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Evaluation of Bait Matrices and Chemical Lure Attractants for Brown Tree Snakes

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ABSTRACT: Brown tree snake introduction to the snake-free island of Guam has severely impacted its native terrestrial vertebrates and economy. These nocturnal predators have extirpated forest birds, caused drastic reductions of lizards, and there is a major concern that snakes could be dispersed to other areas through Guam's cargo transportation systems. Techniques to deter the spread of snakes include cargo inspection with detector dogs and live trapping using live mice as lures to reduce snake densities in cargo handling areas. Maintaining mice in traps is expensive, and an inanimate lure would be highly desirable. Oral baiting with dead neonatal mice (DNM) treated with acetaminophen is another technique being implemented for reducing snake populations. An objection to DNM is that they decompose and become putrid after 3-4 days. Consumption of 21 candidate bait matrices was compared to DNM in free-choice tests in individually caged snakes under laboratory conditions. Consumption of dead quail chicks and geckos was equivalent to DNM. Processed canned meat products, stew beef, and chicken were 30-50% as effective as DNM. Baits not consumed included dog blood, beef liver, and quail eggs. Under field conditions with live trapped snakes, consumption of 7 bait matrices was compared to DNM in 1-choice tests. DNM were preferred (55% consumption) over all other baits. Consumption of plastic lizards, chicken fat, and mealworms was 33%, 14%, and 13%, respectively. Odoriferous chemicals (n = 23), characteristic of decomposing animal flesh, were evaluated for their ability to attract snakes in the field. Chemicals were individually evaluated by placing them on tofu baits in PVC tubes (bait stations). Snake activity around the bait stations was monitored with infrared videography. Some of the 23 chemicals (i.e., L-methionine, 3-methyl-1-butanethiol) appeared to attract snakes to the tubes. However, no tofu baits treated with chemicals were consumed, whereas 100% of the snakes that visited tubes baited with DNM consumed the dead mice. These results, along with analysis of videos, indicate that chemicals may act as long distance lures but are insufficient for stimulating and initiating consumptive or appetitive search behaviors at close range.

KEY WORDS: bait stations, baits, *Boiga irregularis*, brown tree snake, chemical lures, snake control, videography

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INTRODUCTION

The U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (WS) initiated a brown treesnake (*Boiga irregularis*) operational control program on the island of Guam in 1993 to reduce dispersal of snakes through outbound sea and air cargo (Hall 1996, Vice and Vice 2004). These invasive snakes were probably transported to Guam after World War II in cargo shipments from the Admiralty Islands north of Papua New Guinea. By the 1980s, they had colonized the entire island, with 50 - 100 snakes per ha in some areas (Savidge 1987, Fritts 1988, Rodda *et al.* 1992). Brown tree snakes have caused severe ecological and economic damage on Guam, including extirpation of forest birds, electrical power outages, life-threatening bites to infants, and predation on domestic animals (Savidge 1987, Fritts and McCoid 1991, Fritts *et al.* 1990, 1994; Fritts and Chiszar 1999). Snakes have dispersed from Guam to other Pacific islands and the continental United States, and there is a major concern that snake populations established in sensitive locations could cause problems similar to those observed on Guam (Fritts *et al.*1999, McCoid *et al.* 1994).

The WS program uses three primary methods for reducing snake populations in cargo areas: 1) live trapping using a live mouse lure, 2) hand capture from fence lines, and 3) canine inspection of cargo (Engeman and Vice 2001, Vice and Vice 2004). Live mice are highly effective trap lures, providing both visual and odor stimuli that promote appetitive foraging behavior (Shivik and Clark 1997, Shivik 1998). As many as 3,775 traps are in continuous use by the WS program, and maintaining this number of mice with food and water is labor intensive and poses logistical restraints (D. S. Vice, pers. commun.). The development of an inanimate lure replacement for live mice would improve trapping efficiency. Investigations have been conducted on several inanimate trap lures, but research results have not been encouraging (Clark 1997, Shivik and Clark 1999*a*).

Oral toxicant baiting with acetaminophen-treated dead neonatal mice (DNM) is also used to reduce snake populations (Savarie *et al.* 2001, EPA Reg. No. 56228-34). DNM are effective baits for brown tree snakes but have limitations. It is expensive to ship frozen DNM and maintain them frozen until use. In the field, they putrefy after 3 - 4 days and become less acceptable as baits.

