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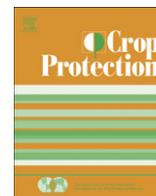
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Witmer, Gary W.; Snow, Nathan P.; and Burke, Patrick W., "Evaluating commercially available rodenticide baits for invasive Gambian giant pouched rats (*Cricetomys gambianus*)" (2010). *USDA National Wildlife Research Center - Staff Publications*. 1072.
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Evaluating commercially available rodenticide baits for invasive Gambian giant pouched rats (*Cricetomys gambianus*)

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ARTICLE INFO

Article history:

Received 2 December 2009

Received in revised form

15 May 2010

Accepted 17 May 2010

Keywords:

Gambian giant pouched rat

Cricetomys gambianus

Rodenticide

Efficacy

Invasive species

ABSTRACT

Gambian giant pouched rats (*Cricetomys gambianus*) are native to Africa, but they are popular in the pet industry in the United States. They were reservoir hosts during a monkeypox outbreak in the Midwestern United States in 2003. A free-ranging population became established on Grassy Key in the Florida Keys, apparently because of a release by a pet breeder. These rodents could cause significant damage to agricultural crops should they reach the mainland. Research under controlled conditions was needed to identify effective rodenticides for Grassy Key or other cases where an invasion of Gambian rats might occur. We tested 2 formulations of diphacinone baits and 1 formulation each of brodifacoum, zinc phosphide, bromethalin, and chlorophacinone baits with captive Gambian rats in multiple-choice food trials. Both the brodifacoum and zinc phosphide rodenticide baits were highly effective (100% mortality). Also, brodifacoum and zinc phosphide treatments performed similar to the Environmental Protection Agency's standard for toxicants of (i.e., 90% mortality in laboratory trials). The chlorophacinone, diphacinone, and bromethalin baits did not appear to be very effective at killing Gambian rats ($\leq 50\%$ mortality) in our study. Effective tools to combat Gambian giant pouched rats have been identified in a laboratory trial. Further field testing of commercially available brodifacoum and zinc phosphide baits may prove useful for controlling the potentially invading Gambian rats.

Published by Elsevier Ltd.

1. Introduction

Introduced rodents pose a serious threat to the native flora and fauna of islands (Howald et al., 2007). Rodents can be very prolific on islands where they have few, if any, predators, and their omnivorous foraging has led to the endangerment or extinction of numerous island species (Witmer et al., 1998). Most seabirds that nest on islands have not evolved in the presence of sympatric predators and are, therefore, very vulnerable to introduced rodents and other species introductions. There has been a concerted worldwide effort to eradicate introduced rodents from islands with numerous successes (Howald et al., 2007; Moors and Atkinson, 1984). These efforts have relied heavily on the use of various rodenticides (Howald et al., 2007; Witmer et al., 2007).

Gambian giant pouched rats (*Cricetomys gambianus* Waterhouse) have become established on Grassy Key in the Florida Keys

(Perry et al., 2006). These rodents are native to a large area of central and southern Africa (Kingdon, 1974) and because of their large size (2.8 kg; 1 m length), they are used as a high-protein food source (Ajayi, 1975). Gambian rats are omnivorous; and in their native range they consume vegetables, insects, crabs, snails, palm fruits, and palm kernels (Ajayi, 1975). Although no food-habit studies have been conducted for the free-ranging Gambian rats on Grassy Key, the region contains many dietary options for Gambian rats, both native and non-native. For example, some plants available are Australian pine (*Casuarina equisetifolia* L.), Brazilian pepper trees (*Schinus terebinthifolius* Raddi), sea grape shrubs (*Coccoloba uvifera* L.), and papaya (*Carica papaya* L.; Long and Lakela, 1971; FNAI, 1990). Other examples of likely food sources are tree snails (*Drymaeus multilineatus* Albers; Townsend et al., 2005), land crabs (*Cardisoma guanhumii* Latreille; Gifford, 1962), and eggs of various nesting birds (Jewell, 2002).

