# STUDIES IN THE EPIDEMIOLOGY OF INFECTIOUS MYXOMATOSIS OF RABBITS

## II. FIELD EXPERIMENTS, AUGUST–NOVEMBER 1950, AND THE FIRST EPIZOOTIC OF MYXOMATOSIS IN THE RIVERINE PLAIN OF SOUTH-EASTERN AUSTRALIA

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(With 3 Figures in the Text)

In the first paper of this series (Fenner, Marshall & Woodroofe, 1953) the serological techniques associated with surveys of the wild rabbit population of Australia for antibodies to myxoma virus were described, and an account was given of the results of such surveys carried out in 1951–2 in seventeen localities in eastern Australia.

During the next year investigations were concentrated at two of these localities, at each of which regular observations were carried out throughout the year by officers of the Wildlife Survey Section of the Commonwealth Scientific and Industrial Research Organization. Descriptions of these form the basis of the third and fourth papers of this series (Myers, Marshall & Fenner, 1954; Marshall, Dyce, Poole & Fenner, 1954).

Only a brief account (Ratcliffe, Myers, Fennessy & Calaby, 1952) has been given of the early experiments which preceded the 'escape' of the virus from the experimental sites in 1950. As this took place in an area in which continuous studies have since been carried out (Myers *et al.* 1954) it is desirable to record here in some detail the early field experiments in this area, and observations made during the first epizootic of 1950–1.

In the years 1937–41, Bull & Mules (1944) carried out several field experiments with myxomatosis, using rabbit populations confined in 90-acre netted enclosures. Effective spread from warren to warren was only obtained in one experiment, when the rabbits were infested with the stickfast flea (*Echidnophaga myrmecobii* Rothsch.) which they had shown was able to transmit the disease. These field experiments were followed, in 1942 and 1943, by a series of field trials carried out under natural conditions in the semi-arid pastoral country of South Australia. When predators were few, and the rabbits infested with the stickfast flea, warren colonies into which the disease was introduced were either exterminated or greatly reduced in population; but there was no evidence of natural inter-warren spread.

As Aragão (1943) had shown that myxomatosis in South America was probably maintained among the local wild rabbits (*Sylvilagus braziliensis*, syn. *minensis*) by mosquito bites, and Bull & Mules (1944) had demonstrated that several Australian

species of mosquitoes could transmit the disease, a series of field trials was under-taken between January and November 1950, on six sites in the well-watered Murray Valley.

### Field experiments at four sites in the Eastern Riverina*

Following earlier experiments at Gunbower, Victoria, started in January 1950, and simultaneously with another large trial at Wymah, New South Wales, in the foothills east of Albury (Fennessy, 1954), a series of field experiments was initiated at four sites in the Corowa region of the Eastern Riverina during August 1950, in order to assess the factors affecting the spread of myxomatosis in natural rabbit populations.

The sites chosen represented three of the four major habitats of rabbit infestation within the region. These included stands of pine (*Callitris glauca* R.Br.) in sandy soil (two Balldale sites); rocky granitic hills (Coreen site); and the old dumps of rock refuse ('mullock heaps'), left as relics of the gold-mining days, in open grazing country (Rutherglen site). The fourth major habitat, river flat and frontage country of the Murray River, with its billabongs and swamps, was not considered at the time owing to the annual flood, then rising.

Four factors which appeared to be of epidemiological importance were studied: rabbit density; predation, which might have a selective effect on diseased members of the population; the behaviour of sick rabbits, particularly their movements away from their warrens; and the presence of possible insect vectors.

### METHODS

#### Population counts of rabbits

The rabbit populations on all the experimental sites were predominantly warren-dwelling: that is to say the situation was not complicated by the presence of numbers of 'surface living' rabbits harbouring in hollow logs, etc., such as are found in many Australian habitats. Observations made during the course of a previous field study had indicated that rabbits in undisturbed populations, after their first appearance from below ground in the early evening, spend a period of 20–30 min. on the surface, or within a few yards of their warrens.

