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Repellent Tools for Invasive Species Control in Military Cargo

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Executive Summary

The Department of Defense is tasked with the logistics of supporting military readiness concurrent with protecting natural resources by not inadvertently transporting invasive species via shipments of cargo and personnel. No suitable chemical repellent tool is available to prevent invasive brown treesnakes (*Boiga irregularis*) from entering cargo or to forcibly evict snakes from shipping containers at U.S bases in Guam where exotic brown treesnakes are currently found. Three formulations of isophorone repellents were tested upon live brown treesnakes, cane toads (*Bufo marinus*), giant African land snails (*Achatina fulica*), and house mice (*Mus musculus*) to investigate isophorone efficacy for deterring wildlife movements into cargo and for repelling wildlife out of cargo. Isophorone epoxy paint reduced snake, toad, and mouse entrances into treated shipping containers, whereas isophorone soaked pads and granular formulations did not affect animal movements. Isophorone epoxy paint reduced brown treesnakes entering cargo containers by 23%. However, the small repellent effect on snakes would not provide an effective alternative to current interdiction methods. Isophorone would need to be integrated with other control and exclusionary methods to be an operationally effective management tool. Further, the effectiveness of the product over time, the cost of reapplication, and the cost effectiveness of this approach would have to be evaluated before this method could be adopted operationally.

Background

Guam is home to the Joint Region Marianas, Andersen Air Force Base and Naval Base Guam, as well as one of the most destructive non-native species known, the brown treesnake (*Boiga irregularis*). This accidentally introduced species has exterminated nearly all of Guam's native vertebrates and resulted in substantial economic losses, primarily as a result of Guam's strong dependence on transportation.

Control and containment of the brown treesnake is integral to facilitating the Department of Defense's operational readiness throughout the Pacific region. The most important management objective remains preventing snakes from entering the transportation system and dispersing to other at risk areas in the Mariana Islands and beyond. Brown treesnakes are

common in and around cargo staging areas on Naval Base Guam, Andersen AFB, and the commercial port. Apra Harbor, where Naval Base Guam and the commercial port are located, handles about 2 million tons of cargo a year, a figure that will increase dramatically with the continued military expansion on Guam. Snakes occasionally arrive in these outdoor staging sites and warehouses within material destined to be shipped out (e.g., military household moves), but more often, they enter directly from surrounding areas, taking daytime refuge in outbound cargo and associated facilities. A significant amount of cargo from Navy Base Guam/Andersen is loaded into shipping containers (e.g., CONEX, MILVAN,) at the naval wharves, and when full, containers are sealed and loaded for ocean transport (figure 1). The Marine Corps, upon relocation to Guam, will likely use ISO type containers for support on Guam and for off-island training activities. A significant amount of cargo from Naval Base Guam/Andersen is also transported by air, but containers are not generally used. Air cargo is instead placed on pallets or slip-sheets that can be shrink-wrapped or netted (figures 2 and 3).

Live trapping and spotlight searches of cargo staging areas by USDA Wildlife Services Guam are central to intercepting snakes before they enter cargo staging areas. Once inside these areas, detection relies almost solely on use of USDA snake detector dogs. Chemical methods for preventing snakes from entering into cargo would complement and enhance the existing interdiction program. No chemical repellents have yet been identified that adequately deter brown treesnakes from entering cargo areas, entering the cargo inside these areas, or eliciting exit of cargo by snakes already hiding in cargo. Clark and Shivik (2002) identified 7 essential oils, 4 listed in EPA FIFRA table 25b, that elicited strong locomotory responses from brown treesnakes. The 4 listed essential oils beginning with the most effective were: cinnamon oil, cedarwood oil, rosemary oil, and citronella oil. However, neither these nor additional chemicals have yet been tested for efficacy in preventing entry or forcing brown treesnakes from cargo containers. In this study we will be investigating isophorone as a chemical deterrent/repellent for brown treesnakes. Isophorone is a commercially available nuisance wildlife deterrent reported to repel Cuban treefrogs and perhaps other species (Johnson et al. 2010).

