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Biological and Microbial Control

Scaling up the deployment of *Psidium cattleyanum* biocontrol in a Tropical Forest

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It can be challenging to deploy and monitor biocontrol for invasive species, particularly in remote forest settings. One of the most abundant and disruptive invasive tree species in Hawai'i and across the Pacific is *Psidium cattleyanum* Sabine (strawberry guava), which replaces native vegetation and negatively impacts forest structure and ecosystem services. A Brazilian leaf-galling insect named *Tectococcus ovatus* Hempel (Homoptera: Eriococcidae) is an approved biocontrol for strawberry guava, but its introduction to date has been restricted to areas with established road and trail networks using ground-based methods. The aim of our study was to develop and test aerial methods of deploying *T. ovatus* within the larger goal of landscape-scale deployment. We also investigated using very high-resolution aerial imagery to detect *T. ovatus* galls on infected leaves to monitor post-deployment inoculation success and quantify spread. We found drone-based aerial deployment using a small 4-unit system was nearly 5 times faster than a ground-based approach in a heavily invaded lowland tropical forest setting and produced a statistically larger inoculation success rate one year after deployment, likely due to more central and higher placement within the targeted canopies. We found aerial imagery to be a useful tool to determine inoculation success and quantify spatiotemporal spread. We also found that larger-capacity drone platforms and conventional helicopters can successfully deploy *T. ovatus* onto individual targeted tree canopies and that landscape-scale deployment operations are feasible using these methods. Our findings are relevant to others working to deploy and monitor biocontrol in forested ecosystems worldwide.

Keywords: Tectococcus ovatus, invasive species, drone, strawberry guava, helicopter

Introduction

Invasive species are a global concern, but they present a particularly serious threat to communities and native ecosystems on islands (Bodey et al. 2023), including in the isolated archipelago of Hawai'i (Cordell 2021). Two-thirds of seedlings now found in Hawaiian forests are non-native species, and the most abundant of these is *Psidium cattleyanum* Sabine (strawberry guava) (Potter et al. 2023). Strawberry guava can form dense monotypic stands in Hawaiian forests, altering fundamental ecosystem characteristics and processes involving forest structure (Denslow et al. 2024), water balance (Takahashi et al. 2011, Safeeq and Fares 2014), and light regime (Rosam et al. 2024).

In an attempt to combat the detrimental effects of strawberry guava invasion, in 2012 an approved biocontrol agent, *Tectococcus ovatus* Hempel (Homoptera: Eriococcidae), was introduced to Hawai'i (Johnson 2016). *Tectococcus ovatus* is a host-specific leaf-galling insect from Brazil that is expected to significantly suppress strawberry guava growth and reproduction (Wessels et al. 2007, State of Hawai'i 2011). Since the initial introduction of *T. ovatus* in 2012, additional releases have taken place across the state of Hawai'i and visible galls can now be found in lowland forests near human population centers (Johnson, unpublished data). The insect disperses as wind-blown eggs and first instar crawlers, requiring new developing foliage where the crawler settles and induces gall formation. Within several weeks, each female, confined singly within a conical gall, will mature and begin producing eggs, which are pushed out in a matrix of wax filaments from a narrow opening or emerge as newly hatched crawlers (Vitorino et al. 2000). Following introduction to a new site, 2 to 4 yr are required before galling levels begin to increase significantly and spread beyond the inoculated trees (Johnson, unpublished data). To date, *T. ovatus* deployment has occurred solely via ground-based methods, using slingshots, hand-throws, or extendable poles to place bolas of freshly harvested strawberry guava leaves containing *T. ovatus* galls into the canopy of ungalled strawberry guava trees. To slow down the spread of strawberry guava across the state, particularly in more remote, upper-elevation forests of high conservation value, *T. ovatus* needs to be deployed at a landscape scale. One means of achieving this is via aerial deployment. The primary motivation for this work was to develop and test aerial methods of deploying *T. ovatus*, within the larger goal of statewide introduction across Hawaiian forests.

There is a long legacy of conventional aircraft operations for the aerial release of insects (Dowell et al. 2005). The most extensive aerial releases by conventional aircraft have been as part of sterile insect technique releases for fruit flies (Wong et al. 1992, Vargas et al. 1995, Enkerlin et al. 2015, Garcia et al. 2020, Conway et al. 2024) and mosquitoes (Alphey et al. 2010, Dobson 2021, Oliva et al. 2021). There have also been releases of insects as biocontrol agents such as parasites and parasitoids (Wong et al. 1992, Sivinski et al. 2000). Conventional aircraft operations can cover large areas quickly but can be expensive and involve risk to human life (Dyck et al. 2021).

Drone releases of insects are increasing rapidly, and operations have included fruit flies (Moses-Gonzales et al. 2021), mosquitoes (Bouyer et al. 2020, Lin et al. 2024), lacewings (Del Pozo-Valdivia et al. 2021), and moth larvae for weed biocontrol (Lake et al. 2021). Drones have also been used to release and distribute biocontrol for invasive *Persicaria perfoliata* (mile-a-minute weed) (Park et al. 2018) and *Lythrum salicaria* (purple loosestrife) (Naharki et al. 2024). While insect release via drone is a rapidly growing field, challenges remain associated with limitations in flight time, carrying capacity, and deployment area (Moses-Gonzales and Brewer 2021). In the United States, the operation of drones for biocontrol releases can fall outside of the scope of agricultural aircraft operations if it consists solely of the release of live insects (14 CFR § 137.3). Operations that do fall within the scope of agricultural aircraft operations must clear additional regulatory hurdles (Rodriguez 2021). Additionally, drone operation by government entities (federal, state, or local) may experience further restrictions regarding aircraft sourcing (Salinas and Lewandowski 2024) and operational considerations (Food, Conservation, and Energy Act of 2008 § 1619).

