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Monitoring the spread of myxoma virus in rabbit *Oryctolagus cuniculus* populations on the southern tablelands of New South Wales, Australia. I. Natural occurrence of myxomatosis

J. C. MERCHANT, P. J. KERR, N. G. SIMMS AND A. J. ROBINSON*

CSIRO Sustainable Ecosystems and Pest Animal Control Cooperative Research Centre, GPO Box 284, Canberra, ACT 2601, Australia

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SUMMARY

A survey of rabbit populations in the southern tablelands of New South Wales, Australia, was carried out to establish the pattern of occurrence of myxomatosis in preparation for a deliberate release of myxoma virus. Myxomatosis was first detected in December and cases were found on most sites through to May. The serological profiles of rabbit populations suggested that their susceptibility to myxoma virus was generally low in winter and highest in spring and summer reflecting the presence of increasing numbers of susceptible young rabbits. This was consistent with the pattern of rabbit breeding, as determined from the distribution of births and reproductive activity in females and males, which occurred maximally in spring and early summer. The serology and age structure of rabbit populations on sites suggested that some rabbit populations can escape an annual myxomatosis epizootic. Although fleas were present on rabbits throughout the year and therefore not considered to be a limiting factor in the spread of myxomatosis, their numbers peaked at times coincident with peak rabbit breeding. It was concluded that mid to late spring was an optimal time for a deliberate release.

INTRODUCTION

Over the past 50 years, biological control has been a successful approach to rabbit control in Australia. Myxoma virus, introduced in 1950, has had a major impact on rabbit numbers and is estimated to hold the population to approximately 50% of pre-1950 levels [1]. Soon after its introduction, the spread of myxomatosis in Australia was shown to be dependent on the presence of mosquitoes [2–4] while in Europe, particularly where mosquitoes are not abundant, the European rabbit flea (*Spilopsyllus cuniculi*) was found to be the main vector [5, 6]. Based on the European experience, the European rabbit flea was introduced into Australian rabbit populations in 1968 and it is considered to have increased the range and efficacy of myxoma virus [7–9]. In 1993 the Spanish rabbit flea (*Xenopsylla cunicularis*) was introduced into arid regions of Australia where the European rabbit flea could not establish [1, 10–12]. The effect of the Spanish rabbit flea is still being assessed. The introduction of rabbit calicivirus (RCV) into the rabbit population in 1995 has caused high mortalities in rabbits over wide areas of Australia, particularly in the arid and semi-arid regions, and continues to have a major effect [13]. Insects, including mosquitoes, fleas, blow flies and bush flies, are also involved in the spread of this virus [14, 15].

Despite these successes, wild rabbits remain a major pest in many areas. The development of resistance to myxoma virus and the uncertain future for RCV especially in wetter areas means there is a continuing need for new methods of control. One method being considered is virally vectored immunocontraception

* Author for correspondence.

(VVIC) [16]. The concept of VVIC is to release into a target pest population a recombinant virus containing a gene for a key component of the pest's reproductive system such that animals mount an immune response to that component and are rendered infertile. Support for the feasibility of this concept has been obtained in a mouse model where, using mousepox virus as the vector, the gene for zona pellucida protein C [17] can cause infertility in infected mice. Also, considerable progress has been made using myxoma virus and rabbit zona pellucida protein B to sterilize rabbits [18]. Myxoma virus is the infectious agent that has been chosen to develop a VVIC strategy for rabbits.

The aim of the VVIC project for rabbits is to achieve control over large special scales in areas where it is costly and often impractical to use toxins or non-disseminating organisms delivered by baits. A potential impediment to the success of VVIC for rabbits would be the inability of a released recombinant virus to spread, to compete with strains of virus circulating in the field (field strains) and to persist. To be successful the virus would need to have either a selective advantage over field strains or be released at an intensity and frequency such that it would become the predominant strain.

We have completed a series of experiments aimed at the question of whether or not it is possible to impose a myxoma virus of choice on populations of rabbits in the field. The study was divided into three components: (a) a survey of the district where the release was planned to determine the most appropriate time of year for a release, (b) selection of a strain for release and (c) release and monitoring of spread. We report here on the first of these components and describe key aspects of the epidemiology of myxoma virus, including rabbit abundance, annual patterns of breeding, abundance of European rabbit fleas, occurrence of myxomatosis and serological status to myxoma virus in rabbit populations in the southern tablelands of New South Wales (NSW).