Gambian rats are known to cause substantial losses to food crops in Africa (Fiedler, 1988). There is a concern that this species could cause substantial agriculture damage if it were to reach the mainland USA and become established (Peterson et al., 2006). Additionally, there is also a concern about this species posing a disease threat as they have been known to carry monkeypox and

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various other diseases transmissible to humans and livestock (Perry et al., 2006; Fiedler, 1988). There was an outbreak of monkeypox in the Midwestern USA in 1993 that was linked to infected Gambian rats that had been brought into the country for the exotic pet industry (Centers for Disease Control and Prevention, 2003). This was the first monkeypox outbreak in the western hemisphere (Enserink, 2003). Fortunately, a sample from the free-ranging Gambian rat population on Grassy Key was found to be seronegative for monkeypox by the Centers for Disease Control and Prevention (Perry et al., 2006). Preliminary work with the Grassy Key invasive rodent population (monitoring, preliminary rodenticide testing) was presented (Engeman et al., 2006) and an eradication strategy was designed and implemented in 2007. Initially, a 2% zinc phosphide rodenticide bait (with the active ingredient mixed with peanut butter, grains, and molasses) was used because a preliminary trial on Grassy Key with a few Gambian rats suggested that it would be effective (Engeman et al., 2007). The rodenticide bait was placed in a grid of bait stations across the entire island. The eradication effort is continuing, but there were difficulties in achieving success (Engeman et al., 2007). Hence, additional effective rodenticide baits are needed to control and eradicate introduced Gambian giant pouched rats. Although many commercial rodenticide baits are registered by the United States Environmental Protection Agency (EPA) for commensal rodent control (Witmer and Eisemann, 2007), none were registered for Gambian rats and we found no evidence of any testing of rodenticides for efficacy with Gambian rats. We chose to test commercial rodenticides already available in the USA and already registered by the EPA for commensal rodents because we knew that managers could more readily obtain a new registration for use of these materials on the invasive Gambian rats as long as they were proven to be efficacious on that species. Even in Africa, few rodenticides are available or used and there has been little efficacy testing (Fiedler, 1994).

We used a standardized efficacy protocol (e.g., Schneider, 1982) under indoor, controlled conditions. Because free-ranging rodents usually have numerous food items available to them, it is important that rodenticide baits be attractive and palatable, as well as efficacious when presented with an alternative food type. We tested the efficacy of six commercially available rodenticide baits on captive, wild-caught Gambian rats from the Florida Keys. We hypothesized that one or more of the test rodenticides would be consumed and highly efficacious ($\geq 80\%$ mortality) when presented with alternative food types (i.e., multiple-choice efficacy trial).

2. Methods

In our trials, we only used free-ranging Gambian rats live-trapped on Grassy Key, Florida, or the first-generation offspring of those animals to make inference to the population on Grassy Key. We housed the captured rats and any subsequent offspring in metal rack cages at the Invasive Species Research Building of the United States Department of Agriculture's (USDA) National Wildlife Research Center (NWRC) in Fort Collins, Colorado. The Gambian rats were held in individual cages, measuring $60 \times 50 \times 45$ cm (Allentown Caging Equipment Co., Allentown, NJ). The Gambian rats were allowed several weeks to acclimate to the room, cages, and foods before the trial began. Animals were fed a maintenance diet consisting of a rodent pelleted chow (Lab Diet 5008, PMI Nutrition International LLC, Brentwood, MO) supplemented with nuts and fruit. Gambian rats are known to feed on fruit and nuts in their native range (Ajayi, 1975). While we did not monitor the amount of maintenance diet consumed by test animals, the diet was well accepted and all rats maintained or even gained weight during the study.

