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Fred Kraus

University of Michigan, fkraus@umich.edu

Randal S. Stahl

USDA-APHIS-Wildlife Services, randal.s.stahl@aphis.usda.gov

William Pitt

U.S. Department of Agriculture, Animal Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Hilo, HI

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Thermal fumigation provides a simple and effective solution for sanitizing cargo from invasive snakes

Fred Kraus · Randal Stahl · William Pitt

Received: 18 June 2014 / Revised: 12 September 2014 / Accepted: 20 October 2014 / Published online: 13 November 2014
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Abstract The Brown Treesnake (*Boiga irregularis*) is invasive in Guam and threatens to be dispersed by military and civilian transportation activities to other islands in the Pacific, where it could be expected to inflict similar damages. Prevention of inadvertent export of snakes in cargo and vehicles currently relies on trained canine detection teams, which are expensive to use and unable to detect all snakes. Hence, there has long been interest in developing effective and cheaper means of fumigating cargo to remove snakes. A companion study has shown that chemical fumigation is unlikely to be readily developed into a practical tool. Here, we demonstrate that these snakes are readily induced to quit test refugia by application of streams of heated air. Many parameters affect snake response times, but we find that application of relatively low temperatures (48–52 °C) at moderate delivery rates (3.4 m³/min) is sufficient to induce exit of these snakes within 5 min. Development of a portable heat-delivery system based on these findings has great potential to ensure snakes do not unintentionally stow away to other locations

in cargo, munitions, vehicles, or airplane wheelwells. Application of such technology can be done on Guam as well as at locations receiving cargo or vehicles from that source, providing an additional layer of security in ensuring these snakes do not colonize additional locations outside their native range.

Keywords Alien species · *Boiga irregularis* · Brown treesnake · Hot air

Key message

Effective cargo fumigants are needed to sanitize transportation networks against invasive snakes so as to avoid spreading them to new locations. We earlier found a variety of proposed chemical fumigants to be ineffective in meeting this goal. Here, we show that streams of moderately heated air are sufficient to quickly induce brown treesnakes to leave experimental refugia. Development of this technology can provide a new and useful tool for ensuring these snakes do not invade areas beyond Guam.

Communicated by J. Jacob.

F. Kraus (✉)
Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA
e-mail: fkraus@umich.edu

R. Stahl
U.S. Department of Agriculture, Animal Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA

W. Pitt
U.S. Department of Agriculture, Animal Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, PO Box 10880, Hilo, HI 96721, USA

Introduction

The brown treesnake (*Boiga irregularis*) was inadvertently introduced to Guam soon after World War II in returning materiel and has since caused a series of negative ecological and economic impacts, including loss of native species, alterations of community structure and food webs, damage to the electric-power and agricultural industries, and envenomation of infants (Fritts et al. 1987, 1990, 1994; Savidge 1987; Fritts and McCoid 1991, 1999; Rodda et al. 1997; Fritts and Rodda 1998; Fritts and Chiszar 1999;

Burnett et al. 2006; Rodda and Savidge 2007; Shwiff et al. 2010). Expectations are that similar results would attend subsequent introduction of the snake to other oceanic islands. This concern arises because the snakes are nocturnal and seek dark refugia in which to shelter during the day (Fritts 1988), and cargo, shipping containers, and transport vessels can provide ready daytime refugia. Consequently, considerable effort has been expended by the U.S. Department of Agriculture's Wildlife Services program since 1993 to confine the snake to Guam by ensuring that cargo and vessels leaving the island are snake free (Engeman and Vice 2002). Major components of this program are reduction of snake populations near port areas and inspection of outbound cargo and vehicles to ensure absence of snakes. Presently, the only means for detecting snakes hiding in outbound cargo is with detector dogs trained to locate snakes (Engeman et al. 1998, 2002; Vice et al. 2009). This technology is fairly effective (Engeman et al. 1998, 2002; Vice et al. 2009) but requires highly trained personnel and dogs, and the program is correspondingly expensive. Additional tools for ensuring absence of snakes in outbound cargo would therefore be of great benefit, particularly tools such as chemical or heat repellents or fumigants that would require comparatively little training for use (Brown Tree Snake Control Committee 1996). As well, having a quick, cheap, and reliable method in other Pacific ports to treat that portion of arriving cargo not liable to inspection prior to leaving Guam would provide those islands with an additional layer of protection from the snakes. Discovery of such new management tools has grown particularly urgent with the impending increase in U.S. military activities on Guam (Robertson 2011) designed to provide a rapid-response platform for the Pacific Island Region and beyond.

Certain chemicals are known to elicit avoidance behavior when directly applied to snakes (Clark and Shivik 2002; T. Mathies and W. Pitt, unpubl. data) or to their refugia (Nishimura 1999), suggesting that one or more of these could be developed into effective fumigants for treating outbound cargo for brown treesnakes. Consequently, we began a series of trials to determine the efficacy and operational feasibility of chemical fumigation, primarily with essential oils. Our results have shown several of the more promising of these chemicals to be of uneven efficacy and to suffer from logistical limitations that make them difficult or impossible to use as effective fumigants under operational conditions (Kraus et al., submitted). However, during attempts to improve evaporation rates for some of these chemicals, we discovered that application of heated streams of air could elicit rapid response from brown treesnakes. We therefore changed the focus of our studies to investigate the efficacy of active thermal treatments, the results of which we report herein.