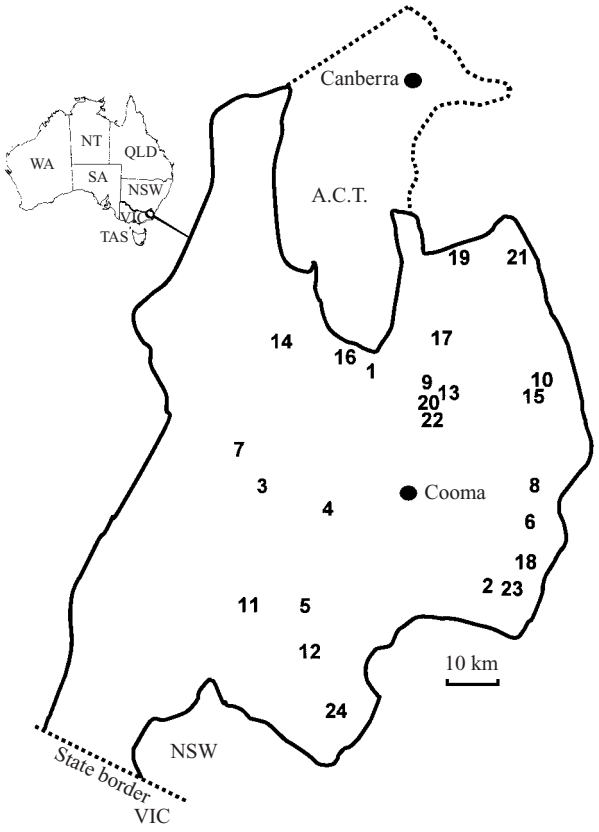

Fig. 1. Map of Cooma RLPB district with locations of the 24 sites sampled. The site number indicates the order in which it was visited in the sequence 1 to 24.

METHODS

Study area and sites

Twenty-four sites were selected within the NSW district administered by the Cooma Rural Lands Protection Board (RLPB) south of Canberra, Australian Capital Territory (ACT) (Fig. 1). Map coordinates for potential sampling sites were generated randomly and then relayed to inspectors of the Cooma RLPB who suggested suitable locations close to these points. Property owners at these sites were then contacted for permission to collect rabbits.

Sampling was carried out between July 1994 and May 1995. The procedure involved the selection of three sites that were then sampled over a 3-week period. An interval of 3 weeks followed and then the process was repeated on three new sites until 24 sites had been sampled. Each site was visited once only. This sampling strategy was chosen as there were insufficient resources to revisit each site on a regular basis. We made the assumption that by pooling the results, the strategy chosen would reflect the epidemiology of myxomatosis in the district as a whole rather than on individual sites. Nevertheless, in the case of serological analysis, broader conclusions were able to be made regarding the epidemiology on individual sites.

Animal sampling

Rabbits were collected on each site by shooting and ferreting to obtain a cross-section of ages. The target

per site was 30 rabbits but due to variable rabbit numbers, samples ranged from 17 to 31.

For each rabbit a blood sample (approximately 1 ml) was taken by cardiac puncture as soon after death as practicable and placed in a vial without anticoagulant. The body weight was recorded, the breeding status of female rabbits was noted (pregnancy and/or lactation) and flea numbers were estimated by combing fleas from the head of the rabbit onto a white board and counting them until no more appeared. The testes of males were assessed and categorized as undescended or internal, descended but small, medium or large. One eyeball was removed and preserved in at least 10 volumes of 10% buffered neutral formalin for subsequent dissection to remove the eye lens for use in age estimation.

Rabbits were examined for lesions indicative of active myxomatosis. When clinical signs of the disease were found, eyelid and lesion samples were taken using sterile scalpel blades, placed in phosphate buffered saline and glycerol (1:1, PBS:glycerol), held for 24–48 h at −20 °C and then stored at −70 °C for subsequent analysis for the presence of myxoma virus [36].

Rabbit abundance

The abundance of rabbits on each site was assessed after sunset by counting rabbits by spotlight [1] over two 1 km transects initiating at the same point and set at right angles. Transect location was selected in daylight on the basis of topography and proximity to warrens where sampling was to be carried out.

Laboratory procedures

Coagulated blood samples were centrifuged at 13 000 ***g*** for 5 min in a microfuge and serum pipetted into a separate tube and stored at −20 °C. The level of antibody specific for myxoma virus was measured using an ELISA [19] and each sample recorded as either seropositive, seronegative or of equivocal status.

To estimate the age of each rabbit, the lens was dissected from the formalinized eyeball and dried in an oven at 80 °C until a constant weight was achieved. An estimate of age was derived from the equation: $-57 + 181{\cdot}4/\log_e(314/\text{lens weight in mg})$ [20].

Estimation of susceptibility to infection with myxoma virus

Susceptibility to infection with myxoma virus was defined as the sum of all seronegative rabbits plus those rabbits less than 80 days old that were seropositive or of equivocal antibody status. Antibody in rabbits less than 80 days old was assumed to be maternal in origin. Maternal antibody to myxoma virus is generally detectable for up to 7 weeks [21] and, in occasional animals, traces can be found as late as 12 weeks after birth using ELISA (P. J. Kerr, unpublished results). Eighty days of age was therefore chosen as a maximum at which antibody found in kittens may be of maternal origin. Regarding susceptibility of animals younger than 80 days of age and with maternal antibody, Fenner and Marshall [21] showed that about one third of 28 day-old kittens with maternal antibody were protected against infection after being exposed to one or two mosquitoes that had probed myxoma virus lesions from a rabbit infected with a field strain and about one third of those that became infected, survived. On the other hand, Sobey and Conolly [22], in a pen trial, could find no difference in survival rate between kittens with maternal antibody and those without when exposed to adult rabbits with myxomatosis in the presence of European rabbit fleas. As kittens in the field would be continually exposed to fleas and therefore to multiple challenges with myxoma virus when myxomatosis was prevalent, we made the assumption that all animals below 80 days of age with or without antibody would be susceptible to infection.

