

# ***In situ* evaluation of an automated aerial bait delivery system for landscape-scale control of invasive brown treesnakes on Guam**

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**Abstract** After decades of biodiversity loss and economic burden caused by the brown treesnake invasion on the Pacific island of Guam, relief hovers on the horizon. Previous work by USDA Wildlife Services (WS) and its National Wildlife Research Center (NWRC) demonstrated that brown treesnake numbers in forested habitats can be dramatically suppressed by aerial delivery of dead newborn mouse (DNM) baits treated with 80 mg of acetaminophen. However, manual bait preparation and application is impractical for landscape-scale treatment. WS, NWRC, and the US Department of the Interior have collaborated with Applied Design Corporation to engineer an automated bait manufacturing and delivery system. The core technology is an aerially delivered biodegradable “bait cartridge” designed to tangle in the tree canopy, making the acetaminophen bait available to treesnakes and out of reach of terrestrial non-target organisms. When mounted on a rotary- or fixed-wing airframe, the automated dispensing module (ADM) unit can broadcast 3,600 bait cartridges at a rate of four per second and can treat 30 hectares of forest at a density of 120 acetaminophen baits per hectare within 15 minutes of firing time. We conducted the first *in situ* evaluation of the ADM in July 2016. Initial acetaminophen bait deployment rates (proper opening of the bait cartridge for canopy entanglement) were low, and mechanism jams were frequent due to internal friction and wind forces; on-site remedial engineering improved these performance measures. Bait cartridge placement and spacing were accurate (average 8.9 m along 9 m swaths) under various flight heights and speeds. Canopy entanglement of properly-deployed acetaminophen baits was high (66.6%). Although only a small proportion (5.9%) of radio transmitter-equipped acetaminophen baits were confirmed to have been taken by brown treesnakes, the baiting density was high enough to make it likely that a significant proportion of brown treesnakes in the area had taken acetaminophen baits. With subsequent improvements in system reliability, the automated bait cartridge manufacturing and delivery system is poised to transition from research and development to operational field implementation. Applications include reduction of brown treesnake numbers around transportation infrastructure and within core habitats for the reintroduction of native birds extirpated by this troublesome invasive predator.

**Keywords:** invasive species suppression, invasive vertebrate predator, public-private partnership, scaling up, technical innovation, toxic baits

## **INTRODUCTION**

The brown treesnake (*Boiga irregularis*) is a nocturnal, arboreal predator that was probably introduced on the island of Guam after World War II as a passive stowaway in cargo from the Admiralty Islands north of New Guinea (Rodda & Savidge, 2007; Richmond, et al., 2014). Lacking natural predators on Guam, the population of brown treesnakes irrupted, reaching as many as 50–100 brown treesnakes per hectare in some areas (Rodda, et al., 1999). Brown treesnakes colonised the entire island of Guam (54,930 ha) in about 20–30 years (Savidge, 1987). The brown treesnake has been – and continues to be – a threat to the economy and ecology of Guam, and is currently the subject of a cooperative programme to control brown treesnake populations on the island and prevent its spread throughout the Pacific Basin and other vulnerable locations (Clark, et al., 2018). Owing to the significant ecological and economic damages caused by the brown treesnake on Guam, the potential for the brown treesnake to be spread to other Pacific Islands, including Hawai‘i, is of great concern (Shwiff, et al., 2010).

Landscape-scale suppression of brown treesnakes is desirable in habitats adjacent to transportation network infrastructure (e.g., cargo terminals), to reduce the risk of accidental transport to other vulnerable ecosystems, and within key habitats for the recovery of Guam’s native wildlife. Because of the great amount of inaccessible and topographically challenging forest habitat on Guam, aerial delivery of brown treesnake suppression tools is key to the management of this species on a landscape scale. Dead newborn mice (DNM) dosed with 80 mg of acetaminophen have proven to be safe and effective baits for lethal control

of brown treesnakes (Savarie, et al., 2001; Johnston, et al., 2002; Clark, et al., 2012) and are registered with the US Environmental Protection Agency (EPA) as an approved pesticide (Registration No. 56228-24, Revised 06/2018). To be effectively delivered to the forest canopy where they are available to foraging brown treesnakes and inaccessible by terrestrial non-targets, the baits must be coupled with a ‘flotation device’ intended to entangle in foliage (Savarie & Tope, 2004).

Through a previous project, the US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Wildlife Services National Wildlife Research Center (NWRC) has demonstrated that brown treesnake abundance in Guam’s forests can be suppressed via the aerial application of DNM baits adhered to paper streamers (Dorr, et al., 2016). During this prior study, baits were hand-prepared and hand-broadcast from a helicopter. While this method of treatment proved effective on a small scale (two 55 ha plots), manual bait preparation and application is economically impractical for larger landscape-scale treatments. In scaling up to meet the challenge of landscape-scale control of brown treesnakes, one of the principal logistical concerns is the obvious need to automate both bait production and the aerial dispensing of baits. In response to this need, NWRC, primarily funded by the US Department of the Interior Office of Insular Affairs, has partnered with a private, small business engineering company (Applied Design Corporation, Boulder, Colorado) to develop a brown treesnake suppression system that offers the capability to achieve precise distribution of thousands of baits in a matter of minutes, through a fully-integrated

solution that encompasses bait cartridge production, an aerial bait cartridge dispensing system, and supporting infrastructure and logistics for practical manufacturing, storing, and flight-line handling of bait cartridges.