The counts taken during the course of this work were of colonies on the surface of their warrens, or, where timber made individual warren counts difficult, counts made at a slow even walk over a standard transect. The distribution of the warrens, on each site, was recorded on a large-scale map, which was used to determine the course of a transect that would provide the best sight coverage of the area, while taking advantage of available cover.

The counts on any site were always carried out in precisely the same manner, and always in the early evening of warm, sunny days when it was considered that conditions favoured the maximum above-ground activity on the part of the rabbits. Before starting a count, the area was always observed for a few minutes to

* The region in which these experiments were carried out, and in which the epizootic discussed in the latter part of this paper developed, constitutes the south-eastern corner of the Riverine Plain of Butler (1950). For convenience and brevity, it will be referred to in the text as the 'Eastern Riverina'.

ensure that the population had not been disturbed and appeared to be behaving normally.

Warren density was measured as the number of warrens per acre. The number of rabbits per acre of warren area, and the number of rabbits per active 'lead' (i.e. burrow opening) were recorded to provide indices of rabbit population density.

The measurement of changes in rabbit populations is of the utmost importance in assessing mortalities caused by myxomatosis. As the methods used during the experiments described here form the basis of future work (Myers *et al.* 1954) it is desirable to give consideration to their reliability. In the later investigations, standard transects, rather than warren population counts, had to be relied on, the transects being designed to 'sample' the area, not to 'cover' it.

In the absence of an accurate determination of the absolute population of rabbits on a given area, there can be no really satisfactory check on the reliability of sight counts as indices of population densities and change. The variability of counts over periods during which the population observed can be regarded as for all intents and purposes stable should, however, give an indication of their worth for the purposes required.

Tidswell (1908) used sight counts in his experiments with pasteurellosis in a rabbit population enclosed in a fenced-in area. His counts, over periods when the population was presumed to be stable, are included in Table 3, in which are also set out the counts made on three of our experimental sites. It will be seen that our counts show a markedly lower variability than Tidswell's, which were made at longer intervals and irrespective of weather conditions.

It may be presumed that the low variability of our counts is a reflexion of the special precautions taken to avoid obvious sources of error; and it is considered that when these precautions are taken, sight counts should provide indices that can be used to determine changes in population density with adequate accuracy.

In our myxomatosis work, it is necessary to distinguish clearly between a 95 % and 99·5 % mortality in a rabbit population, while the distinction between a 90 and 95 % kill is rarely of great importance. The overestimation of a 90 % kill by 5 %, however, would involve an error of the order of 100 % in the count of the surviving population, which is very much greater than one need postulate for our methods. Similarly, confusion between a 98 and a 99·5 % mortality involves a 300 % error in the post-epizootic count.

In our epidemiological field studies, no more is usually required than indices of relative population density. It is believed, however, that in our smaller experimental sites where conditions for observation were exceptionally favourable (e.g. at Rutherglen) the population counts obtained approximate to the absolute number of rabbits in the area.

### *Inoculation of rabbits*

Rabbits for inoculation were obtained at Coreen and Balldale by ferreting, and at Rutherglen by digging out and live-trapping on their feeding grounds. On each site care was taken to see that the infection was well dispersed throughout the

population. Subsequent observations of the rabbits, recognizable by bare inoculation sites, showed that this had been achieved.

All rabbits caught were inoculated subcutaneously with 1·0 ml. of a glycerolated liver-lung extract from a diseased rabbit, supplied by the New South Wales Veterinary Research Station, Glenfield. This animal had been inoculated with virus derived from the strain B of Martin (1936). Three caged wild rabbits inoculated at Balldale with a 1/10,000 dilution of this virus suspension developed typical lesions on the 7th day and died on the 9th day.

In the second trial at Rutherglen (Rutherglen II), the rabbits were continuously driven underground, during the daylight hours, from the 7th day after the inoculations when they showed typical signs of the disease. It was hoped thereby to increase the frequency of contact between diseased and healthy rabbits and thus to encourage the spread of myxomatosis through the population.

### Assessment of predator activity

Direct measurement of the rate of predation was not attempted. In its place, regular examinations of the trial sites were carried out for indications of predator activity. In the case of the second trial at Rutherglen, searches were made daily.