We conducted a series of laboratory and field evaluations to assess feasibility of developing an alternative bait to replace DNM. Our two primary objectives were to: 1) determine snake acceptance of candidate bait matrices using DNM as the positive reference under both laboratory and field conditions, and 2) determine if odoriferous chemicals applied to tofu bait promote foraging and appetitive behavior.

METHODS
Study Sites

Laboratory and field studies were conducted on Andersen Air Force Base, Guam. Field studies with bait matrices were conducted in second-growth forested areas on the southwest end of the flight line and Tarague Beach. Field studies with chemical lures applied to tofu baits were conducted in forested plots (≈500 - 1,500 m × 130 m) bounded by access roads in the Conventional Munitions Storage Area.

Laboratory Studies with Bait Matrices

Brown tree snakes were live-captured by WS personnel in modified crawfish traps with 1-way doors on both ends, using a live mouse attractant housed inside a protective interior cage (Linnell *et al.* 1998). Snakes were transported to an outdoor roof-covered area with open sides and caged individually in plastic cages approximately 38 × 28 × 23 cm high for at least 3 days before testing. Each cage contained a double-sided plastic bowl and water was provided *ad libitum.* A 2.9-cm hole through the outside wall of the bowl allowed it to be used as a hide shelter. Cages were cleaned weekly or as needed. Two hundred forty-eight snakes were tested (88 males, 124 females; 28 snakes were disposed of before sexing or snout-vent length measurement, and 8 small snakes could not be reliably sexed; mean body mass = 82.0 g, SD = 42.4, *n* = 248; mean snout-vent length = 890.7 mm, SD = 138.0, *n* = 219). Twenty-two baits were evaluated (Table 1). Two prey item baits were tested; euthanized geckos (*Lepididactylus lugubris*) were captured on Guam and euthanized quail chicks (surrogates for wild bird hatchlings) were obtained from a commercial source. Liquid baits, such as commercial edible pork blood, dog blood, and beaten chicken egg yolks and whites, were soaked into polyurethane foam. Foam baits were cut into sizes approximating DNM. Other commercial test baits included fresh thawed shrimp, stew beef, beef liver, canned processed meats, chicken meat and fat, plastic eggs (mimics for native bird eggs), thawed mealworms, hard-boiled quail eggs (surrogates for native bird eggs), apples, and grapes. Each night, 1 - 3 test baits with a DNM (positive control and surrogate for wild prey rodents) were offered to individual snakes by suspending the baits from thread inside the front of each cage. Bait consumption (bait-take) was recorded the next day and any remaining baits were removed. Snakes were re-tested at 5- to 7-day intervals. Consumption of test baits was compared to the number of DNM consumed.

Field Studies with Bait Matrices in Live Capture Traps

Eight bait matrices were evaluated: 1) DNM, the positive control, 2) plastic lizards, 3) chicken fat (freshly rendered chicken fat mixed with 33% paraffin), 4) thawed mealworms, 5) wax polymer dog meal (prepared by a commercial bait formulator), 6) polyurethane foam, 7) beef tallow (made with commercial tallow mixed with 10% paraffin), and 8) hard-boiled quail eggs (Table 2). Baits were suspended by cotton threads in live traps with a live mouse lure protected in an interior holder (Linnell *et al.* 1998) and offered no-choice to captured snakes. Four traplines were run with traps at 20-m intervals along the forest edge adjacent to roads and trails. Three traplines contained 40 traps with 8 bait treatments of 5 traps each, and 1 trapline contained 32 traps with 8 bait treatments of 4 traps each. Baits were randomly assigned to traps within each trapline. Traps were checked daily for 5 days to remove captured snakes and to record bait consumption. Multiple snake captures in a trap (Rodda *et al.* 1999) were designated as 1 snake capture for calculating percentage of baits consumed. Baits were replaced as needed if they were eaten by snakes, ant- or maggot-infested, consumed by ants, or spoiling.

