AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523
BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
Batch 42

1. SUBJECT CLASSIFICATION

A. PRIMARY
Serials — Y-AH10-0000-0000

B. SECONDARY
Food production and nutrition--Pests of plants

2. TITLE AND SUBTITLE

Vertebrate damage control research, annual progress report, calendar year 1976

3. AUTHOR(S)

(101) Denver Wildlife Research Ctr.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1976	29 p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Interior

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*

(Research summary)

9. ABSTRACT

This annual research report contains papers on: rodenticide evaluations; bait formulation studies; effects of vampire bat parasitism on bovine milk production; effects of the anticoagulant diphenadione on suckling calves; nonlethal electric barrier development; bait-additive development; edible oils; effects of ultrasound devices on rats; evaluation of devices for studying baitshyness in rats; importation of African red-billed weaver finches (Quelea quelea); diphenadione residue studies in milk; bait-additive development; rice oil; developments in wildlife telemetry; field instrumentation for recording food preference of rats; and assistance for laboratory instrumentation. There are no general methods, conclusions, or recommendations.

10. CONTROL NUMBER
PN-AAC-788

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Pest control

13. PROJECT NUMBER

14. CONTRACT NUMBER
PASA RA(ID)1-67 Res.

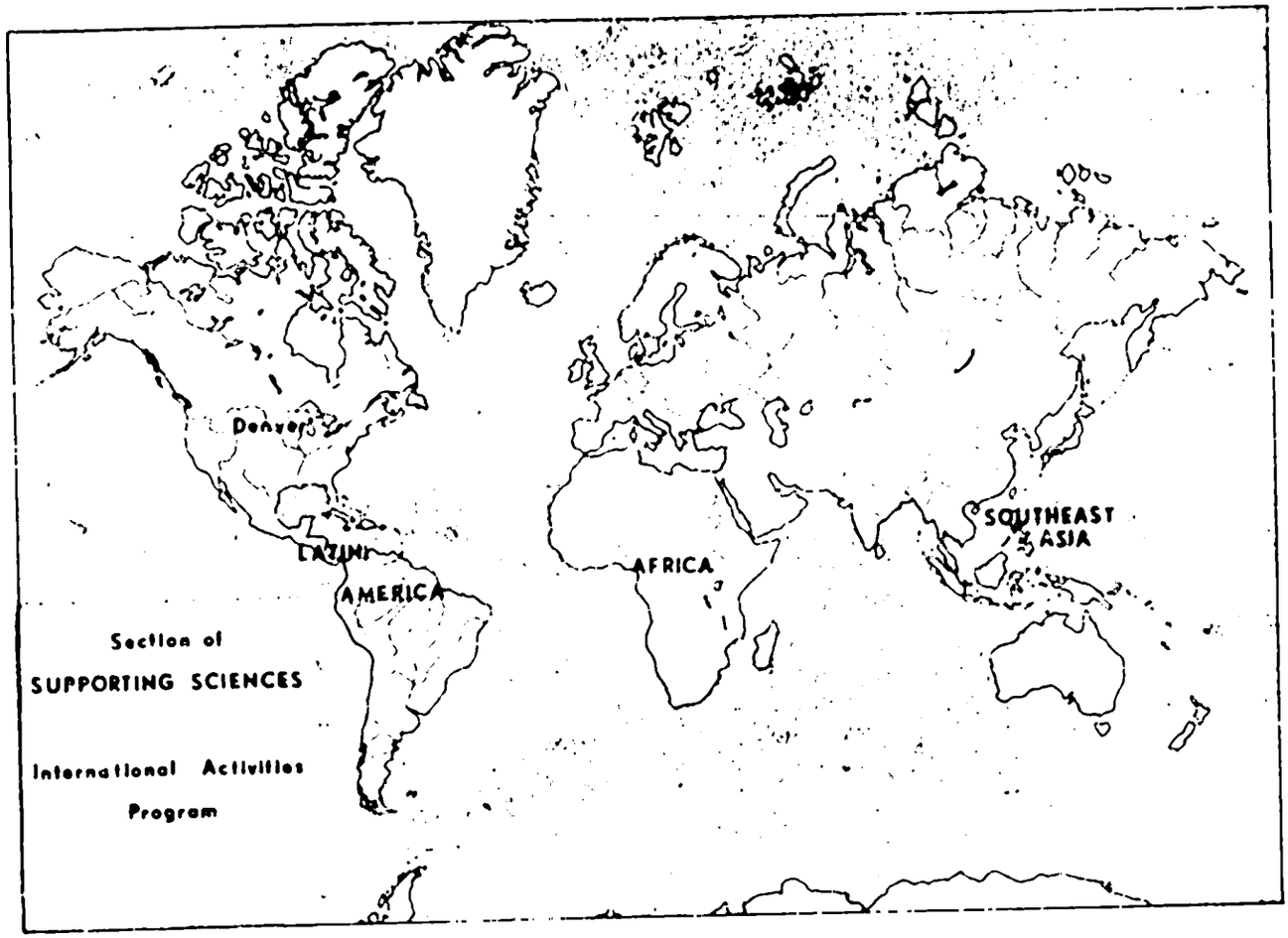
15. TYPE OF DOCUMENT

AID 590-1 (4-72)

PA-84 RA(ID)1-67 Res.
PN-AAC-758

VERTEBRATE DAMAGE CONTROL RESEARCH

WILDLIFE RESEARCH CENTER



1975 ANNUAL REPORT

A Cooperative Program
of

U.S. DEPARTMENT OF
THE INTERIOR
Fish and Wildlife Service
Division of Population Regulation Research
Denver Wildlife Research Center

U.S. AGENCY FOR
INTERNATIONAL DEVELOPMENT
Bureau of Technical Assistance
Office of Agriculture and Fisheries
Washington, D C

SECTION OF SUPPORTING SCIENCES*

1975 ANNUAL PROGRESS REPORT

PRINCIPAL INVESTIGATORS

R. Daniel Thompson, Peter J. Savarie, Stephen A. Shumake,
Roger W. Bullard, Melvyn Garrison, Ray T. Sterner, A. Lawrence Kolz
Brad Johns and Donald J. Elias

This research was supported by funds provided to the U.S. Fish and Wildlife Service by the U.S. Agency for International Development under PASA RA(ID) 1-67.

