MONITORING BAIT ACCEPTANCE IN BRUSH-TAILED POSSUM POPULATIONS: DEVELOPMENT OF A TRACER TECHNIQUE

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(Received for publication 3 June 1981; revision 17 September 1981)

ABSTRACT

Rhodamine B dye, Lissamine green dye, and demethylchlorotetracycline were evaluated as tracers for monitoring bait acceptance by brush-tailed possum (**Trichosurus vulpecula** Kerr) populations. Demethylchlorotetracycline left no detectable trace in possums, even at high dosages. Lissamine green was not persistent enough externally and did not stain the gut tissue. Rhodamine B marked animals for 7 days when used at 0.5% concentration, surface-coated on baits, and can be used at up to 1% concentration without reducing the palatability of the bait. Rhodamine B was therefore selected as a suitable tracer.

INTRODUCTION

This work was conducted as a prerequisite to another study (Morgan in prep.) in which was estimated the proportion of possums in various populations that would accept different types of bait. Tracers have been used in several baiting studies (Lindsey *et al.* 1971; Nass *et al.* 1971; Nelson & Linder 1972; Sullins & Verts 1978). They have also frequently been used to study the movements of animals. Comprehensive reviews of the literature on this subject have been given by Taylor & Quy (1973) and Evans & Griffith (1973).

A successful tracer for field use in possum bait-acceptance studies had to:

(1) Be effective as a marker for a minimum of 7 days, thus allowing time for capture of a sample of possums after baiting;

(2) Not affect the palatability of the carrier bait;

(3) Not affect the behaviour of the possum, particularly in relation to feeding;

(4) Be inexpensive in large quantities since the proposed field use would eventually involve addition of tracer to several tonnes of bait concurrently.

SELECTION OF MATERIALS

Whilst radioisotopes offered the potential of a quantifiable technique (Spragg & Fox 1974) they were considered to be too hazardous and, like many of the otherwise apparently suitable chemical dyes, too costly for bulk usage.

Demethylchlorotetracycline (DMCT) has been a useful tracer in a variety of vertebrates. Single doses of 10 mg/kg body weight administered orally to coyotes (*Canis latrans*) produced fluorescence under ultraviolet light that was noticeable in the mandible and which lasted for at least 5 months (Linhart & Kennelly 1967). A single oral dose of 50 mg/kg body weight produced fluorescence in the jaw of laboratory white rats in less than 1 day and the fluorescence remained visible under ultraviolet light for up to 6 months (Crier 1970). Other tetracyclines have been used to label teeth in fur seals (Yagi *et al.* 1963) and bones and scales of fish (Weber & Ridgway 1962). Hence it was expected that DMCT might prove to be a successful marker in the possum.

Rhodamine B, a red dye that fluoresces orange under ultraviolet light, was selected for evaluation since it has been used successfully in bait acceptance studies. Evans & Griffith (1973) fed 100 g of carrot treated with a 0.1% solution of Rhodamine B to black-tailed jack rabbits (*Lepus californicus*) and cottontail rabbits (*Sylvilagus nuttallii*). The black-tailed jack rabbits remained marked around the mouth, paws, belly, anus, and urogenital openings for 7 days and the cottontail rabbits were marked around the anus and urogenital openings for 15 days. Sullins & Verts (1978) found marking of internal organs in Beldings ground squirrels (*Spermophilus beldingi*) that fed on oat groats treated with 2.5 g Rhodamine B/kg.

Lissamine green dye is used in poisoning operations against rabbits and possums in New Zealand as a means of deterring birds from eating the carrot bait (Caithness & Williams 1971). Even at the concentration of 0.01% used operationally, contaminant stains appear to be persisent. Since it is also inexpensive it was considered worthwhile evaluating this dye for use as a tracer.

METHODS OF TESTING

Rhodamine B, DMCT, and Lissamine green were separately evaluated in terms of their effect on bait palatability and their persistence as tracers. Bait materials to which these chemicals were added were either chopped carrot or a pellet bait manufactured specifically as a possum bait.

The three chemicals were applied to baits as a surface coat in various concentrations using water as a solvent.

Palatability Tests

In order to mark possums effectively it was necessary to find the maximum concentration of each chemical that could be added as a surface coat without impairing the palatability of the bait. This was investigated in three separate trials in each of which three treatments (0.1% weight/weight, 1%, and 3% surface concentration) of the pellet bait and a non-treated control were offered to 11 possums kept in a pen measuring 5 × 30 m. The baits were laid out at three plots of each treatment, the

plots being 5 m apart. Twenty baits were laid at each plot and fresh water and other food (apples, silver beet, and turnip) were also offered freely. The total numbers of baits taken were recorded in the mornings and fresh baits laid. The trials were conducted for 3 consecutive nights. Data were normalised by arcsine transformation and analysed by two-factor (i.e., night : concentration) analysis of variance and the least significant difference (LSD) test.