### Automated Bait Manufacturing System (ABMS)

Many of the functional details of the three-stage ABMS are currently considered proprietary information pending application for US and foreign patent protection. The descriptions provided below will suffice as a basic functional explanation.

The first of three bait cartridge manufacturing stations is the Gluer/Placer Station (Station 1) where the DNM are distributed on moulded pulp paper trays and an 80 mg acetaminophen tablet is adhered to the DNM via a hot-melt adhesive. At the final stage of Station 1, the individual capsules containing the acetaminophen tablet and DNM are cut from the paper trays and fed into a transport cassette for transfer to the Assembly/Winder Station (Station 2). Hereafter, a DNM with an adhered acetaminophen tablet will be referred to as an “acetaminophen bait”.

At Station 2, the capsule is folded and held closed by pinching at the paper hinge between the two capsule halves. This pinched paper hinge, hereafter referred to as the ‘tang,’ is inserted into a slotted pressed pulp paper end cap. One end of a biodegradable plastic ribbon is adhered to the endcap and the entire assembly is rotated until the ribbon is wound around the length of the capsule in a ‘barber pole’ fashion. The terminal end of the ribbon is then adhered to the paper capsule. An exterior cardboard tube is placed over the wound assembly, with the end cap tightly pressed into the tube; this entire resulting assembly, comprised of the acetaminophen bait, capsule, streamer, and end cap, enclosed within the external tube, is referred to as a “bait cartridge”. The entire bait cartridge (Fig. 1) is biodegradable.

The final manufacturing station is Packaging (Station 3). Completed bait cartridges are automatically fed to the packaging station, where they are gathered and placed into a corrugated plastic case (900 bait cartridges per case). Filled cases are shrink wrapped, placed on a shipping pallet, and frozen. A complete pallet of 40 cases holds 36,000 bait cartridges, enough to treat 300 ha at the current EPA-approved maximum application rate of 120 acetaminophen baits/ha.

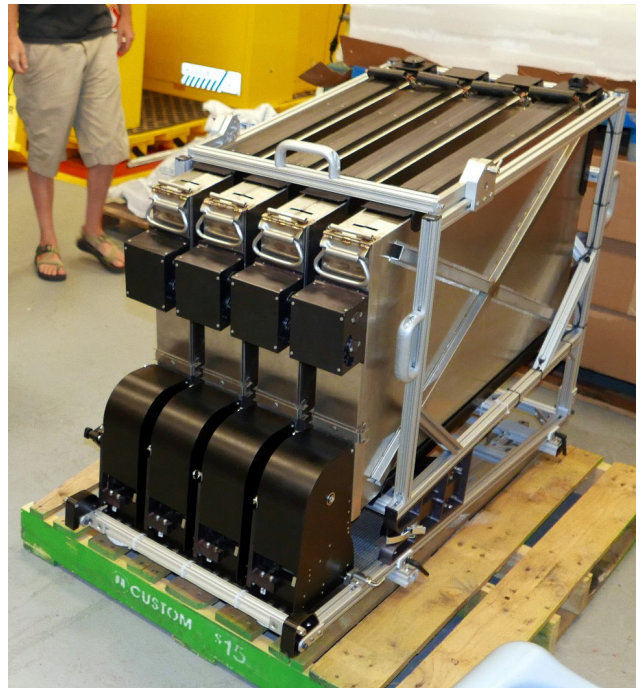


**Fig. 1** When deployed, the bait capsule and outer bait cartridge tube are joined by a length of unfurled ribbon intended to entangle in the forest canopy when applied aerially.

### Automated Dispensing Module (ADM)

The Automated Dispensing Module (ADM; Fig. 2) is comprised of three main components: 1) four magazines; 2) an electro-mechanical firing unit on a tilt-plate; and 3) a frame, which holds the power supply battery, the computer control module, and integrates the other components into a single functional ADM. The frame is mounted within the hold of the aircraft.

Each magazine is comprised of a body with two halves hinged at the back, allowing the payload area to be fully exposed, and a faceplate. The opened magazine receives the contents of one case (900 bait cartridges). Upon loading, the bait cartridges receive a final inspection for manufacturing imperfections or shipping damage which may adversely affect smooth feeding through the magazine and into the firing unit (Fig. 3).



**Fig. 2** The ADM is comprised of four firing units and four 900-cartridge magazines along with an onboard battery and control electronics (not visible).



**Fig. 3** Bait cartridges are inspected for manufacturing imperfections or shipping damage that might impede smooth feeding and ejection.