Following the deployment of an agent, post-release monitoring is required to determine the success and efficacy of biocontrol releases (Schaffner et al. 2020). For *T. ovatus* in Hawai'i, ground-based monitoring has been used to visually detect the presence of galls on strawberry guava leaves, either through naked-eye observations or the use of binoculars. This method requires the observer to be in close proximity to the tree in question and leaves in the upper canopy may be obscured from view. With these limitations in mind, we were interested in determining if *T. ovatus* galls could be detected from high-resolution aerial imagery. Remote sensing has been used to detect leaf damage and monitor biocontrol success in other settings (Abd El-Ghany et al. 2020, Görlich et al. 2021, Manganiello et al. 2021, Pane et al. 2021), but not for the detection of *T. ovatus*. Leaf galls in other species have been detected from laboratory-based optical imagery using deep transfer learning (Ahmed et al. 2023, Ayaz et al. 2023), though it is unclear if this approach has been applied to aerial drone images collected in the field.

Materials and Methods

Study Sites

This study occurred across 3 different locations on eastern Hawai'i Island, two within forest reserves managed by the Hawai'i Division of Forestry and Wildlife (Site A within the Upper Waiākea Forest Reserve and Site B within the Ola'a Forest Reserve) and another (Site C) located on private land outside the town of Kea'au (Fig. 1). Invasive strawberry guava is commonly found across all 3 sites along with other invasive tree species including Albizia (*Falcataria moluccana*) and Octopus Tree (*Schefflera actinophylla*) within a vegetation canopy containing remnant native 'Ōhi'a lehua (*Meterosideros polymorpha*) trees. Invasive liana species were also present, including Stink Vine (*Paederia foetida*) and Passion Flower (*Passiflora* sp.). The understory vegetation was dominated by Uluhe (*Dicranopteris linearis*), a native fern species. Annual precipitation in the forest reserve sites ranged from 6,273 mm per year (Site A) to 5,911 mm per year (Site B), while Site C receives 3,684 mm per year (Longman et al. 2024).

Documenting Spread from an Established Inoculation Site

In September 2017, a 50 × 50 m site within Upper Waiākea Forest Reserve (Site A, Fig. 1A) was treated with *T. ovatus* via slingshot applications of galled leaves into approximately 25 large trees, as part of an ongoing demographic study of the impacts of strawberry guava biocontrol (Denslow et al. 2024). On 26 August 2021, images were collected over the site using a small multirotor drone (Inspire 2, SZ DJI Technology Co., Ltd., Shenzhen, China) equipped with an RGB camera with a 4/3 sensor (Zenmuse X5S with 15 mm MFT lens, SZ DJI Technology Co., Ltd.). Flight surveys were conducted manually following a pre-designed cross pattern radiating out from the center of the plot (Fig. 1A) with nadir photos taken at set way points every 10 m at an altitude of 8 to 10 m above the canopy with automatic settings for focus and white balance. These surveys captured images (5,280 × 3,956 pixels) with a ground sampling distance of approximately 0.22 cm px^{-1}. No geometric correction, radiance correction, or reprojection was performed on the aerial photos. Photos were retaken on 8 August 2024, using another small multirotor drone (Matrice 300 RTK, SZ DJI Technology Co., Ltd.) equipped with an RGB camera with a full-frame sensor (Zenmuse P1 with 35 mm MFT lens, SZ DJI Technology Co., Ltd.). These surveys captured images (8,192 × 5,460 pixels) with a ground sampling distance of approximately 0.10 cm px^{-1}. Photos from each of the 2 surveys were manually assessed by 2 independent geospatial analysts to determine the amount of imaged strawberry guava canopy cover within the photo and the presence of *T. ovatus* galls on any visible strawberry guava leaves. Manual assessment was performed by opening individual photos in IrfanView 64 (irfanview.com), zooming in to observe individual leaves, and scanning the image in a systematic grid search pattern. Individual photos took 1 to 3 min to scan, depending on the complexity of the forest structure and the number of imaged leaves. Detection results were recorded in a spreadsheet, including pixel locations of detected gall clusters. While artificial intelligence was not used in this process, the results from this work are being used as training data for a convolutional neural network detection algorithm. To determine the presence of any statistically significant clustering of *T. ovatus* galls within the imaged area, the

