over the period of a year in spite of a normal breeding season, and that whereas before the flea reached high numbers there was a steep increase in the rabbit count during each breeding season, there has been no such rise since.

With the introduction of the flea to assist in rabbit control in Australia it seems that its most immediate use would be in maintaining low rabbit populations following 1080-poisoning as illustrated by the results from Longford. It is not clear from the data whether the reintroduction of virulent viruses into areas where the flea has been established is of value in rabbit control. However, it has been demon-

strated that virulent virus can be reintroduced and maintained for some time in the field, and it is suggested that such reintroduction might be most useful from a control point of view during the breeding season, when both the flea and susceptible rabbits are abundant and the colder weather will favour a high mortality.

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REFERENCES

Allan, R. M. (1956). A study of the populations of the rabbit flea *Spilopsyllus cuniculi* (Dale) on the wild rabbit *Oryctolagus cuniculus* in north-east Scotland. *Proceedings of the Royal Entomological Society of London* **31**, 145.

Armour, C. J. & Thompson, H. V. (1955). Spread of myxomatosis in the first outbreak in Great Britain. *Annals of Applied Biology* **43**, 511.

Fenner, F. & Marshall, I. D. (1957). A comparison of the virulence for European rabbits (*Oryctolagus cuniculus*) of strains of myxoma virus recovered in the field in Australia, Europe and America. *Journal of Hygiene* **55**, 149.

Fenner, F. & Chapple, P. J. (1965). Evolutionary changes in myxoma virus in Britain. An examination of 222 naturally occurring strains obtained from 80 countries during the period October–November 1962. *Journal of Hygiene* **63**, 175.

Fenner, F., Poole, W. E., Marshall, I. D. & Dyce, A. L. (1957). Studies in the epidemiology of infectious myxomatosis of rabbits. VI. The experimental introduction of the European strain of myxoma virus into Australian wild rabbit populations. *Journal of Hygiene* **55**, 192.

Fenner, F. & Ratcliffe, F. N. (1965). *Myxomatosis*. Cambridge University Press.

Lazarus, M. (1956). The toxicity and relative acceptability of some poisons to the wild rabbit *Oryctolagus cuniculus* (L). *C.S.I.R.O. Wildlife Research* **1**, 96.

Lockley, R. M. (1954). The European rabbit flea, *Spilopsyllus cuniculi*, as a vector of myxomatosis in Britain. *Veterinary Record* **66**, 434.

Lord, R. D. (1959). The lens as an indicator of age in Cottontail rabbits. *Journal of Wildlife Management* **23**, 358.

Marshall, I. D. (1959). The influence of ambient temperature on the course of myxomatosis in rabbits. *Journal of Hygiene* **57**, 484.

Mead-Briggs, A. R. & Rudge, A. J. B. (1960). Breeding of the rabbit flea, *Spilopsyllus cuniculi* (Dale): requirement of a 'factor' from a pregnant rabbit for ovarian maturation. *Nature, London* **187**, 1136.

Myers, K. (1954). Studies in the epidemiology of infectious myxomatosis of rabbits. II. Field experiments August–November 1950 and the first epizootic of myxomatosis in the Riverina Plain of south-eastern Australia. *Journal of Hygiene* **52**, 47.

Myers, K. & Gilbert, N. (1968). Determination of age of wild rabbits in Australia. *Journal of Wildlife Management* **32**, 841.

Mykytowycz, R. (1956). The effect of season and mode of transmission on the severity of myxomatosis due to an attenuated strain of the virus. *Australian Journal of Experimental Biology and Medical Science* **34**, 121.

Rivers, T. M. & Ward, S. M. (1937). Infectious myxomatosis of rabbits. Preparation of elementary bodies and studies of serologically active materials associated with the disease. *Journal of Experimental Medicine* **66**, 1.

Rowley, I. (1968). Studies on the resurgence of rabbit populations after poisoning. *C.S.I.R.O. Wildlife Research* **13**, 59.

Sobey, W. R., Conolly, D. & Adams, K. M. (1966). Myxomatosis: a simple method of sampling blood and testing for circulating soluble antigens or antibodies to them. *Australian Journal of Science* **28**, 354.

SOBEY, W. R., CONOLLY, D. & ADAMS, K. M. (1967). Myxomatosis: The preparation of myxoma virus for inoculation via the eye. *Australian Journal of Science* **30**, 233.

SOBEY, W. R. & MENZIES, W. (1969). Myxomatosis: The introduction of the European rabbit flea *Spilopsyllus cuniculi* (Dale) into Australia. *Australian Journal of Science* **31**, 404.

VAUGHAN, N. E. M. & VAUGHAN, J. A. (1968). Some aspects of the epizootiology of myxomatosis. *Symposia of the Zoological Society of London* **24**, 289.