Theoretical considerations suggest that application of heat should elicit escape behavior from snakes exposed to lethal temperatures. All reptiles have narrowly delimited critical thermal maxima (Heatwole 1976) and are sensitive to avoiding those limits, which are usually only a few degrees above preferred body temperatures (Brattstrom 1965; Huey and Stevenson 1979). The upper lethal temperature for the brown treesnake has been determined to be 41 °C (Christy et al. 2007), which is relatively low. Consequently, introduction of heated air into refugia would appear to have the potential to serve as an effective inducement for snakes to leave those refugia. Preliminary work on use of heat as a control method for brown treesnakes was initiated in the late 1990s. The sole study (Perry and Vice 2007) examined upper temperatures attained during transit of standard 20-foot cargo sea containers leaving Guam in an effort to determine whether passive thermal heating of these containers could be a reliable fumigation method for these snakes. They found that maximum lethal temperatures were often attained in these containers but could not be guaranteed, with temperatures typically non-lethal in containers packed with cargo (most containers leaving Guam are empty) or protected from sunlight due to cloudy conditions or internal stacking of containers on ships. Further investigation of heat as an operational tool for brown-treesnake control has not been pursued. Importantly, this earlier work was focused on using temperature as a potential fumigant, being designed to use temperature extremes to kill snakes in situ. Our investigations modify this focus to inquire whether temperature can be used to impel snakes to leave refugia.

Materials and methods

Test site and animals

We conducted tests on Guam in a warehouse on Andersen Air Force Base at ambient air temperatures (26–31 °C). Snakes were retained in a communal cage for at least 1 day prior to use, were kept in shade at all times, and had drinking water-provided *ad libitum*. We determined the sex of each snake by probing for hemipenes, measured its snout-vent length to the nearest 1 mm, and transferred it to an individual 14.2 L container that served as a test refugium. We then gave snakes at least 1 h to calm down prior to testing, although most snakes were left overnight in the container prior to testing.

Test apparatus

We tested snakes inside an experimental refugium consisting of a commercially available translucent

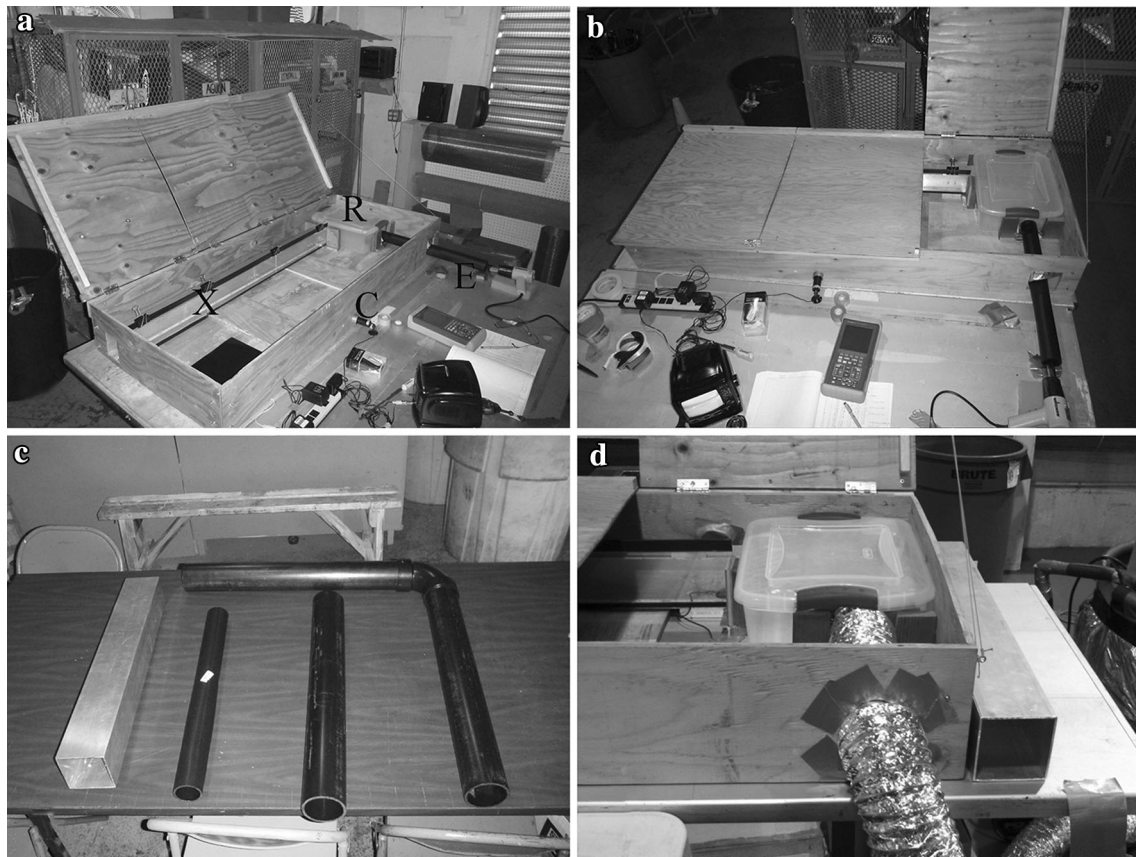


Fig. 1 View of **a** the opened test apparatus showing the test refugium (R), entry tube (E), aluminum exit tube (X), and infrared camera (C) connected to a video monitor; **b** the semi-closed test apparatus showing two of the three top doors that close so as to allow tests to be conducted in darkness and with heat gun placed at entrance of entry

tube; **c** the types of entry tubes used in the four 2013 treatments; from left to right: aluminum tube, 2 × 24" ABS tube, 3 × 27" ABS tube, and 3 × 54" ABS tube with 90° elbow; and **d** entry-pipe configuration using a commercial mylar dryer hose placed flush to the entry hole of each test refugium for all 2014 trials

polyethylene container measuring 17 × 33 × 42 cm and having a volume of 14.2 L. We drilled a 7.5-cm-diameter hole at one end of the container and covered it with hardware cloth. During experimental trials, we placed this hole next to the end of the inlet pipe through which the stream of heated air was introduced (Fig. 1b, d). On the side of the refugium container near the opposite end, we drilled a second 7.5-cm-diameter exit hole that abutted flush onto an aluminum exit pipe faced with glass and having dimensions 7 × 10 × 152 cm and a volume of 10.4 L (Fig. 1a, b). The length of the exit pipe was somewhat more than half the length of the longest dimension of a commercial “463 L” pallet (274 × 224 cm; height restrictions vary among aircraft types), ensuring that successful exit of this length of pipe would signal likely exit of containers and pallets under operational conditions, even for snakes sequestered in the center of cargo containers. Air volume of the refugium, entry pipe, and exit tube summed to 31 L. We then fixed the test container, exit pipe, and approximately half of the input pipe inside a wooden box with closable lid to allow

the experiments to be conducted in darkness (Fig. 1b). By housing the section of the apparatus containing a snake and its potential escape route in a darkened box, a realistic challenge to exit is presented similar to that occurring in real cargo. Because brown treesnakes are nocturnal, a snake inside the apparatus would be expected to be reluctant to exit the darkness within the pipe and enter into a well-lit room. Thus, as under field conditions, a snake will have to choose between opposing adverse stimuli: light versus repellent. We fitted an infrared camera connected to a video monitor into the front of the box so we could monitor activities of the test subjects; we placed a small infrared lamp in the corner opposite the refugium to provide illumination (Fig. 1a, b).