Changes in susceptibility over the course of the study were examined by modelling susceptibility as a binary response for each rabbit, but excluding: all rabbits less than 80 days of age (assumed susceptible); all rabbits with an equivocal response to antibody testing; sites 3, 17 and 23, due to no recent exposure to myxomatosis.

Firstly a generalized linear model with binomial error and logit link [23] was fitted to adjust the probability of susceptibility for the effect of age, hence obtaining site means adjusted for age effects. Estimated ages were log transformed before fitting this model, as the distribution of ages was highly positively skewed. Secondly a generalized additive model [24] was fitted to account for the age effects (log transformed) obtaining a smooth trend over time. This amounted to fitting a spline on 4 degrees of freedom through the 21 adjusted site means.

RESULTS

Rabbit abundance

The relative abundance of rabbits on the 24 sites as determined by spotlight counts ranged from 0·5 to 59·5

Table 1. *Mean number of rabbits counted per km on spotlight transects, by site and month*

Site no.	1	2	3	4	5	6	7	8	9	10	11	12
Month	Jul	Jul	Jul	Aug	Aug	Aug	Oct	Oct	Oct	Dec	Dec	Dec
No. of rabbits	10·0	4·0	7·5	38·0	5·5	24·0	9·0	27·5	3·5	**2·0**†	**13·0**	**7·5**
Site no.	13	14	15	16	17	18	19	20	21	22	23	24
Month	Jan	Jan	Jan	Feb	Feb	Feb	Apr	Apr	Apr	May	May	May
No. of rabbits	**9·5**	**59·5**	**7·5**	**41·5**	8·0	**8·5**	**0***	8·0	6·0	1·5	5·0	**3·5**

* No rabbits were seen on the spotlight transect but rabbits with myxomatosis were found during sampling.
† Rabbit numbers in bold type indicate those sites where cases of myxomatosis were found in the sample.

rabbits per km (Table 1). On 17 of the 24 sites less than 20 rabbits per km were recorded, on 2 sites there were between 20 and 30 while on the remaining 5, rabbit numbers ranged from 38 to 59·5 per km.

Rabbit ages and breeding

Of 675 rabbits collected, for which ages were derived from dried eye lens weights, 445 (65·9%) were less than 1 year old, 152 (22·5%) were between 1 and 2 years old and the remaining 78 (11·6%) were over 2 years old. Seasonal trends in breeding were examined using the birth months calculated from the estimated ages of the 445 rabbits aged 1 year or less (Fig. 2*a*). Although births occurred throughout the year, a distinctly seasonal pattern of breeding activity was indicated with most births occurring from late winter to early summer. From January to June, 84 births (18·9%) were recorded and from July to December, 361 (81·1%). Birth months calculated for rabbits aged 1–2 years old and greater than 2 years old show that of these older rabbits only marginally higher proportions were born in the second half of the year, 57·6% and 57·7% respectively. However, birth dates for rabbits greater than 2 years of age must be treated with caution as estimates of age calculated from eye lens weight become increasingly inaccurate after this time.

An independent measure of the main breeding season was obtained from assessments of breeding status of adult females and males. The pattern of breeding in adult female rabbits >1400 g was examined by assessing the numbers of females in two reproductive groups, (A) either lactating or pregnant or both and (B) neither lactating nor pregnant (Fig. 2*b*). Only rabbits greater than 1400 g were included in the assessment to avoid including well grown but sexually immature rabbits. This selection criterion was also applied to male rabbits (see below). These data show that while breeding occurs throughout the year there is a seasonal influence reflected in the varying proportions of breeding females. The proportion of non-breeding females peaked in May (84·6%) while the proportion of breeding females peaked in October (92·8%). A seasonal breeding response in males >1400 g body weight was indicated by the varying proportions of males in each of four testis-size groups (Fig. 2*c*). The proportion of males with enlarged testes (82·6%) peaked in October, coincident with major breeding activity in females (Fig. 2*b*).

Flea numbers

European rabbit fleas were found on rabbits on all sites but numbers were highest from July to January when most rabbit breeding occurred (Table 2). Although the mean number of fleas were generally higher on females, in 7 out of the 8 months for which there were data, the differences were statistically significant only in August, October and January. The seasonal distribution and average flea numbers per rabbit are shown for males and females in Figure 2*d*.

Seasonal occurrence of myxomatosis

Clinical signs of myxomatosis were first recorded in the first week of December and subsequently on 8 of 9 sites sampled in December, January and February and on 2 of 6 sites sampled in April and May (Table 3) (Fig. 3*a*). In total, 57 (8·4%) rabbits of the 675 rabbits sampled from the 24 sites had clinical myxomatosis. The 57 infected rabbits represented 20·7% of the 275 rabbits sampled from sites where myxomatosis was found.