U.S. DEPARTMENT OF
THE INTERIOR
Fish and Wildlife Service
Division of Wildlife Research
Denver Wildlife Research Center

U.S. AGENCY FOR
INTERNATIONAL DEVELOPMENT
Bureau of Technical Assistance
Office of Agriculture and Fisheries
Washington, D. C.

* Results incomplete and not for publication, release, or use without the authority of the Director, Denver Wildlife Research Center.

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

TABLE OF CONTENTS

	Page
ABSTRACTS	
Pharmacology and Physiology	1
Behavioral Biology	2
Biochemical Development	3
Bioelectronics	4
REPORTS	
Pharmacology and Physiology	
I. Rodenticide Evaluations	5
II. Bait Formulation Studies	8
III. Effects of Vampire Bat Parasitism on Bovine Milk Production	8
IV. Effects of the Anticoagulant Diphenadione on Suckling Calves	11
Behavioral Biology	
I. Nonlethal Electric Barrier Development	12
II. Bait-additive Development: Edible Oils	17
III. Effects of Ultrasound Devices on Rats	19
IV. Evaluation of Device for Studying Baitshyness in Rats	20
V. Importation of African Red-billed Weaver Finches (*Quelea quelea*)	22
Biochemical Development	
I. Diphenadione Residue Studies in Milk	22
II. Bait-additive Development: Rice Oil	24
Bioelectronics	
I. Developments in Wildlife Telemetry	26
II. Field Instrumentation for Recording Food Preference of Rats	26
III. Assistance for Laboratory Instrumentation	27

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

ABSTRACTS

PHARMACOLOGY AND FORMULATION

I. Rodenticide Evaluations

A. Difenacoum feeding tests in anticoagulant resistant and non-resistant wild Norway rats (*Rattus norvegicus*). A bait containing 0.005% difenacoum killed 100% of all resistant and non-resistant wild Norway rats. Resistant rats consumed 6.5 to 14.3 mg/kg difenacoum. These same rats had previously survived warfarin baits of which they consumed 15 to 28 mg/kg.

B. Effectiveness of bromadiolone as a rodenticide in wild Norway rats. The acute single dose oral LD_{50} of bromadiolone in both male and female rats is 1.4 mg/kg. Free-choice feeding tests using an oat groat bait containing 0.005% bromadiolone killed 90% of the males and 100% of the females.

II. Bait Formulation Studies

A. Single lethal baits for Polynesian rats (*Rattus exulans*). In a series of bait acceptance tests with caged Polynesian and Norway rats, zinc phosphide treated corn and oat groat tablets were offered to determine the effectiveness of single lethal bait particles. These tablets were made with a dry binder (Nu-Tab) that has a sweet taste. The results indicate that this carrier bait with the sweet binder could be effective against rodents in sugarcane fields of Hawaii and other regions of the world.

III. Effects of Vampire Bat Parasitism on Bovine Milk Production

Parasitism by vampire bats (Desmodontidae) has been alleged to severely affect livestock health and milk production in Latin America, even where rabies is not present. To explore this question, milk production and blood indicators were measured in 58 Holstein cows from a dairy herd near Quito, Ecuador, and 28 mixed-breed cows from a dairy herd near Managua, Nicaragua, before and after vampire bat parasitism (averaging 2-4 fresh bites per cow per night) was reduced 97-100% by systemic treatment. In Ecuador the climate was mild, the cows were well managed and healthy, and milk production was high before treatment; in Nicaragua the

opposite was true. After treatment, blood packed-cell volumes increased significantly ($P < 0.05$) in both herds but milk production and hemoglobin counts increased significantly only in Nicaragua. These findings indicate that moderately severe vampire bat parasitism has little or no influence on the milk production of otherwise healthy cows in a mild climate but is detrimental to health and milk production in cows already under stress from poor diet, harsh climate, and other parasites. Thus, where bat-borne rabies is not a factor, the economic benefits of vampire bat control may be only marginal for healthy, well-managed cows but of definite value for low-producing, poorly managed cows in tropical environments.

IV. Effects of the Anticoagulant Diphenadione on Suckling Calves

Intraruminal injection of diphenadione for vampire bat control caused only negligible changes in prothrombin times in adult cattle but significantly increased clotting times in suckling calves. Although single injections of Vitamin K_3 attenuate this effect, anticoagulant treatment of calves less than 4 months old is discouraged pending further investigation.

BEHAVIORAL BIOLOGY

I. Nonlethal Electric Barrier Development

A. Laboratory Tests of Nonlethal Electric Barrier Effectiveness

An observation chamber was constructed and used sucessfully to evaluate an 18-inch double electrode barrier design. Observations were made for 34 consecutive days using this design, with polyethylene insulators substituted for the porcelain type. These less expensive insulators were equally as effective as the porcelain.

B. Field Test of Nonlethal Electric Barrier Effectiveness in the Philippines

Encouraging results were obtained with the prototype nonlethal electric barrier in a small scale field test in the Philippines. Comparisons of damage between test and control quarter hectare rice plots showed 95 percent less damage in the area protected by the barriers.

II. Bait-additive Development: Edible Oils

Seven commercially available grain and vegetable oils were evaluated as additives for enhancing bait ingestion by ricefield rats. Results were compared to consumption of bait containing a rice-oil extract. This rice-oil preparation significantly enhanced consumption as compared to soybean, corn, palm kernel, and coconut oils, but was not significantly preferred over rice containing peanut, linseed or high oleic safflower oils. Since safflower oil is reportedly tasteless and odorless, we concluded that ricefield rat preferences for oil-treated bait might be attributed to texture rather than flavor enhancement.

III. Effects of Ultrasound Devices on Rats

Three ultrasound devices generating 20 KHz, 20-30 KHz, and 40 KHz signals were evaluated for rat repellency. The devices were set up inside sound insulated chambers located within separate 10 x 10 ft enclosures. The enclosures were connected by small passageways. Four days of baseline and 10 days of ultrasound data were then collected using eight ricefield rats. All three ultrasound devices initially decreased the activity and food consumption of the rats. This study is continuing and more data are required before statistical analyses can be performed.