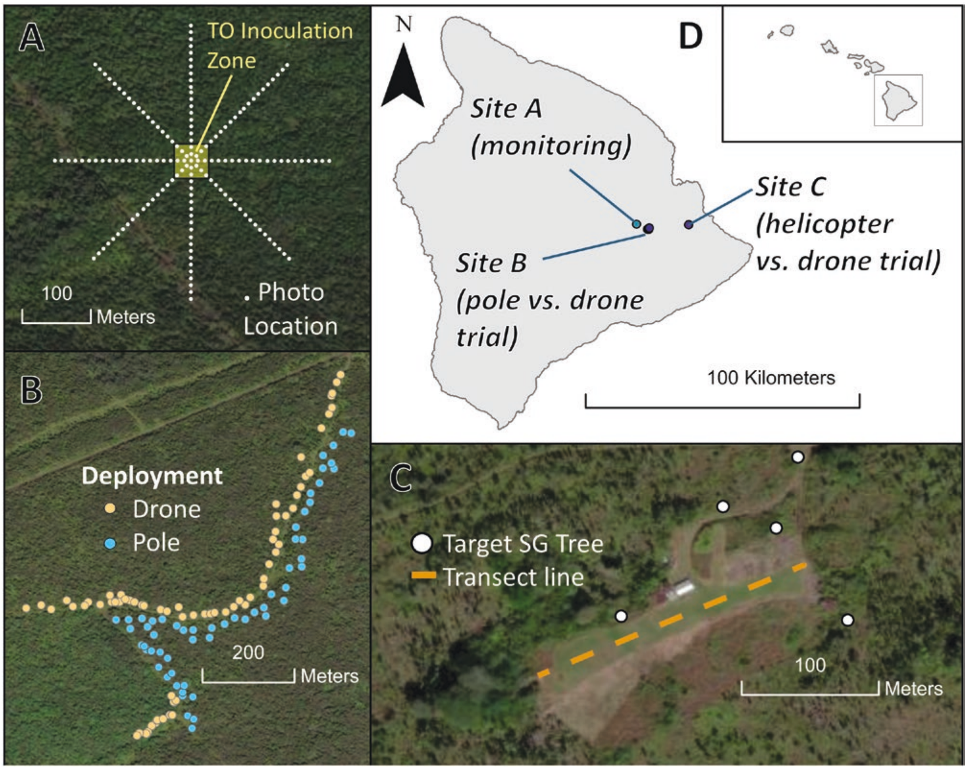

Fig. 1. Study site locations on Hawai‘i Island. (A) Long-term photo monitoring Site A within the Upper Waiākea Forest Reserve. (B) Pole vs. drone deployment experimental trial Site B within the Ola‘a Forest Reserve. (C) High-capacity *T. ovatus* release trial Site C via helicopter and drone. (D) Map of site locations on Hawai‘i Island.

Global Moran's Index (Moran 1950) was calculated to measure spatial autocorrelation. Moran's Index varies from −1 to + 1, with positive values indicating a significant clustering pattern, values near zero representing a random distribution, and negative values representing a dispersed distribution pattern. Moran's Index was calculated within ArcGIS Pro 3.1.2 (ESRI, Redlands, California, USA).

Ola‘a Forest Reserve T. ovatus Deployment Trials

To measure differences in the efficiency and efficacy of ground-based vs. aerial deployment of the *T. ovatus* biocontrol, we conducted field trials with two different delivery systems for placing bolas within target strawberry guava canopies at Site B. Each bola consisted of 30 cm of jute twine, with a bundle of 2 to 4 *T. ovatus*-galled strawberry guava leaves tied to each end (Fig. 2A). The *T. ovatus*-galled leaves were harvested from a nearby site where stands of strawberry guava had been mechanically mowed (approx. 18 mo prior) to near ground level and allowed to resprout in the presence of colonizing *T. ovatus*. Brightly colored biodegradable flagging was also tied on to each end of the bola to make it easier to detect following deployment. Target strawberry guava trees were identified within 25 m of a secondary road in the Ola‘a Forest Reserve and inoculated with bolas by either a ground-based pole method (southeastern side of the road) or an aerial drone (northwestern side) (Fig. 1B).

The pole method used an extendable pole (maximum reach of 12 m) with a custom two-prong flexible attachment which held a bola and allowed it to be lifted and placed into branches overhead (Fig. 2C, D). Pole deployment teams consisted of 2 to 3 field technicians. A stopwatch and datasheet were used to record the time elapsed to hike to each tree and successfully deploy the *T. ovatus* bola, providing a measure of the number of successful placement attempts and deployment time per tree. Placement attempts were considered successful if the bola was caught and remained in the upper branches of the target strawberry guava tree, and unsuccessful if the bola fell through the canopy to the ground or subcanopy branches or vegetation. During pole deployment a drone, the DJI Mavic 2 Pro or DJI Mavic 3 Multispectral (SZ DJI Technology Co., Ltd.) was positioned above the target tree to collect a sequence of video and still photographs to record the exact placement location of the bola, to determine if there were any pre-existing *T. ovatus* galls in the target tree, and to assist with future monitoring efforts.

The aerial deployment method used a custom bola deployment device mounted to a small drone (Matrice 200 V2, SZ DJI Technology Co., Ltd.) equipped with an RGB camera with a 4/3 sensor (Zenmuse X5S with 15 mm MFT lens, SZ DJI Technology Co., Ltd.) (Fig. 2E, F). The system, capable of carrying and remotely releasing 4 individual bola units, was designed by the UH Hilo Spatial Data Analysis & Visualization (SDAV) Research laboratory in collaboration with Easybotics Inc. (Hilo, Hawai‘i, USA). Flight crew members consisted of a drone pilot, bolas payload operator, and a visual spotter/data recorder. After the drone pilot flew to the target tree, lowered the platform to a height of < 5 m above the canopy, and started recording a nadir perspective video, the payload operator would release a bola. As in the pole method, placement attempts were considered successful if the bola was caught and remained in the upper branches of the target strawberry guava tree, and unsuccessful if the bola fell through the canopy to the ground or subcanopy branches or vegetation. In some instances, bola placement appeared to be successful before being blown off the canopy by the drone propwash. In these instances, the drop was considered unsuccessful and another attempt made. A stopwatch and datasheet