Persistence Tests

The general details applying to persistence tests involving all three substances are given below; specific details appear in the Results section.

A total of 45 captive possums housed in individual 45 × 65 × 45-cm cages were used for testing the persistence of the three chemicals over various time periods. Animals were each given one treated bait just before dusk at the beginning of a test. This is the minimum intake of bait that must leave a detectable trace; the chemical control of possum populations is based on the control model that each bait contains a lethal dose of poison (Peters 1975). A variety of food consisting of apples, silver beet, turnip, carrots, and stock food pellets was provided once each possum had eaten the test bait. Fresh water was also supplied daily after cages had been cleaned.

Possums were inspected at various periods after eating the test bait. This was done by either anaesthetising with ether, or by killing with an overdose of ether or by peritoneal injection of 2 ml sodium pentabarbitone. Anaesthetising was satisfactory for inspection of the paws and mouth but it was necessary to kill the animals in order to search the gut for traces of the chemicals. A high-intensity (7000 W/cm^2) longwave (366 mm) ultraviolet light was used to search for external traces of Rhodamine B and internal traces of both Rhodamine B and DMCT.

Throughout the course of the experiments with captive animals care was taken to monitor any unusual behaviour that might have been caused by the chemicals. For example, it was anticipated that DMCT, like other antibiotics when given in large dosages, might unsettle an animal's digestive system causing loss of appetite and scouring. However, no obvious changes in behaviour were noticed during any of the experiments.

RESULTS AND DISCUSSION

Demethylchlorotetracycline

All baits of the different treatments offered to possums in the palatability test were eaten, suggesting DMCT is tasteless or not unpleasant to possums even at high concentrations. This being so, a range of concentrations up to approximately 5% was subsequently used in persistence tests.

To test for persistence of DMCT four possums were each given a single dose of 10 mg DMCT/kg body weight and four others were given a single dose of 50 mg/kg. The dose was applied as a surface coat to pellet baits weighing 3.5 g and was adjusted to the weight of each animal. Concentrations on baits thus varied from 0.71% for the lightest possum weighing 2.5 kg and receiving a 10 mg/kg dose, up to 4.86% for the heaviest possum weighing 3.4 kg and ingesting 50 mg/kg. Gross analysis of bone tissue throughout the skeleton failed to reveal any fluorescence under ultraviolet light

in animals that were killed at periods of 24, 48, and 72 hours after eating treated bait. Microscopic analysis (at magnification ×40) of sagitally sectioned teeth from an animal that was killed at 72 hours also failed to reveal any fluorescence under ultraviolet light. Since DMCT has been found to fluoresce in bone tissue in such widely different animals as mammals and fish it was surprising that the possum did not show a similar reaction.

In veterinary use, recommended dosages of orally administered tetracyclines are 10–50 mg/kg body weight daily (Stecher 1968). The clinical symptoms associated with excessive dosages given to humans are gastro-intestinal disturbance, hypersensitivity, photosensitivity, and dizziness (Avery 1976). Weber & Ridgeway (1962) noted that dosages of 250 mg/kg body weight often killed sockeye salmon (*Oncorhyncus nerka*) whilst Stehn *et al.* (1980) found that 400 mg/kg body weight killed all pine voles (*Pitymys pinetorum*) that were fed on vegetation after dosing.

Since all baits were eaten by the 11 possums during the palatability test, it is fair to assume that large doses of DMCT were consumed by all individuals. A mean intake of 809 mg/kg body weight (mean body weight being 2.9 kg) was calculated for the 3-day period. Thus, not only did the individual dosages of 50 mg/kg body weight produce no fluorescence, but very large doses ingested over a 3-day period in the palatability trial also caused no obvious symptoms of adverse reactions.

Since the possum obviously has a very high tolerance to DMCT whilst displaying none of the fluorescence reactions found in other animals, it must metabolise the antibiotic in a rapid or as yet unexplained manner.

Lissamine Green

The mean quantities of baits in each treatment taken nightly are shown in Table 1. The total numbers taken differed between nights and significantly less of the 3% treatment was eaten during the trial (Table 2). The ability of possums to detect the 3% concentration was not apparent on the first night, but on the second and third nights significantly less ($p < 0.05$) of the 3% treatment was eaten (as shown by LSD tests of the significant Night × Concentration interaction). This suggests that the animals' behaviour is modified by some kind of metabolic feedback rather than by a purely sensory response. If this is so, it would be tempting to accept the 3% concentration as the maximum for bait acceptance experiments, thus increasing the tracer's persistence in or on the possum. However, to allow a safety margin the 1% concentration was selected as the maximum quantity that could be used without affecting bait acceptance.