We investigated isophorone products manufactured under the brand name of “Sniff’n’Stop”. We evaluated the capability of three isophorone formulations to achieve two objectives: (1) prevent snakes from entering cargo, and (2) prevent snakes from entering cargo areas. In

addition to brown treesnake, we also evaluated isophorone repellents upon the following three invasive species: cane toads (*Bufo marinus*), African giant land snails (*Achatina fulica*), and house mice (*Mus musculus*) (figure 4). Amphibians, mice, and snails are all known to respond to airborne chemical stimuli (Brondeau et al. 1990, Chase and Boulanger 1978, Johnson et al. 2010, R.L. Doty 1986, Smith and Boswell 1970).

Methods

Isophorone formulations have not yet been evaluated specifically for repelling brown treesnakes. We investigated three isophorone formulations manufactured by Sniff'n'Stop©: epoxy paint, impregnated pads, and a granular formulation. All three isophorone formulations were tested outdoors under natural conditions. Isophorone has a molecular weight of 138.21 g/mole, is airborne at standard temperature and pressure, and thus should be detectable via classical olfaction, and perhaps via the vomeronasal systems of the snake, mouse, and toad. Work was conducted in the field on Guam from mid-August to mid-September 2010 using four invasive species in the Pacific region: brown treesnakes, cane toads, giant African land snails, and house mice. Total number of individuals tested of each species was: 255 brown tree snakes (120 paint, 135 pads, and 35 granules), 40 house mice, 20 cane toads, and 20 African giant land snails.

To test objective 1, we used two-part epoxy paint and pads containing isophorone. Objective 2 was tested using a barrier strip of isophorone impregnated granules. However, instead of placing a barrier around a cargo area we placed the barrier along a cargo doorway and assessed if brown treesnakes were reluctant to cross the barrier to escape the container. Paint containing isophorone was tested on all four species, whereas the isophorone treated pads and granules were only tested upon brown treesnakes.

The following experimental apparatus was constructed to test objective 1 using isophorone epoxy paint. Isophorone, in a two-part epoxy paint, was applied to the inside of a standard 20-ft intermodal shipping container. A second adjacent control container was painted with the carrier (figure 5). Twenty separate mesh tubes were suspended between two metal shipping containers. The end of each tube entered inside each container and was capped. An entrance port on each tube at midpoint between the two containers (outside) allowed us to place a test

subject into each tube. Subjects were placed in tubes in the evening. Subjects could choose which container to take refuge within and these data were recorded the following morning. Because all 4 species used are primarily nocturnal, most individuals were expected to take refuge in a container before dawn, rather than remain in its tube outside containers.

An isophorone pad testing apparatus was constructed using four pairs of large cardboard boxes with interconnecting mesh tubes (figure 6). Treatment boxes contained isophorone pads placed at the end of each tube. Control boxes did not have pads. Individual snakes (9 per night) were placed within the outside tubes at dusk and the locations of the test subjects were recorded in the morning.

Four trials of 12 snakes were conducted to assess the repellent potential of the granular formulation. Isophorone granules were laid out as a “barrier” strip that snakes were given the choice to cross or not cross (figure 7). The barrier strip was 55cm wide and 5-10 mm deep. Brown treesnakes were held in restraining tubes and released inside the CONEX at night while a door across from the barrier strip was left open 25 cm (figure 8). Brown treesnake presence or absence from the container was recorded in the morning. Snake aversion or indifference to the granular isophorone strip was recorded with an IR-illuminated video camera.

Data were analyzed separately by species and formulation type using SYSTAT software. Chi-squared tests were used to assess species response to the treatments and Fisher exact tests were used to analyze potential associations between snake gender and treatment responses. Other data will be presented as summary statistics. Test subjects that remained outside the treatment and control containers were not included in the statistical analysis, as it is uncertain if they encountered and subsequently responded to the isophorone material.

Results

<u>Isophorone 2-part epoxy paint</u>

We placed 120 brown treesnakes (20 per night) into the isophorone-painted metal shipping container test matrix from August 24th to August 31st, 2010. Forty-four snakes sought daytime refuge within the treated containers, 69 were in the control containers, 6 were outside within

the tubes and one snake had either escaped or evaded detection (table 1). The isophorone paint significantly reduced the number of snakes seeking refuge within the treatment container (χ^2, p = 0.019). Six snakes remained outside within the tube "T's" where they were released. It is uncertain if these snakes moved following emplacement. There was not a significant association between snake gender and response to the isophorone paint (χ^2, p = 0.279).

We tested 40 house mice (20 each night) for their response to isophorone paint on September 7th and 9th 2010. This test proved technically difficult in the field setting, as 5 tubes were knocked down by a feral cat during the second trial and those mice were therefore not included in the trial. Of the remaining 35 mice, significantly more mice (n = 21) were within the control container in the morning than were inside the treatment container (6, χ^2, p = 0.004).

