He Kōrero Paihama
Possum Research News

Issue 15 *June 2001*

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Manaaki Whenua
Landcare Research

Plant-based Contraceptive Bait for Possums - a Step Closer

The control of possum fertility is one exciting, humane alternative to well-established methods of lethal control that is under evaluation. Recently, the humble potato was trialed at Lincoln to demonstrate that genetically modified plants have potential for development as a bait to lower possum fertility and may one day join the arsenal of techniques available to control possum populations.

Scientists from Landcare Research and the Marsupial Cooperative Research Centre (Marsupial CRC) recently completed a contained-laboratory trial using gentically modified potatoes grown by the Boyce Thompson Institute for Plant Research in the United States. The Boyce Thompson Institute is a world leader in developing plant-based vaccines for humans. In the trial, possums were fed pieces of potato containing a foreign protein, in this case, part of a protein from a bacterium.

Antibodies to the foreign protein were subsequently found in the possums' blood, gut and reproductive tracts, indicating that the antibodies had permeated throughout the possums' systems.

The result is a boost for Landcare Research and the Marsupial CRC's plans for a similar trial in containment involving feeding possums carrots that contain a protein called ZP3, derived from the coating of the female possum's eggs. Scientists believe baits made from plants containing ZP3 will act as a vaccine that causes the possum's immune system to treat its own eggs as foreign bodies, and make antibodies against them. This birth control method is called immunocontraception, as it essentially immunises possums against conceiving.

The leader of this research, Janine Duckworth, believes the potato trial results are encouraging news for the team, as they work towards a 60-70 % reduction in possum fertility with immunocontraceptive baits. The positive results indicate the considerable potential for the use of plants as a delivery system for a biological control agent for possums. The successful development of immuno-contraception should be a useful and humane addition to current possum control measures.   Using immunocontraceptive baits after poisoning operations will slow the rate at which possum populations rebuild. This means toxic control will be needed less frequently, resulting in less environmental contamination, and reduced risk to non-target species.

A possum eating a piece of potato.

The plant baits themselves will be environmentally friendly. The plants will be processed so that they can neither grow nor spread genetic material to other plant life. Also, the parts of the possum protein contained in the carrots will be specific to marsupials, and should not affect any other animals.

The carrots are currently being grown in the USA, and their importation and the trial have recently been approved by the Environmental Risk Management Authority (ERMA). The carrots will arrive in New Zealand soon, in time for testing on possums in strict containment during the current possum-breeding season.

This research was funded by the Foundation for Research, Science and Technology and the Marsupial CRC.

***Janine Duckworth** and **Phil Cowan** work on fertility control in possums. **Amanda Walmsley** and **Dwayne Kirk** work with plant vaccines at the Boyce Thompson Institute for Plant Research.*

Guest Editorial

This year marks the 10th anniversary of the establishment of the National Science Strategy Committee (NSSC) for Possums and Bovine Tuberculosis Control. This committee was established in October 1991 by Government following a report by Dr A J Allison in July 1991 titled “Towards a national strategy for science. The problem of possums and bovine and cervine tuberculosis”.

The Cabinet Committee on Enterprise, Growth and Employment agreed to establish the NSSC “to enhance co-ordination of all research programmes on possums / bovine Tb control”.

In the last decade there have been many changes in the research programme, in the level of investment in research on possum and tuberculosis control, within the NSSC itself, in the possum “industry”, in public interest in possum control, in the generation and accessibility of information, and in the linkages between science and the possum industry.

Since the establishment of the NSSC, an extensive research programme on novel methods to control possums, loosely described as “biocontrol” of possums, has been established, research on new tuberculosis vaccines has been initiated, new toxins introduced, more cost-effective ways to use established toxins developed, public attitudes to possum control studied, and increased investment in research achieved.

Particularly noteworthy has been the establishment of cooperative research programmes between New Zealand researchers and their overseas counterparts. The most notable of these is the partnership of Landcare Research with other Marsupial Cooperative Research Centres and the collaboration between AgResearch and MAFF (UK).

Following a recommendation from the NSSC, Cabinet approved funding to establish a “biocontrol” research programme to control possums. This area of research has become a major investment theme with over $6 million committed annually.

As research has progressed, new issues have arisen. These include safety of using toxins, quality assurance programmes for industry, compilation of toxin databases, concern about animal welfare, the use of genetic modification in control technologies, and the need for community consultation on new control methods.