Carcasses of dead rabbits usually bore some evidence of the predator responsible —decapitation by the fox (*Vulpes vulpes*, L), the fur-plucks of the little eagle (*Hieraaëtus morphnoides*, Gould) (Calaby, 1951), and the cleaned pelt, turned inside out, of the feral cat (*Felis cattus* L.). In addition, at Rutherglen and Coreen, feral cats were found to carry their catches to lairs. In these places the pelts could be counted and removed at each visit.

Evidence of predation by nocturnal birds was obtained when regurgitated crop-pellets of the barn owl (*Tyto alba*, Scopoli) were collected. These were identified and examined by a colleague, Mr J. Calaby.

The presence of fresh scats and the characteristic smell of the fox were noted whenever present.

Predation was directly observed on two occasions only, at Rutherglen, both by the little eagle.

### Insect vectors

Catches of adult biting insects were for the most part limited to those coming to human subjects. The number biting the bare arm per minute was used as the index of density. Observations were also made on rabbits in the field for the presence of biting insects.

During the later phase of the work sampling of mosquito larvae began. This was done on a mass collection basis with a limit of 15 min. collecting in any one breeding place. In order that the collections should be comparable, 5 min. were given to the collection of surface-resting larvae, 5 min. to deep sweeping with a net-covered scoop, and 5 min. to collecting from the surface after muddying the water. Often the last procedure drove larvae to the surface after the first two methods had proved fruitless.

## Identification of individual rabbits

The inoculated rabbits at Balldale and Coreen were earmarked by a combination of punches which indicated the particular warren from which they were taken.

At Rutherglen, where observations were carried out daily, it was also found possible to recognize individual rabbits on some or all of the following characteristics, size, colour, position within warren (i.e. constant use of the same burrow) and stage of development of myxomatosis.

## RESULTS

Details of the localities, the nature of the experimental sites, and rabbit densities are shown in Table 1; and the inoculation procedure, the results of introduction of the virus, the activity of predators and the presence of insect vectors in the localities are set out in Table 2.

Table 1. *Sites of field experiments and rabbit densities*

| Locality | Site | Warren area (acres) | No. of warrens | Warrens per acre | No. of rabbits (round figures) in counts | Rabbits per acre of warren area | Active leads | Rabbits per active lead |
|---|---|---|---|---|---|---|---|---|
| herglen I | Mine dump. Mullock heap in cleared pasture country. No timber | 13 | 14 | 1·07 | 700 | 54 | 800 | 0·9 |
| herglen II, ginning of periment | Mine dump. Mullock heap in cleared pasture country. No timber | 13 | 15 | 1·15 | 900 | 70 | 810 | 0·9 |
| herglen II, end experiment | Mine dump. Mullock heap in cleared pasture country. No timber | 13 | 15 | 1·15 | 1000 | 77 | 850 | 1·2 |
| een | Rocky hill, granite residual. Sparse timber (*Eucalyptus* sp.) | 17 | 26 | 1·5 | 400 | 24 | 600 | 0·7 |
| ldale A | Stand of native pine (*C. glauca*) in light, sandy soil | 17 | 150 | 8·8 | 300 | 18 | 1250 | 0·2 |
| ldale B | Stand of pine (*C. glauca*) and box (*Eucalyptus* sp.) in sand | 1·6 | 24 | 15 | 150 | 94 | 550 | 0·3 |

## The second experiment at Rutherglen

The most detailed observations were made during the second experiment at Rutherglen, and a brief account will be given of them. The results at the other localities were essentially similar, the disease failing to gain momentum, and either dying out or approaching this end-point during the period of observation.

The Rutherglen trials were carried out on the site of an old deep-alluvial gold mine which took the form of a conical hill 60 ft. high surrounded by lesser hills of sludge and water-washed quartz. The dense rabbit population dwelt in two large warren complexes, one in the hill itself, the other on the flatter ground at its base.