IV. Evaluation of Device for Studying Baitshyness in Rats

A continuous, automatic weighing system for measuring the continuous bait intake of rats was tested. The device monitors and records the minute-by-minute bait consumption of rats. Consumption weights are accurate to ± .06 g.

V. Importation of African Red-billed Weaver Finches (*Quelea quelea*)

A field station has been established in Arusha, Tanzania. To assist the station in attaining its goal of reducing small grain crop damage by quelea, the first shipment of quelea was imported to the Denver Center in July. Various laboratory studies are planned and a few initial observations have been made.

BIOCHEMICAL DEVELOPMENT

I. Diphenadione Residue Studies in Milk

The final residue data for the 1973-74 study of diphenadione residues in milk are reported. The 1 and 5 mg/kg levels were each tested

on three lactating cows. Low levels of residues (< 0.03 ppm) were found at 12, 24 and 48 hours posttreatment in the cows dosed at 5 mg/kg but none were found in the milk of cows dosed at 1 mg/kg.

II. Bait-additive Development: Rice Oil

In attempting to control the release of rice aroma volatiles, we discovered that the Freon 11 Soxhlet extract of rice is an excellent bait enhancer. Extraction methods, bait preparation, and a preference testing procedure were developed. Then the effects of concentration and experience on food acceptance were measured on *Rattus rattus mindanensis*. These studies provided basic information for further investigation of rice oil as a bait additive.

BIOELECTRONICS

I. Developments in Wildlife Telemetry

Radio telemetry instrumentation for tracking quelea is being developed for use in 1976.

II. Field Instrumentation for Recording Food Preference of Rats

Food preference tests with ricefield rats positively demonstrated the utility of coulometric techniques for data storage in a field recording device. A description of this experiment and equipment has been accepted for publication.

III. Assistance for Laboratory Instrumentation

Electronics personnel aided in various animal behavioral experiments by measuring and recording parameters associated with audio sounds, ultrasonics, and electrical shock.

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

PHARMACOLOGY AND PHYSIOLOGY

I. RODENTICIDE EVALUATIONS

A. Difenacoum Feeding Tests in Anticoagulant Resistant and Non-resistant Wild Norway Rats (*Rattus norvegicus*)

Introduction

Difenacoum, 3-(3-p-diphenyl-1,2,3,4-tetrahydro-naphth-1-yl)-4-hydroxycoumarin, is an oral anticoagulant that has an action similar to that of warfarin. Difenacoum, however, has one major advantage: it is effective against anticoagulant resistant rats. Initial test results with difenacoum in anticoagulant resistant rats were conducted in Great Britian. The results presented here are the first test results obtained with resistant rats in the United States.

Results and Discussion

Resistant rats were supplied by Dr. Alan Bowerman, New York State Rodent Control Bureau. The resistant rats had previously survived feeding tests in which they consumed 15 to 28 mg/kg of warfarin. Non-resistant rats were obtained from a colony maintained at the Denver Wildlife Research Center. Individually caged rats were offered a single cup containing 30 grams of oatmeal bait (90% oatmeal, 10% glycerol) treated with 0.005% difenacoum for 6 consecutive nights. No other food source was available and there was no prebaiting. Cups were replenished daily with fresh bait and food consumption recorded. Two groups of rats consisting of eight non-resistant and eight resistant rats were tested. Each group of eight rats was comprised of four males and four females.

There was 100% mortality in both groups, with all rats dying 5 to 8 days after exposure to the treated bait (Table 1). The non-resistant rats consumed 6.5 to 14.3 mg/kg of difenacoum, and the resistant rats consumed 7.1 to 14.0 mg/kg. The data on difenacoum in resistant rats are interesting, especially when one compares the sublethal dose of warfarin to the dose of difenacoum which was lethal to all the resistant rats.

Table 1. Mortality in non-resistant and resistant wild Norway rats given 0.005% difenacoum in a bait.

Type of rat	Sex	Difenacoum consumed, mg/kg	# deaths/ # tested	Days to death
Non-resistant	M	9.6-11.2	4/4	7-8
"	F	6.5-14.3	4/4	5-8
Resistant	M	7.1- 9.5	4/4	6-8
"	F	10.8-13.9	4/4	7-8

B. Effectiveness of Bromadiolone as a Rodenticide in Wild Norway Rats

Introduction

Bromadiolone is a brominated 4-hydroxy coumarin derivative manufactured by LIPHA Company, Lyon, France. It is also claimed to be effective against warfarin-resistant rats but has not been tested in resistant rats by the Denver Wildlife Research Center.

Results and Discussion

The acute single dose oral LD_{50} of bromadiolone in both male and female wild Norway rats is 1.4 mg/kg and animals died 4-8 days posttreatment.

Free-choice feeding tests with individually caged rats were very successful. Rats were offered two separate food cups for 6 consecutive nights. Untreated cups contained 20 g of oat groat bait. The treated cups contained 20 g of commercially prepared oat groats treated with 0.005% bromadiolone. Position of the untreated and treated cups were reversed each night to overcome any position preference by the animals. Bait was replenished daily and food consumption recorded. Survivors were given lab chow and observed for 21 days. Results are given in Table 2.

Table 2. Six (6) day free-choice feeding test results in wild Norway rats with 0.005% bromadiolone in oat groats bait.

Sex	Mortality	Time to death (days)	Bait eaten, mean (range)		Chemical consumed Mean, (range) mg/kg
			Treated	Untreated	
Male	9/10*	6 - 8	53.87 (16.7-70.9)	19.03 (4.2-54.9)	7.49 (3.6-11.1)
Female	10/10	6 - 10	51.04 (34.6-65.2)	13.13 (5.7-28.4)	9.92 (7.8-14.4)

* The sole survivor ate 15 g of treated bait only on the 4th test night. On the remaining test nights, no treated bait was consumed. Survivor consumed the equivalent of 3.6 mg/kg LM-637.

II. BAIT FORMULATION STUDIES

A. Single Lethal Baits for Polynesian Rats (*Rattus exulans*)

Introduction

To improve the effectiveness of baiting programs in sugarcane fields, a tableted baiting system in which a single bait particle could deliver several LD_{50}'s of zinc phosphide is being studied. One major problem with the use of such a system would be the high moisture content in the cane field that could cause rapid disintegration of the tablets. The purpose of these studies was to develop an acceptable tableted bait that could be moistureproofed and still retain its acceptability to the rats.