A magazine can be loaded with a case of bait cartridges and prepared for flight in two to three minutes. Once four magazines are prepared, they are loaded into the aircraft-mounted ADM frame (Figs. 4 and 5). The bait cartridge exit door on each magazine is then opened, allowing bait cartridges to flow into the firing unit feed chute. A full payload of 3,600 bait cartridges is sufficient to treat 30 ha of forest at the maximum application rate. At full performance, this area can be treated at 120 acetaminophen baits/ha within 15 minutes of firing time (Fig. 6). An additional set of magazines allows for the next payload to be prepared while the current payload is being applied.

A payload manager and the pilot are the only personnel aboard the aircraft. As directed by the payload management software, the computer control module engages the firing units within the ADM to fire bait cartridges at the proper rate to match the aircraft's current ground speed and intended acetaminophen bait application rate. The payload management software detects when a port is jammed or a magazine is empty and increases the firing rate of the other three ports to maintain the desired bait cartridge delivery rate.



**Fig. 4** Magazines are loaded into the helicopter-mounted ADM frame.



**Fig. 5** Complete ADM with loaded magazines mounted in a McDonnell-Douglas MD 500D helicopter.

Aerial navigation is achieved by following a preprogrammed mission plan in the payload management software, which details the transects to be flown. The pilot is provided with an LCD display, a “virtual lightbar,” that provides realtime feedback as to whether the aircraft is on the prescribed flight path and what corrective movements are needed to return to the path. The payload manager manually toggles on bait cartridge firing when over the treatment area, and toggles it off when the flight path is complete. After an ‘ag turn’ (an aerial maneuver to quickly reverse directions) the next flight path is flown in the opposite direction. This is repeated until the payload is expended or the treatment area has been fully covered.

### Objectives

This report describes the first *in situ* evaluation of this system through the experimental treatment of 110 hectares of forest on Guam. The major objectives were to evaluate: 1) the ground support work flow and performance of the automated dispensing module in-flight; 2) the precision of spatial coverage of the treatment area; and 3) the proper deployment of bait cartridges into the forest canopy and the fate of acetaminophen baits once distributed into the environment.

## MATERIALS AND METHODS

### Study site

The evaluation was conducted over 110 ha of secondary forest on the Marbo Annex of Andersen Air Force Base (typically referred to as “Andy South”) in Yigo, Guam, at approximately 13.508°N, 144.873°E. This site was selected because: 1) there is low risk to threatened or endangered species; 2) the habitat is representative of much of Guam’s forests; and 3) it is on a closed military facility with restricted public access.

### ADM performance

We assessed the performance of the ADM through two trial applications of acetaminophen baits, simulating operational applications for brown treesnake control. The first application was initially scheduled to be completed on 19 July 2016, during which 13,200 acetaminophen



**Fig. 6** Bait cartridges dispensed in flight.

baits would be applied over the 110 ha treatment area (120/ha). A second application was scheduled to occur three days later. For the purposes of this report, we define an “application” as a treatment of an area with aerially-distributed acetaminophen baits within the usage restrictions described in the EPA label.

A McDonnell-Douglas MD 500D (Fig. 5) and pilot were contracted from Hansen Helicopters (Tamuning, Guam) to perform aerial bait cartridge delivery. GoPro video cameras (GoPro, Inc., San Mateo, California) were positioned at various locations on the helicopter to document and evaluate bait cartridge ejection and deployment success.

On the night prior to flight operations, bait cartridge cases required for the next day’s application were removed from the freezer to thaw and were stored overnight in an air-conditioned workspace to minimise condensation. The plastic wrapping on the cases were left intact to ensure that all condensation would occur on the external surface of the plastic wrap rather than on the paper-based bait cartridges themselves.

### Bait cartridge coverage

Bait cartridge spacing trials were conducted to determine the accuracy and evenness of bait cartridge distribution at varying flight heights and airspeeds. Three lanes of approximately 200 m were delineated with orange traffic cones within an open grassy area at the treatment site. The helicopter, traveling at 50 knots, distributed bait cartridges along each flight line at heights of 25 m, 50 m, and 100 m above ground level. A ground crew attempted to locate all bait cartridges and measured their distance from the ideal flight path and the distance to the next bait cartridge along that path. A second round of transects was flown, this time at 60 knots, to determine the effect of airspeed on accuracy and spacing.

The completeness and the evenness of the spatial coverage of the treatment area was determined by recording the GPS flight paths in the payload management software, and generating coverage maps. Flight path segments were highlighted where the ADM unit was firing.

### Acetaminophen bait fate

Methods for monitoring of radio transmitter-equipped baits were modified from procedures established by Dorr, et al. (2016). During each treatment, a subset of baits was prepared containing small 1.0 g VHF radio transmitters (Holohil BD-2H with internal helical antennae, Holohil Systems Ltd., Carp, Ontario, Canada) implanted in the acetaminophen bait DNM. Transmitter-equipped bait cartridges were placed directly in the ADM firing port unit so that they would be deployed simultaneously at the beginning of the flight path, to be followed by bait cartridges without transmitters.