The rodenticides we tested had varying amounts of active ingredients, but all are currently registered for use with commensal rodents. We randomly assigned 6 Gambian rats to each treatment group. The treatments included baits containing one of the following: 2.0% zinc phosphide on oats (Zinc Phosphide on Oats, USDA Animal Plant and Health Inspection Service, Riverdale, MD), 0.0025% brodifacoum pellets (CI-25 pellets, Bell Laboratories, Inc., Madison, WI), 0.005% chlorophacinone pellets (Rozol Pellets, Liphatech, Inc., Milwaukee, WI), 0.01% bromethalin blocks (Fastrac Blox, Bell Laboratories, Inc., Madison, WI), 0.005% diphacinone pellets (Ramik Green, HACCO, Madison, WI) or 0.005% diphacinone blocks (Ramik Mini Bars, HACCO, Madison, WI). The control group was fed only the maintenance diet. The treatment groups of Gambian rats also received the maintenance diet throughout the trial. The maintenance diet was replenished daily. All rats were at least 6 months of age (i.e., sexually mature) at the beginning of the study. Each group of rats contained both sexes, but the ratio varied because of the actual number of females and males available for the study. We compared the average weights of Gambian rats among each group with an analysis of variance (ANOVA; Proc GLM, SAS Institute, Cary, NC).

On day 1 of the 7-day, multiple-choice feeding trial we added the respective rodenticide baits along with maintenance diet. We placed 100 g of the appropriate rodenticide bait into the cage inside a ceramic bowl. For the rodenticide blocks (diphacinone and bromethalin), we initially added two of the blocks (44–56 g), because additional blocks could readily be inserted into the cages later, as needed. Rodenticide baits and maintenance foods were replenished as needed (based on a visual observation each day of the amounts remaining) so that rats always had all types of provisions available. Rodenticide bait consumption was monitored by weighing the initial bait when the trial began and any bait that was replenished, then we subtracted the weights of any bait that accumulated below the cages and any bait that remaining in the cage after the seventh day (end of rodenticide exposure period). All rodenticide baits were removed at the end of the seventh day and surviving rats were put into clean cages and were fed the maintenance diet. We compared the average proportions of rodenticide baits consumed among the treatment types with an analysis of variance (ANOVA; Proc GLM, SAS Institute, Cary, NC).

All rats were examined daily and the condition of the rats and any mortality was recorded. We conducted necropsies on all rats, during which time we recorded weight and any signs of anticoagulant poisoning for the anticoagulant rodenticides (Stone et al., 1999). Rats that remained alive after the trial were observed for another 10 days, then were euthanized and necropsied as described above. Any mortality that occurred in that 10 day period was recorded. We compared the average weights of rats within each treatment group with an ANOVA (Proc GLM, SAS Institute, Cary, NC). After necropsy, all carcasses from the study were incinerated at NWRC.

The EPA standard for desired efficacy of rodenticide baits in a laboratory trial is 90% mortality (Schneider, 1982). We compared the efficacy of each treatment type to the EPA standard using Fisher's Exact, chi-squared tests (Proc Freq, SAS Institute, Cary, NC).

3. Results

The mean weights of Gambian rats among the various treatment types did not vary ($F_{32} = 0.90$, $P = 0.349$). The percent of total bait consumed among treatment types did significantly differ ($F_{33} = 6.22$, $P = 0.018$; Table 1). The treatment types tested had varying degrees of efficacy for poisoning Gambian rats (Table 1). The zinc phosphide and brodifacoum baits were highly efficacious (100% mortality) and only a small amount of the bait needed to be

Table 1
Summary of rodenticide efficacy trial with Gambian giant pouched rats, 2007–2009.