We conducted two sets of experiments using different heating and air-blowing equipment:

- (1) We conducted initial trials in April 2013 using commercial heat guns to introduce the heat down the entry tube and into the test refugium. Treatments used either a Master Appliance PH1500 heat gun set at a

Table 1 Test conditions for different types and configurations of entry tube in the first round of trials conducted in 2013

Treatment	Tube type	Total air volume of system (L ³)	Conductivity (W/mK)	Heat gun temperature (°C)	Air-flow rate (m ³ /min, cfm)
1	Aluminum	6.58	204–210	400	0.45, 16
2	3 × 27" ABS	3.13	0.17–0.19	400	0.45, 16
3	3 × 54" ABS	6.68	0.17–0.19	400	0.45, 16
4	2 × 24" ABS	1.24	0.17–0.19	538	0.57, 20

temperature of 400 °C and a flow rate of 0.45 m³/min (16 cubic feet/min (cfm)), or a Wagner HT1000 heat gun with a temperature setting of 538 °C and a flow rate of 0.57 m³/min (20 cfm) (Table 1).

- (2) Results from our first round of trials suggested that snakes were responding more to rate of temperature increase than to absolute temperature per se (see Results below). Consequently, for our second round of trials in January 2014, we sought to test a system of heat delivery using much higher air-flow rates and much lower temperatures. For these experiments, we generated hot air by blowing ambient-temperature air through a heat exchanger connected with an open circulating pump system to an insulated, heated-metal water reservoir containing approximately 110 L of water. Heat was provided by a 1,000 W immersion heater (Humboldt, Elgin, IL) inserted into the reservoir and three 1,000 W band heaters (Grainger, Lake Forest, IL) attached to the outside of the reservoir. We adjusted heat output to maintain water temperature at ~80 °C in the reservoir. We pumped the water through the heat exchanger using an external impeller pump (SHURflo, Cypress, CA) at a maximum flow rate of 14 L/min. The heat exchanger had a face surface area of 0.093 m² and a maximum rated air capacity of 11.3 m³/min. A variable-speed squirrel-cage blower (Dayton Electric Mfg., Niles, IL) forced air through the heat exchanger. We directed air away from the heat exchanger using a 10-cm-diameter flexible aluminum-foil duct attached to a manifold enclosing the exchanger coil. The opposite end of this duct was then connected to the test refugium (Fig. 1d).

Test procedure

At the start of each test, we introduced one of the 14.2 L containers in which a snake had been acclimated into the enclosing test apparatus, placed the entry-pipe flush with the side of the cage at the entry opening, and closed the box, placing the snake in darkness. We varied experimental procedures slightly depending on the different heating and blowing equipment used in the two sets of trials:

In the initial treatments in 2013, we varied the length, diameter, and constituent material of the entry pipe (Fig. 1c; Table 1) to assess how these parameters would affect times to snake response. These parameters are relevant to real-life transport situations in which cargo and vehicular refugia may be expected to vary in composition and convolutedness of pathways to escape. All treatments used 20 male and 20 female snakes.

For these initial trials using heat guns, we could not obtain temperatures simultaneously with snake response times, so, following the conclusion of the snake trials, we used a PR-11 series resistance thermal device (RTD; Omega Engineering, Inc., Stamford, CT) monitored with a Fluke 97 Scopemeter to obtain heating profiles of the test system under each experimental entry-tube configuration (Table 1). We measured heating profiles of the container air, container sidewall, and distal end of exit tube separately for a period of time sufficient to cover almost all snake reaction times. For the trials involving the aluminum, 3 × 27" ABS, and 3 × 54" ABS entry tubes (Fig. 1c), we recorded these temperature profiles for 420 s; for the smaller 2 × 24" ABS tube temperature profiles, we needed to record for only 120 s because of the more rapid heating rate with that entryway configuration.

In subsequent trials in 2014, we standardized the entryway using a collapsible 10-cm-diameter mylar dryer duct (conductivity = 0.15 W/mK) as entry tube for all experimental treatments (Fig. 1d). Treatments therefore did not vary types of entryway, as in 2013; however, we investigated two additional variables. First, we examined the effect of light flux density outside the test apparatus on times of snake exit by either (a) using ambient light levels in the warehouse or (b) increasing illumination by placing an incandescent desk lamp outside the exit tube. We measured light levels (Table 3) with an Extech EasyView 30 photometer. Secondly, we investigated the effect of refugium air volume on snake response times using either (a) the standard 14.2 L test refugia or (b) filling most of the space of these refugia with closed cell polyisocyanurate foam blocks so as to reduce air volume to 3.3 L. Each of these treatments used air-flow rates of 3.4 m³/min (120.2 cfm), air temperatures of 52–54 °C, and 40 snakes (20 males, 20 females).

We also conducted two sets of treatments to confirm that snake reactions were not simply a response to airflow.

These were paired trials in which we first tested 5 males and 5 females for response to air flow alone. We then let each snake rest undisturbed for at least 1 h before testing them again using the same air-flow rate but with the addition of the heat. These treatments used air-flow rates of either (1) 3.4 m³/min (120.2 cfm) or (2) 4.6 m³/min (161.4 cfm) and air temperatures of 52–54 °C. These were the low and high settings on the air blower, respectively.