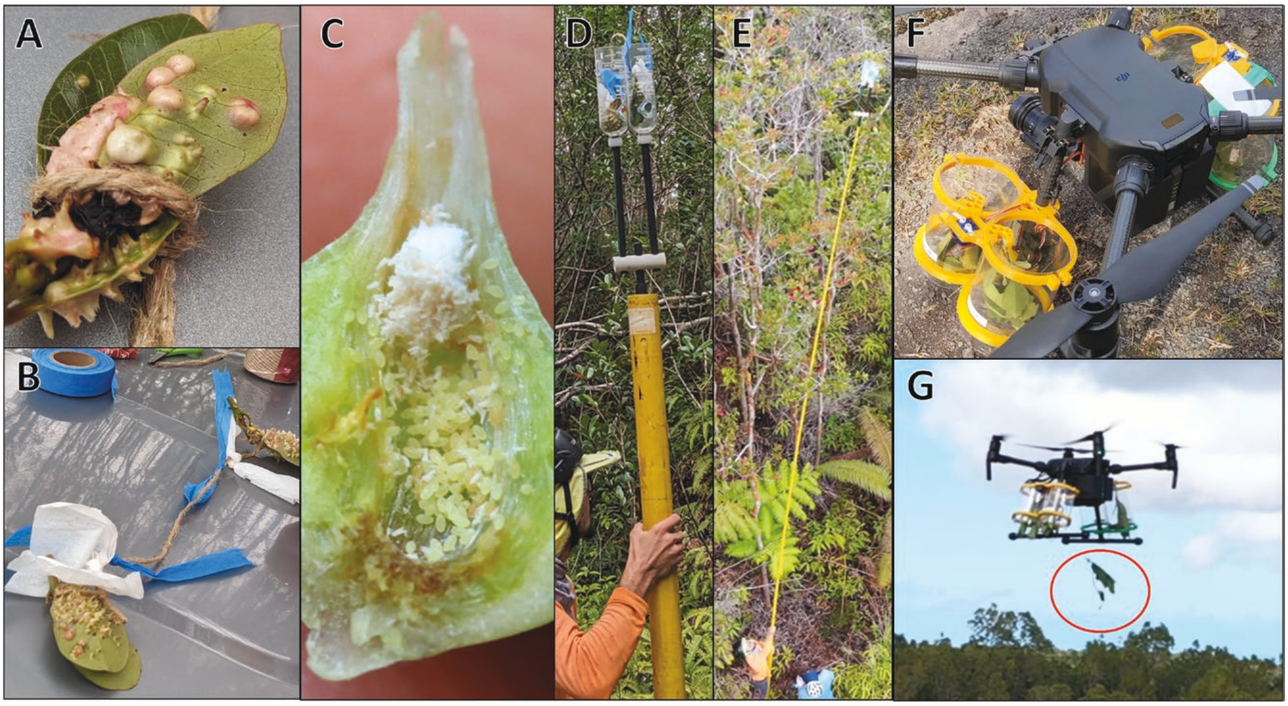

Fig. 2. (A) *T. ovatus*-galled *Psidium cattleyanum* Sabine (strawberry guava) leaves with mature galls tied to one end of a bola. (B) Bola with *T. ovatus*-galled leaves and biodegradable flagging for increased visibility. (C) Mature gall of *T. ovatus* split open to reveal numerous eggs (yellow) and nymphal crawlers (pinkish). Photo by JB Friday. (D) Photo of the extendable pole used to deploy *T. ovatus* bolas via the ground-based method. (E) Drone photo of the extendable pole set for placement; target tree is on the left side of the photograph. (F) Photograph of the 4-unit bolas deployment system on the ground. (G) Drone in the air, red circle shows bola in mid-drop.

were used to record the time elapsed to successfully deploy the *T. ovatus* bolas and the number of successful drops per flight (with a maximum of 4), providing a measure of the number of placement attempts and the deployment time per tree. Aerial releases were conducted under an operating certificate for commercial agricultural aircraft operations granted to the UH Hilo SDAV Research Laboratory by the U.S. Federal Aviation Administration. Bolas were deployed across the experimental site using both methods over 4 field days from 20–25 April 2023.

To determine if there were statistical differences in the hang success rate of the 2 different methods, a Shapiro-Wilk normality test to determine if the data were normally distributed was applied to the recorded datasets, followed by an F test to compare variances and then a Wilcoxon rank sum test with continuity correction. The same statistical tests were applied to the recorded amount of time required to deploy the *T. ovatus* bolas by the 2 different methods. These and all subsequent analyses were performed using R (version 4.4.2) (R Core Team 2024) .

For both the pole and aerial approaches, immediately following a successful deployment, a series of high-resolution geo-tagged still photographs were collected over each bola, beginning at an altitude of 5 m with a GSD of 0.06 cm, often capturing only a portion of the tree canopy, and progressing upwards to an elevation of 30 (GSD of 0.49 cm) to place the target bola and strawberry guava tree into a broader context within the study site to assist with future monitoring. The highest-resolution images for each strawberry guava tree included in the study were manually scanned by 2 different geospatial analysts to determine if there were any pre-existing *T. ovatus* galls in any of the target strawberry guava trees included in the study using the same assessment method described above.