| Treatment (% active ingred.) | n | Deaths | Percent mortality | Mean body wt. (kg) | SE | Mean bait consumed (g) | SE | Proportion bait consumed | SE | Mean days to death | SE |
|----------------------------------|---|--------|-------------------|--------------------|------|------------------------|-------|--------------------------|------|--------------------|------|
| Zinc phosphide on oats (2.0%) | 6 | 6 | 100 | 1.28 | 0.08 | 3.8 | 2.82 | 0.03 | 0.02 | 1.3 | 0.49 |
| Brodifacoum pellets (0.0025%) | 6 | 6 | 100 | 1.37 | 0.05 | 89.0 | 12.71 | 0.39 | 0.05 | 12.2 | 1.27 |
| Chlorophacinone pellets (0.005%) | 6 | 3 | 50 | 1.47 | 0.08 | 89.7 | 29.46 | 0.30 | 0.10 | 11.7 | 1.96 |
| Bromethalin blocks (0.01%) | 5 | 1 | 20 | 1.55 | 0.10 | 25.58 | 2.56 | 0.21 | 0.03 | 14.0 | – |
| Diphacinone blocks (0.005%) | 6 | 1 | 17 | 1.50 | 0.09 | 92.6 | 22.72 | 0.30 | 0.05 | 12.0 | – |
| Diphacinone pellets (0.005%) | 6 | 0 | 0 | 1.47 | 0.08 | 106.2 | 16.19 | 0.35 | 0.05 | NA ^a | – |
| Control | 6 | 0 | 0 | 1.44 | 0.03 | NA | – | NA | – | NA | – |

^a NA = not applicable.

consumed (0.03%, 3.78 g, of the zinc phosphide bait and 0.39%, 89.02 g, of brodifacoum bait on average). The average time to death was approximately 1 day for animals tested with zinc phosphide and 12 days for brodifacoum baits.

The chlorophacinone rodenticide bait resulted in 3 dead rats (50% mortality) and the bromethalin rodenticide bait resulted in only 1 dead rat (20% mortality; Table 1). Neither of the diphacinone baits were efficacious (<17% mortality) despite the animals having eaten the greatest amounts of these baits (Table 1).

Compared to the EPA standard of 90% mortality, only zinc phosphide, brodifacoum, and chlorophacinone were not significantly different (i.e., $X^2 \geq 0.05$; Table 2). All other rodenticides tested were significantly less effective than the EPA standard mandates. We expect that the chlorophacinone bait (50% mortality) also was less effective than the EPA standard, but the small sample sizes made this difficult to detect.

4. Discussion

In the preliminary outdoor rodenticide trial in Florida before the eradication effort began, relatively few Gambian rats were used, weather conditions made the monitoring of bait consumption difficult, and the 2-choice trials were conducted with horse sweet mix (grains and molasses) as the alternative food (Engeman et al., 2006). In those trials, it appeared that the alternative food was not very palatable to the Gambian rats, therefore the results of those trials were suspect. However, those trials suggested that zinc phosphide was the rodenticide of choice and subsequently was used in the eradication effort. However, the eradication effort has been slow in achieving of success, and other rodenticides are being considered for follow-up baiting.

In our multiple-choice food trials with Gambian rats, we found that both zinc phosphide (an acute rodenticide) and brodifacoum (a second-generation anticoagulant rodenticide) formulations tested were efficacious rodenticides. Gambian rats seemed particularly sensitive to the rodenticide zinc phosphide because they consumed small amounts of bait, yet 5 of 6 animals were dead in less than 1

day. Our findings reconfirm that zinc phosphide was an acceptable rodenticide for the initial eradication effort (Engeman et al., 2007), but because some Gambian rats are still remaining on Grassy Key, we suggest attempting to remove any remaining animals with an alternative rodenticide. Our findings suggest that brodifacoum should be an effective alternative. Brodifacoum has been used successfully to eradicate various species of rats (*Rattus* spp. Fischer) from islands throughout the world (e.g., Taylor and Thomas, 1989; Empson and Miskelly, 1999; Dolan et al., 2003; Orueta et al., 2005; Howald et al., 2007).

One hypothesis for why the eradication on Grassy Key with zinc phosphide was not initially successful is bait shyness which is defined as “a cautious attitude toward food (and poison bait) experienced previously with harmful effects” (Rzoska, 1953). Zinc phosphide can generate rodent avoidance due to sublethal toxicosis and learned aversion, thereby decreasing the acceptance and efficacy (Sterner, 1994, 1999). Sterner (1994) suggests that bait shyness toward zinc phosphide is more likely in larger rodents (e.g., prairie dogs [*Cynomys* spp. Rafinesque]) because they must ingest larger amounts of bait to receive a lethal dose, probably over a long time span. Gambian rats are even larger than prairie dogs, therefore bait shyness may be more problematic with them.