For all experiments in 2014, we measured temperature using three 24 gauge type-T thermocouples (Omega Engineering, Inc., Stamford, CT) recorded with Signal Express software using a NI-DAQ interface containing a thermocouple input module (National Instruments, Austin, TX). We collected temperature data for the three thermocouples simultaneously at 1 s intervals across the duration of each trial. We exported data to MS Excel following each trial for subsequent analysis.

Data

In the 2013 trials, we used a stopwatch to record to the nearest second times to (1) first snake movement, (2) first frantic movement, (3) exit of snake from test container (refugium), and (4) exit of snake from test apparatus (distal end of exit tube). We subsequently recorded heating profiles for six replicates and used them to derive polynomial, least-squares-fit equations of temperature to response time for each treatment. We estimated relevant temperatures during snake reaction times from the equations for refugium air temperatures because the refugium walls and the exit tube never heated sufficiently to pose a thermal danger to the snakes. Therefore, we presumed that the (higher) air temperature was driving snake behavior.

For the 2014 trials, we again recorded to the nearest second times to (1) first snake movement, (2) first frantic movement, (3) exit of snake from test container (refugium), and (4) exit of snake from test apparatus, except that for the treatment involving the reduced refugium air volume we could only measure times for (3) and (4) because the foam blocks used to reduce refugium volume prohibited observation of snake behavior within the refugium. We analyzed temperatures at times of first snake movement, first frantic movement, and exit from refugium using the thermocouple data for air temperatures inside the refugium; temperatures of the sidewall of the refugium climbed too slowly to present a threat to snakes prior to their exiting the refugium and so were ignored by us. We analyzed temperatures at time of exit from the test apparatus using data taken from the thermocouple placed at the exit of the test apparatus.

We used the measured air temperature and the refugium-wall temperature to calculate a thermally equivalent temperature (T_e) that mathematically describes the total radiant and convective energy exchange between the snake and its

environment as the wall temperature of a black-body cavity containing the snake:

$$T_e = T_a + r_e (R_{\text{abs}} - \varepsilon \sigma T_a^4) / \rho c_p$$

(Campbell 1977 : Eq.7.17),

where T_a is the air temperature, R_{abs} is the total amount of long and short wave radiation, ε is emissivity of the air, σ is the Stephan–Boltzman constant, ρ is the density of the air, c_p is the specific heat of air, and r_e is a parallel resistance term combining both sensible and radiative transfer resistances. The minimum radiant environment corresponds to the long-wave radiation transfer (IR) of heat from the refugium walls to the snake as the studies are conducted in the dark (Campbell 1977: Eq. 7.11). The emissivity (ε_s) term is an average across all surface types, and a_L is the absorptivity coefficient for long-wave radiation by the snake. This value is added to the temperature contribution from the air. We calculated T_e for each time point in each trial for which both air and refugium-wall temperatures were measured. We calculated the rate of change of T_e (dT_e/dt) by dividing the change in T_e between trial onset and time to exit the refugium by that time interval. We calculated these values for each trial across all treatment groups. We also calculated total heat input in kJ, using the equation $Q = m \times C_p \times dT$, where Q = heat capacity, m = air mass (kg), C_p = specific heat of air on a unit mass basis kJ/(kg \times C), and dT = change in temperature in °C (Olmsted and Williams 1997).

We used Mann–Whitney U tests with Bonferroni correction to test for response-time and response-temperature differences between the sexes; we used the Mann–Whitney U or Kruskal–Wallace tests without correction to test for differences between treatments.

Results

2013 trials with heat guns

Streams of heated air invariably elicited attempts by the snakes to exit the test apparatus. Most such attempts were successful, although some were not. No sexual differences in response time occurred for any of the experiments; hence, response-time data are not here partitioned by sex. As expected, snake response times were quicker when the air volume of the entry tube was reduced and when the entry tube was non-metallic (Table 2). Standard deviations of these reaction times and their corresponding temperatures were, however, quite large. Nonetheless, in most trials (102/160), snakes exited the test apparatus within 5 min. In 13 instances, failure to exit was because the snake died in the refugium or in the exit tube; in five instances, snakes coiled near the end of the exit tube but

Table 2 Snake response times and estimated refugium air temperatures at these times by experimental treatment

Tube type	Time (s) to first movement	Temp (°C) at first movement	Time (s) to first frantic movement	Temp (°C) at first frantic movement	Time (s) to exit refugium	Temp (°C) at exit from refugium	Time (s) to exit apparatus	% exiting by 300 s (% dead)
Aluminum	123.6 ± 11.81 (8–279)	58.0 ± 1.855 (33.9–75.6)	243.1 ± 10.46 (140–390)	72.6 ± 0.750 (63.3–81.1)	237.9 ± 15.92 (150–310)	72.5 ± 1.193 (64.7–77.1)	304.6 ± 19.92 (167–818)	57.5 (2.5)
3 × 27" ABS	71.8 ± 8.42 (9–223)	50.8 ± 1.850 (33.8–74.5)	174.3 ± 15.82 (30–415)	66.9 ± 1.654 (41.1–82.2)	173.5 ± 15.12 (38–427)	67.1 ± 1.576 (43.7–83.0)	204.2 ± 15.50 (53–437)	72.5 (12.5)
3 × 54" ABS	113.9 ± 10.65 (9–237)	50.0 ± 1.417 (33.0–62.8)	276.9 ± 14.05 (108–499)	64.4 ± 0.831 (50.9–76.3)	271.7 ± 14.08 (103–503)	63.9 ± 0.847 (50.2–76.7)	330.0 ± 15.28 (136–524)	32.5 (10.0)
2 × 24" ABS	19.5 ± 2.96 (3–80)	38.1 ± 1.473 (29.8–68.2)	51.1 ± 5.45 (15–129)	53.8 ± 2.711 (35.8–92.5)	57.6 ± 4.88 (19–129)	57.0 ± 2.428 (37.8–92.5)	80.1 ± 7.60 (25–255)	92.5 (7.5)

Values are mean ± SD (range). Temperatures are not estimated for time to exit apparatus because of the exit's great distance from the refugium air from which these temperatures were taken

refused to leave the apparatus. However, all snakes exited the refugium (test container) except for eight that died trying.