We tested 20 cane toads for their response to the isophorone paint on September 1st and 4th 2010. The same 20 toads were used in four trials on 4 consecutive nights. Cane toads proved to be quite sedentary, with the majority of responses (44 of 75) being to remain at the release point. Five toads escaped their enclosures. Of the remaining toads that were actively exploring the experimental structure, significantly more (23 of 75) sought refuge within the control containers than in the isophorone treated containers (8 of 75, χ^2, p = 0.007).

We tested 20 giant African land snails for their response to the isophorone paint on September 2nd, 10th, and 11th 2010. The same 20 land snails were used in 3 trials on 3 non-consecutive nights. Giant African land snails also proved to be sedentary, as 29 of 60 were still located at the release site the following morning. One snail escaped its enclosure. Of the remaining snails that were actively exploring the experimental structure, significantly more (24 of 60) sought refuge within the isophorone treatment containers rather than in the control containers (6 of 60) (χ^2, p = 0.001).

<u>Isophorone saturated pads</u>

We introduced 135 brown treesnakes (9 per night) into the isophorone pad testing apparatus from September 4th to September 18st, 2010. All snakes choose either the control or the treatment refuge and there were no escapees (table 1). Sixty-three snakes sought refuge in the isophorone treatment boxes and 72 were found in the control boxes. There was not a

significant refuge selection difference induced by the isophorone pads (χ^2, p = 0.439). There was not a significant association between snake gender and responses to the isophorone pads (χ^2, p = 0.169).

Isophorone granule barrier

We used 47 brown treesnakes to test the capability of isophorone granules to create a barrier to brown treesnake egress from metal cargo containers. The snakes were released within the test container on 4 consecutive nights (12 per night). Twelve of the snakes were used as a control and all 12 left the container by morning. Next, three trials assessed whether brown treesnakes would cross a 55cm wide isophorone granule barrier and leave a metal shipping container with a partially open door (25cm).

The sampling during the first night was compromised by a feral cat. The strip of granules was disturbed and a chewed snake carcass was within the container. The remaining 11 snakes had left the container. Data for this night was removed from analysis due to the negative motivation for remaining within the container with a feral cat. The cat was trapped before conducting the next trial. During the next two trials (11 and 12 snakes, respectively) all 23 snakes exited the container by morning.

Discussion

The 2-part epoxy paint containing isophorone significantly affected refugia selection by all 4 species. Snakes, mice, and toads were all significantly less likely to select isophorone painted refugia than the controls whereas snails were attracted by the treatment paint. The 2-part epoxy paint carrier for isophorone likely had no effect on the test results, as controls were also painted with non-isophorone 2-part epoxy paint. The isophorone paint stimulus elicited only a marginal aversive response from the snakes (44 versus 68), but more strongly repelled mice (6 versus 21) and toads (8 versus 23, table 1).

Overall 39% of brown treesnakes were in the isophorone treated container in the morning and 61% were in the control. Isophorone reduced brown treesnakes entering cargo containers by 23%. We did not test the effectiveness of the product over an extended period of time, but repellency would likely decrease as volatile chemicals, responsible for the aversive response,

are dissipated. We found no association between snake gender and responses to the isophorone paint and pads. The gender of the test subjects was only determined for brown treesnakes.

Neither the isophorone pads nor the granules were effective in repelling brown treesnakes. Isophorone pads were ineffective at preventing snakes from entering simulated cargo containers. Some of the tested snakes entered treatment containers and sought refuge at the end of perforated tubes right up against the isophorone pads (see figure 6, lower photo). Isophorone granules were not effective at preventing brown treesnakes from exiting cargo containers and thus would not be a suitable material to use as a chemical barrier preventing snakes from entering cargo staging areas.

Significantly more mice sought refuge within the control container than in the isophorone epoxy paint treated container. The presence of the feral cat did not impact mouse response to the isophorone treatment. On the night that the cat knocked down 5 of 20 experimental tubes 2 mice sought refuge in the treatment container and 11 were in the painted control, whereas on the cat-free night 4 mice were in the treatment refuge while 10 were in the control refuge. Overall 22% of mice were in the isophorone treated container in the morning and 78% were in the control. Thus, there was a 56% reduction in mice entering cargo containers treated with isophorone as compared to a no-effect 50-50 test result.