Although resources have always constrained the activities of the NSSC, among its achievements have been the organisation of annual workshops and publication of their Proceedings, the publication of an Annual Report to include research databases, research highlights, mini reviews on possums and Tb, a science strategy, and a bibliography of publications and reports. It also publishes a regular Possum Research Newsletter.

In the last decade, possum industry changes have impacted on the NSSC. These include the establishment of the National Possum Control Agencies, the Animal Health Board’s National Tb Management Strategy, the Department of Conservation’s possum control strategy, regional councils’ pest management strategies, the introduction of Quality Assurance systems into industry, the holding of pest management conferences and technology transfer workshops, the development of new possum product industries, and the increasing professionalism of contracting animal-pest-control companies. All of these have had research and science implications requiring improved linkages between science providers and technology users.

The introduction of new toxins, the development of superior bait stations, and the use of the Residual Trap Catch index as a performance measure have been some of the benefits from these linkages.

The research investment into new Tb vaccines is resulting in improved vaccines, and the possibilities of reducing possum fecundity have been demonstrated. Ongoing investment in research will be required to develop, test and commercialise these opportunities. The rewards will be great.

We still have Tb – but there are indications that the prevalence in possums is decreasing in many regions of New Zealand. Vector control towards Tb eradication remains an expensive operation and without the introduction of more cost-effective methods, the cost to New Zealand to become Tb-free will remain high. We still have possums and there is little prospect that, with the exception of on offshore islands, these will be eradicated.

In summary, NSSC’s 10 years have overseen some significant outcomes from research into possum and bovine Tb control. Technology users have already benefited from some of the research programmes. Research into novel methods to control possums and to eradicate Tb shows promise of major future benefits for New Zealand’s twin problems of Tb and possum ecological damage.

***Doug Wright** is the Convenor, National Science Strategy Committee for Possums and Bovine Tuberculosis Control.*

Fertility Control in Possums - Results from a Large-scale Field Experiment

Fertility control offers a potentially useful tool for the management of possums. However, its effectiveness in possums is untested, particularly as it influences how populations respond to reductions in birth rate. Dave Ramsey and his team evaluated this response using data from a large-scale field experiment on the effects of fertility control on the population dynamics of possums.

The experiment was carried out at six study sites at two different locations (Orongorongo and Turitea valleys) in podocarp / hardwood forest in the lower North Island. Beginning in late 1995, possums were captured, tagged, and released at each site, using cage traps at 30-m spacing on a 12-ha grid set in January, June and September each year. In early 1996, the team imposed artificial reductions on the birth rates on four of the six populations by surgically sterilising either 50% or 80% of the adult females present, with sterilisation treatments adjusted annually to maintain sterility levels in each treatment. As the effect of the surgery on possum survival was unknown, sham operations (no sterilisation) were undertaken on the two controls and two 50%-sterility sites to balance the level of surgical manipulation across the two replicates of each treatment.

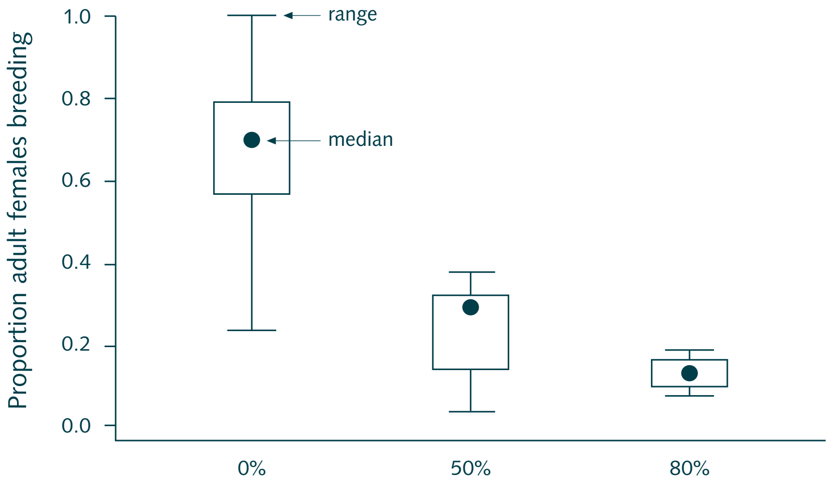


Fig. 1. *Proportion of female possums breeding in replicates with 0%, 50% and 80% of the population surgically sterilised. The box represents the upper and lower quartiles.*