Methods

Two carrier baits, corn and oat groats, were used in a series of bait acceptance studies with Polynesian and Norway rats. A dry binder, Nu-Tab, was mixed with the baits at a 50/50 ratio. Zinc phosphide was added to the mixture at 4% and a Stokes' single punch machine equipped with 1/2-inch-diameter punches was used to form the tablets. The tablets weighed approximately 300 mg each. Coating of tablets was accomplished by dipping heated tablets into melted paraffin. Tablets were offered to individually caged animals.

Results and Discussion

Results are shown in Table 3.

The incorporation of the sweet-tasting dry binder, has enhanced acceptance of both non-treated and treated tableted baits by both species of rats.

III. EFFECTS OF VAMPIRE BAT PARASITISM ON BOVINE MILK PRODUCTION

Introduction

The initial study in this series (see 1974 report) was conducted in the highlands of Ecuador, with a dairy herd of registered Holstein cows, under good management and husbandry practices and in a climate ideally suited for dairying. We found no increase in milk production despite a dramatic reduction in vampire parasitism. A subsequent study was carried out this year in Nicaragua. The

ıble 3. Consumption of zinc phosphide treated tablets by Norway and Polynesian rats.

lumber ımals	Species	Type tablets	Tablets offered each rat		Average tablets consumed		Number of deaths
			Control	Treated	Control	Treated	
5	Polynesian[a]	Oat groats	5	-	5	-	-
3	Polynesian[a]	Oat groats	-	5	-	2-1/2	3
3	Polynesian	Oat groats	-	5	-	2-1/4	3
6	Polynesian	Corn	5	-	5	-	-
6	Polynesian	Corn	-	1	-	1	6
3	Norway	Corn	-	2	-	1-3/4	2[b]
3	Norway	Corn[c]	-	2	-	1	3
5	Polynesian	Corn[c]	-	1	-	1	5

Regular diet of laboratory chow was removed.

One non-eater.

Tablets coated with paraffin.

lower elevation and hotter climate offer a more stressful environment. The ranch was much smaller and the resources and facilities available provided for a less than optimum management and husbandry operation for the herd of nondescript mixed-breed cows.

Methods

The procedures followed were similar to those used in the Ecuador study. The systemic (rumen injection) method of vampire control was used. Fresh vampire bite counts were made immediately prior to treatment and at 30 and 60 days posttreatment. Milk production records were maintained for each of the animals in the study for 15 days prior to treatment and for 60 days thereafter. Blood samples were collected just before treatment and again on the 30th and 60th day afterward.

Results and Discussion

Vampire parasitism was again dramatically reduced. Pretreatment bites averaged 2.09/cow. Thirty days later the average was 0.07 bites/cow (96.85% reduction) and by 60 days after treatment vampire parasitism had been completely eliminated. The other parameters examined showed similar responses in both studies, with the exception of milk production. Hematocrit and hemoglobin both increased following treatment. These increases were of a slightly greater magnitude in Nicaragua than in Ecuador. Milk production remained virtually unchanged in Ecuador but showed an appreciable increase of about 16% by the 60th day after treatment in Nicaragua.

Conditions relative to the two study sites, the management and husbandry practices followed and the animals themselves ranged from one extreme to the other. Many variables, some known and some unknown contributed to or had an effect on these results. On the basis of the information presently available we believe that dairy animals which are healthy, well cared for and under mild climatic conditions will not exhibit any marked change in milk production or other response as a result of reduced vampire parasitism. On the other hand, animals in more typical tropical conditions which are already under stress from such factors as climate, inadequate or marginal diet, hemoparasite or other infections as well as blood loss to vampires will show definite responses when the degree of stress is lessened. If this hypothesis is valid it would indicate that control of vampire bats would be of significant benefit to those ranchers and farmers who need the most help -- the small operator with few animals and the least resources to invest in their care and management.

IV. EFFECTS OF THE ANTICOAGULANT DIPHENADIONE ON SUCKLING CALVES

Introduction

The systemic method of vampire bat control has been proven both highly effective and safe when used on mature cattle. However, reports of the deaths of several calves following treatment with diphenadione in Nicaragua and of one during earlier experiments at Denver Wildlife Research Center prompted concern over the potential hazard involved to young calves. This study was designed to (1) obtain further information on the effects of diphenadione on young calves, (2) determine if single IM injections of Vitamin K_3 would be of value if calves did exhibit higher susceptibility and (3) determine what effect the Vitamin K_3 would have on the toxicity to vampire bats of blood from the treated animals.

Methods

Twenty animals (10 adults and 10 suckling calves) were given intraruminal injections of 2 ml/100 kg of Motomco Suspension Vampiracida Difenadiona (equivalent to 1 mg/kg of Diphenadione). In addition, immediately after the administration of diphenadione, five of the calves received a single intramuscular injection (1 mg/kg) of Vitamin K_3 (Menadione sodium bisulfite, Haver-Lockhart Laboratories) and the remaining five calves of the control group received an intramuscular injection of physiological saline at the same dosage level. Blood samples were collected from each animal immediately prior to treatment and at 24-hour intervals up to 144 hours posttreatment. Prothrombin clotting times were determined by the Sera-Tek Prothrombin Reaction Chamber Technique. At the 24-hour collection, approximately 40 mls of blood were drawn from each calf. It was defibrinated and offered to each of 10 captive vampire bats for a single 24-hour feeding period. Five bats received blood from Vitamin K_3 treated calves and five bats received blood from the control calves.

Results and Discussion

Analysis of variance revealed significant differences for the main effect of cattle groups ($F=6.52$; $df=2/17$). Significant differences ($F=8.28$; $df=6/101$) were also found for the main effect of time (hours). The analysis of variance also indicated significant differences for the cattle group and time interaction effect ($F=3.66$; $df=12/101$). Statistical analysis of the 24-hour blood toxicity trial with vampire bats revealed no significant difference in toxicity of blood from the Vitamin K_3 calves or blood from the control group.