Post-deployment Monitoring

In addition to the images of target trees collected during bolas placement, an automated mapping flight was conducted over the study area to create a base orthomosaic. This flight was conducted on 5 May 2024 with a small multirotor drone (Mavic 3 Multispectral, SZ DJI Technology Co., Ltd.) connected to an RTK base station (R10, Trimble, Westminster, CO, USA) at an altitude of 94 m (GSD 2.55 cm/px) with a side and forward overlap of 90%. The coordinates of the RTK base station were determined by occupying the site for 5 h and processing through the National Geodetic Survey Online Positioning User Service (NGS OPUS) with a final estimated accuracy of 5 cm.

The resulting orthomosaic and digital surface model/3D point cloud, together with the tree-specific deployment photos, were used to accurately locate the 3-dimensional coordinates of each deployed bola within the RTK-georeferenced coordinate space. These coordinates were then entered into flight planning software (UgCS v 5.5.0, SPH Engineering, Riga, Latvia), and a semi-automated flight mission was generated to assist in repeatable data collection for future monitoring efforts. This was particularly important as the high-visibility biodegradable colored flagging faded or was lost over time. Target trees were revisited and imaged using the semi-automated flight mission at 6 and 12 mo after treatment and the collected imagery was manually scanned by 2 different geospatial analysts to determine if there were any visible *T. ovatus* galls present and if the bolas were still apparent using the same assessment method described above.

To determine if there were statistical differences in the amount of *T. ovatus* galling produced by the 2 different deployment methods, a Shapiro-Wilk normality test, *F* test to compare variances, and a

Wilcoxon rank sum test with continuity correction were applied to the recorded gall datasets collected at 6 mo and 1 yr after treatment.

Higher-capacity T. ovatus Deployment Trials

Two additional aerial deployment methods were also developed and studied at Site C to assess their ability to carry out larger landscape-scale missions, a higher-capacity attachment for use with larger drones, and hand-release via manned helicopter. Sixteen-unit and 54-unit drone attachment deployment systems (Fig. 3) were developed by the University of Hawaiʻi at Hilo Spatial Data Analysis & Visualization (SDAV) Laboratory in collaboration with Easybotics LLC in Hilo, Hawaiʻi, USA. The systems are controlled remotely and consist of servos, electronics, and 3D-printed frames containing individual cylindrical cells used to hold the bolas in isolation, so they deploy without tangling. The units are attached to a drone via a vertical carbon rod and connection system (Perroy et al. 2022) and flown over the target tree, guided by a live videofeed from a downward-looking camera. The 54-unit version is named Kūhualūlū to reflect its Hawaiian origins and the concepts of vertical movement (Kū), yield (hua), and scattering or sowing (lūlū).

The helicopter deployment method entailed hand-throwing bolas at target trees from the rear seat of a Hughes 500 helicopter (Fig. 4). Three different bolas storage systems to minimize pre-deployment tangling were tested: stacked plastic cups stored in a covered box, a 20-compartment small parts storage container (DWST14830, DeWalt Industrial Tool Company, Towson, Maryland, USA), and a custom system consisting of 2 wooden slats 0.6 m in length, connected in the center via 2 threaded bolts with interior spacers inserted between the slats. Forty bolas were sequentially loaded between the slats, which were then secured with rubber bands attached to the ends.

Tests of targeting accuracy and swath width were conducted for the high-capacity Kūhualūlū and helicopter aerial systems at a testing location in Keaʻau, Hawaiʻi (Fig. 1C). Five strawberry guava trees were selected prior to the test flights and identified with flagging tape and ground markers to make them clearly visible from the air. Ten bolas were dropped on each tree via the 2 different methods, and data on drop success or failure recorded. All 3 bolas storage systems were used to assess their utility during the helicopter test flight. To compare bolas hang success between the helicopter hand throw and Kūhualūlū deployment methods, a Shapiro-Wilk normality test, *F* test to compare variances, and a Welch Two Sample *t*-test were applied to the recorded datasets.

In addition, a 200-m linear transect was set up along the flying field and marked with bright orange marking paint. Each aircraft flew at a continuous speed 5 m/s (approximately 10 knots) down the transect line and bolas were released, aiming to strike the line, at a height of 5 m for Kūhualūlū and 10 m for the helicopter. Following each flight, an RTK-enabled mapping drone (Matrice 300) flew over the area to create an orthomosaic with a GSD of 1.0 cm, fine enough to detect the dropped bolas resting positions. The orthomosaic was then used to measure the perpendicular distance of the bolas' resting positions from the painted target transect line. To determine if there were statistical differences in the spread of perpendicular distances from the target line produced by the 2 different deployment methods, an *F*-test to compare 2 variances was applied to the Kūhualūlū and helicopter datasets.