Apart from 1996, when many possums failed to breed over large areas of New Zealand, Dave's team were able to demonstrate that the sterility levels selected suppressed the birth rate in each treatment so that an average of 0.3, and 0.14 of females breed on the 50%, and 80% sterility sites, respectively, compared with 0.7 on control sites (Fig. 1). Of particular interest, though, was whether these reductions in the birth rate translated to reductions in recruitment at each site, i.e., the number of animals born that survived to enter the breeding population. If such recruitment into the breeding population can be reduced to less than those leaving it (dying), then the population will

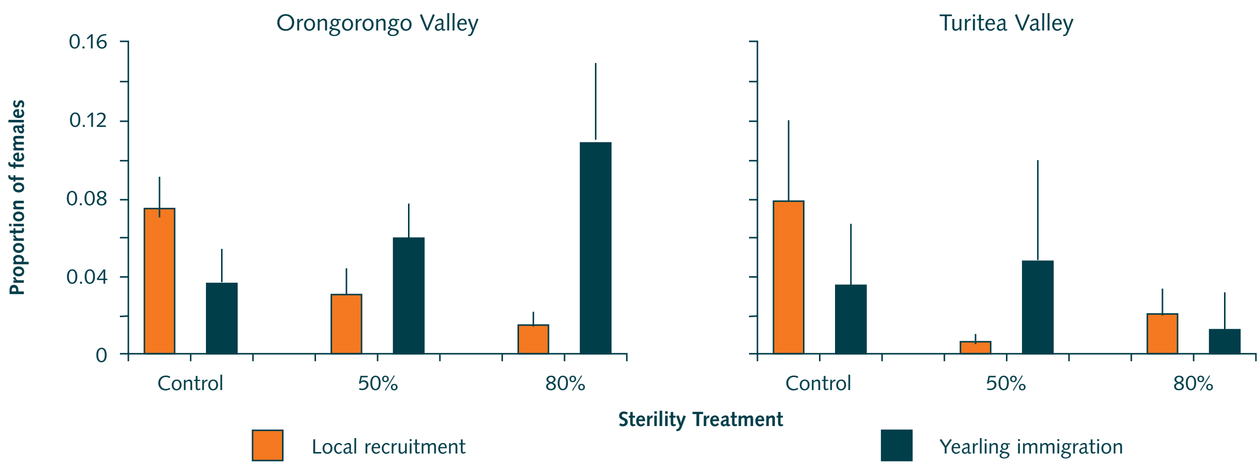


Fig. 2. *The proportional contribution of local recruitment and yearling (1 year old) immigration of female possums to year-to-year population change for each sterility treatment. The top of the bars indicate means and the lines the standard error.*