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Since this dump was surrounded by high-value grazing land, it was completely fenced, enclosing an area of almost 13 acres. The warren complex on the flat had expanded outside the fence, however, and free movement from the enclosure to the

Table 2. *Dates and numbers of rabbits inoculated, predator activity, activity of biting insects, and results of introduction of myxoma virus*

| Locality | Rabbit population (counts) | No. inoculated | Date inoculated | Predator activity | | Biting insects | History o myxomatos |
|---|---|---|---|---|---|---|---|
| | | | | Types of predators | Predator selection | | |
| Rutherglen I | 700 | 27 | 7. ix. 50 | Feral cat (1), little eagle (1 pair), fox (rare) | Small kittens | *A. theobaldi,* 2/10 min. | Apparently died out |
| Rutherglen II | 900 | 66 | 27. x. 50 | Feral cat (1), little eagle (1 pair), fox (rare) | Small kittens 1 adult (healthy) killed by fox | *A. annulipes,* 1/10 min. | Incidence low when observ tions ceased 16. xii. 50 |
| Coreen | 400 | 48 | 10. ix. 50 | Feral cat (2), fox (2–3), little eagle (1 pair) | Small kittens. 4 adults killed by foxes, including 1 diseased animal | *A. theobaldi,* 4/10 min. | Apparently died out |
| Balldale A | 300 | 29 | 17. ix. 50 | Feral cat (2), barn owl (1 pair), fox (infrequent), little eagle (1–2 pairs) | Small kittens. 1 adult killed by fox | *A. theobaldi,* *A. alboannulatus, A. sagax* 20/10 min. | Apparently died out. Epizootic commenced early December |
| Balldale B | 150 | 33 | 9. ix. 50 | | | | |

Table 3. *Population counts made on successive favourable nights (see text) at Balldale, Coreen and Rutherglen; and similar counts by Tidswell (1908).*

| Balldale A mid August 1950 | Balldale B mid August 1950 | Coreen early Sept. 1950 | Rutherglen I late August early Sept. 1950 | Rutherglen II mid October 1950 | Rutherglen III mid December 1950 | Tidswell July 1907 | Tidswell August 1907 | Tidswel August 1907 |
|---|---|---|---|---|---|---|---|---|
| 289 | 153 | 372 | 721 | 963 | 967 | 46 | 69 | 145 |
| 334 | 141 | 412 | 673 | 904 | 1031 | 63 | 94 | 125 |
| 314 | 131 | 401 | 652 | 827 | — | 64 | 100 | — |
| — | — | 420 | 715 | 897 | — | 52 | 67 | — |
| — | — | — | 701 | 930 | — | 49 | 55 | — |
| — | — | — | 721 | — | — | 53 | 69 | — |
| — | — | — | 747 | — | — | — | 91 | — |
| — | — | — | 681 | — | — | — | 64 | — |
| — | — | — | 714 | — | — | — | 91 | — |
| — | — | — | 712 | — | — | — | — | — |
| — | — | — | 701 | — | — | — | — | — |
| — | — | — | 683 | — | — | — | — | — |
| Mean 312 | 141 | 401 | 702 | 904 | — | 54 | 78 | — |
| Range 45 | 22 | 60 | 95 | 136 | 54 | 18 | 33 | 20 |
| Coeff. of variation | | 3·3% | 1·1% | 2·5% | | 5·6% | 6·9% | |

outside paddocks was possible, via burrows beneath the fence. Three coloured rabbits (one yellow, two pie-bald white and agouti) were seen to move in and out in this way, on many occasions.