The ages of affected rabbits ranged from 55 to 480 days but 54 (94·7%) were less than 1 year of age and

Table 2. *The burden of fleas on rabbits expressed as percentages per month of sampling*

No. of fleas	Jul	Aug	Oct	Dec	Jan	Feb	Apr	May
0	7·7	11·4	9·4	11·5	11·6	6·3	22·2	17·3
1–25	57·6	52·3	57·6	65·6	74·4	87·3	58·3	75·9
26–50	14	10·2	5·9	10·3	3·5	1·3	13·9	2·3
51–75	3·3	13·6	7·1	4·6	2·3	2·5	4·2	3·4
76–100	4·4	3·4	2·4	2·3	3·5	1·3	1·4	1·1
>100	13	9·1	17·6	5·7	4·7	1·3	0·0	0·0
n	91	88	85	87	86	79	72	87

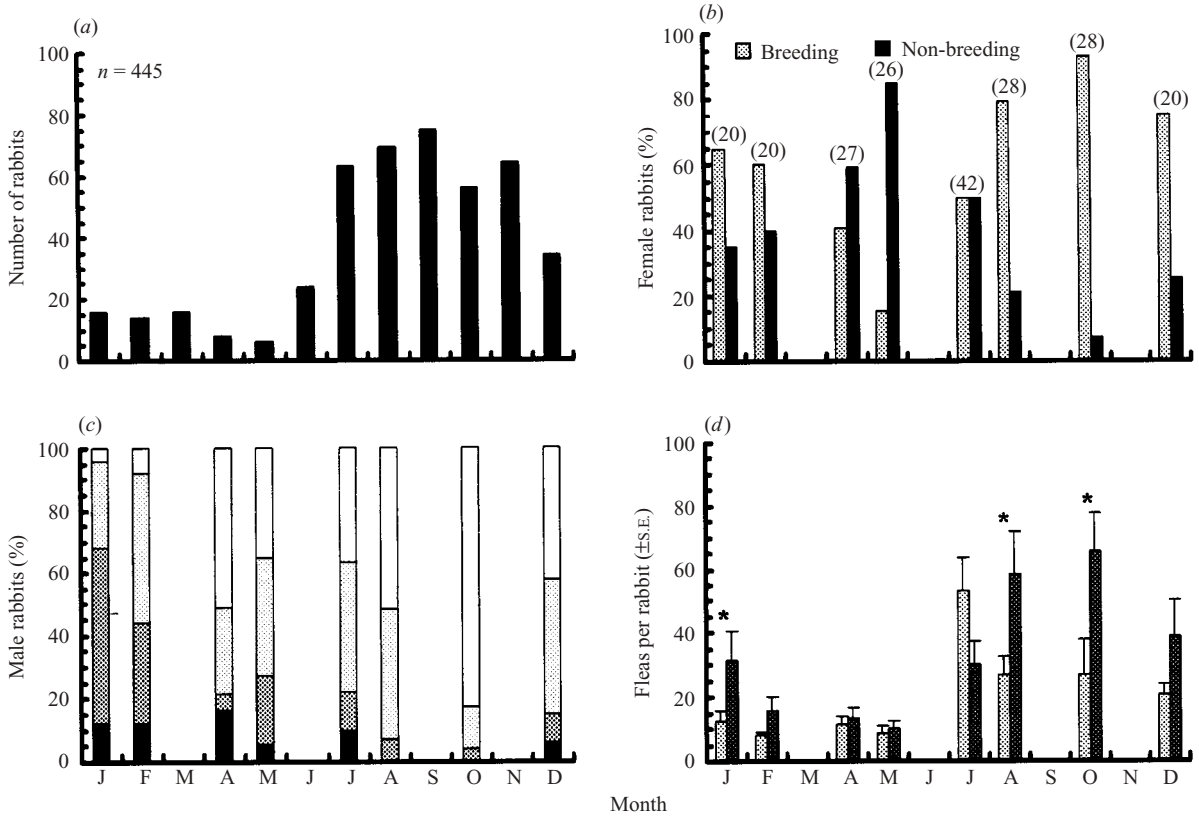

Fig. 2. Month of birth and breeding status of rabbits and occurrence of the European rabbit flea in the Cooma RLPB district. (*a*) Estimated month of birth for rabbits less than 1 year of age, (*b*) breeding status of female rabbits greater than 1400 g body weight (totals for each sample are in parentheses), (*c*) breeding status of male rabbits greater than 1400 g body weight as indicated by testis size (internal testes, black bar; testes descended but small, dark hatch; medium sized testes, light hatch; and large testes, open bar) and (*d*) seasonal distribution of fleas on male (light hatch) and female (dark hatch) rabbits. Samplings are marked (*) where there was a significant difference ($P \leqslant 0{\cdot}05$) in flea numbers between males and females.

these had a mean age of 149·7 ± S.E. 8·87 days. The mean ages of affected rabbits in relation to site and month of sample are presented in Figure 3*b*.