An acetaminophen bait is considered properly “deployed” when the inner capsule assembly slides out of the outer cardboard tube, unfurling the ribbon to allow entanglement in the forest canopy. While some acetaminophen baits may deploy on impact with treetops, the system is designed for the acetaminophen bait to deploy in the air immediately upon ejection of the bait cartridge from the ADM.

Immediately after being aerially distributed, field technicians with handheld VHF receivers located the transmitter-equipped baits and recorded: bait cartridge location, position (in tree/vegetation or on ground), type of vegetation the bait cartridge was suspended from, height above ground, whether the bait cartridge was actually seen or its location was estimated, whether the acetaminophen

bait was properly deployed and the DNM available for take by a brown treesnake, whether the acetaminophen tablet was still adhered to the mouse, and other notes about the circumstances of the condition and location of the acetaminophen bait and its availability for take by a brown treesnake.

If a DNM became separated from the bait cartridge and was on the ground but still had the acetaminophen tablet attached, it was considered intact and available for take by a brown treesnake. If the acetaminophen bait did not deploy properly and the DNM was not available to be taken, the bait cartridge and transmitter were recovered and that trial was ended. After deployment-day data were collected, the transmitter-equipped baits were left to determine the fate of acetaminophen baits over the next 48–72 hours. On each day following the application, each transmitter was re-located and the following data were recorded: whether the acetaminophen bait was still present and viable, whether the acetaminophen tablet was still attached, whether the acetaminophen bait was consumed by a brown treesnake or a non-target, whether the brown treesnake or non-target was alive or dead, whether the transmitter had moved to a new location, and other notes about acetaminophen bait location and condition.

If acetaminophen baits were unconsumed and still viable, they were left for another night and located again the next day. If acetaminophen baits had been consumed by a brown treesnake or non-target that was still alive, it was left undisturbed and relocated daily to establish survival or time to death. While tracking transmitters, technicians were alert for carcasses of any dead organisms, including those that had ingested transmitter-equipped baits. Global Positioning System (GPS) locations and notes on the location and condition of carcasses were recorded. Carcasses were collected and stored frozen for future analytical chemistry to verify acetaminophen exposure.

## RESULTS

### ADM performance

The first application of bait cartridges commenced on schedule on 19 July 2016. Ground operations and logistical support proceeded according to plan. However, crew and video observations indicated poor ADM performance in two primary categories: 1) bait cartridge feed/ejection reliability and 2) percentage of bait cartridges properly deploying in flight. These problems with system performance resulted in frequent flight stoppages to resolve bait cartridge jams and address other engineering challenges. As a result, additional flight days on 20, 22, 23, 25, and 26 July were required to achieve the first complete coverage of the treatment area.

Reliable bait cartridge ejection was hampered in three primary manners: 1) mechanical jams in the firing unit; 2) “starvation” of the firing unit feed ramp (bait cartridges not arriving at the firing position from the magazine); and 3) impediment of ejection by aerodynamic forces. These are not distinct processes, with multiple possible interactions among them. These issues were resolved with a variety of on-the-fly field improvements, with the causes and effects noted for future ADM design improvements.

Acetaminophen baits that do not deploy from the bait cartridge constitute a waste of resources (because the toxicant is inaccessible to snakes) and a fruitless toxic input into the environment. While we did not expect 100% deployment, observations by ground crew and video camera evidence indicated that initial acetaminophen bait deployment rates were unacceptably low at far less than 50%. Acetaminophen bait deployment issues generally fell

into two categories: inadequate rotational energy imparted by the firing unit to overcome external air resistance effects and internal friction between the sliding components of the bait cartridge.

Air resistance effects were largely mitigated by employing adjustable baffles near the firing unit ejection ports to disrupt ejection-inhibiting air currents. Internal friction effects were traced to excessive friction between the bait cartridge capsule ‘tang’ and end cap. As manufactured, the tang (folded paper hinge) of the interior clamshell is seated in the slot of the end cap to prevent rotation of the internal assembly during manufacturing and unwinding of the ribbon during shipping and handling. However, it was discovered that the tension of the ribbon wound around the clamshell capsule caused the inner assembly to rotate slightly and the tang to twist against the sides of the slot in the end cap. This friction, along with the taut wind of the ribbon, created a ‘locking’ force, holding the entire assembly together and resisting the available centrifugal force which would otherwise deploy the acetaminophen bait properly. We determined that tearing off the paper tang would relieve the friction against the end cap slot, greatly increasing the deployment rate. For the second application, all bait cartridges were prepared by manual removal of the paper tang.

After system modifications were made, the second application was re-scheduled for 29 July 2016 (three days after the completion of the first application in accordance with EPA label restrictions). During this application, bait cartridge ejection and acetaminophen bait deployment were far more reliable. Bait cartridge jams in firing ports were less frequent and were promptly cleared. The only significant delay occurred when an ejector unit bearing broke; a temporary bushing replacement was fabricated and the ADM was returned to service within a few hours. Aside from this stoppage, the entire second application was completed within 2.5 hours.