*Spread and effect of the disease*

Daily counts of diseased rabbits were made and carcasses were counted and removed. These observations are shown in Fig. 1. Four natural generations of the disease were recognizable, each smaller than its predecessor. Analysis of the daily observations indicated that the maximum numbers of infected rabbits present during the second, third and fourth generations were 16, 12 and 8. Individual recognition was less easy in the larger first generation, which was estimated at 35.
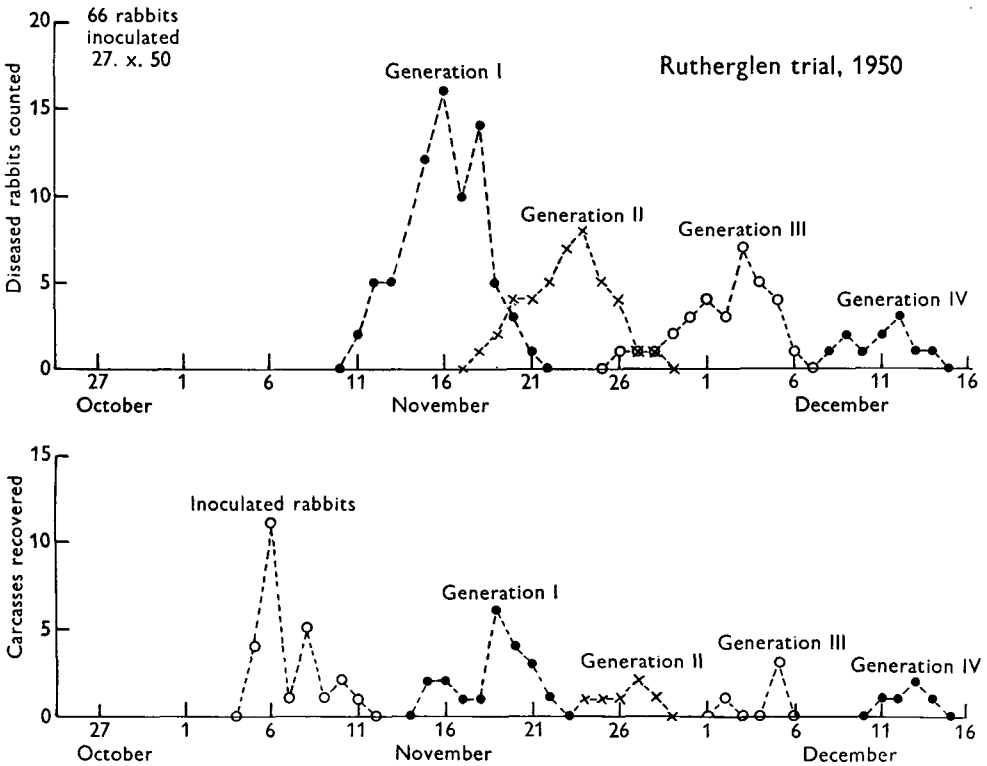


Fig. 1. Daily counts of diseased rabbits and the collected carcasses of rabbits dying on the surface of the ground during the second trial at Rutherglen.

Since 66 rabbits were inoculated, it is possible to calculate the ratio of infection from generation to generation as the following:

| Inoculated rabbits to 1st natural generation | 0·53 |
| First natural generation to second | 0·46 |
| Second to third | 0·75 |
| Third to fourth | 0·66 |

The intervals between the peaks of the first and second, and second and third generations (8 and 9 days) agree reasonably well with what one would expect, knowing that a rabbit begins to become highly infective for vectors on the seventh or eighth day.

When intensive observations ceased on 15 December, the initial population of 700 rabbits had increased to 1000, the increase having occurred over 14 weeks.

During this period, known predation and coccidiosis (see below) had accounted for approximately 400 kittens. Naturally acquired myxomatosis was known to have killed about 70 rabbits during the same period.

### The behaviour of sick rabbits

In Table 4 successive daily sightings of individually recognizable sick rabbits, still living within their own warrens, are listed. No doubtful sightings are included, so that these observations indicate the minimum number of diseased rabbits behaving in this manner. Since it was decided that the maximum numbers of