Prothrombin clotting times of each animal increased following treatment with diphenadione. Means (±SD) of the prothrombin clotting times for each cattle group by respective sampling periods are shown in Table 4. Diphenadione caused a threefold increase in clotting times for calves relative to adults and administration of Vitamin K_3 to diphenadione treated calves caused a general reduction in the magnitude of this effect.

Evidence accumulated to date indicates that: (1) young calves, lacking a fully functional rumen and primarily dependent on milk for sustenance are susceptible to diphenadione poisoning; (2) single intrasmuscular injections of Vitamin K_3 given at the time of treatment with diphenadione apparently foreshortened prothrombin clotting time recovery periods but do not offer complete protection against diphenadione poisoning in young calves; and (3) blood from calves treated with diphenadione and Vitamin K_3 is equally toxic to vampire bats as that from animals treated only with diphenadione. On the basis of these conclusions, we recommend that, until a satisfactory antidotal procedure is developed, the diphenadione rumen injection technique of vampire bat control not be used on cattle less than 4 months of age.

BEHAVIORAL BIOLOGY

I. NONLETHAL ELECTRIC BARRIER DEVELOPMENT

A. Laboratory Tests of Nonlethal Electric Barrier Effectiveness

Introduction

Lethal electric barriers designed to kill rats for crop protection are being used in the Philippines. Although these lethal barriers are reported to be more effective than other control methods, such as baiting and trapping, the electric current levels used with these devices pose a serious hazard to humans working in rice paddies. In addition, the use of lethal barriers involves considerable labor for removal of electrocuted rats; if a rat is killed and remains in contact with the electrodes, the barrier becomes ineffective. Routine inspection and removal of dead rats must be performed in order to keep the barrier working. For these reasons, we have attempted to develop a nonlethal electric barrier for use with Underwriters Laboratories safety-tested livestock fence chargers.

Table 4. Mean prothrombin clotting times in seconds (± SD) for cows and calves, at different intervals after treatment with diphenadione.

Animal age and treatment group	Hours after treatment							Group average
	0	24	48	72	96	120	144	
Adults								
Diphenadione	14.1(0.8)	16.6(1.3)	24.4(3.1)	30.5(7.1)	21.1(4.0)	18.1(2.6)	15.8(1.7)	20.1(5.8)
Calves								
Diphenadione + Vitamin K_3	14.8(1.0)	21.7(4.0)	55.4(29.7)	90.9(43.2)	77.8(68.9)	41.0(19.2)	20.5(6.0)	46.8(41.8)
Calves								
Diphenadione + saline	14.3(0.5)	21.8(4.4)	60.8(21.0)	102.0(37.2)	132.0(65.0)	124.2(115.0)	122.0(180.4)	82.3(49.8)
Average, all animals	14.3(0.8)	19.2(3.9)	41.3(24.1)	63.5(43.2)	62.9(64.1)	50.3(69.8)	44.7(97.4)	

Further laboratory development of our prototype design was necessary to reduce installation costs, evaluate the potential under different use situations, and increase the safety factors.

Methods

A laboratory observation chamber (8 x 2 x 4 ft) was constructed to observe the climbing behavior of 15 individually-tested ricefield rats. All animals were food-deprived and a food cup containing 10 g of hulled rice was located on the protected side of an 18-inch double electrode barrier. Porcelain insulators were used to hold the electrode wires clear of the grounded 1/2-inch chicken wire backing. Each rat was tested in two 30-minute sessions and all approaches to the barrier were recorded on videotape for subsequent detailed analyses of rat-electrode-wire contacts.

In a subsequent test, four electrical barriers designed to prevent movement of rats into a 4 x 4 ft area were set up within four 10 x 10 ft rat enclosures. All barriers were charged with one "Scorpion" Model 8509-B electric fence charger. Polyethylene insulators were substituted for the porcelain type used in the prototype design. Four ricefield rats were released into the unprotected part of each 10 x 10 ft enclosure. Rats were allowed free access to food, water, and cover outside the 4 x 4 ft test areas; water, cover, and 100 grams of rice were also placed within each barrier-protected area. Food consumption was measured daily in each enclosure, both inside and outside the 4 x 4 protected areas. Photocells, set up inside these protected areas, were used to monitor rat activity.

Results and Discussion

The 15 rats tested in the laboratory observation chamber made a total of 37 attempts to cross the barrier. During these attempts, the rats each received from 1-5 shocks. There were a total of four successful barrier crossings by two rats. One animal learned to hang on to the outside of each porcelain insulator and avoid the electrode wires, and the other rat was apparently unaffected by the shock voltage used. Most rats (n = 32) were shocked by the lower set of wires but some animals (n = 5) reached the upper wires before receiving their first shock.

Comparative food consumption data for the rat enclosure test with polyethylene insulators is shown in table 5. During the first 19 test days, a free-food condition was in effect and barrier effectiveness, based on reduced feeding activity, ranged from 89.3 to 97.7 percent. A barrier defect was noted on Day 14 and subsequently repaired. From day 20 to day 34 of the test, the rats

Table 5. Performance of nonlethal electrical barrier using polyethylene insulators.

Days	Consumption from unprotected feeders (g/day) $\bar{X}$ ± S.E.	Consumption from barrier protected feeders (g/day) $\bar{X}$ ± S.E.	% Reduction in feeding $\bar{X}$ ± S.E.
	Ad lib food provided outside barrier area		
1-5	216.0 ± 9.7	5.3 ± 3.3	97.7 ± 1.4
6-10	231.8 ± 8.2	10.8 ± 6.4	95.5 ± 2.7
11-15*	238.5 ± 23.9	18.5 ± 17.8	89.3 ± 10.4
16-19	192.8 ± 11.0	4.3 ± 4.2	97.5 ± 2.5
	One-half food ration provided outside barrier area		
20-24	190.3 ± 16.6	0.0 ± 0.0	100.0 ± 0.0
25-29	218.3 ± 18.0	0.0 ± 0.0	100.0 ± 0.0
30-34	231.8 ± 13.9	0.0 ± 0.0	100.0 ± 0.0

* One of the four barriers was re-wired during this interval due to suspected short-circuiting of electrodes.

were only offered one-half of their normal food ration outside the barrier (approximately 10 g of rice/rat/day). The same barriers proved to be 100 percent effective in reducing feeding activity during this 14-day interval.