Even after the above-mentioned modifications, only 37.3% of acetaminophen baits (571 out of a sample of 1,528 bait cartridge ejections observed on video) deployed immediately, as intended. Bait cartridges could only reliably be observed for about a third of their trajectory to the canopy, and some certainly deployed lower in the airstream. Still more would have deployed upon impact with the canopy or the ground. Nonetheless, we determined that improvement is needed in the reliability of aerial deployment of acetaminophen baits. Though there is no way to be certain of the actual deployment rates, we presume the realised acetaminophen bait deployment rate to be <50% for the overall acetaminophen bait application period.

### Bait coverage

Bait cartridge placement and spacing was tested on 28 July 2016. Wind conditions during all flights were recorded at 0 to 1 on the Beaufort scale (0 = < 1 km/h, calm, smoke rises vertically; 1 = 1–5 km/h, light air, wind motion visible in smoke). When air movement was detectable, it was moving north to north-northwest. Flight direction was west to east or east to west. Bait cartridge distributions over trial flight paths are depicted in Fig. 7.

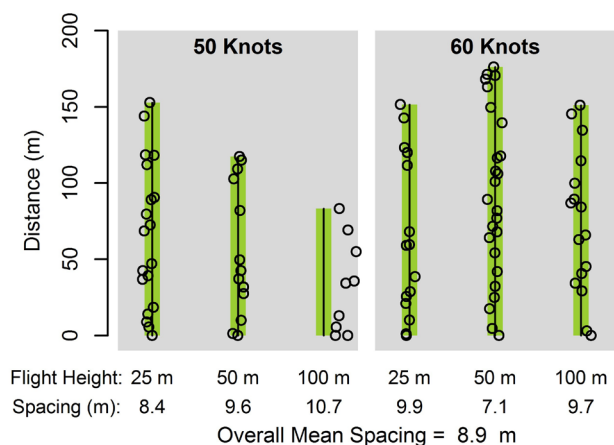
Placement along target flight paths and within 9-m swaths was very accurate and consistent. The one exception was the run at 100 m flight height at 50 knots airspeed; these results are inconsistent with the other five, and we consider this to be an anomalous lapse in pilot flight accuracy. Results do not appear to be influenced by the difference between 50 and 60 knots airspeed. Likewise, accuracy of placement along paths did not appear to be influenced by flight height. The most challenging combination of higher

flight speed (60 knots) and highest flight height (100 m) resulted in an acceptable distribution pattern. Spacing between bait cartridges along a given flight path was highly variable, but the mean overall spacing of 8.9 m was virtually identical to the target spacing of 9 m.

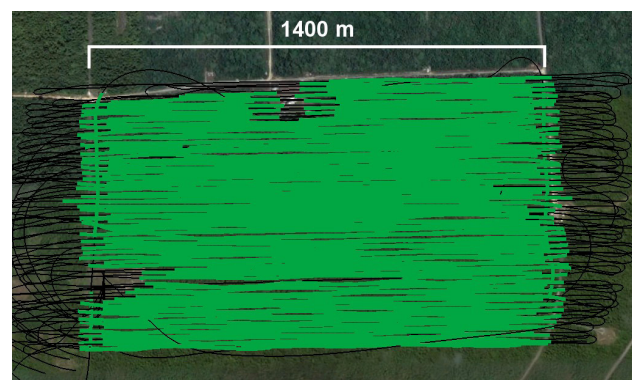
Due to frequent flight stoppages during the first application, the full site coverage was achieved piecemeal over several days, with the entire area being treated by the 6<sup>th</sup> flight day. While the appropriate number of bait cartridges was deployed, the evenness of transect spacing was of reduced importance compared to overcoming the engineering challenges. The second application of acetaminophen baits was relatively uninterrupted. Flight paths were flown as planned which, along with increased pilot and payload manager experience, resulted in a much more even treatment (Fig. 8).

### Acetaminophen bait fate

On 26 July 2016, 28 transmitter-equipped baits were broadcast over the treatment site. On 29 July 2016, an additional 23 were broadcast, for a total of 51 transmitter-equipped baits. The conditions of acetaminophen baits on the day of deployment are summarised in Table 1. Of the



**Fig. 7** Bait cartridge spacing and placement results. The centre line for each flight path indicates the target line, over which the pilot flew and bait cartridges were dispensed. Green boxes around the centre lines indicate 4.5 m on each side of the centre line, for a 9 m swath (the ideal flight path spacing for applications at 120 baits/ha). ‘Spacing’ is the average distance from one bait cartridge to the next one along the flight path (target spacing was 9 m).