Table 4. *Daily sightings of diseased rabbits—Rutherglen II*

| | Warren complex 1 | | | | | Warren complex 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Second generation* | | | | | | | | |
| 19 Nov. | A* | B | — | — | — | — | — | — | — |
| 20 Nov. | A | B | D | — | — | C | — | — | — |
| 21 Nov. | A | B | D | — | — | C | — | — | — |
| 22 Nov | A | B | D | — | — | C | E | — | — |
| 23 Nov. | A | B | D | f* | — | C | E | — | — |
| 24 Nov. | A | B | D | f | I | Dead | E | g | h |
| 25 Nov. | — | — | D | f | — | — | | g | h |
| 26 Nov. | — | — | — | f | I | | | g | h |
| 27 Nov. | — | — | — | — | I | | Dead | Dead | |
| 28 Nov. | — | — | — | — | I | | | | |
| Sightings | 6 | 6 | 6 | 4 | 4 | 4 | 3 | 3 | 3 |
| | *Third generation* | | | | | | | | |
| 26 Nov. | — | — | — | | | m | — | — | — |
| 27 Nov. | — | — | — | | | m | — | — | — |
| 28 Nov. | — | — | — | | | m | — | — | — |
| 29 Nov. | 1 | — | — | | | m | — | — | — |
| 30 Nov. | 1 | n | — | | | — | 0 | — | — |
| 1 Dec. | 1 | n | — | | | — | 0 | — | — |
| 2 Dec. | 1 | n | — | | | — | Dead | — | — |
| 3 Dec. | 1 | n | R | | | — | | q | — |
| 4 Dec. | — | — | R | | | — | | q | s |
| 5 Dec. | — | — | R | | | — | | — | s |
| 6 Dec. | — | — | R | | | — | | — | s |
| 7 Dec. | — | — | — | | | — | | — | — |
| Sightings | 5 | 4 | 4 | | | 3 | 2 | 2 | 3 |

\* A, etc. (capital letters) indicate individual adult rabbits. f, etc. (small letters) indicate individual kittens.

diseased rabbits present in the second and third generations were 16 and 12 respectively, it can be seen that the rabbits actually identified as remaining within their warrens formed more than 50 % of their respective generations.

Failure to observe rabbits after several days of illness presumably denoted death in the burrow.

### The activity of predators

Observations at Rutherglen showed that three types of predator were active. Two of these, a feral cat and a pair of little eagles killed between them two or three

small kittens daily, mostly healthy. As far as could be ascertained, the third predator, a fox, visited the site on three occasions only and made one kill, a healthy adult. About ten kittens died each week above ground with symptoms of acute coccidiosis.

*The activity of potential insect vectors*

During August, September and October the only winged blood-feeding insect taken at Rutherglen was the mosquito *Aedes theobaldi* (Taylor) in very low numbers, biting during the day. Towards the end of November, occasional specimens of *Anopheles annulipes* (Walk.) were caught biting in the evening.

The only ectoparasite found was the louse *Haemodipsus ventricosus* (Denny) in small numbers on every rabbit examined (Mykytowycz, 1951).

At Balldale, from August to October, dense populations of the day-biting mosquitoes *Aedes theobaldi*, *A. alboannulatus* (Macq.) and *A. sagax* (Skuse) were present, but no epizootic developed during the period of their activity.

*Comment*

Previous work (Martin, 1936; Bull & Mules, 1944) has shown the need for close contact between sick and healthy rabbits for the transmission of the disease by contact infection. Apparently contact between the members of the same warren colony is not adequate to ensure regular transmission. Even at the high density of 1·2 rabbits per active lead, no epizootic developed.

In these trials, in contrast to those of Bull & Mules, it was obvious that predation did not exert any control over disease performance. Predation, on the whole, was light and almost wholly directed against the youngest age group.

Most sick rabbits remained as members of their own warren community for the greater part of, or all, the period during which they might be infective, so that the failure of the disease to spread could not be ascribed to movement of the sick rabbits away from their warrens.

The failure of the *Aedes* group of mosquitoes at Balldale to spread the disease, although in high density, suggested their inefficiency as vectors (later confirmed). In the light of later knowledge (Myers *et al.* 1954) it appears that the population of *Anopheles annulipes* at Rutherglen must have been below the threshold density necessary for effective transmission of myxomatosis to occur.

The final trial at Rutherglen was carried out under conditions which would favour the initiation of an epizootic transmitted by contact, or by the oral or respiratory routes, namely an extremely high population density, no selective predation of diseased animals, and a high proportion of sick rabbits remaining within their warrens until death. Under those conditions, however, the ratio of infection from generation to generation was approximately 0·6, and the incidence of myxomatosis in the population diminished to a very low level. As subsequent developments show, the one factor lacking was transmission by flying insects.