Serology

The data on serum antibody status are presented as the numbers of rabbits >80 days old either seropositive, seronegative or equivocal for each site and month of sampling (Table 3). The number of animals ⩽80 days of age and all considered susceptible are also included in Table 3. Combining these data, the proportion of animals susceptible could be calculated. Of 257 rabbits considered to be susceptible, 242 (94·2%) were less than 1 year old (mean age 140·9 days ± S.E. 5·82, range 10 to 364 days), 14 were 1–2 years old and 1 was over

Table 3. *Myxoma virus-antibody status of rabbits >80 days old, presence of rabbits ⩽80 days old, and cases of active myxomatosis in rabbits collected on 24 sites in the Cooma RLPB district*

Site no.	Month	Sample no.	>80 days of age			⩽80 days of age	Active myxomatosis
			Positive	Negative	Equivocal		
1	Jul	30	25	4	1	0	0
2	Jul	30	30	0	0	0	0
3	Jul	30	4	23	0	3	0
4	Aug	30	15	7	0	8	0
5	Aug	28	17	3	0	8	0
6	Aug	30	15	7	0	8	0
7	Oct	25	14	4	0	7	0
8	Oct	30	10	11	0	9	0
9	Oct	30	13	12	0	4	0
10	Dec	26	19	3	1	2	6
11	Dec	30	15	12	2	3	2
12	Dec	30	17	8	01	4	6
13	Jan	29	23	3	0	3	8
14	Jan	30	16	11	0	3	9
15	Jan	27	16	1	2	8	11
16	Feb	30	25	4	0	1	3
17	Feb	25	8	17	0	0	0
18	Feb	25	12	13	0	0	5
19	Apr	17	15	1	0	1	1
20	Apr	30	27	1	0	2	0
21	Apr	25	24	1	0	0	0
22	May	30	26	4	0	0	0
23	May	27	4	21	0	2	0
24	May	30	20	8	2	0	6

2 years of age. Non-susceptible rabbits less than 1 year old averaged 219·9 days ± S.E. 5·23, range 82–354 days, and were significantly older than their susceptible counterparts (Obs $t = 9{\cdot}91$, D.F. = 441, $P > 0{\cdot}001$).

Analysis of the data for the younger seropositive rabbits revealed that on 21 of the 24 sites, 16 were less than 200 days old, 4 were between 200 and 300 days and the remaining rabbit was 328 days old. Rabbits on these sites had presumably experienced an epizootic of myxomatosis in the previous year. The three remaining sites 3, 17 and 23 (Table 3), sampled in July, February and May, had not experienced an epizootic the previous year as the ages of the youngest seropositive rabbits on these sites were calculated as being 473, 446 and 560 days old respectively. These sites were excluded in modelling susceptibility.

There was a highly significant effect of log age on susceptibility (deviance = 113·98, 1 D.F., $P < 0{\cdot}001$), with younger rabbits being more susceptible. There was also a highly significant difference between susceptibility on the 21 sites after adjustment for age effects (deviance = 95·84, 20 D.F., $P < 0{\cdot}001$). As the mean log age for the rabbits included in the analysis was 5·751, corresponding to age 314·5 days, adjusted means for each site were calculated for rabbits of age 300 days and are presented in Figure 4.

When a smooth trend over time was fitted instead of site differences, it was also highly significant (deviance = 39·91, 4 D.F., $P < 0{\cdot}001$), meaning that there were significant changes in susceptibility over the course of the study. The fitted trend for rabbits of age 300 days is also presented in Figure 4. It rose to a peak around mid-October and then declined to a minimum in March–April.

DISCUSSION

This study showed that in the year from July 1994, myxomatosis in the Cooma RLPB district in the southern tablelands occurred mainly in the summer months. The strategy of sampling each site only once over the 12 months meant that the timing of appearance of myxomatosis on each site could not be determined, but pooling the results from each site to

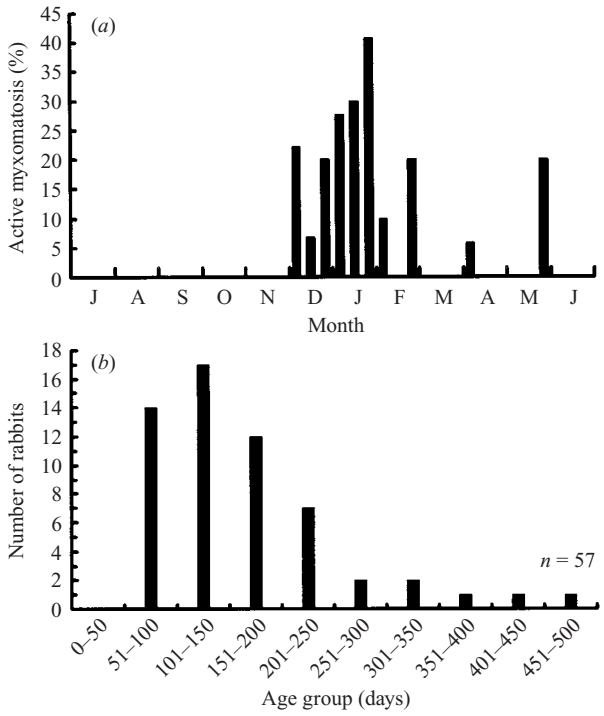

Fig. 3. Occurrence of myxomatosis in the Cooma RLPB district. (*a*) Seasonal distribution of active cases of myxomatosis (% per site) and (*b*) age distribution of the 57 rabbits showing clinical signs of myxomatosis.