Field Studies with Chemically-Treated Tofu Baits

DNM (the positive control), tofu treated with DNM (T-DNM), and tofu pieces, each treated with one of 23 odiferous chemicals characteristic of decomposing flesh, were evaluated (Table 3). Tofu baits approximated the size of a DNM. T-DNM baits were prepared by allowing tofu to come in contact with DNM for over 24 h. Chemicals were at least reagent grade (Sigma-Aldrich, St. Louis, MO). Solid chemicals were dissolved in acetone (1 mg/ml). Approximately 100 µg of test chemical was applied to each tofu bait. DNM, T-DNM, and chemically-treated tofu were tested separately in open-ended 10.1 × 30.5-cm PVC tubes hung about 1.5 m high at 20-m intervals in trees and shrubs along the forest perimeter adjacent to roads. Three blank tubes without any treatment served as the control. A transect contained 4 randomly assigned treatment tubes, including at least 1

Table 1. Baits consumed by caged brown tree snakes when compared to euthanized dead neonatal mice (DNM).

Baits offered		No. offerings *	No. consumed		Ranking index
A	B		A	B	B/A
DNM	Quail chick	50	25	28	1.1
DNM	Gecko	50	37	36	1.0
DNM	Canned Spam™	40	26	12	0.5
DNM	Canned Treet™	20	13	7	0.5
DNM	Stew beef	50	32	10	0.3
DNM	Raw chicken	20	14	4	0.3
DNM	Canned sausage	20	11	2	0.2
DNM	Chicken fat	53	32	3	0.1
DNM	Plastic lizard	53	32	3	0.1
DNM	Shrimp	20	11	1	0.1
DNM	Plastic egg	20	13	1	0.1
DNM	Pork blood	20	13	1	0.1
DNM	Wax dogmeal	53	32	0	0.0
DNM	Polyurethane foam	20	13	0	0.0
DNM	Mealworm	50	32	0	0.0
DNM	Dog blood	20	11	0	0.0
DNM	Egg white & yolk	20	13	0	0.0
DNM	Beef liver	10	6	0	0.0
DNM	Quail egg	20	15	0	0.0
DNM	Apple	10	8	0	0.0
DNM	Grape	10	7	0	0.0

* number of each bait matrix offered to snakes

Table 2. Baits consumed by brown tree snakes captured in live traps under field conditions. Baits were offered no-choice in live traps containing a caged live mouse lure unavailable to snakes.

Bait	No. Snakes Captured	No. Baits Consumed	% Consumption[a]
Dead neonatal mice	11[b] (12)[c]	6	55
Plastic lizards	6	2	33
Chicken fat	7	1	14
Mealworms	15[b] (20)[c]	2	13
Wax dogmeal	12	1	8
Polyurethane foam	12	1	8
Beef tallow	12	0	0
Quail eggs	10[b] (14)[c]	0	0

[a] calculation based upon number of traps that captured a snake
[b] number of traps that captured a snake
[c] total snakes captured include multiple captures per trap

DNM treatment, and was run for 4 days. Each tube was monitored daily for about 24 h with a 12V infrared video camera system (Supercircuits, Liberty Hill, TX) similar to that described by Shivik and Gruver (2002), except cameras were mounted about 1.5 - 2 m high in vegetation and 1 - 2 m from the tube, and a 12V deep-cycle battery powered the system. The presence of bait was recorded daily, and batteries and video tapes were changed daily. Tapes were scored for presence of snakes and non-target animals. The time of each snake visit was recorded, and multiple visits were recorded only when there was a 15-min or greater interval between snake observations. Means and standard errors of visitation times were based on the total time and number of snake visits for each treatment.

RESULTS

Laboratory Studies with Bait Matrices

Of the 22 baits evaluated, brown tree snake consumption of euthanized geckos, euthanized quail chicks, and DNM was similar under laboratory conditions (Table 1). The overall consumption of DNM ranged from 50 - 80%. Consumption of quail chicks and geckos was 56% and 72%, respectively. Canned meat products, stew beef, and raw chicken meat consumption was 20-50% as effective as DNM. A few baits were eaten occasionally, such as chicken fat, plastic lizard, shrimp, plastic egg, and pork blood. Nine other baits, including hard-boiled quail eggs, were not eaten.

Field Studies with Bait Matrices in Live Capture Traps

Snakes captured in live traps using a live mouse lure ate 6 of the 8 bait types in varying quantities (Table 2). Snakes consumed 55% of the DNM and DNM were preferred over all other baits. Plastic lizards, chicken fat, mealworms, wax dogmeal, and foam baits were eaten by a few captured snakes. Beef tallow and hard-boiled quail egg baits were not consumed. Traps baited with DNM, mealworms, and quail eggs had multiple snake captures.