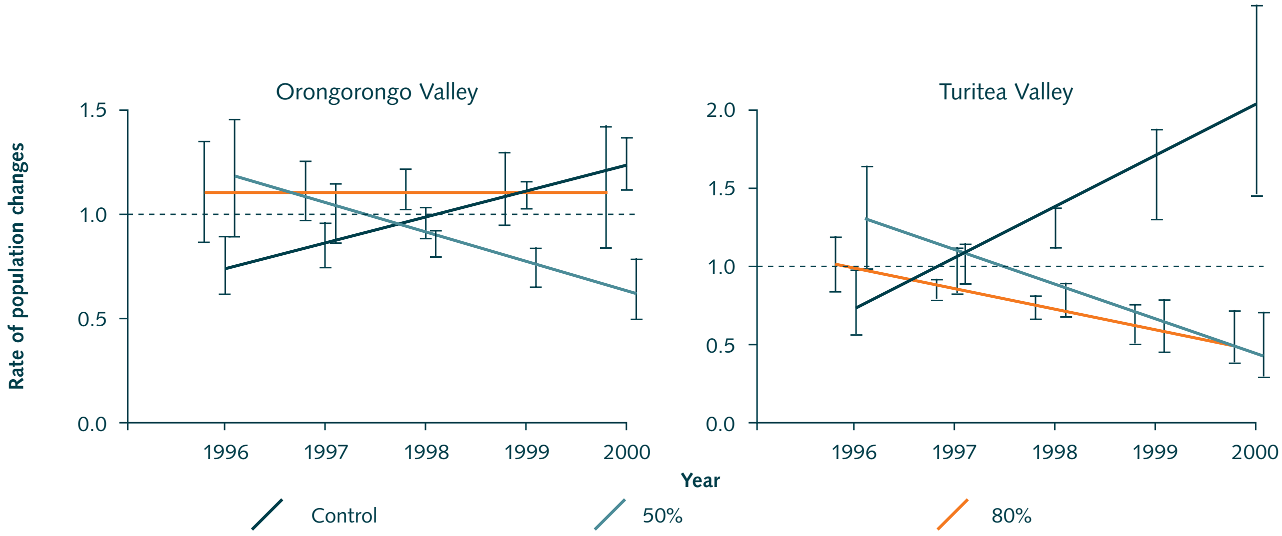


Fig. 3. *Trends in the rate of population change for each sterility treatment. A rate of change equal to 1 implies the population is neither increasing nor decreasing. The vertical lines are the 95% confidence intervals.*

decrease. For simplicity, the team focused on the female portion of the population only, as variation in the recruitment rate of females usually has a greater influence on the overall dynamics of the population than recruitment rates of males.

However, recruitment into a population results from both the breeding of resident females (local recruitment) and immigration. Comparing local recruitment among populations that vary in size is problematical. One way to make these comparisons is to determine their relative contribution to year-to-year population change. Initial results show that the sterility treatments applied reduced the contribution of local recruitment to annual population change by an average of 0.57 and 0.77 on the 50% and 80% sterility treatments, respectively. However, in the Orongorongo Valley, immigration was the dominant component of recruitment on the 50% and 80% sterility sites, and compensated almost perfectly for the reduction in local recruitment due to the sterility treatments (Fig. 2). In the Turitea Valley, immigration made a smaller contribution to population change.

The net effects of recruitment and mortality to the study populations can be summarised by looking at the trend in annual population change (Fig. 3). In the Orongorongo populations, only the 50% sterility site showed a negative trend in population change over the duration of the study, while the 80% sterility site showed little change due largely to immigration. On the Turitea sites where immigration had a more modest impact, sustained negative trends in population change were recorded on both the 50% and 80% sterility sites.

Clearly, immigration has the potential to cancel out the effects of fertility control in small areas. Such control will therefore need to be applied over large areas, where immigration will be a less significant component of the population dynamics. More importantly, results from the experiment indicate that even moderate levels of fertility control can significantly reduce the level of local recruitment into populations of possums, leading to population decline. Fertility control has therefore the potential to provide considerable benefits in the war against possums.

This work was funded by the Ministry of Agriculture and Forestry.

***Dave Ramsey**, **Aaron Miller**, **Ruth Fleeson** and **Kathryn Knightbridge** work on the effects of fertility control on possum populations.*