*The epizootic of December* 1950–*February* 1951 *in the Eastern Riverina*

Intensive observations on the Balldale and Coreen sites ceased in November when the disease appeared to have died out. In December 1950, however, an

epizootic broke out at Balldale. Control measures by the landholder prevented a study of the disease on this site, but very soon diseased rabbits appeared on the low-lying flats of the Murray River, 8 miles to the south. The subsequent remarkable dispersal of the disease westwards along the Murray River and northwards to Queensland have been described by Ratcliffe *et al.* (1952).

The lack of data on pre-epizootic populations prevented any quantitative assessment of the effect of the epizootic on the river-side rabbit populations; and attention was therefore concentrated upon a study of the insects which might be associated with the production of the epizootic.

### The mosquito fauna

During the months of January and February 1951, when the epizootic was at its height, the mosquito population of the river-flats was completely dominated by two species, *Culex annulirostris* Skuse and *Anopheles annulipes*. Wide-scale sampling of larvae yielded 1369, 88·4 % *Culex annulirostris*, 11·6 % *Anopheles annulipes*.
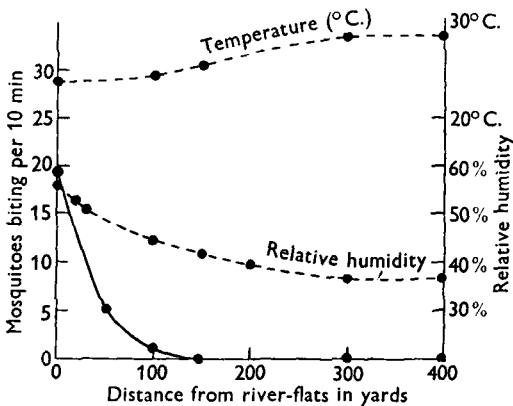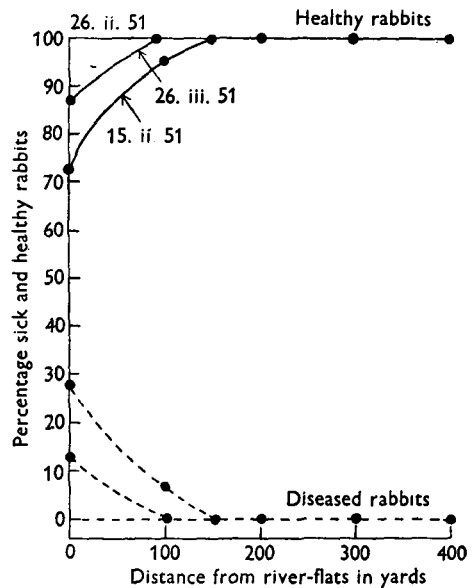


Fig. 2.



Fig. 3.

Fig. 2. Adult mosquito activity on a typical summer evening at 9 p.m. on river frontage country. Adult density drops sharply with the lower relative humidities and higher temperatures of the habitats away from the river.

Fig. 3. Counts on three occasions during the later stages of an epizootic on river-flat country. Compare with Fig. 2 for correlation of high mosquito density and disease incidence.

These two species appeared to be the only mosquitoes breeding in the region at the time, and active as adults throughout the epizootic areas.

The epizootic was confined to the actual flats and frontage country of the Murray River and the distribution of the mosquito populations of the region was similarly restricted (Figs. 2, 3). Both the adult mosquito density and disease incidence

dropped sharply as soon as the river-flats gave way to the higher, drier ground on either side. From a study of a series of flats, delimited by the meanderings of the river, a rough correlation was obtained between the kill and the measured area of breeding waters of the two mosquitoes. Mortality from myxomatosis was greater where the exposed rabbit populations lived close to large areas of mosquito-breeding waters.

The Murray River reached the peak of its annual flood on 31 October 1950. The numerous permanent and semi-permanent lagoons of the river-flats were thus still subject to strong currents in early November and could not have become suitable for mosquito breeding on a large scale until later in the month. Subsequent observations have shown that after the annual flood there is an unproductive period before the lagoons become well vegetated and suitable for the breeding of *Culex annulirostris* and *Anopheles annulipes*.