Another hypothesis why zinc phosphide was not initially successful on Grassy Key could be the required use of the bait stations. Bait stations are commonly designed and used so that they facilitate bait access by the target species, while excluding non-target animals (e.g., Erickson et al., 1990; Phillips et al., 2007). However, designing a bait station large enough for Gambian rats, while still excluding any non-target individuals (e.g., children, pets, raccoons [*Procyon lotor* Storr], opossums [*Didelphis virginiana* Linnaeus], etc.) was challenging, and potentially some Gambian rats did not access the bait. Gambian rats are known to be intelligent rodents with a keen sense of smell (e.g., they can be trained to detect land mines [Wines, 2004] and to detect tuberculosis-infected sputum samples from humans [Nickerson, 2008]), therefore, they may learn to avoid bait stations or bait smells, especially if they experience adverse effects. Additionally, not all landowners on Grassy Key were amenable to the eradication efforts and did not grant access to their lands. Therefore, Gambian rats could be provided refuge in those locations, away from the bait stations. To ensure a successful eradication, removing any Gambian rats from those refuge areas will be necessary.

Of the other rodenticides tested (chlorophacinone and diphacinone, first-generation anticoagulant rodenticides; and bromethalin, a chronic rodenticide), all were not acceptably efficacious. The one Gambian rat that died from the rodenticide bromethalin did not die until 14 days after exposure to the toxin. This is not an unusual result because this rodenticide is a chronic toxicant with slow-acting effects when consumed as a food bait (Timm, 1994). Anticoagulants are also known to be slow-acting toxicants. The 3 rats that died from the first-generation anticoagulant chlorophacinone died, on average, in 11.7 days. This is similar to the days

Table 2
Rodenticide efficacy trials with Gambian giant pouched rats, 2007–2009, compared to Environmental Protection Agency standards (i.e., $\geq 90\%$ mortality) for desired efficacy in laboratory trials.

| Treatment (% active ingredient) | X^2 | p |
|----------------------------------|-------|-------|
| Zinc phosphide on oats (2.0%) | 0.64 | 1.000 |
| Brodifacoum pellets (0.0025%) | 0.64 | 1.000 |
| Chlorophacinone pellets (0.005%) | 3.20 | 0.118 |
| Bromethalin blocks (0.01%) | 7.35 | 0.017 |
| Diphacinone pellets (0.005%) | 8.60 | 0.008 |
| Diphacinone blocks (0.005%) | 12.34 | 0.001 |
| Control | 12.34 | 0.001 |

to death with the second-generation anticoagulant brodifacoum: 12.2 days. While second-generation anticoagulants such as brodifacoum are more toxic than the first-generation anticoagulants, their mode of action and time to lethal effect are similar. However, we showed that the first-generation anticoagulants were not effective, while the second-generation anticoagulant was effective.

Gambian giant pouched rats pose serious threats as an invasive rodent in the USA because of the established, free-ranging population on Grassy Key and because of their popularity in the exotic pet industry. Based on the results of this study, it appears that zinc phosphide and brodifacoum rodenticide baits are effective rodenticides to use in Gambian rat eradications. Gambian rats do not seem to be particularly sensitive to chlorofacinone, diphacinone, and bromethalin rodenticide baits and, hence, these rodenticides cannot be recommended for control or eradication efforts.

Acknowledgements

This study was conducted under the approved NWRC Project: Development and assessment of methods and strategies to monitor and manage invasive mammalian vertebrate species with emphasis on rodents. The study protocol, QA-1428, was approved by the NWRC Institutional Animal Care and Use Committee on April 6, 2007. Reference to trade names does not imply U.S. government endorsement of commercial products or exclusion of similar products with equal or better effectiveness. We thank USDA Wildlife Services-Florida operational staff for funding and logistical support during the Gambian rat work in Florida and the rodenticide efficacy study conducted in Fort Collins. We also thank the State of Florida and the US Fish and Wildlife Service for support in the Gambian rat eradication effort in Florida.

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