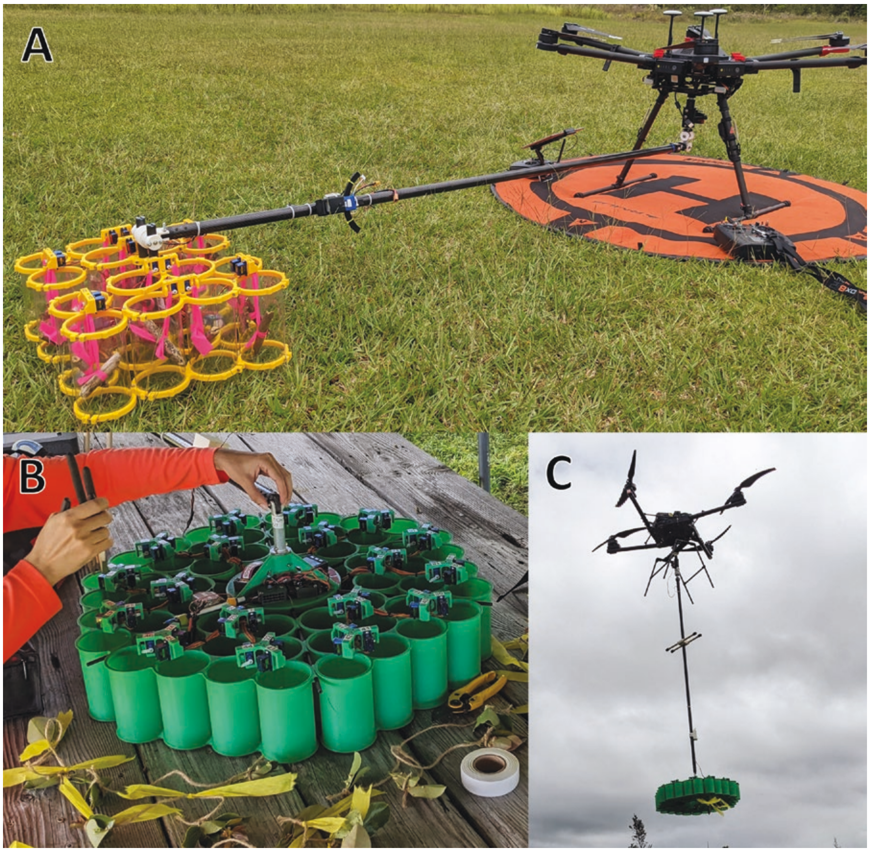

Fig. 3. Examples of higher capacity *T. ovatus* release attachments for aerial deployment via drone developed for this project. (A) 16-unit bolas release system attached to a DJI Matrice 600 drone. (B) Preparing the 54-unit Kūhualūlū system with bolas prior to flight. (C) 54-unit Kūhualūlū in flight with the Freefly Alta X drone.

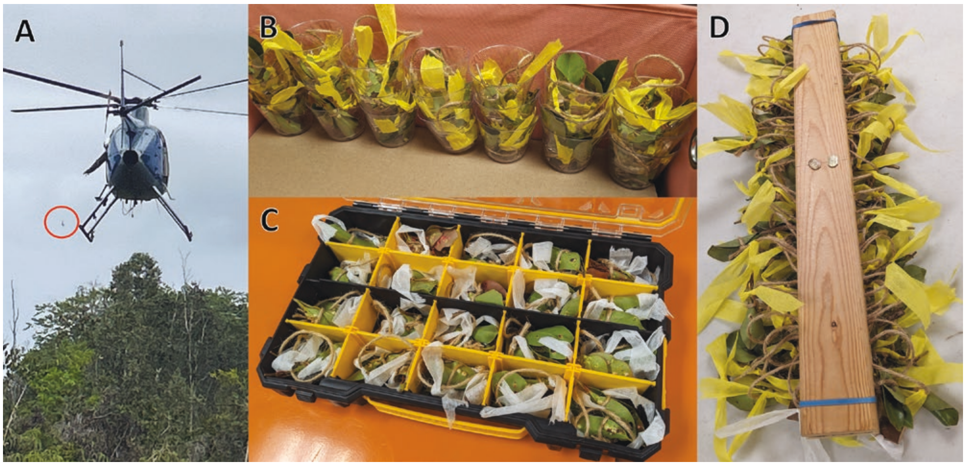

Fig. 4. Examples of different units tested for efficient and safe tangle-free storage and release of *T. ovatus* via aerial release by helicopter. (A) Helicopter hand-release of *T. ovatus*. (B) Examples from stacked cup bolas storage system test. (C) Store-bought storage system to hold 20 bolas. (D) Custom system for compactly storing 40 bolas tangle-free within 2 wooden slats.