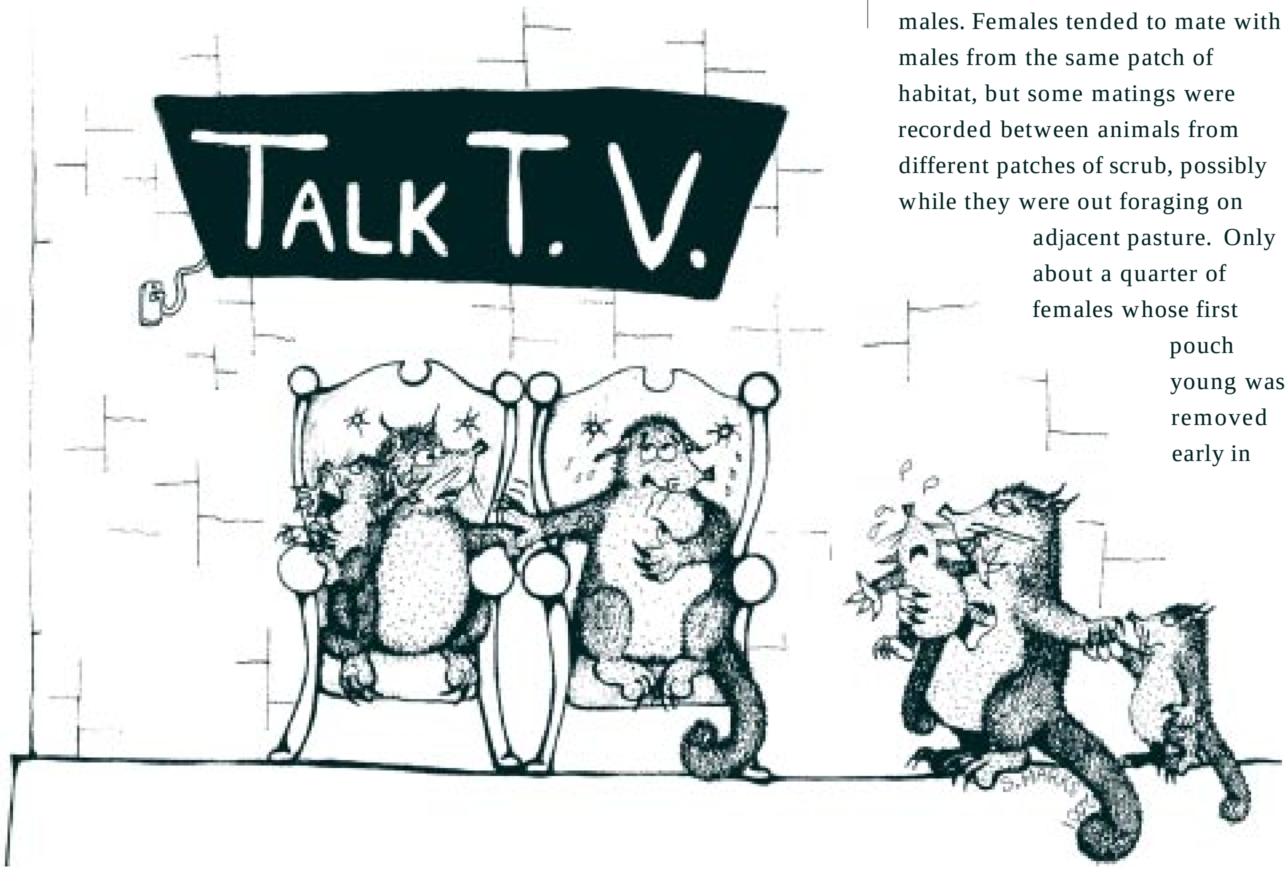
DNA Fingerprinting Reveals Possums' Sex Lives

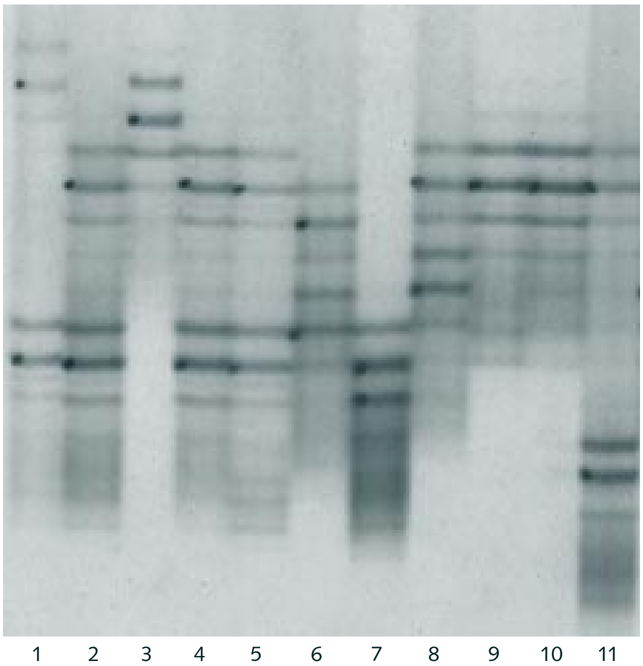
Better knowledge of the mating system of possums is important for two reasons — first, to improve our understanding of the epidemiology of bovine Tb, since interactions between possums are thought to be the main mode of possum-to-possum Tb transmission; and second, to assist with the assessment of systems for biological control of possums, since the rate of sexual contact between individuals will directly influence the rate of spread of a sexually transmitted vector.

A trans-Tasman team from the Marsupial Cooperative Research Centre used DNA fingerprinting to carry out a paternity analysis on a population of about 180 possums on farmland with pasture and isolated patches of scrub and shelter belts at Bridge Pa, Hawke's Bay. Most of the pouch young were removed from females early in the breeding season, and then all possums including additional pouch young were killed at the end of the breeding season. Blood and tissue samples were collected from all possums. The key questions addressed in the study were what was the degree of variation in male reproductive success, what were the characteristics of successfully breeding males, and if females lose their pouch young, how often do they mate again with the same male?