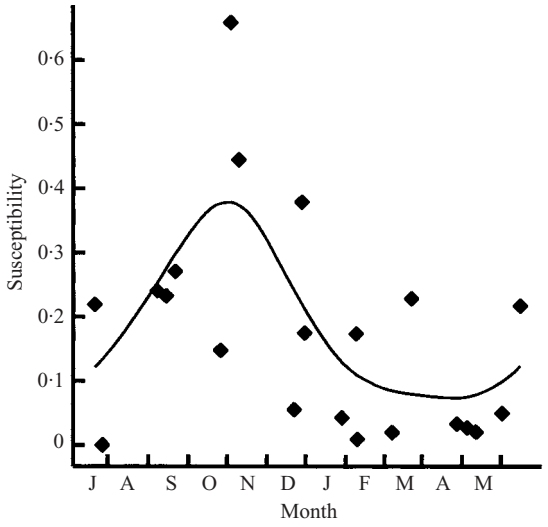

Fig. 4. The estimated probability of susceptibility of rabbits, adjusted to age 300 days, for the 21 sites that had experienced an epizootic of myxomatosis in the previous 12 months in relation to time of sampling, and the smooth-fitted trend of susceptibility for rabbits of age 300 days.

determine the pattern of myxomatosis for the district as a whole was valid as the sites were chosen randomly and all were typical of this bioclimatic region.

Between 1969 and 1973 intensive studies on the prevalence of myxomatosis on sites in the southern tablelands revealed epidemics in winter [25–28], spring [28] and summer/autumn [29, 30]. During this time the European rabbit flea was introduced or appeared naturally on the sites under study and it was speculated that the flea would alter the seasonal pattern of myxomatosis epizootics from winter to summer. However, at that time, winter epidemics were recorded in the presence [26, 28] and absence [27, 29] of the flea and interpretation of the data regarding the influence of the flea on natural patterns of myxomatosis was difficult. Also, apart from the Snowy Plains site [25, 29], the sites were manipulated in some way with measures such as ripping, fumigation, the application of 1080 and fencing which may have altered the natural patterns of spread of myxoma virus.

A better indication of the effect of the introduction of the flea on the seasonal patterns of myxomatosis was gained by analysing Pasture Protection Board records over the years 1959–64 and 1980–6 [31]. These data showed that in the southern tablelands there was a shift from records of myxomatosis starting in winter and peaking in summer to records of fewer cases in winter and spring and a peak in summer/autumn. This supports earlier speculation that the introduction of the flea would tend to shift the epidemics to summer. The pattern found in the current study is also consistent with this view and indicated that spring might be an opportune time to release a virus. Nevertheless, the data gathered in the present study and the records collected in the district over the past 25 years show that myxomatosis can occur at any time of year and, in some instances, can miss a year on some sites. In order to gain a better insight into an optimal time to release a virus, the serological status and age structure of the population and breeding patterns were determined.

Serological analyses, through which the history of myxoma virus infection on a site could be traced back for at least 12 months, showed that the level of potential susceptibility of the rabbit population on a site increased markedly in spring and early summer. This was not unexpected in that it is related to the strong seasonal pattern of breeding and the recruitment of young susceptible rabbits in spring. However, as well as susceptible young rabbits there are also more susceptible adult rabbits present in spring as the trend depicted in Figure 4 is age-adjusted.

Although rabbits can breed throughout the year, reproductive activity increases significantly through

spring and early summer as evidenced by increasing numbers of pregnant and lactating females and males with large testes as well as significant increases in the numbers of births. This breeding pattern has changed little from data collected in the southern tablelands from 1972 to 1974 [30]. There was an apparent lag between the appearance of susceptible animals and the appearance of myxomatosis reinforcing the view that there is an opportunity for a release in mid to late spring and that the window of opportunity may be one to two months. Furthermore, on three of the 24 sites where the population appeared to have escaped a myxomatosis epizootic the previous year, most rabbits were susceptible and the window of opportunity for a release on such sites is, potentially, even wider.

The observation that myxoma virus can become locally extinct for a period of a year or more is interesting. This is a similar pattern to that reported prior to the flea being introduced [25, 27] and indicates that the presence of the flea is not necessarily sufficient to maintain annual outbreaks. It indicates further that annual outbreaks in an isolated group of warrens relies on the introduction of virus from the outside and argues against the idea of myxoma virus latency, promulgated in the 1970s [32].