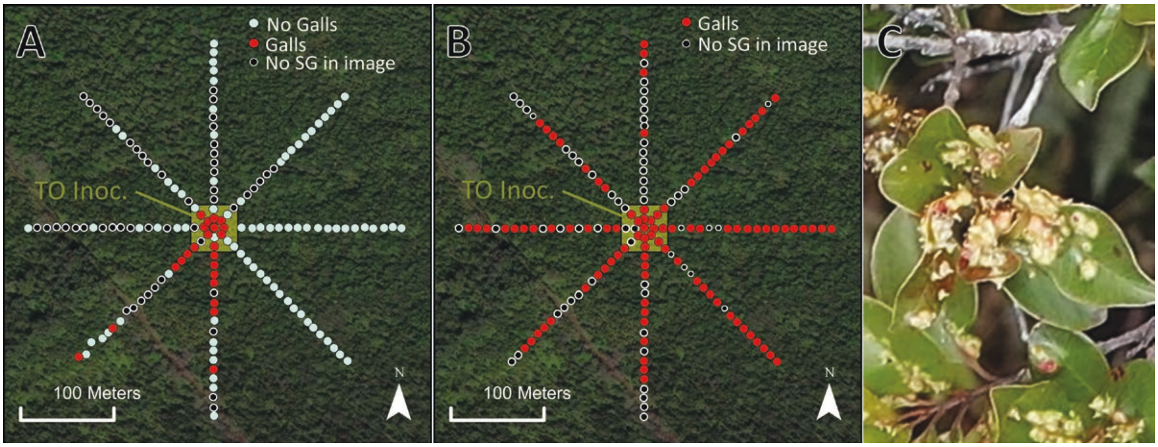

Fig. 5. Long-term monitoring of a *T. ovatus* release site for the detection of *Psidium cattleyanum* Sabine (strawberry guava) trees and *T. ovatus* leaf galls via drone-based high resolution aerial imagery. (A) Results from image interpretation of strawberry guava tree locations and *T. ovatus* gall detections from 8/26/2021 flight. (B) Results from image interpretation of strawberry guava tree locations and *T. ovatus* gall detections from 8/30/2024 flight. (C) Aerial photo example from 2024 imaging flight showing extensive visible *T. ovatus* galls.

Results

Results from Cross-shaped Monitoring 2017 Forest Release Site

At Site A, inoculated with *T. ovatus* in 2017, 23.1% of the photos containing imaged strawberry guava trees taken in 2021 had visible *T. ovatus* galls (Fig. 5), with all but one of the strawberry guava trees imaged within 10 m of the central point positive for *T. ovatus*. At distances further from the center, there was a strong directional pattern in inoculation success, with trees to the south and southwest exhibiting *T. ovatus* galls, including out to 200 m. This pattern was significantly clustered, with a measured Moran's Global Index value of 0.671, z-score of 8.98, and a P-value of < 0.01. No trees in any other direction exhibited galls. At the same site re-imaged during the 2024 photo survey, 100% of the strawberry guava trees exhibited *T. ovatus* galls.

Results from Pole vs Drone Tests

During the Olaʻa forest reserve field trials, *T. ovatus* bolas were deployed onto 129 trees: 62 by the pole method and 67 by the 4-bolas drone method. The hang success rate differed between methods (Shapiro-Wilk $W = 0.44188$; $P < 0.0001$; $F = 2.8039$, num df = 66, denom df = 62; $P < 0.0001$; Wilcoxon $W = 1649$, $P = 0.0005$) with the pole method at 97.6 (± 2.0) and the drone method at 86.6 (± 5.5). Average treatment time between methods was also significantly different (Shapiro-Wilk W = 0.93535; $P < 0.0003$; $F = 0.051905$, num df = 25, denom df = 61; $P < 0.0001$; Wilcoxon $W = 31.5$, $P < 0.0001$), with the 4-bolas drone method reducing average treatment time by 77%, 2.8 min (± 1.2) vs. 12 min (± 5.2). The maximum amount of time needed to successfully place a bolas on a tree was 27 min for the pole method and 5 min for the drone method.

None of the treated trees had pre-existing *T. ovatus* galls, based on a close examination of the low-altitude drone photos collected during deployment. A subset of 80 trees located along the primary road running through the study area were included for monitoring (39 pole trees and 41 drone trees) and low altitude (< 3 m above the canopy) images via a programmed RTK flight plan were collected at 6- and 12-mo post-treatment. At the 6-mo mark, there was no statistical difference in inoculation success between the two methods (Shapiro-Wilk $W = 0.58031$; $P < 0.0001$; $F = 1.2069$, num df = 38, denom df = 38; $P = 0.565$; Wilcoxon $W = 858$, $P = 0.3327$), but there was a significant difference at the 1 yr mark, with treatment by drone having a significantly higher rate of detectable inoculation (Shapiro-Wilk $W = 0.62681$; $P < 0.0001$; $F = 0.80383$, num df = 37, denom df = 36; $P = 0.5116$; Wilcoxon $W = 936$, $P = 0.004029$) (Fig. 6).

In reviewing deployment video footage, we observed that bola placement via the pole method was almost always (97% of the time) on the edge of a strawberry guava canopy (Fig. 7) and often on lower branches given the limited vertical extent of the pole. The drone method, in contrast, placed bolas in the center and upper branches of the target strawberry guava canopy more than half the time.

Results from Higher Capacity *T. ovatus* Deployment Trials

There was no statistical difference in hang success rate between the Kūhualūlū high-capacity drone attachment and hand throws from the helicopter (Shapiro-Wilk $W = 0.88984$; $P = 0.1689$; $F = 0.36733$, num df = 4, denom df = 4; $P = 0.3555$; Welch $t = 0.32$, df = 6.5893; $P = 0.7589$), with Kūhualūlū achieving 71.6% (± 21.5) and hand throws reaching 68.0% (± 13.0). In the linear transect targeting accuracy tests, the helicopter hand release method was found to have a significantly larger variance in perpendicular distances from the target line ($F = 6.6689$; num df = 24, denom df = 28; $P < 0.0001$). The maximum distance from the target line was 8.24 m for the helicopter method, compared to 2.83 m for the Kūhualūlū system. Bolas landing within 2 m of the target line accounted for 93% of the Kūhualūlū drops, with 62% landing within one meter; whereas 68% of drops from the helicopter landed within 2 m, and 28% within one meter (Fig. 8).