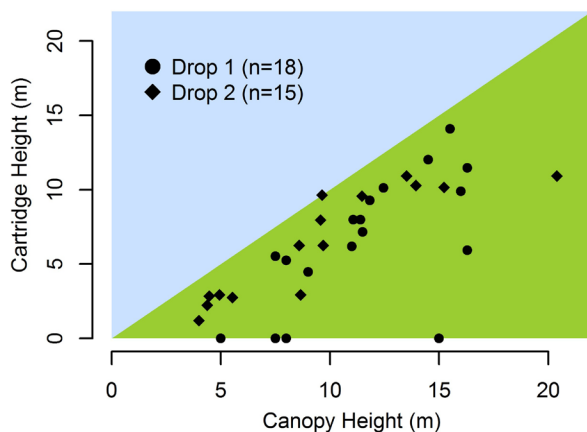
**Fig. 8** Flight paths from the second bait application. Green swaths (portion of the flight paths where bait firing was actuated) are depicted at 9 m width, the optimal bait cartridge spacing for the 120 acetaminophen baits/ha application rate.

51 transmitter-equipped baits, 92.2% deployed from the bait cartridges, with the acetaminophen baits available to brown treesnakes.

Thirty-four bait cartridges (66.6%) tangled in the canopy as intended (Fig. 9). Thirteen (25.5%) were on the ground, but open and available to be taken by ground-foraging brown treesnakes. Four (7.8%) were not deployed (closed) on the ground, making the bait and toxicant unavailable to the brown treesnake. During each application, one transmitter-equipped cartridge was in the canopy but could not be confirmed to have deployed; we consider it unlikely that an unopened bait cartridge would be caught in the canopy, so assumed that these acetaminophen baits deployed.



**Fig. 9** Desired canopy entanglement and acetaminophen bait exposure.



**Fig. 10** Hanging height of the bait cartridge (y-axis) in relation to the height of the canopy at that location (x-axis).

Of the 47 opened bait cartridges, two from each application had the acetaminophen tablet detached from the DNM, making it an ineffective acetaminophen bait. In total, 41 of the 51 acetaminophen baits (80.4%) had acetaminophen tablets attached to the DNM and were available for take by a brown treesnake. This should be viewed as the overall successful bait deployment rate for this sample of baits.

Of the 51 transmitter-equipped baits, canopy height and acetaminophen bait height data were available on 33 acetaminophen baits that successfully deployed (18 from Application 1 and 15 from Application 2). The hanging height of the acetaminophen bait with respect to the canopy height is represented graphically in Fig. 10. In a linear regression, canopy height and acetaminophen bait height were significantly correlated ( $p < 0.001$ , adjusted  $R^2 = 0.694$ ; the four bait cartridges on the ground were not included in the regression). These results show that the majority of deployed acetaminophen baits were entangled within a few metres of the top of the canopy.

Deployed and intact acetaminophen baits were re-checked daily, with very few confirmed takes by brown treesnakes or non-target organisms (Table 2). Of the 51 transmitter-equipped baits, three (5.9%) were confirmed by visual sighting to have been taken by brown treesnakes, or 7.3% of the 41 transmitter-equipped baits known to be available and intact. The 95% binomial confidence interval (logit parameterisation) for the estimated take rate of 5.9% is 1.9% to 16.7%; given the small number

**Table 1** Status of transmitter-equipped bait cartridges following ejection from ADM. “Deployed” means the inner capsule completely exited the outer tube and the acetaminophen bait was available for take by a brown tree snake. “Intact” means the acetaminophen tablet was still attached to the bait mouse and available to be taken by a brown treesnake.

Bait cartridge status	Application 1 (n=28)	Application 2 (n=23)	TOTAL (n=51)
Opened in canopy*	19 (67.9%)	15 (65.2%)	34 (66.6%)
Opened on ground	7 (25.0%)	6 (26.1%)	13 (25.5%)
Not deployed	2 (7.1%)	2 (8.7%)	4 (7.8%)
Unknown	1 (3.6%)	1 (4.3%)	2 (3.9%)
Total deployed*	26 (92.3%)	21 (91.3%)	47 (92.2%)
Total known deployed and intact**	23 (82.1%)	18 (78.3%)	41 (80.4%)

\*Assumes that “unknown” bait cartridges in canopy were deployed; \*\*Does not assume “unknown” baits were intact.

**Table 2** Transmitter-equipped acetaminophen baits taken by target (brown treesnake) or non-target species.

Species	Application 1	Application 2	Total
Brown tree snake	1	2	3
Monitor lizard	1*	0	1
Marine toad	0	2*	2
Unknown	0	1	1

\*Transmitter recovered in faeces

of acetaminophen baits equipped with transmitters, the actual rate of acetaminophen bait take by brown treesnakes could vary widely. All three transmitters were regurgitated prior to death, so no transmitters were recovered in brown treesnake carcasses. All three transmitters taken by non-targets were later found in faeces; it is unclear whether any of these animals succumbed to acetaminophen toxicosis.

All vertebrate carcasses encountered during field activities were collected. This included three brown treesnakes and one marine toad (*Rhinella marina*).

## DISCUSSION

### ADM performance

Future improvements to the ADM will focus on: baffling of the airstream around the ejector ports to prevent interference with ejection; improved feeding of bait cartridges from redesigned magazines and; increased energetic impact imparted to the bait cartridge at the instant of firing in order to improve ejection and deployment reliability. Engineering modifications to the ABMS will further address the non-deployment issue through tighter quality control on bait cartridge imperfections and abatement of tang friction through a redesigned end cap.