The transmission of myxoma virus is dominated by the presence or absence of insect vectors [3]. A range of biting insects can act as vectors but the European rabbit flea is probably the main vector in the southern tablelands [26, 29]. Although mosquitoes were not abundant during the period of this survey, they are probably occasionally responsible for long distance spread between isolated populations but it is the flea that is the vector most likely to aid spread within populations. Although male and female rabbits are infested from an early age, female fleas are reproductively adapted to the hormonal profile of the blood of breeding female rabbits [33, 34]. This was reflected in this study in that there was a seasonal trend in flea numbers with the highest numbers being found on female rabbits in spring and summer. However, the numbers of fleas found on rabbits sampled throughout the year indicated that flea abundance was unlikely to be a limiting factor in the spread of myxoma virus if it was introduced onto a site at any time of year.

Population density is another factor that might influence the rate of spread of myxoma virus but it was not our intention in this study to relate density to the occurrence or rate of spread of myxomatosis. The purpose of measuring density on sites was to locate regions in the district that could be used for a release in the succeeding year. Nevertheless, the spot-light transects did show that the density of rabbits across the district ranged widely from low (0·5/km) to high (59·5/km) probably due to time of year and the effectiveness of rabbit control measures. From this survey, sites 14 and 16 were considered suitable release sites and subsequently these and two other sites of similar rabbit density nearby were used [35].

In conclusion, the data presented here indicate that mid to late spring is the optimal time for a deliberate myxoma virus introduction in the Cooma RLPB district. A release at this time would be more likely than at other times to pre-empt the emergence of naturally occurring field strains but late enough to allow the build-up of a supply of susceptible young rabbits capable of sustaining an epizootic of myxomatosis. It was recognized that there will be variation in the optimal time for a release from site to site and year to year as indicated by the data collected in this study but the information obtained provide a basis for a trial release and monitoring of a readily identifiable field strain.

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REFERENCES

1. Williams CK, Parer I, Coman BJ, Burley J, Braysher ML. Managing vertebrate pests: rabbits. Canberra: Bureau of Resource Sciences/CSIRO Division of Wildlife and Ecology, Australian Government Publishing Service, 1995: 284.
2. Fenner F, Day MF, Woodroofe GM. The mechanism of the transmission of myxomatosis in the European rabbit (*Oryctolagus cuniculus*) by the mosquito *Aedes aegypti*. Aust J Exp Biol Med Sci 1952; **30**: 139–52.
3. Fenner F, Ratcliffe FN. Myxomatosis. Cambridge: Cambridge University Press, 1965: 379.
4. Myers K. The ecology of the mosquito vectors of myxomatosis (*Culex annulirostris* Skuse and *Anopheles annulipes* Walk.) in the eastern Riverina. J Aust Inst Agric Sci 1955; **21**: 250–3.
5. Allan RM, Shanks PL. Rabbit fleas on wild rabbits and the transmission of myxomatosis. Nature 1955; **175**: 692.