The DNA fingerprinting was carried out using microsatellite markers. These are small, apparently non-functional regions within an animal's DNA containing repeated sequences of the nucleotide base pairs that make up DNA. Individuals differ in the number and type of repeats at different regions within their DNA. Six microsatellite markers were identified for possums, with between 7 and 16 variants for each. DNA was extracted from the tissue or blood samples collected from the possums. Analysis using all six markers gave a greater than 99% chance of identifying the correct father.

Surprisingly, only about half of the mature males successfully fathered a young. Among the successful males, about half fathered one young, with successively fewer fathering multiple young, up to a maximum of four. Successfully breeding males tended to be older and larger than non-breeding males. Females tended to mate with males from the same patch of habitat, but some matings were recorded between animals from different patches of scrub, possibly while they were out foraging on adjacent pasture. Only about a quarter of females whose first pouch young was removed early in





The 11 verticle gel lanes each contain the microsatellite profile (dark bands) of a single possum. Individuals which do not share major bands (e.g., those in lanes 3, 5 and 6) can be excluded as being related as parents or offspring. When major bands are shared, such as in lanes 9 and 10, these possums are likely to be related to each other.

the breeding season remated with the same male, suggesting that such behaviour is relatively uncommon.

Together, these results suggest that possums show a low level of polygyny, that is males mating successfully with more than one female. This finding has important implications for the transmission of both Tb and sexually transmitted biological control vectors. The low incidence of repeated matings between the same adults and the relatively high proportion of males involved in breeding will tend to maximise transmission of infectious agents from the female to the male population. However, the generality of these findings will need to be confirmed in a range of different habitats.

This work was funded by the Foundation for Research, Science and Technology, the Australian Research Council and the Marsupial CRC.

Phil Cowan *works on the ecology and fertility control of possums.* ***Andrea Taylor*** *carried out the genetic analysis, with assistance from* ***Belinda Fricke*** *(not shown) and* ***Des Cooper*** *at Macquarie University. Landcare Research and Macquarie University are participants in the Marsupial Cooperative Research Centre.*

Spring- (or Double-) Breeding in Possums

The possum breeding season in New Zealand typically commences about March and usually ends by November, although there is some variation with region and year. As with many mammals from temperate regions, the onset of breeding is controlled by hours of daylight (photoperiod). Studies on captive possums have shown that changing from a long- to a short-day photoperiod brings forward the onset of breeding, while changing from a short- to a long-day photoperiod terminates breeding. Other factors, such as physical condition of females (as indicated by higher mean body weights), have been shown to be correlated with earlier breeding, suggesting that nutritional status can influence the number of young born.

Typically, female possums in New Zealand mature at 1–2 years of age and give birth to a single young (twins are rare). The median date of birth is from late April to mid-May. In some localities or at certain times

Female with backrider.

a second pulse of breeding occurs, generally between August and November, and this is referred to as spring- (or double-) breeding. In Australia, up to 38% of possums breed twice a year. This has generally been attributed to reproductive failure during autumn or during lactation, or to double-breeding by successful females in good habitat.

Previously in New Zealand, spring-breeding has been thought to be largely restricted to colonising, and thus rapidly increasing, populations (when food resources and other essential habitat factors such as den sites are plentiful). Wayne Fraser has re-examined this assumption by looking at historical and recent data, mostly gathered before widespread possum control (see Table). He has found that the percentage of females with spring births in colonising and rapidly increasing populations is high, but that this percentage declines markedly as those populations approach peak numbers and then crash.