Seventy-four percent of cane toads took refuge within the control container. This 3:1 result is the same as seen in Cuban treefrog isophorone deterrent tests by Johnson et al. 2010. It is notable that the majority of toads did not leave the point of release during the first night. It may prove difficult to find a chemical repellent capable of eliciting the exit of cane toads that have already found refuge within cargo shipments.

Snails were 4 times more likely to enter the isophorone treated container than the epoxy paint control. Isophorone epoxy paint appeared to act as an attractant for the giant African land snails. Snail baits used by researchers are generally food related such as beer or sugar and yeast mixtures or cabbage, taro, and papaya leaves (Smith and Boswell 1970, Vanitha et al. 2008). Snails have also been reportedly attracted to sheets of newspaper laid on the ground,

but it is unknown if they are attracted to the paper or the ink (Joshi and Cruz 2001). There is no known record of snail attraction to isophorone epoxy paint, although other invertebrates have shown an attraction to isophorone as an indicator of potential mates or food supply or hosts (Ingvarsdottir et al. 2002, Ishida et al. 2008). It may be fruitful to continue this line of research to investigate using isophorone epoxy paint in snail traps for the benefit of farmers. The giant African land snail is an economically destructive introduced pest in many tropical localities, including Hawaii and other Pacific islands (Mead 1979, Karnatak et al. 1998, Raut and Barker 2002), with limited technology available for controlling their populations.

The benefit of applying isophorone paints alone to all shipping containers leaving Guam to inhibit brown tree snake dispersal (23% reduction) would not justify the initial costs and maintenance of the deterrent treatment since the small repellent effect on snakes would not provide an effective alternative to current interdiction methods. Further, it is unclear how long this effect would last as the paint ages and volatile compounds are lost. An economic analysis would need to be performed to determine the cost/benefit of painting the inside of cargo containers with isophorone paint and requirements for reapplication. Isophorone deterrents alone, although promising, would not be profoundly effective in preventing brown tree snakes, house mice, and cane toads from entering cargo, and if used would need to be integrated with other quarantine measures to maximize their benefit.

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Table 1. Summary table of species responses to isophorone treatments. Granules were not a deterrent to exiting container. Majority of toads and snails that were sedentary during the trials. Also note that the giant African snails showed an affinity for the isophorone paint.

Organism	Isophorone Treatment	# Animals tested	Selected refuge type				repeated measures
			In Treatment	In Control	Outside	Escape	
Snake	Paint	120	44	69	6	1	no
Snake	Pads	135	63	72	0	0	no
Snake	Granules	23	0	-	23	-	no
Mouse	Paint	40	6	21	6	3	no
Toad	Paint	20	8	23	44	5	yes
Snail	Paint	20	24	6	29	1	yes

Table 2. Statistical results for species responses to isophorone treatments. Isophorone epoxy paint significantly affected refuge selection for all 4 species. Isophorone impregnated pads and granules had no effect on brown tree snake movements. All snakes crossed over the granule barrier and exited shipping container.

Organism	Isophorone Treatment	Selected refuge				Chi Square p-value	repeated measures?
		Actual		expected (H_o)			
		In Treatment	In Control	In Treatment	In Control		
snake	paint	44	69	56.5	56.5	**0.019**	No
snake	pads	63	72	67.5	67.5	0.439	No
snake	granules	0	23	23	0	1.000	No
mouse	paint	6	21	13	13	**0.004**	no
toad	paint	8	23	15.5	15.5	**0.007**	Yes
snail	paint	24	6	15	15	**0.001**	Yes

Table 3. No significant association was observed between snake gender and isophorone response.

Snake	Isophorone Treatment	Selected refuge				Chi Square p-value	Fisher exact test
		Actual		expected (H_o)			
		Treatment	Control	Treatment	Control		
male	paint	25	32	22.2	34.8	0.279	0.336
female	paint	19	37	21.8	34.8		
male	pads	35	31	30.8	35.2	0.147	0.169
female	pads	28	41	32.2	36.8		

Figure 1. Typical CONEX shipping container

Figure 2. Fully-loaded 463L pallet, plastic wrapped and exposed to elements.

Figure 3. Fully-loaded 463L pallet being loaded onto military aircraft.

Figure 4. Photos of species tested.