The electric fence charger was turned off on day 34. No barrier crossings were detected by the photocells and no food was consumed for 6 additional days (day 40). These data indicate that failure of a fence charger in the field may not lead to immediate resumption of rat damage. Apparently, the rats became conditioned to avoid fence contact after a few weeks of exposure.

B. Field Test of Nonlethal Electric Barrier Effectiveness in the Philippines

Introduction

During August and September 1975, we undertook a small-scale field test of the nonlethal electric barrier design described under Part 1.A.

Methods

The study was conducted in central Luzon, Philippines, in collaboration with the Rodent Research Center. The test site was adjacent to a barrio (small farming community) and consisted of four quarter-hectare plots each containing 10-week-old rice. Two plots were fenced and two were not fenced for comparison of differences in rice damage at harvest. The fencing consisted of bamboo stakes and 14 inch high, 1/2 inch mesh chicken wire. Alternating polyethylene and porcelain insulators were attached to the bamboo stakes on the outside of the test plots, and were used to hold two ground wires and two electrode wires. The ground and electrode wires were spaced 6 inches apart. A commercial livestock fence charger served as the source of current.

Results and Discussion

After the fence was energized night observations were made at selected locations along the barrier using an infrared video camera. Most of the observed rat activity took place during the first evening. The rats made a considerable amount of noise upon contact with the electrodes. Most rats were shocked by the lower set of wires, but some managed to reach the upper set of electrodes before jumping off the barrier. Approximately 30 contacts were observed, representing multiple attempts by about

seven rats. Additional observations were made on the second night but no activity was seen and burrows located nearby appeared to be unoccupied. A few animals were seen moving from an older rice paddy, but these rats veered away from the fence and moved towards the unfenced plots.

Two weeks before harvest, a damage survey was made. In the two unfenced plots, damage was 13.65 and 13.39 percent as compared to only 0.60 and 0.78 percent respectively in the protected plots. Thus, the prototype barrier design afforded 95 percent crop protection in the quarter-hectare test plots. Larger scale field tests using 2-hectare plots are currently underway.

II. BAIT-ADDITIVE DEVELOPMENT: EDIBLE OILS

Introduction

Certain vegetable and grain oils can enchance consumption when added to a rodenticide bait. However, to date, no systematic study in which various edible oils were compared under controlled conditions for their effect on bait consumption has been reported.

In the current work, rice oil was compared to seven commercially available oils in an attempt to determine whether texture, odor, or taste properties account for ricefield rat preference for oil-treated bait.

Methods

Eight groups of six rats each were tested using rice treated with either soybean, corn, peanut, linseed, palm kernel, high-oleic-safflower, coconut, or rice oil. Each animal was offered a choice between untreated ground rice and ground rice containing 10 percent oil. The choice conditions were controlled by individual preference devices that alternated the positions of the untreated and oil-treated rice every 5 minutes for 17.5-hour test periods. Each rat underwent six daily test periods.

Results and Discussion

The test results are shown in Table 6. As can be seen, preferences for each oil increased during the first 3 test days and then stabilized. A statistical comparison among oil treatments indicated that the rice oil was preferred to soybean, corn, palm kernel, and coconut oil mixtures ($P < .05$). No differences in preference were

Table 6. Mean percent preference of independent groups (n = 6/group) of ricefield rats for rice containing oil-treated versus untreated rice.

Day	Oil							
	Soybean	Corn	Peanut	Linseed	Palm kernel	High-oleic-safflower	Coconut	Freon-11-extracted-rice oil
1	54.6±32.8	61.8±30.3	55.6±24.9	71.3±21.6	64.8±19.3	65.9±38.4	25.9±32.8	83.9± 6.8
2	60.8±29.3	67.1±34.8	79.9±12.3	88.1±14.4	78.8±12.4	80.2±23.7	66.8±35.8	97.9± 2.3
3	76.6±36.1	76.8±38.6	83.0±12.6	88.4±12.9	77.5±11.7	82.3±25.0	71.1±38.5	98.3± 3.9
4	75.0±34.2	75.3±38.6	84.9±15.0	90.3±17.2	79.7±10.3	82.3±25.0	69.9±35.7	96.2± 7.0
5	72.6±35.1	78.8±38.9	88.1±13.9	88.5±18.0	71.0±15.2	88.3±21.5	87.2± 7.3	93.8±14.9
6	79.3±36.0	78.2±39.1	89.3±14.8	86.0±19.5	72.3±20.2	89.6±22.2	64.1±37.1	94.1±13.1
Mean ±S.D.	69.8±33.9	73.0±36.7	80.1±15.6	85.4±17.3	74.0±14.9	81.4±26.0	64.2±31.2	94.0± 8.0

noted between the rice oil and the remaining oils (peanut, linseed, and high-oleic-safflower).

Since high-oleic-safflower oil is reportedly odorless and tasteless to humans, it is very possible that oily texture in combination with high caloric value are the main factors causing ricefield rat preference for these edible oils.

III. EFFECTS OF ULTRASOUND DEVICES ON RATS

Introduction

Rats often invade food storage warehouses, grain elevators, and other facilities where the use of rodenticides may be strictly regulated or even prohibited. In these situations, rat-proofing procedures must be carried out and the resident population of rats must often be reduced using costly nonchemical methods such as trapping. Although several commercially manufactured ultrasonic devices for repelling rats have been marketed, their efficacy in semi-field situations has never been adequately assessed. Likewise, the conditions under which these ultrasound devices produce their maximum effects have never really been defined. For example, it should be more difficult to repel rats that are food deprived, have resided in the area previously, or have been exposed to ultrasound for long periods. Hence, we have begun to evaluate the potential rodent-repellent effects of certain ultrasonic sound devices under the aforementioned conditions.

Methods

Three ultrasound generators were evaluated in a 36-ft-diameter test building. The devices operated at 20 KHz (118 dB), 20-30 KHz (103 db), and 40 KHz (116 dB). Each generator was placed in a 2 x 2 x 2 ft sound-insulated test chamber within a 10 x 10 ft enclosure. A fourth sound insulated test chamber contained no generator and served as a control area. The four 10 x 10 ft enclosures were connected by 4 ft passageways so that any of the eight rats on test could visit any feeding site.