Estimated air temperatures in the refugium at times of initial snake response, first sign of frantic behavior, and exit from the refugium also show great variance (Table 2), but certain patterns are clear. The test refugium attained higher temperatures before eliciting snake responses when the air stream passed through material of high conductivity (aluminum), and much lower temperatures were needed to elicit snake responses when the air volume of the entryway was smaller, passed via a low-conductivity conduit, or air-flow rate was faster. This latter effect was most pronounced for the 2 × 24" ABS tube (Table 2).

These data suggested that snakes might be responding not so much to absolute ambient temperature as to rate of increase in temperature. We confirmed this by contrasting rates of temperature increase (dT/dt) among the four treatments, finding that trials with the 2 × 24" ABS entry tube heated the refugium much more quickly than did trials under the other treatments ($H = 91.36$, $df = 3$, $p < 0.001$), consistent with that treatment providing the shortest response times and lowest response temperatures (Table 2).

2014 trials using blower and heat tank

Streams of heated air under the new experimental configuration invariably elicited attempts by the snakes to exit the test apparatus. All but two such attempts were successful within 5 min (Table 3). No sexual differences in response times occurred for any of the treatments; hence, response data are not here partitioned by sex. Nor did we find any correlation of response time or temperature to body length (graph not shown).

The first, baseline, treatment utilized ambient light levels in the warehouse and the unmodified refugium volume. Hence, they were directly comparable to the set of treatments from 2013 in terms of light levels and refugium size but differed in having higher air-delivery rates and much lower temperature of the stream of air, as well as type of entry tube. As expected, these trials produced more rapid-response times (Table 3) and lower response temperatures (Table 4) than seen in trials from 2013, with the exception of the low-volume 2 × 24" ABS tube, which was approximately comparable (Table 2). The 2014 trials also had consistently lower variance of results (cf. Table 2 vs. Tables 3, 4). Increased light levels outside the exit tube did not increase exit times or temperatures of the snakes (Tables 3, 4). But reduced air volume in the refugium did result in significantly more rapid exit times (Table 3) and slightly lower temperatures at times of exit (Table 4). Across all treatments, temperatures at time of exit from the

Table 3 Response times of snakes under (1) standard experimental treatment, (2) higher light levels, and (3) confined refugium space

Treatment	Light level (lux)	Air flow (m ³ /min)	Refugium volume (L)	Time (s) at first movement	Time (s) to first frantic movement	Time (s) to exit refugium	Time (s) to exit apparatus	% exiting by 300 s (% dead)
1	49.3	3.4	14.2	39.8 ± 4.538 (5–110)	97.1 ± 6.708 (27–174)	101.4 ± 6.871 (16–196)	144.3 ± 7.034 (74–259)	100 (5)
2	171.0	3.4	14.2	46.5 ± 4.525 (8–143)	99.2 ± 6.618 (45–218)	103.5 ± 5.984 (32–251)	143.8 ± 7.130 (71–259)	100 (0)
				$p = 0.138$	$p = 0.850$	$p = 0.898$	$p = 0.899$	
3	49.3	3.4	3.3	NA	NA	31.9 ± 2.114 (14–67)	115.9 ± 5.624 (46–196)	100 (0)
						$p < 0.0001$	$p = 0.004$	

All times are mean ± SD (range). Significance values for Mann–Whitney tests are given in relation to Treatment 1

Table 4 Response temperatures of snakes under (1) standard experimental treatment, (2) higher light levels, and (3) confined refugium space

Treatment	Light level (lux)	Air flow (m ³ /min, cfm)	Refugium volume (L)	Temp (°C) at first movement	Temp (°C) to first frantic movement	Temp (°C) to exit refugium	Temp (°C) to exit apparatus
1	49.3	3.4, 120.2	14.2	47.9 ± 0.576 (38.1–53.5)	51.6 ± 0.329 (47.0–53.8)	51.6 ± 0.307 (44.8–54.3)	41.7 ± 0.319 (36.1–44.4)
2	171.0	3.4, 120.2	14.2	49.0 ± 0.496 (40.3–53.8)	52.3 ± 0.263 (48.4–54.5)	52.0 ± 0.236 (48.9–54.3)	40.6 ± 0.190 (37.4–42.5)
				$p = 0.073$	$p = 0.159$	$p = 0.268$	$p = 0.0007$
3	49.3	3.4, 120.2	3.3	NA	NA	49.3 ± 0.668 (34.8–53.5)	38.7 ± 0.358 (33.9–43.2)
						$p = 0.0027$	$p < 0.0001$

All temperatures are mean ± SD (range). Significance values for Mann–Whitney tests are given in relation to Trial 1

refugium lay within a narrow range in the upper 40 s to lower 50 s °C (Table 4), with exit times varying depending on refugium volume (Table 3). Rates of temperature increase are approximately equivalent to those seen in the 2013 trials, but total temperature change was considerably lower except in comparison to the trial using the low-volume 2 × 24" ABS tube (Table 5).