Table 3. Results of bait tubes containing dead neonatal mice (DNM), tofu treated with DNM (T-DNM), or tofu treated with chemicals.

Treatment	CAS No.	No. Tests	No. Tubes Visited	% Tubes Visited	No. BTS Visits	Visits per tube	Mean(s)[a], SEM	% Bait Take[b]
DNM	–	69	39	57	84	2.2	331, 39	100
T-DNM	–	24	15	62	25	1.7	436, 95	0
3-methylthio-2-butanone[c]	53475-15-3	4	1	25	1	1	1161, –	0
methyl thiobutyrate[c]	2432-51-1	4	1	25	1	1	667, –	0
ethyl-2-mercaptopropinate[c]	19788-49-9	5	1	20	1	1	612, –	0
2-ethylthiophenol[c]	4500-58-7	4	2	50	2	1	551, 359	0
L-methionine[c]	63-68-3	8	4	50	8	2	372, 162	0
3-methyl-1-butanethiol[c]	541-31-1	4	1	25	3	3	356, 177	0
dimethylamine	124-40-3	4	3	75	3	1	315, 77	0
2-methyl-1-butanethiol[c]	1878-18-8	8	3	38	4	1.3	271, 56	0
n-butyric acid	107-92-6	4	1	25	1	1	222, –	0
ethyl isovalerate	108-64-5	4	1	25	3	3	142, 36	0
butyric acid ethyl ester	105-54-4	4	1	25	1	1	124, –	0
thio-2-napthol[c]	91-60-1	4	2	50	2	1	119, 98	0
4-methylthio-2-butanone[c]	34047-39-7	4	1	25	1	1	119, –	0
1-hexanol	11-27-3	4	2	50	2	1	110, 76	0
acetaldehyde	75-07-0	4	1	25	2	2	106, 38	0
L-cysteine[c]	52-90-4	4	1	25	1	1	86, –	0
thiolactic acid[c]	79-42-5	4	1	25	2	2	85, 48	0
pyrazine ethanethiol[c]	35250-53-4	4	1	25	2	2	58, 4	0
dimethyl disulfide[c]	624-92-0	4	1	25	2	2	35, 14	0
1-propanethiol[c]	107-03-9	4	0	0	–	–	–	0
2-phenylethanol	60-12-3	4	0	0	–	–	–	0
methyl-2-thiofuroate[c]	13679-61-3	4	0	0	–	–	–	0
4,5-dimethylthiazole[c]	3581-91-7	3	0	0	–	–	–	0
Blank tube	–	3	2	67	2	1	211, 14	0

[a] amount of time per snake visit
[b] calculated on number of tubes visited by snakes
[c] sulfur compound

Field Studies with Chemically-Treated Tofu Baits

The percentage of tubes visited by snakes ranged from 0-67% (Table 3). The blank tube had the highest percentage of visits (67%), but only 3 tubes were tested. Fifty-seven percent (39/69) of the DNM and 62% (15/24) of the T-DNM tubes were visited. No tofu baits treated with DNM or chemical lures were consumed by snakes, whereas 100% of the snakes that visited tubes baited with DNM consumed the dead mice. About 4,700 hr of video tape were scored and no non-target animals were observed.

The highest snake visits per tube was 3 each for 3-methyl-1-butanethiol and for ethyl isovalerate (Table 3). Snake visits per tube were 2.2 for DNM and 1.7 for T-DNM. Mean times per snake visit were highly variable, and the time for the blank tube (211 s) was greater than 14 of the 23 chemical treatments. T-DNM and 6 chemical treatment mean times per visit were greater (range 356 s - 1,161 s) than the time for DNM (331 s).

DISCUSSION

Brown tree snakes consume a broad variety of prey items in their urban and forest habitats on Guam including birds and their eggs, rodents, skinks, geckos, and lizard eggs, with lizards constituting the major portion of their prey (Savidge 1988, Rodda and Fritts 1992). Because animate baits would not be practical for snakes, this study focused on inanimate baits. Although evaluations of inanimate baits under both laboratory and field conditions (with snakes captured in live traps) are unnatural conditions, the results do give an indication of brown tree snake bait preference.