Each sound chamber contained feeders with a total of 200 g of ground rat chow. Lab chow biscuits, water, and cover were also available outside the sound chambers in each 10 x 10 ft enclosure. The eight rats were allowed 4 days to adapt to the passageways, enclosures, and chambers. The devices were then turned "on" for 10 days, with food consumption and chamber entrance-exit activity measured during

successive 23-hour periods (i.e., 1 hour/day allowed for maintenance of the equipment and animals).

Results and Discussion

The current data represent the initial phase of a work unit designed to answer questions about habituation effects, novelty effects and food-deprivation effects of ultrasound. Briefly, the following is a summary of preliminary results: (1) all three devices decreased rat activity during the 10 test days, (2) food consumption was eliminated using all three devices during the first 23-hour period, and (3) food consumption was decreased with all three devices throughout the 10-day test period. Tables 7 and 8 present the daily activity and food consumption data obtained. As can be seen, activity and food consumption began to recover toward the end of the 10 days.

IV. EVALUATION OF A DEVICE FOR STUDYING BAITSHYNESS IN RATS

Introduction

One of the most important problems affecting the use of acute rodenticides is baitshyness. This refers to the decreased acceptance of a rodenticide bait once sublethal quantities have been consumed. To date, studies designed to quantify aspects of baitshyness have been greatly limited by food-intake-measurement procedures. Without exception, these studies have involved the use of daily bait consumption data, an approach which obscures changes in rat feeding patterns produced by sublethal effects of rodenticides.

During the past year, a Scientific Electronic Weighing System was evaluated as a device for recording the minute-by-minute bait-consumption patterns of rats.

Results and Discussion

Preliminary results with the device have been encouraging. Minute-by-minute food intake measurements accurate to ± .06 g are possible with the system. Although equipment problems with magnetic tape storage of data were encountered, a program for automatically entering and analyzing these data via computer was prepared. Research to assess the bait consumption patterns of ricefield rats fed select rodenticide/bait mixtures is now underway.

Table 7. Rat activity during the 4-day adaptation period and during 10 days of the ultrasound test period.

Day	Photocell Breaks (Rat Activity)			
	Control	20 KHz	20-30 KHz	40 KHz
	Adaptation Period			
1	182	110	174	122
2	152	109	380	98
3	207	151	329	83
4	189	129	294	113
	Ultrasound Period			
5	138	29	6	27
6	225	22	40	24
7	205	11	210	11
8	90	39	117	13
9	70	18	161	97
10	164	2	68	9
11	216	7	115	4
12	298	3	93	15
13	245	45	132	7
14	208	53	42	87

Table 8. Food consumption during the 10 days of the ultrasound test period.

Day	Food Consumed (g)			
	Control	20 KHz	20-30 KHz	40 KHz
5	16	0	0	0
6	23	3	6	3
7	73	0	15	0
8	24	2	3	2
9	19	1	12	4
10	34	0	9	30
11	49	2	8	1
12	64	0	11	0
13	55	8	28	0
14	52	29	8	21

V. IMPORTATION OF AFRICAN RED-BILLED WEAVER FINCHES (*Quelea quelea*)

Introduction

A field station has been established in Arusha, Tanzania by the Section of International Programs. This station will be used to conduct research aimed at reducing quelea damage to small grain crops in Africa. Essential laboratory research activities in support of the field station required the establishment of suitable facilities at the Denver Center and subsequent importation of Quelea.

Results and Discussion

The U.N. Food and Agriculture Organization (FAO) office in Dakar, Senegal, cooperated with the Denver Center by capturing and shipping the birds to the U.S. One hundred and ninety-five Quelea were imported on July 16, 1975. This importation required considerable effort to meet applicable U.S. federal regulations regarding the importation of a bird classed as an injurious species. The birds passed a 90-day quarantine in good health with only a few deaths soon after arrival.

Initial work with the birds has involved (1) photographing them to record plumage changes under laboratory conditions, (2) collection of literature and making personal contacts regarding breeding of Quelea in captivity, (3) conducting various experiments with different methods of attaching radio transmitters, and (4) providing the birds with various weaving materials for behavioral observations.

BIOCHEMICAL DEVELOPMENT

I. DIPHENADIONE RESIDUE STUDIES IN MILK

Introduction

In 1973 we began a two-part study to determine if mammillary transfer of diphenadione occurs in lactating cows. After finding trace residues at 12, 24 and 48 hours posttreatment in the milk of cows that had been given 2.75 mg/kg doses, the study was expanded to include 1 and 5 mg/kg doses. Prothrombin clotting times for 1, 2.75 and 5 mg/kg dose levels and milk residues for the 2.75 mg/kg dose were reported in the 1973 and 1974 Annual Reports. Milk and blood plasma residue data for the 1 and 5 mg/kg doses are reported here.

Methods

Three lactating cows were given 5 mg/kg and three received 1 mg/kg doses of diphenadione. Each cow had a suckling calf. Milk and blood samples were collected from each cow immediately pretreatment, at 12 hours, and then at 24-hour intervals up to 144 hours post-treatment. Sample aliquots were analyzed by a previously developed glc method.

Results and Discussion

Small quantitites of diphenadione were detected in the 12, 24, and 48 hour milk samples from cows given 5 mg/kg doses. No residues were detected in milk from cows dosed at 1 mg/kg (Table 9). The presence of diphenadione in milk from cows dosed at 2.75 mg/kg (1973 Annual Report) and 5 mg/kg but not 1 mg/kg indicates that mammillary transfer of the drug is dependent on the blood plasma level (Table 10). Apparently the blood threshold level for mammillary transfer of detectable quantities into the milk (0.005 ppm) lies between 1.49 and 2.17 ppm (1973 Annual Report). This phenomenon is similar to the dose dependency reported in the literature for other anti-coagulants. The same dose dependency exists for prothrombin clotting times (1974 Annual Report).

Residue levels in the milk from cows dosed at 5 mg/kg were only slightly higher than those from cows given 2.75 mg/kg. As discussed in the 1973 Annual Report, this means a person would have to drink in excess of 30 gallons of 12, 24 or 48 hour posttreatment milk from overdosed animals to obtain the 2.5-mg minimum daily dosage used in human anticoagulant therapy. If cows are given the recommended 1 mg/kg doses there are no detectable residues.