However, data Wayne collected recently from a long-term study in the Waihaha catchment (Pureora Conservation Park, central North Island) suggest that spring-breeding can be induced by population changes following harvesting and control. Possums colonised the Waihaha catchment about 40 years ago and vegetation surveys in the area suggest that they reached peak numbers in the late 1970s. Although other post-peak populations in New Zealand exhibit little or no spring-breeding, about 8% of adult females sampled from the Waihaha catchment in 1989–94 had spring births, apparently a result of

Table: *Percentages of adult female possums with spring births according to population status. Only those females from populations sampled between August and March were included; body weight of pouch young was used to assess month of birth.*

Location	Year	Population status	No. of adult females	% of females spring - breeding
Arawhata Valley (mid)	1997	colonising	19	52.3
Copland Valley (upper)	1978	colonising	6	50.0
Copland Valley (upper)	1985	increasing	39	35.9
Copland Valley (mid)	1978	pre-peak	29	6.9
Copland Valley (mid)	1985	pre-peak	63	3.2
Copland Valley (lower)	1978	post-peak	51	0.0
Copland Valley (lower)	1985	post-peak	32	0.0
Waiatoto Valley (mid)	1997	post-peak	29	3.4
Waipara Valley (lower)	1997	peak	58	0.0
Waihaha catchment	1989–94	pre-control	303	8.3
Waihaha catchment	1994–99	post-control	62	56.5

intensive commercial trapping for skins throughout the 1980s. More dramatic still is the marked increase in spring-breeding recorded by Wayne following a large-scale aerial poisoning (1080-carrot) operation, including all of the Waihaha catchment, in winter 1994 (which resulted in a 92% kill). Clearly, the increased spring-breeding recorded was a response to reduced competition for food resources (and possibly favourable den sites), as well as the noticeable vegetation recovery observed following control. The rapidity with which possums can "compensate" for such changes has significance for ongoing and future possum control — as artificially reduced populations are able to increase their reproductive rate. This has important implications for the assumptions on which possum- and Tb-control models are based.

***Wayne Fraser** works on the ecology, impacts, and management of ungulates and possums.*

Research update: Successful Use of Released Pigs to Detect Tb in Wildlife

In the May 2000 issue of Possum Research News, Graham Nugent, Jackie Whitford, and Nigel Young outlined a trial being undertaken to test a new concept in bovine Tb surveillance – the use of released pigs to detect Tb in possums and other wildlife. To do that, 17 Tb-free feral pigs were fitted with radio transmitters and released in February 2000 into a West Coast forest known to contain infected possums and deer. The pigs were radio-tracked regularly, and at approximately 2 monthly intervals after release up to four were killed and necropsied (15 in total) to determine their Tb status. The first two recovered did not have lesions typical of Tb but culture of key lymph nodes showed they were infected. The remaining thirteen had typical Tb lesions, with those released for the longest time tending to be most severely infected. It seems that all of the pigs became infected within a few weeks of release, indicating their usefulness as moderately sensitive indicators of the presence of Tb.

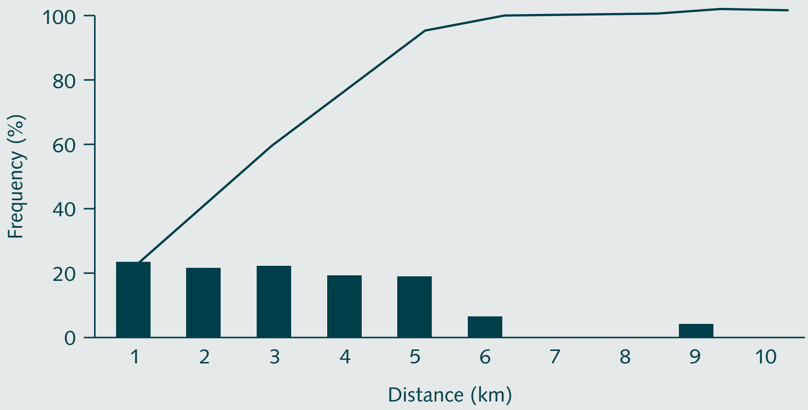


Fig. *The actual (bar) and cumulative (line) percentage of previous locations within one kilometre radius classes around the locations of the 7 pigs still present 6 months after release.*

Forty-one resident feral pigs were also shot in the same area, many of them runnning with the released pigs. By comparison with released pigs, fewer of the resident pigs had typical Tb lesions (37%), but this was mostly because many were piglets 1- 2 months old.

The distribution of distances between the location of pigs about 6 months after release and all previous locations (see fig) indicates that about 95 % of such distances were less than 5 km. The presence of infection in an unmarked feral pig therefore indicates that there is a 95% chance the source of infection is within a 5-km radius around the kill site.

Overall, the trial has demonstrated that the concept is technically feasible, and as a result, vector managers have begun using the method. What is now needed is more information on the sensitivity of released pigs to detect Tb when it occurs more rarely than in this trial.