Figure 5. Standard 20-ft intermodal shipping containers. One container's inside surfaces were painted with Sniff'n'Stop© isophorone epoxy paint and the other container was painted with just the carrier epoxy. Snakes were free to move within mesh tubes and choose to seek daytime refuge inside either darkened container or remain outside.

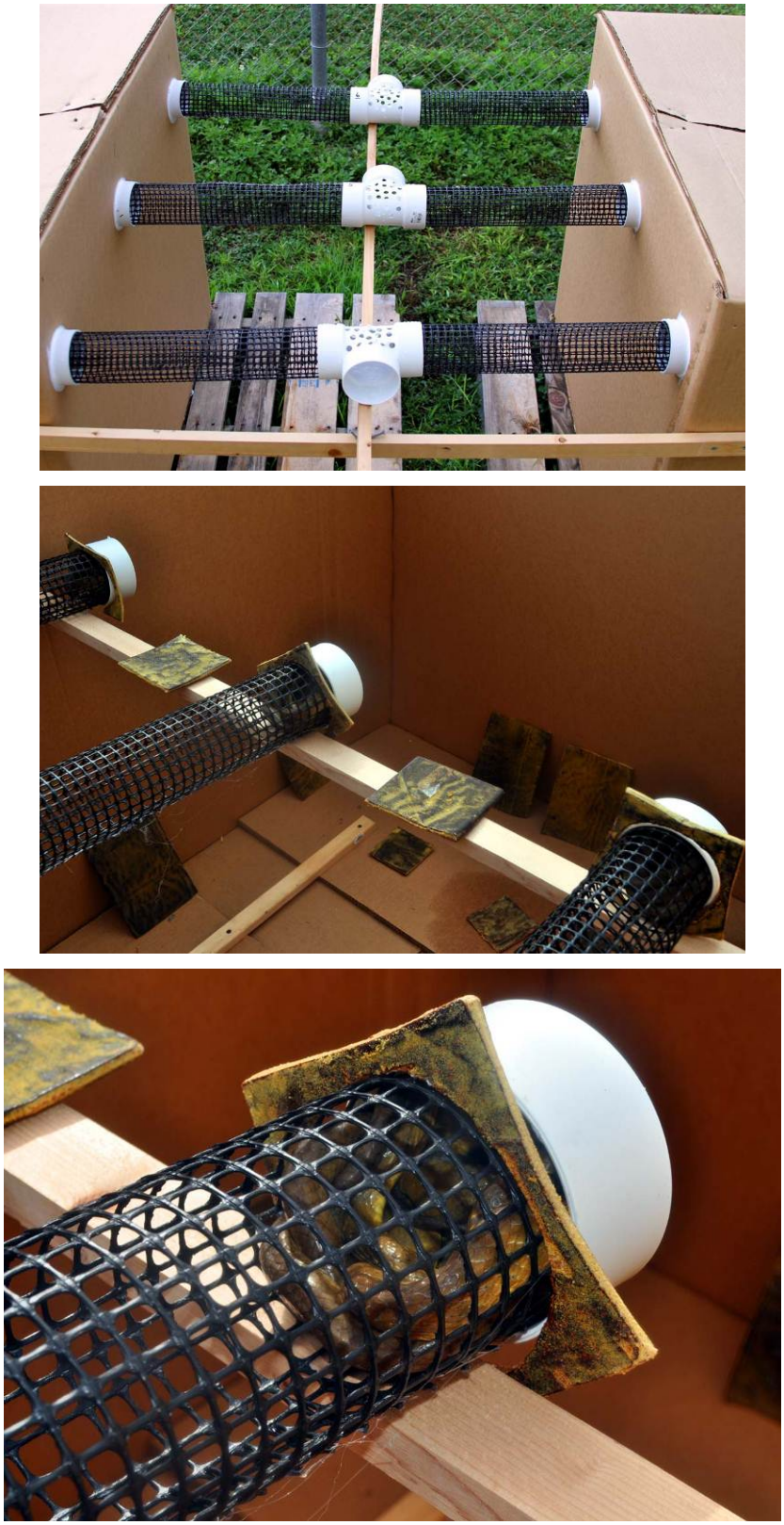

Figure 6. Experimental design for evaluating isophorone impregnated pads using 4 pairs of large cardboard boxes.

Figure 7. Barrier type isophorone treatment.

Figure 8. Brown tree snakes used to test barrier type isophorone treatment.

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