Table 9. Diphenadione residues in milk of test cows (ppm).

Hours posttreatment	ppm found (mean ± SD)	
	1 mg/kg	5 mg/kg
0	<0.005	<0.005
12	"	0.022
24	"	0.029
48	"	0.024
72-144	"	<0.005

Table 10. Diphenadione residues in the blood plasma of test cows (ppm)

Hours posttreatment	ppm found (mean ± SD)	
	1 mg/kg	5 mg/kg
0	<0.01	<0.01
12	0.91	4.06
24	1.49	6.66
48	1.20	2.42
72	0.42	0.86
96	<0.01	0.36
120	<0.01	0.06
144	<0.01	0.05

II. BAIT-ADDITIVE DEVELOPMENT: RICE OIL

Introduction

In attempting to control the release of rice aroma volatiles we discovered that the Freon 11 Soxhlet extract of rice is an excellent bait enhancer. After completing much of the research on rice aroma volatiles as a bait enhancer for *Rattus rattus mindanensis*, we wished to find a means of controlling their release from an exposed bait. Since oils are known to retard release of volatiles, and the familiar food is often preferred, our first choice of test material was rice oil.

Methods

Freon 11 (trichlorofluoromethane) was the solvent used to extract the oils from ground rice. After the 3-hour Soxhlet extraction period most of this volatile solvent was removed by rotary evaporation. The remaining extract was then added to ground rice and mixed in a modified round bottom flask on the rotary evaporator. Three 2-cm deep vertical "ribbed" depressions symmetrically located on the sides of the flask cause tumbling of the material during rotation assuring complete mixing of the components and removal of the remaining traces of the Freon 11.

The principal objectives of the preference tests were to find the optimal concentration and the profile of short-term preference responses. In the tests, 12 animals were each given a choice between untreated ground rice and ground rice with the oil additive. Individual preference devices that alternate the positions

of the foods every 5 minutes for 17.5-hour test periods controlled the choice conditions.

The first test determined which of four concentrations (2%, 4%, 6%, and 10%) is least accepted by ricefield rats. The second test measured the daily preference response of a new group of rats for a 9-day period. These data provided basic information needed for planning future tests.

Results and Discussion

Results of the concentration preference test are shown in Table 11. As can be seen, acceptance increased with concentration. These tests were conducted with rats that had previously been exposed to rice oil preparations. Consequently, their intra-day preference response had stabilized.

Table 11. Food preference response of ricefield rats for increasing rice oil content (mean ± S.D.)

Concentration	Day 1	Day 2
2%	56.12 ± 4.11	54.73 ± 11.06
4%	63.03 ± 17.50	61.00 ± 19.76
6%	68.67 ± 22.87	67.50 ± 13.51
10%	79.92 ± 9.82	80.33 ± 13.32

Prior observations indicated that the preference for rice oil increased during the first few days of testing and then stabilized. This prompted a 9-day test of 10% rice oil to see if the daily response for a new group of rats changed over a period of time. The results (Table 12) show that the response does increase for about 3 days before stabilization occurs. This response is in keeping with the prebaiting policy followed in many rodent control programs.

These tests provided necessary information basic to further investigation of rice oil as a bait additive. Other tests (see Section of Behavioral Biology, p. 16) have shown that rice oil shows considerable potential as a bait additive. The exciting feature of this finding is that solvent extraction of rice is an economically feasible means of producing a bait additive.

Table 12. Daily preference response for a new 10% rice oil bait (Mean ± S.D.)

Day	Acceptance
1	57.18 ± 5.25
2	62.67 ± 10.43
3	76.87 ± 16.43
4	71.66 ± 12.30
5	75.36 ± 17.83
6	71.00 ± 22.90
7	76.58 ± 22.64
8	70.33 ± 20.62
9	79.67 ± 20.67

BIOELECTRONICS

I. DEVELOPMENTS IN WILDLIFE TELEMETRY

Introduction

Work was initiated to develop radio telemetry techniques and equipment to track the movements of Quelea in Africa.

Results and Discussion

AID personnel in Africa anticipate requiring radio tracking equipment during 1976 for the Quelea weaver finch. Quelea are smaller (14 to 20 g) than any birds we have instrumented and new or modified techniques must be devised. At this time, we are measuring the weight lifting capability of the birds and testing different transmitter attachment methods.

II. FIELD INSTRUMENTATION FOR RECORDING FOOD PREFERENCE OF RATS

Introduction

Prototype field instrumentation was developed in 1974 to test the food preferences of ricefield rats (*Rattus rattus mindanensis*). This design has a number of desirable features which readily adapt for counting and timing a variety of biological phenomena. The uniqueness of the equipment is the use of inexpensive coulometric

components for data storage which can be removed from the field portion of the instrument and returned to the laboratory for readout. In this manner, the complexity of the recording instrument is minimized, and continuous monitoring is possible by rotating coulometric cells.

Results and Discussion

The accuracy and field reliability of the food preference recorders were evaluated in a simulated test environment using ricefield rats. These results have been summarized and accepted for publication in the Journal of Wildlife Management in 1976.

In summary, the preference recorders operated with no failures during a 12-day test period, and their timing and count accuracy compared within ±6 percent with readings obtained using conventional electromechanical counters and timers. A statistical comparison relating the actual weights of consumed foods and the time and count data from the preference recorders calculated a high correlation coefficient ($r = 0.87$). It was further shown that a multiple linear regression for predicting the rat's food consumption using both the recorded number of rat visits and feeding time resulted in a coefficient of multiple correlation of $R = 0.92$. With these favorable results, the food preference monitors have been sent to the Philippine's field station.

III. ASSISTANCE FOR LABORATORY INSTRUMENTATION

Introduction

Electronics personnel are frequently requested to perform electrical measurements for biological experiments. This year we contributed time for sound studies and an electric barrier test.

Results and Discussion

The ultrasonic spectrum and sound pressure levels were measured for three types of rat repellent devices being evaluated for efficacy.

A test involving the design of an electric barrier for ricefield rats has involved electronics personnel for wiring assistance, suggestions for personnel safety, equipment modifications, and voltage measurements.