### Bait cartridge coverage

The accuracy of bait cartridge placement along flight lines was encouraging. There was very little air movement during these trials; under windier conditions, bait cartridges distributed from greater heights will be more likely to drift further from the intended flight path.

We attribute high variability in bait cartridge spacing along the flight lines to variability in the times at which acetaminophen baits deployed after being ejected from the ADM. When the acetaminophen bait deploys, wind drag increases greatly and the forward momentum is quickly attenuated, causing the bait cartridge to drop straight down. Acetaminophen baits that deploy later maintain forward momentum longer and will move farther along the flight path before landing. It is expected that bait cartridge modifications that improve acetaminophen bait deployment will also result in less variability in time of deployment, leading to more consistent spacing along flight paths.

Variability in spacing along the flight path does have the potential to affect bait cartridge placement accuracy at the edges of treatment areas where bait cartridge application begins and ends, potentially leading to a small number of bait cartridges landing outside of the desired treatment area. To make up for the inconsistency of bait cartridge density at these edges, it is advisable that another application flight should occur along these edges, perpendicular to the original flight paths, ensuring that the edges get a full treatment in a more controlled fashion, similar to coastal aerial rodenticide applications during island rodent eradications.

Variability in bait cartridge placement along and perpendicular to the flight path will add apparently random "noise" to the locations, as opposed to placing bait cartridges precisely on an idealised 9 x 9 m grid. This variability will not affect the ability to get acetaminophen baits into the movement areas of every brown treesnake. The greatest risk of gaps in coverage might arise from strong changes in wind direction, which might introduce strong biases in bait cartridge drift patterns. This will likely factor in with other considerations leading to recommendations not to apply baits during high wind conditions.

Wind effects at bait cartridge ejection ports and direct sunlight on the bait cartridge counter photogates resulted

in unreliable bait cartridge counts as tabulated by the ADM onboard software. We ensured that EPA label application rate restrictions were not exceeded by confirming that no more than 14.66 cases (13,200 bait cartridges) were applied throughout the treatment area during each application period.

### Acetaminophen bait fate

The proportion of transmitter-equipped baits taken by brown treesnakes was low (5.9%); however, only a very small portion of the bait cartridges distributed (0.19%) were equipped with transmitters. If we assume that half of the acetaminophen baits applied during both applications properly deployed and were viable, then there were 13,200 acetaminophen baits available for take by brown treesnakes. If 5.9% of those acetaminophen baits were taken by brown treesnakes, we would expect approximately 779 brown treesnakes to have taken an acetaminophen bait. If we assume a density of 25 brown treesnakes per hectare in this area (a conservative estimate based on the 25-50/ha range reported by Rodda, et al. 1999), 2,750 brown treesnakes would have been exposed to the treatment. If 779 brown treesnakes took acetaminophen baits and were killed, this would be a brown treesnake mortality of approximately 28% in what was effectively a single treatment (given the low deployment rate). The three acetaminophen baits visually confirmed to have been taken by brown treesnakes were found on the ground, apparently regurgitated. In previous NWRC lab efficacy trials of acetaminophen baits with acetaminophen tablets internally-implanted in the DNM (rather than glued to the exterior), 26% were regurgitated, but 100% of the caged brown treesnakes that regurgitated the acetaminophen bait died within 12 to 36 hours (Savarie, 2002). Based on that result, it is reasonable to assume that the brown treesnakes that had taken and regurgitated acetaminophen baits with transmitters in this study also died.

With respect to deployment and entanglement rates, caution should be taken in considering transmitter-equipped cartridges to be representative of the standard bait cartridges distributed during this evaluation. Machine-assembled bait cartridges were manually unwound and rewound by hand after the implantation of the radio transmitter; this may explain why transmitter-equipped cartridges deployed at a higher rate than those observed on video. The added mass of the transmitter may also have an effect on the forces exerted on various parts of the bait cartridge and acetaminophen bait assembly. However, it is also possible that unopened bait cartridges without transmitters actually did deploy lower in the air column (out of view of the video cameras) or upon impact with the canopy.

The overall reduction of brown treesnake abundance in the treatment area – as inferred from a foraging activity index based on take rates of non-toxic DNM from bait stations – is currently being monitored as a separate study for future publication.

## CONCLUSION

Upon firing from the ADM, bait cartridge ejection and acetaminophen bait deployment reliability was initially low. Performance was improved dramatically with field-improvised remedial measures. It is estimated that <50% of acetaminophen baits deployed from the bait cartridges, resulting in an under-treatment compared to the target application rate of 120/ha. Canopy entanglement of acetaminophen baits that properly deployed was high. Aerial bait cartridge placement and spacing were satisfactorily accurate. Reliability of bait cartridge ejection

and acetaminophen bait deployment will be a critical focus of bait manufacturing and delivery system improvements, increasing per-cartridge effectiveness. Future advancements of this technology may include adaptation for payload management by the pilot alone, incorporation of a longer-lasting artificial bait to replace the DNM, and increases in ejector unit and magazine capacity for greater payload.