Poison Residues in our Meat and Milk

Sodium monofluoroacetate (1080) and brodifacoum are two toxins used in a range of bait carriers to control possums and other vertebrate pests. However, both compounds when consumed in sub-lethal amounts by non-target animals may put at risk humans utilising such species for food. Risks from consuming residues of either toxin in contaminated milk or meat have not been quantified.

Cheryl O'Connor and her team have used sheep as a model for assessing possible contamination of cow's milk by 1080 or brodifacoum. They fed cereal baits containing 1080 to lactating ewes at high and low dose rates, and collected milk and blood samples 4, 12, 24, 48, 72 and 96 hours later. Similar trials were undertaken using brodifacoum delivered orally to lactating ewes at high and low dose rates, and milk and blood was again collected 2, 4, 8, 16, 24 and 32 days later. The "high-dose" exposure was equivalent to a 60-kg sheep eating 3 kg of brodifacoum cereal bait, while the "low-dose" groups were more akin to levels recorded in wildlife in field situations about toxic bait lines.

Residues of 1080 were detected in both the blood and milk of ewes, with concentrations in blood 30 times higher than those found in milk (see Fig). Maximum concentrations of 1080 were detected in the high-dose group's blood and milk 4 hours after administration and low concentrations of the toxin were still detectable in the blood of all the surviving ewes (high- and low-doses) 96 hours after exposure. By comparison, residues of 1080 were detected in the milk of surviving high-dose ewes only at the limit of detection (0.0005 µg / ml) 72 hours after exposure, and were completely absent at 96 hours. Four of the eight low-dose ewes had no detectable 1080 residues in their milk 48 hours after exposure, and all were free of 1080 residues at 72 hours.

Although significant amounts of brodifacoum were detected in the blood of high-dose ewes 2 and 4 days after exposure, only four high-dose ewes showed any traces of brodifacoum in their milk at all, and only one of these was above detectable assay levels (0.01 µg / ml). No brodifacoum was detected in the ewe's milk beyond 4 days.

Brodifacoum and 1080 are thus both transferred to the milk in sheep, but even when the differences in milk production between sheep and dairy cows are considered, the milk residue results reported by Cheryl and her team indicate human poisoning through drinking milk is very unlikely. Estimates for human susceptibility to these poisons indicate a person would have to drink 120 times their weight of milk over a short period of time to receive enough 1080 to kill 50% of consumers, and 25 times their weight for brodifacoum.

The same is not true for contaminated meats, however. Baking possum muscle and liver tissue at 180°C in an oven for 20 minutes failed to remove significant traces of either brodifacoum or 1080. Hence no animals that are thought likely to be contaminated either with 1080 or brodifacoum should be eaten under any circumstances.

This work was funded by the Animal Health Board.

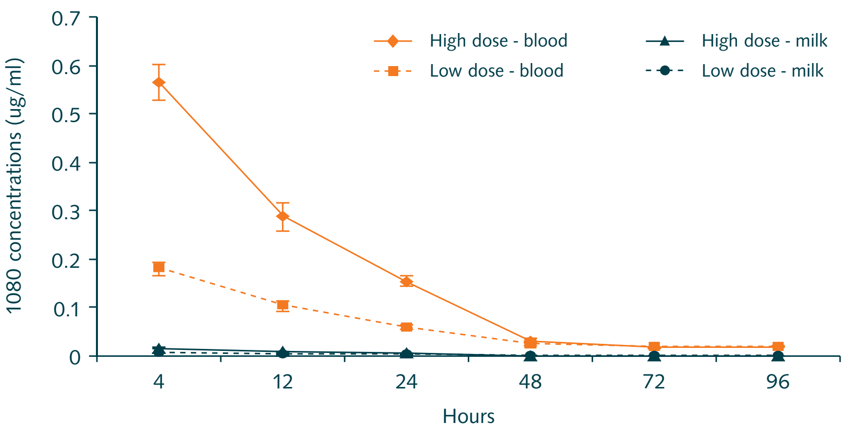


Fig. *Blood and milk 1080 residue concentrations over time*

Cheryl O'Connor, Lynne Milne, Geoff Wright, *and* ***Charles Eason*** *work on the toxicology and welfare of vertebrate pests following the ingestion of toxicants.*

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A Selection of Recent Possum-related Publications

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