TABLE 1—Mean numbers of Lissamine green-dyed and non-dyed baits eaten in 3-night palatability trial

	Dye concentration			Non-dyed
	0.1%	1%	3%	
Night 1	19.9	20.0	20.0	20.0
Night 2	19.3	16.9	9.8	20.0
Night 3	20.0	19.9	7.9	19.9

TABLE 2—Analysis of variance in palatability of Lissamine green-dyed baits

Source of Variation	d.f.	Mean square	F ratio	LSD test† (p = 0.05)
Night (N)	2	0.343	14.29***	2 3 1
Concentration (C)	3	0.529	22.03***	3% 1% 0.1% Control
N × C	6	0.181	7.53***	
Error	24	0.024		

*** $p < 0.01$

† Levels are shown in order of ascending mean value. Those underlined are not significantly different.

Three of the 12 possums that were each given a single bait carrying 1% Lissamine green were killed 48 hours after eating the bait. Two of these animals showed no trace of green dye on the paws or in the gut contents or gut tissue. In the rectum of only the third animal were faecal pellets dyed greenish-brown. Since this dye appeared to pass through the gut rapidly, the remaining nine animals of the treated sample were conserved for other experiments and the use of Lissamine green as a tracer was discarded.

Rhodamine B

The mean quantities of baits in each treatment eaten nightly are shown in Table 3. Rhodamine B had the same effect on the palatability of pellet baits as did Lissamine green. Quantities of bait eaten nightly varied significantly and overall the 3% treatment was significantly less palatable (Table 4) although, again, the difference was not apparent on the first night of the trial but significant ($p < 0.05$) on nights two and three (as shown by LSD tests of the significant Night × Concentration interaction). As for Lissamine green, 1% was chosen as the safe maximum concentration of Rhodamine B for surface coating baits.

The persistence of Rhodamine B dye was then determined using a group of 21 possums; each was given either a dry or a moistened 1% Rhodamine-dyed bait and individuals were killed at various periods after bait administration. Dye was found to persist in the gut for between 2 and 4 days but fluorescence of the dye under ultraviolet light was not seen in the gut. Dry baits marked the fore-paws for 2 days ($n = 5$), but left no trace after 3 days ($n = 2$), or 7 days ($n = 3$). Moistened baits, whether

TABLE 3—Mean numbers of Rhodamine B-dyed and non-dyed baits eaten in 3-night palatability trial

	Dye concentration			Non-dyed
	0.1%	1%	3%	
Night 1	20.0	19.6	19.9	20.0
Night 2	18.1	17.6	6.7	19.1
Night 3	18.9	18.9	13.5	19.9

TABLE 4—Analysis of variance in palatability of Rhodamine B-dyed baits

Source of Variation	d.f.	Mean square	F ratio	LSD test† ($p = 0.05$)
Night (N)	2	0.490	23.42***	2 3 1
Concentration (C)	3	0.344	16.49***	3% 1% 0.1% Control
N × C	6	0.082	3.92**	
Error	24	0.021		

*** $p < 0.01$

** $p < 0.1$

† Levels are shown in order of ascending mean value. Those underlined are not significantly different.

carrot or pellet, marked the fore-paws more effectively. All the animals killed after 2 days ($n = 3$), 3 days ($n = 2$), 4 days ($n = 2$), and 7 days ($n = 4$) still showed detectable traces of dye. Traces were found under ultraviolet light to remain longest as bright orange lines at the proximal end of the claws of the fore-paws. This marking is caused by the possum's habit of manipulating small food items with its fore-paws while sitting on its haunches to eat.

Since it was later found that the field-use of Rhodamine B dye at 1% concentration would be rather expensive, a similar test was conducted to determine if halving the rate of application of the dye would still produce effective persistence. Thirteen possums, comprising those conserved after Lissamine green testing and four additional animals, were each given a single, 0.5% Rhodamine B dyed, moist, carrot bait and were killed 7 days later. All 13 showed fluorescent traces of the dye on the paws and around the mouth under ultraviolet light and several still showed visible red stains on the paws under normal light.

Although the use of Rhodamine B as a tracer does not permit determination of the quantity of bait an individual animal has eaten, some measure of this can be obtained by inspecting gut contents. This should be done if only faint traces are found on the paws of an animal known or thought to have consumed bait recently (i.e., 1 or 2 days previously) as this may be evidence of less than a whole bait having been eaten. A small quantity of Rhodamine B left by only one or two bites of a bait will initially leave only a localised stain amongst the stomach contents and later turn the contents of the intestine red-brown. Consumption of an entire Rhodamine B dyed bait is sufficient to leave a large diffuse stain amongst stomach contents and later colour the contents of the colon and rectum a reddish-purple.

Rhodamine B was selected on the basis of these results as being a satisfactory tracer for determining acceptance of bait by possum populations if dyed baits are moistened before use.

ACKNOWLEDGMENT

I thank Mr R. T. Egan for assistance in the care of captive possums, Mr J. Bell for helpful advice on the use of dyes, and Drs J. D. Coleman, W. Q. Green, and E. B. Spurr for critically reading the manuscript.

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