With this evaluation – and subsequent improvements in system reliability – we consider the concept of automated bait production and aerial delivery to be fundamentally sound. For the first time in the decades-long saga of the brown treesnake invasion of Guam, the prospect of landscape-scale suppression hovers on the horizon.

## DISCLAIMER

The use of trade or corporation names within this report is for the convenience of the user in identifying products. Such use does not constitute an official endorsement or approval of any product by the U.S. Department of Agriculture.

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## AUTHOR CONTRIBUTIONS

S. Siers was the principal investigator for the field evaluation, establishing the study design, coordinating site access and environmental compliance, curating data, executing analyses and data visualisation, and writing the original draft of the manuscript. S. Siers, M. Messaros, C. Clark, and R. Gosnell supervised field evaluation activities. W. Pitt and M. Messaros conceptualised the automated bait cartridge manufacturing and delivery systems. M. Messaros was the chief engineer and developer of the intellectual property associated with the bait cartridge and associated manufacturing and delivery systems. W. Pitt, L. Clark, A. Shiels, J. Eisemann, and S. Siers contributed to programme administration, funding acquisition, and other matters associated with research and development of the automated systems. J. Eisemann coordinated pesticide registration and technology transfer matters, and contributed to on-site coordination of evaluation activities. All authors contributed to review and editing of the manuscript.

## REFERENCES

- Clark, L., Savarie, P.J., Shivik, J.A., Breck, S.W. and Dorr, B.S. (2012). 'Efficacy, effort, and cost comparisons of trapping and acetaminophen-baiting for control of brown treesnakes on Guam'. *Human-Wildlife Interactions* 6: 222–236.
- Clark, L., Clark, C.S. and Siers, S.R. (2018). 'Brown tree snakes: methods and approaches for control.' In: W.C. Pitt, J.C. Beasley and G.W. Witmer (eds.) *Ecology and Management of Terrestrial Vertebrate Invasive Species in the United States*, pp. 107–134. New York: Taylor & Francis.
- Dorr, B.S., Clark, C.S. and Savarie, P. (2016). *Aerial application of acetaminophen-treated baits for control of Brown treesnakes (RC-200925; NWRC study number: QA-1828)*. Environmental Security Technology Certification Program Final Report, Virginia, USA: Department of Defense, Alexandria.
- Johnston, J.J., Savarie, P.J., Primus, T.M., Eisemann, J.D., Hurley, J.C. and Kohler, D.J. (2002). 'Risk assessment of an acetaminophen baiting program for chemical control of brown tree snakes on Guam: Evaluation of baits, snake residues, and potential primary and secondary hazards'. *Environmental Science and Technology* 36: 3827–3833.
- Richmond, J.Q., Wood, D.A., Stanford, J.W. and Fisher, R.N. (2014). 'Testing for multiple invasion routes and source populations for the invasive brown treesnake (*Boiga irregularis*) on Guam: Implications for pest management'. *Biological Invasions* 17: 337–349.
- Rodda, G.H., McCoid, M.J., Fritts, T.H. and Campbell III, E.W. (1999). 'Population trends and limiting factors in *Boiga irregularis*.' In: G.H. Rodda, Y. Sawai, D. Chiszar and H. Tanaka (eds.) *Problem Snake Management: the Habu and the Brown Treesnake*, pp. 236–254. New York: Cornell University Press.
- Rodda, G.H. and Savidge, J.A. (2007). 'Biology and impacts of Pacific island invasive species: 2. *Boiga irregularis*, the brown tree snake (Reptilia: Colubridae)'. *Pacific Science* 61: 307–324.
- Savarie, P.J., Shivik, J.A., White, G.C., Hurley, J.C. and Clark, L. (2001). 'Use of acetaminophen for large-scale control of brown tree snakes'. *Journal of Wildlife Management* 65: 356–365.
- Savarie, P.J. (2002). *Acute oral toxicity of acetaminophen tablets to brown treesnakes, Unpublished report – QA-636*. Fort Collins, Colorado: National Wildlife Research Center.
- Savarie, P.J. and Tope, K.L. (2004). 'Potential flotation devices for aerial delivery of baits to brown treesnakes'. In: R.M. Timm and W.P. Gorenzel (eds.) *Proceedings of the 21<sup>st</sup> Vertebrate Pest Conference*, pp. 27–30. Davis, California: University of California.
- Savidge, J.A. (1987). 'Extinction of an island forest avifauna by an introduced snake'. *Ecology* 68: 660–668.
- Shwiff, S.A., Gebhardt, K., Kirkpatrick, K.N. and Shwiff, S.S. (2010). 'Potential economic damages from introduction of brown tree snakes, *Boiga irregularis* (Reptilia: Colubridae), to the islands of Hawai'i'. *Pacific Science*, 64: 1–10.