We confirmed that snakes in our trials were not merely responding to tactile stimuli from passing currents of air. When we introduced ambient air at 3.4 m³/min (120 cfm), all snakes sat passively in the refugium. In contrast, those same snakes responded quickly when heat was added to the airstream, with eight snakes exiting the test apparatus within 5 min, although one snake died in the refugium (one additional trial had to be discarded because the timer failed to start). For these treatments, temperatures when snakes exited the refugium were 50.4 °C ± 0.457 (range = 47.6–51.6 °C), while those when they exited the test apparatus were 41.2 °C ± 0.848 (range = 37.6–43.7 °C). These results are consistent with those of the experimental treatments discussed above. For currents of unheated air blown at a faster 4.6 m³/min (161 cfm), three snakes moved; one re-coiled and remained quiet in the refugium,

whereas the other two exited the refugium after 245 s and slowly crawled down the exit tube but did not exit the apparatus. None of these snakes exhibited any sign of urgency, stress, or escape behavior. In contrast, when these same snakes had heat applied to the airstream, all but one exited the test apparatus within 5 min and showed typical signs of agitation and urgency (e.g., rapid exploration with head, frequent tail lashing). The sole exception was a snake that could not find the refugium exit, was overcome by heat, and was removed at 180 s before it died. For these higher-airflow trials, temperatures when snakes exited the refugium were 48.2 °C ± 0.524 (range = 44.5–49.7 °C), while those when they exited the test apparatus were 40.6 °C ± 0.614 (range = 38.0–43.2 °C). Again, these exit temperatures are in accordance with those for the main treatments (Table 4).

Discussion

In our experiments, application of streams of heated air inevitably elicited from snakes signs of distress and attempts to escape the refugium into which the air was

Table 5 Comparison of total heat and air inputs, temperature changes, and rates of change of thermally equivalent temperatures (dT_e/dt) among the seven sets of experiments conducted in 2013 and 2014

Year	Trial	Delivery T (°C)	Air flow (m ³ /min)	Time (s) to exit refugium	Air mass used (kg)	Total dT (°C)	Average dT $\int dt$	Total heat input (kJ)
2013	1	400	0.45	237.9 ± 15.92 (150–310)	1.82 ± 0.116 (1.18–2.34)	41.55 ± 1.193 (33.67–46.12)	0.315 ± 0.056 (0.249–0.431)	77.18 ± 6.867 (39.78–108.61)
	2	400	0.45	173.5 ± 15.12 (38–427)	1.33 ± 0.111 (0.32–3.17)	36.11 ± 1.576 (12.67–51.99)	0.482 ± 0.212 (0.192–1.119)	53.97 ± 6.321 (4.04–165.89)
	3	400	0.45	271.7 ± 14.08 (103–503)	2.12 ± 0.105 (0.84–3.81)	32.92 ± 0.847 (19.23–45.67)	0.262 ± 0.071 (0.169–0.496)	73.64 ± 5.442 (16.30–174.74)
	4	538	0.57	57.6 ± 4.88 (19–129)	0.57 ± 0.044 (0.20–1.18)	25.99 ± 2.428 (6.79–61.54)	1.255 ± 0.575 (0.696–2.506)	18.96 ± 2.976 (1.40–73.17)
2014	5	52–54	3.4	101.4 ± 6.871 (16–196)	6.24 ± 0.421 (1.01–12.07)	21.81 ± 0.360 (15.98–26.00)	0.268 ± 0.160 (0.113–1.016)	138.80 ± 9.894 (16.17–255.00)
	6	52–54	3.4	103.5 ± 5.984 (32–251)	6.36 ± 0.369 (1.99–15.55)	22.08 ± 0.216 (19.06–25.34)	0.239 ± 0.089 (0.085–0.602)	142.17 ± 8.517 (38.08–339.52)
	7	52–54	3.4	31.9 ± 2.114 (14–67)	3.72 ± 0.008 (3.67–3.90)	20.09 ± 0.672 (6.79–25.47)	0.674 ± 0.244 (0.232–1.373)	74.97 ± 2.415 (26.60–94.09)

All values are mean ± SD (range)

directed. However, our initial set of trials conducted in 2013 had high variances for both response times and estimated response temperatures (Table 2). Furthermore, response times were longer, and temperatures were higher than would be ideal for use as an operational tool to remove snakes from cargo. Nonetheless, only eight snakes of 160 tested failed to find the refugium exit, and those that failed died trying. Most snakes exiting the refugium also exited the entire test apparatus via the exit tube (142 of 152 = 93 %); although five snakes died in the tube while trying to exit, and another five coiled near the exit but refused to leave the tube. These initial results clearly indicated that heat was a sufficient inducement to flee that brown treesnakes would overcome their aversion to daylight in order to escape it. Thus, it appeared that with further refinement, directed streams of warmed air had potential to force snakes to quit refugia in cargo.

We believed the wide scatter in our 2013 findings to result from reliance on non-optimal equipment. Both types of heat guns used in these tests introduced air at a much slower rate than desirable (0.45 or 0.57 m³/min). Because of this slow delivery rate, much of the emitted energy was dissipated in slowly heating the entry tube and the refugium walls instead of just the refugium air surrounding the snake. As a result, snake reaction times were often slow and covered a broad range of values. Snakes often seemed to delay escape behavior to a point at which they had only a relatively short time before they were overcome by the heat, suggesting that slow rates of heating were not an optimal stimulus to escape. Response times were much shorter when even a slight increase (25 %) in airflow rate

was used in heating the refugium (Tables 1, 2). This is why several snakes died during attempted escape if they could not quickly locate the refugium exit.

Unsurprisingly, response times increased on average when surrounding materials had high conductivity and were able to absorb much of the energy emitted by the heat gun (Table 2). The practical implication of this is that refugia consisting of metal—such as break-bulk cargo—are likely to require longer treatment times, faster flow rates, or higher temperatures to achieve the same effectiveness as shorter treatments for refugia having low conductivities. This would have practical ramifications in developing operational methods for treating assorted types of cargo. Response times were also delayed when the air stream had to follow a more convoluted path, as tested with varying lengths of ABS tube (Table 2). Pathway convolution will vary depending on treatment target, so treatment times will also likely vary across targets. These time requirements will vary with airflow rate and application temperature but can only be determined empirically.

Because snakes were much quicker to respond to increasing temperatures when heated air was introduced at even a marginally faster rate, it seemed likely that their decision to vacate the refugium was cued more toward rate of temperature increase as opposed to absolute temperature—a hypothesis corroborated by our data correlating response times with heating rates among different configurations of entry pathway (Table 2). Thus, delivery of faster streams of air could prove more quickly effective in eliciting exit of snakes from experimental refugia. Tied to this, if we could increase heat-delivery rates sufficiently,

we expected that we could use much lower temperatures than those provided by the heat guns, which were not feasible as operational tools (Table 1). Consequently, we developed a more powerful heat-delivery system using much lower temperatures and faster flow rates.