Except for hard-boiled quail eggs, prey baits (DNM, dead quail chicks, dead geckos) were the preferred choice of brown treesnakes in both laboratory and field trials. DNM were readily eaten by both caged and live-trapped snakes. Euthanized quail chicks and geckos were as well accepted by caged snakes as the young dead mice. Dead geckos were taken by 72% of the caged snakes, an expected result because geckos are part of their normal diet on Guam (Savidge 1988). Two other baits eaten by live-trapped snakes, plastic lizards and chicken fat, were also eaten occasionally by caged snakes. Most likely the plastic lizards mimic their natural prey of geckos and skinks (Savidge 1988, Rodda and Fritts 1992). Quails eggs were not eaten by either live-trapped or caged snakes, perhaps because their scent was removed by boiling.

Savidge (1988) reported that no caged snakes consumed insects but did find plant material and insects, presumably ingested inadvertently, in some necropsied snakes. However, captive neonatal brown tree snakes consumed small grasshoppers (Linnell *et al.* 1997). In the present study, no laboratory caged snakes consumed mealworms or plant material, but 13% of snakes captured in live traps consumed mealworms.

Brown tree snakes exhibit both active search and ambush foraging tactics for prey (Rodda 1992). Evidence that they are also opportunistic scavengers (DeVault *et al.* 2003) comes from videographic analysis, which showed that 100% of snakes visiting DNM carrion-baited tubes consumed young dead mice. Snakes are also attracted to mouse carrion in live traps with capture rates equivalent to live mouse lures (Shivik and Clark 1997), with live mice capturing a larger size class of snakes (Shivik and Clark 1999*b*). A plausible explanation for the attractiveness of carrion is that snakes are prey-deprived because of drastic reductions of avian and mammalian prey and with reliance upon lizards as their primary source of prey (Savidge 1988, Rodda and Fritts 1992).

Snake visits to blank tubes is not surprising, considering that snakes have been found in other artificial structures including aircraft struts and wheel wells, cargo containers, warehouses, homes, and electrical transmission lines (Fritts *et al.* 1990, 1994; Fritts and Chiszar 1999; Vice and Vice 2004). Some snakes have also been captured in empty live traps (Shivik and Clark 1997, Shivik 1998). These patterns of activity attest to their exploratory behavior, which may or may not be related to prey searching.

The 23 odiferous chemicals have organoleptic characteristics of decomposing flesh; 17 are sulfur compounds (Table 3), which are a major group of chemicals identified in decomposing flesh of human cadavers (Vass *et al.* 2004). Seven of the 23 chemicals, including acetaldehyde, dimethyl disulfide, and 1-hexanol, are used in the food industry as flavoring ingredients (Fenaroli 1971). Acetaldehyde, dimethyl disulfide, and 1-hexanol are also 3 of the 13 major volatile components of mouse carrion odor identified by Shivik (1999). A reconstructed formulation using the 13 major components of dead mice was not an effective attractant for capturing snakes in traps. In contrast to whole DNM carrion, none of the 23 odiferous chemicals singly applied, or DNM applied to tofu baits promoted appetitive behavior in the present study. However, snakes consumed tofu wrapped in mouse pelts (Jojola-Elverum *et al.* 2001).

The odor plume of dead mice probably consists of multiple chemical signatures that change over time, and attractiveness of these temporal chemical fingerprints probably also change. Jojola-Elverum *et al.* (2001) reported consumption of 1-day old (24-48 hr) DNM was significantly greater than fresh (<24 hr old) DNM, and that microbial metabolism of the mouse skin produced decomposition odors that were attractive to snakes. The concept of developing a microbial lure is tantalizing but may be too expensive for practical use.

The empirical approach for synthetic brown tree snake attractants in the present study is similar to other studies and all have produced disappointing results. Attractiveness of several agents, including major components of carrion odor such as cadaverine and putrescine, predator lures, bird odors, rotten eggs, and rotten milk, is low (Chiszar *et al.* 1997, Clark 1997, Fritts *et al.* 1989, Shivik 1999, Shivik and Clark 1999*a*). It is highly unlikely that a single chemical will be found to be an attractant for snakes. Coyote attractants have been developed using systemic chemical fractionations and bioassays (Bullard *et al.* 1978*a*,*b*; Kimball *et al.* 2000*a*,*b*), and this approach is suggested for developing lures that elicit snake appetitive prey search behavior.

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