Concomitantly with the main extension of the epizootic along the river-flats during January, outbreaks of the disease occurred many miles away from the river, in the vicinity of drying up swamps and water-holes. By early February, however, the majority of these outlying foci had disappeared entirely, and the epizootic contracted again to the river-flats, with its weedy lagoons and bordering stands of the river red-gum (*Eucalyptus camaldulensis*, Denh.).

By 15 March 1951, evening temperatures on the river frontage (8 p.m.) dropped below 70° F. for the first time, and adult mosquito activity began to decline. By April, evening temperatures registered 50–60° F., adult mosquito activity ceased completely, and with it all evidence of the disease.

## Mosquito habits

Both *Anopheles annulipes* and *Culex annulirostris* are night-biting mosquitoes, becoming active at dusk. When feeding on rabbits they appeared to prefer the sites where the fur was very short—around the eyes, the ears, nose and paws. About three times as many mosquitoes fed on rabbits with myxomatosis as on healthy animals, due apparently to the lethargy of sick rabbits. Twenty counts of mosquitoes feeding on sick and healthy rabbits between 9 p.m. and 10 p.m. on a typical summer evening showed that 8–12 (average 10) were feeding on the sick and 1–4 (average 3) on healthy rabbits. Interrupted feeding, due to flicking with the paws, etc., was much more common on the healthy rabbits.

## Recovery of virus from mosquitoes

During the decline of the epizootic, 168 female *C. annulirostris* caught biting man on river frontage were ground in a mortar and pestle and taken up in 5 ml. normal saline. Of this suspension 0·5 ml. was inoculated subcutaneously into the right flank of a laboratory rabbit. A local lesion appeared after 3 days, and the animal developed typical symptoms of generalized myxomatosis a few days later (Mykytowycz, 1951).

At the same time, three laboratory rabbits were exposed to mosquito biting daily from 8 to 11 p.m. for 14 successive days. Between the exposure periods they were kept in insect-proof cages within a wire enclosure. *C. annulirostris* was observed

to bite the exposed rabbits freely. Two of the animals developed generalized symptoms of myxomatosis on the 14th and 15th days respectively after the first exposure. The third rabbit was not infected (Mykytowycz, 1951).

*Other vectors*

The only other insect which appeared to play a part in the development of the 1950–51 epizootic in the Eastern Riverina was a simuliid fly, *Simulium melatum* Wharton. In early February 1951, this fly was observed feeding on the ears of rabbits at the site of an active myxomatosis outbreak in a thickly wooded bend of the Murray River near Yarrawonga, Victoria. Mykytowycz (1954) was able to demonstrate experimentally that *S. melatum* was acting as a vector in this particular epizootic area. Another simuliid, *S. nicholsoni*, was also active in the area, but was only collected from stock.

*Comment*

The observations described in this paper all point to the importance of winged insect vectors (principally mosquitoes) in the epizootic of myxomatosis that developed in the Eastern Riverina during the summer of 1950–1.

It is apparent that although a relatively recent introduction to Australia, the rabbit contributes substantially to the support of large populations of certain native insect species that have become adapted to it as a source of blood meals.

It appears reasonable to conclude that wherever myxomatosis causes a significant mortality in a rabbit population, a blood-sucking vector is responsible for the spread of the infection. Conversely, where the disease fails to develop within a susceptible host population, no insects or other arthropods capable of acting as vectors are present.

## SUMMARY

An account is given of experiments designed to study the spread of myxomatosis in populations of rabbits living under natural conditions on a number of sites in the Eastern Riverine Plain of south-eastern Australia. In five different trials the disease spread from inoculated rabbits, but failed to gain momentum and died down within a few weeks of its introduction.

In December 1950, when the disease was persisting at low incidence on one test site and seemed to have died out in the others, an epizootic broke out in the neighbourhood which spread, in a few months, over the greater part of south-eastern Australia. The only factor, apart from climatic ones, which could account for this sudden change in the activity of the disease was the development of large populations of two rabbit-feeding mosquitoes, *Anopheles annulipes* and *Culex annulirostris*. A close correlation was demonstrated, on the flats bordering the Murray River, between the distribution of these insects and myxomatosis activity.

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