Results from the second round of trials using that design were far more consistent and compelling in demonstrating the ability of heated air to elicit escape behavior from brown treesnakes. We found average response times to be much shorter in the 2014 trials, temperatures at response times to be much lower, and variance in responses much narrower than in the first round of trials (cf. Table 2 vs. Tables 3, 4). Snake behavior in the second round of trials was also more consistent in indicating stress. In the first experiments using the low-airflow heat guns, even though most snakes exited the apparatus, they often did so in a slow, or even sluggish, fashion. In the later experiments, snakes were clearly stressed by the experience, almost invariably thrashing their tails in displeasure and quickly exploring their environment with rapid back-and-forth head movements. That result was not nearly so common in the first set of experiments.

Obviously, a number of factors contribute to the efficacy of heat in eliciting exit of snakes from test refugia: delivery temperature, air-delivery rate, refugium size, and surrounding materials all contribute to the rapidity of snake response. Our results varying these several parameters may all be interpreted in the unifying language of energy transfer (T_c), its rate of change (dT_c/dt), and total energy input to the system. Values for the seven sets of trials conducted by us make clear that trials imposing higher values of dT_c/dt (Table 5) lead to more rapid exit times (Tables 2, 3) and lower exit temperatures (Tables 2, 4) than do trials with lower values of dT_c/dt . Similarly, more rapid delivery of air also reduces snake response times, especially if refugium space is more restricted (Table 5). Both results suggest that what primarily accounts for rapid snake exit is the ability to deliver heat sufficiently rapidly that boundary-layer resistance between the snake and its environment is reduced. Because of this resistance, heat input and air speed interact so that as air speed increases, resistance decreases, and the snake is less isolated from thermal stimuli. Thus, with low wind speeds, it takes a longer time for snakes to absorb sufficient heat to behaviorally respond; consequently, they do so at surrounding temperatures that are much higher (total dT in Table 5) than when wind speeds are higher. With higher wind speeds, lower temperatures may be used to unsettle snakes because boundary-layer resistance to heat transfer from the air is smaller and, therefore, more of the input heat energy transfers to warming the snake instead of just flowing past it. Because wind speeds increase with increasing air-delivery rates and with decreased refugium size, under

those conditions, low delivery temperatures are effective at displacing snakes in shorter times, as seen in the 2014 trials (Table 5).

Our results make clear that if heated air can be applied into refugia of small to modest size, such as typify most cargo and vehicular refugia, snakes can be reliably induced to vacate such refugia within 5 min (and often much less) using air at temperatures of only 48–52 °C. These treatment times are sufficiently short for the method to have relevance as an operational tool, and the temperatures are sufficiently low that few, if any, cargo goods would be damaged by the treatment. Indeed, the temperatures approximate ambient summertime highs in the U.S. Southwest. It seems possible that even more rapid exit of snakes from refugia could be had by increasing air-flow rates even more. In support of this, our paired trials at a higher air-flow rate led to exit of snakes at temperatures approximately 2 °C cooler than those obtained using the standard treatment. How much lower treatment temperatures may be reduced by increasing air-flow rates remains to be determined empirically, although 41 °C would obviously be the lower theoretical limit possible inasmuch as it is the snake's upper lethal limit. But present results suggest that use of this approach for quick operational ejection of snakes from cargo and vehicles is likely feasible.

Under what circumstances might active thermal fumigation be applied operationally to reduce the risk of snake transport from Guam? That risk inheres mainly to exported cargo and the vehicles on which they are moved. For application of heated air streams to drive snakes out of these spaces, sufficient airspace is needed to allow for the free flow of air in the target space so as to heat all potential refugia. Hence, it seems reasonable to expect that the method could be most relevant for treating airplane wheelwells, break-bulk cargo, munitions, and transported vehicles. Standard palletized or containerized cargo likely presents greater challenges in that tightly packed cargo may not allow for efficient airflow to reach all internal refugia, although it remains to be determined what, if any, flow rate might be used to meet that challenge. However, even should more passive means of heating be needed to treat palletized/containerized cargo, application of streams of heated air could still address a variety of currently unmet quarantine needs. Historically, airplane wheelwells have been particularly recalcitrant to effective inspection because of hydraulic and electrical lines that provide innumerable hiding places for snakes and the difficulty of visually inspecting that environment. Transported vehicles and break-bulk cargo present many of the same problems. And munitions are often unavailable for inspection by the civilian authorities providing inspection services of out-bound cargo and vehicles on Guam. In each of those

circumstances, refugium airspaces are sufficiently abundant that thermal fumigation with rapidly delivered streams of heated air may be operationally feasible if a sufficiently powerful, portable delivery system is developed.

Such an operational tool would provide three additional advantages over passive thermal methods during trans-shipment. First, because this method relies on eliciting exit of snakes from refugia, snakes would still be on Guam after application of the method, decreasing their chances of leaving the island. Second, it avoids relying on a method that cannot discriminate whether snakes discovered dead at the receiving port died due to the treatment or from other causes. Third, it would provide additional information on the circumstances in which snakes enter cargo refugia on Guam instead of trying to infer those details once snakes are found in receiving ports. This would augment the rather sparse data available on cargo-refuge use by snakes obtained using canine teams (Vice and Vice 2004) and could potentially hone interdiction efforts.

Author contributions

FK and RS designed the research and collected and analyzed the data; WP obtained funding for the research; FK wrote the manuscript; all authors read, edited, and approved the manuscript.

Acknowledgments This study was approved as NWRC Protocol QA-1837 and funded by the Department of Interior's Office for Insular Affairs. Support for initial work on this project was provided to FK by Mississippi State University's Department of Wildlife, Fisheries & Aquaculture. We thank C. Clark, J. Guerrero, T. Salas, and D. Vice for assistance on Guam, and the AAFB snake crew for providing us with test animals and for their kind tolerance of our invasion of their work space.