6. Mead-Briggs AR, Vaughan JA. The differential transmissibility of myxoma virus strains of differing virulence grades by the rabbit flea *Spilopsyllus cuniculi* (Dale). J Hyg 1975; **75**: 237–47.
7. Sobey WR, Menzies W. Myxomatosis: the introduction of the European rabbit flea *Spilopsyllus cuniculi* (Dale) into Australia. Aust J Sci 1969; **31**: 404–5.
8. Sobey WR, Conolly D. Myxomatosis: the introduction of the European rabbit flea *Spilopsyllus cuniculi* (Dale) into wild rabbit populations in Australia. J Hyg 1971; **69**: 331–46.
9. Shepherd RCH, Edmonds JW. Myxomatosis: changes in the epidemiology of myxomatosis coincident with the establishment of the European rabbit flea *Spilopsyllus cunicili* (Dale) in the Mallee region of Victoria. J Hyg 1978; **81**: 399–403.
10. Cooke BD. Factors limiting the distribution of the European rabbit flea *Spilopsyllus cuniculi* (Dale) (Siphonoptera) in inland South Australia. Aust J Zool 1984; **32**: 493–506.
11. Cooke BD. Spanish rabbit fleas, *Xenopsylla cunicularis*, in arid Australia: a progress report. In: Statham M, ed. 10th Australian Vertebrate Pest Control Conference. Hobart, Tasmania, 399–401.
12. Robertshaw JD, Gould WJ. Queensland Spanish rabbit flea – releases and results. In: Statham M, ed. 10th Australian Vertebrate Pest Control Conference. Hobart, Tasmania: Department of Primary Industry and Fisheries, Tasmania, 402–7.
13. Anonymous. National RCD Monitoring and Surveillance Program and Epidemiology Program: Final report to the RCD Management group, November 1998. Canberra: Bureau of Rural Sciences, 1998.
14. CSIRO. Field evaluation of RCD under quarantine. Project CS 236. Australian and New Zealand Rabbit Calicivirus Disease Program: a report of research conducted in 1996 by CSIRO Division of Animal Health and Wildlife and Ecology. Sydney: Meat Research Corporation, Undated.
15. Asgari S, Hardy JR, Sinclair RG, Cooke BD. Field evidence for mechanical transmission of rabbit haemorrhagic disease virus (RHDV) by flies (Diptera: Calliphoridae) among wild rabbits in Australia. Virus Res 1998; **54**: 123–32.
16. Tyndale-Biscoe CH. Fertility control in wildlife. Reprod Fertil Dev 1991; **3**: 339–43.
17. Jackson RJ, Maguire DJ, Hinds LA, Ramshaw IA. Infertility in mice induced by a recombinant ectromelia virus expressing mouse zona pellucida glycoprotein 3. Biol Reprod 1998; **58**: 152–9.
18. Kerr PJ, Jackson RJ, Robinson AJ, et al. Infertility in rabbits (*Oryctolagus cuniculus*) alloimmunized with the rabbit zona pellucida protein ZPB either as a purified recombinant protein or expressed by recombinant myxoma virus. Biol Reprod 1999; **61**: 606–13.
19. Kerr PJ. An ELISA for epidemiological studies of myxomatosis: persistence of antibodies to myxoma virus in European rabbits (*Oryctolagus cuniculus*). Wildl Res 1997; **24**: 53–65.
20. Myers K, Gilbert N. Determination of age of wild rabbits in Australia. J Wildl Manage 1968; **32**: 841–9.
21. Fenner F, Marshall ID. Passive immunity in myxomatosis of the European rabbit (*Oryctolagus cuniculus*): the protection conferred on kittens by immune does. J Hyg 1954; **52**: 321–36.
22. Sobey WR, Conolly D. Myxomatosis: passive immunity in the offspring of immune rabbits (*Oryctolagus cuniculus*) infested with fleas (*Spilopsyllus cuniculi* Dale) and exposed to myxoma virus. J Hyg 1975; **74**: 43–55.
23. Dobson AJ. An introduction to generalized linear models. London: Chapman and Hall, 1990: 174.
24. Hastie TJ, Tibshirani RJ. Generalized additive models. London: Chapman and Hall, 1990: 335.
25. Dunsmore JD, Williams RT, Price WJ. A winter epizootic of myxomatosis in sub-alpine south-eastern Australia. Aust J Zool 1971; **19**: 275–86.
26. Williams RT, Fullagar PJ, Kogan C, Davey CC. Observations on a naturally occurring winter epizootic of myxomatosis at Canberra, Australia in the presence of rabbit fleas (*Spilopsyllus cuniculi* Dale) and virulent myxoma virus. J Appl Ecol 1973; **10**: 417–27.
27. Williams RT, Fullagar PJ, Davey CC, Kogan C. Factors affecting the survival time of rabbits in a winter epizootic of myxomatosis at Canberra, Australia. J Appl Ecol 1972; **9**: 399–410.
28. Fullagar PJ. Observations on myxomatosis in a rabbit population with immune adults. Aust Wildl Res 1977; **4**: 263–80.
29. Dunsmore JD, Price WJ. A non-winter epizootic of myxomatosis in sub-alpine south-eastern Australia. Aust J Zool 1972; **20**: 405–9.
30. Parer I, Libke JA. Biology of the wild rabbit *Oryctolagus-cuniculus* L. in the southern tablelands of New South Wales, Australia. Wildl Res 1991; **18**: 327–42.
31. Parer I, Korn TJ. Seasonal incidence of myxomatosis in New South Wales Australia. Aust Wildl Res 1989; **16**: 563–8.
32. Williams RT, Dunsmore JD, Parer I. Evidence for the existence of a latent myxoma virus in rabbits *Oryctolagus cuniculus* (L.). Nature 1972; **238**: 99–101.
33. Mead-Briggs AR. The reproductive biology of the rabbit flea *Spilopsyllus cuniculi* Dale and the dependence of this species upon the breeding of its host. J Exp Biol 1964; **41**: 371–402.
34. Rothschild M, Ford B. Breeding of the rabbit flea (*Spilopsyllus cuniculi* Dale) controlled by the reproductive hormones of the host. Nature 1964; **201**:103–4.
35. Merchant JC, Kerr PJ, Simms NG, Hood GM, Pech RP, Robinson AJ. Monitoring the spread of myxoma virus in rabbit *Oryctolagus cuniculus* populations on the southern tablelands of New South Wales, Australia. III. Release, persistence and rate of spread of an identifiable strain of myxoma virus. Epidemiol Infect 2003; **130**: 135–47.
36. Kerr PJ, Merchant JC, Silvers L, Hood GM, Robinson AJ. Monitoring the spread of myxoma virus in rabbit *Oryctolagus cuniculus* populations on the southern tablelands of New South Wales, Australia. II. Selection of a strain of virus for release. Epidemiol Infect 2003; **130**: 123–33.