References

- Brattstrom BH (1965) Body temperatures of reptiles. *Am Midl Nat* 73:376–422
- Brown Tree Snake Control Committee (1996) The brown tree snake control plan. Final report. Aquatic Nuisance Species Task Force, Brown Tree Snake Control Committee, U.S. Fish and Wildlife Service, Honolulu
- Burnett K, Kaiser B, Pitafi BA, Roumasset R (2006) Prevention, eradication, and containment of invasive species: illustrations from Hawaii. *Agric Resour Econ Rev* 35:63–77
- Campbell GS (1977) An introduction to environmental biophysics. Springer, New York
- Christy MT, Savidge JA, Bischof R, Rodda GH (2007) Can temperature be used as a tool for limiting brown treesnake invasion via transportation pathways? In: Witmer GW, Pitt WC, Fagerstone KA (eds) Managing vertebrate invasive species: proceedings of an international symposium. USDA/APHIS/Wildlife Services, National Wildlife Research Center, Fort Collins, pp 246–256
- Clark L, Shivik J (2002) Aerosolized essential oils and individual natural product compounds as brown treesnake repellents. *Pest Manag Sci* 58:775–783
- Engeman RM, Vice DS (2002) Objectives and integrated approaches for the control of brown tree snakes. *Integr Pest Manag Rev* 6:59–76
- Engeman RM, Vice DS, Rodriguez DV, Gruver KS, Santos WS, Pitzler ME (1998) Effectiveness of detector dogs for locating brown tree snakes in cargo. *Pac Conserv Biol* 4:256–260
- Engeman RM, Vice DS, York D, Gruver KS (2002) Sustained evaluation of the effectiveness of detector dogs for locating brown tree snakes in cargo outbound from Guam. *Intern Biodeterior Biodegrad* 49:101–106
- Fritts TH (1988) The brown tree snake, *Boiga irregularis*, a threat to Pacific islands. *US Fish Wildl Serv Biol Rep* 88(31):1–36
- Fritts TH, Chiszar D (1999) Snakes on electrical transmission lines: patterns, causes, and strategies for reducing electrical outages due to snakes. In: Rodda GH, Sawai Y, Chiszar D, Tanaka H (eds) Problem snake management: the habu and brown tree-snake. Comstock Publ, Ithaca, pp 89–103
- Fritts TH, McCoid MJ (1991) Predation by the brown tree snake *Boiga irregularis* on poultry and other domesticated animals in Guam. *Snake* 23:75–80
- Fritts TH, McCoid MJ (1999) The threat to humans from snakebite by snakes of the genus *Boiga* based on data from Guam and other areas. In: Rodda GH, Sawai Y, Chiszar D, Tanaka H (eds) Problem snake management: the habu and brown treesnake. Comstock Publ, Ithaca, pp 116–127
- Fritts TH, Rodda GH (1998) The role of introduced species in the degradation of island ecosystems: a case history of Guam. *Ann Rev Ecol Syst* 29:113–140
- Fritts TH, Scott NJ Jr, Savidge JA (1987) Activity of the arboreal brown tree snake (*Boiga irregularis*) on Guam as determined by electrical outages. *Snake* 19:51–58
- Fritts TH, McCoid MJ, Haddock RL (1990) Risks to infants on Guam from bites of the brown tree snake (*Boiga irregularis*). *Am J Trop Med Hyg* 42:607–611
- Fritts TH, McCoid MJ, Haddock RL (1994) Symptoms and circumstances associated with bites by the brown tree snake (Colubridae: *Boiga irregularis*) on Guam. *J Herpetol* 28:27–33
- Heatwole H (1976) Reptile ecology. University of Queensland Press, Brisbane
- Huey RB, Stevenson RD (1979) Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Amer Zool* 19:357–366
- Nishimura M (1999) Repellents and use of prey items for delivering toxicants for control of habu (*Trimeresurus flavoviridis*). In: Rodda GH, Sawai Y, Chiszar D, Tanaka H (eds) Problem Snake Management: Habu and Brown Tree Snake Examples. Cornell University Press, Ithaca, pp 158–167
- Olmsted J III, Williams GM (1997) Chemistry: the molecular science. William C. Brown Publishers, Dubuque
- Perry G, Vice DS (2007) An evaluation of passive thermal fumigation from brown treesnake control in surface transportation from Guam. In: Witmer GW, Pitt WC, Fagerstone KA (eds) Managing vertebrate invasive species: proceedings of an international symposium. USDA/APHIS/Wildlife Services, National Wildlife Research Center, Fort Collins, pp 224–233
- Robertson JM (2011) Status report: the Guam military buildup. *Mil Eng* 672:73–74
- Rodda GH, Savidge JA (2007) Biology and impacts of Pacific Island invasive species. 2. *Boiga irregularis*, the brown tree snake (Reptilia: Colubridae). *Pac Sci* 61:307–324
- Rodda GH, Fritts TH, Chiszar D (1997) The disappearance of Guam's wildlife: new insights for herpetology, evolutionary ecology, and conservation. *BioSci* 47:565–574

- Savidge JA (1987) The ecological and economic impacts of an introduced snake on Guam and its threat to other Pacific islands. *Pac Life Environ Stud* 3:29–34
- Shwiff SA, Gebhardt K, Kirkpatrick KN, Shwiff SS (2010) Potential economic damage from introduction of brown treesnakes, *Boiga irregularis* (Reptilia: Colubridae), to the islands of Hawai'i. *Pac Sci* 64:1–10
- Vice DS, Vice DL (2004) Characteristics of brown treesnakes *Boiga irregularis* removed from Guam's transportation network. *Pac Conserv Biol* 10:216–220
- Vice DS, Engeman RM, Hall MA, Clark CS (2009) Working dogs: the last line of defense for preventing dispersal of brown treesnakes from Guam. In: Helton WS (ed) *Canine ergonomics: the science of working dogs*. CRC Press, Boca Raton, pp 195–204