As a thought experiment for what a large-scale aerial *T. ovatus* deployment operation might entail, we selected the ~17,000 ha Watershed Reserve Section of the Hilo Forest Reserve on east Hawai'i Island as a candidate area. This area was established by Governor's Proclamation on 24 July 1905, for the purpose of watershed protection and contains a mixture of intact and heavily strawberry guava-invaded 'Ōhi'a lehua forest. Three different grid spacings, 100 m, 500 m, and 1,000 m, were used to generate candidate drop locations across the Forest Reserve over the extent of the densely infested strawberry guava area within a GIS program (Fig. 9).

Realistic average transit speeds between points, plus 30 s to secure a successful drop based on our results from this study, were used to estimate the total amount of time required to deploy *T. ovatus* at the 3 different grid spacings (Table 1). While the smallest grid spacing is untenable from a logistical and cost perspective, the 500 m spacing could be realistically accomplished in 2 fuel cycles, and the 1 km spacing could be completed in less than a single fuel cycle. Scaling up the 1 km grid approach across the estimated 475,000 forested hectares statewide potentially affected by strawberry guava (State of Hawai'i 2011), it would require 4,750 drops and 92.5 h of helicopter time, not including additional time for ferrying and refueling.

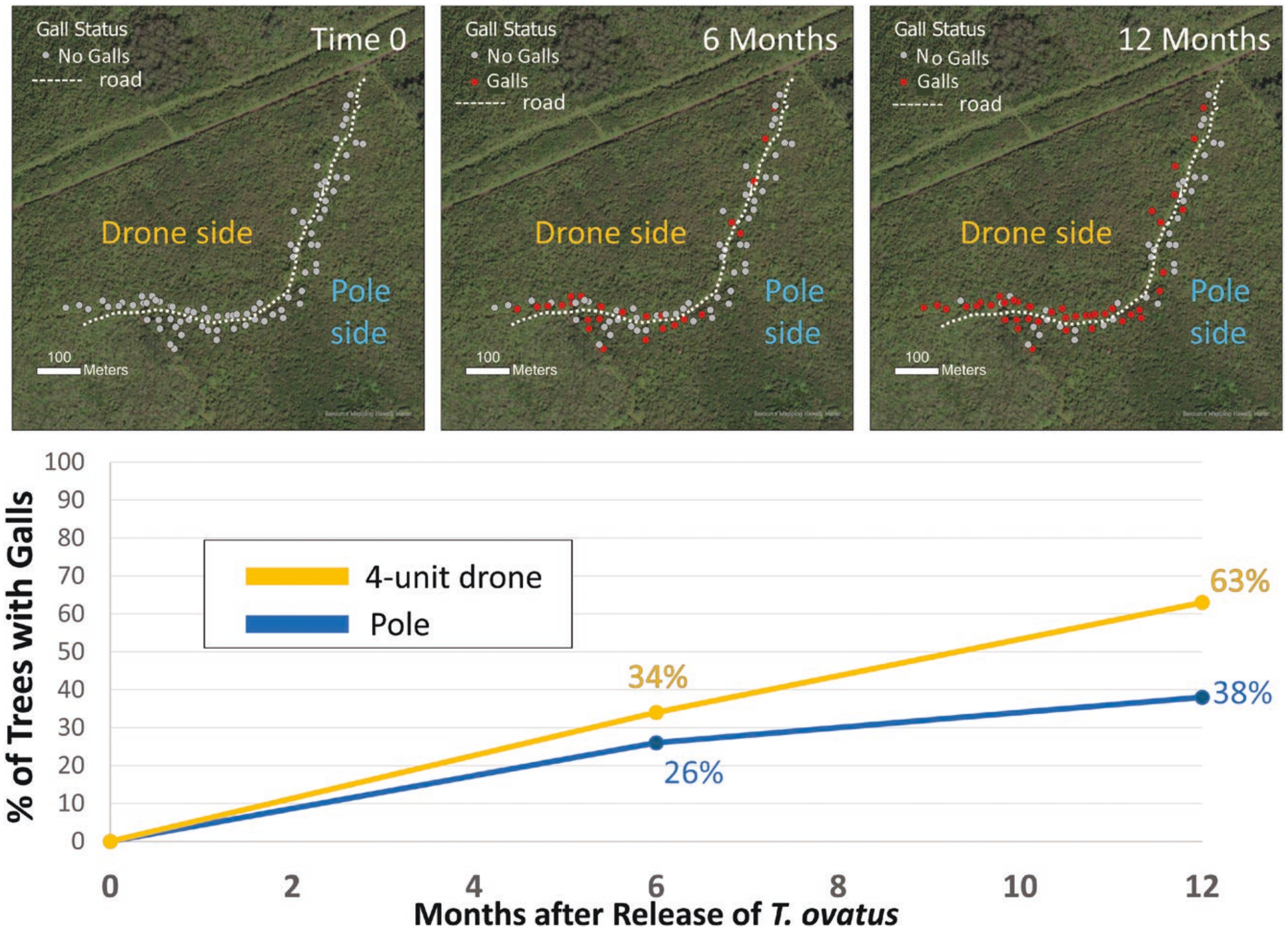

Fig. 6. (Top) Spatial pattern of *T. ovatus* gall development over time at Site B. (Bottom) Percentage of treated trees exhibiting *T. ovatus* galls over time following release of *T. ovatus* via either 4-unit drone (yellow) or ground-based pole method (blue).

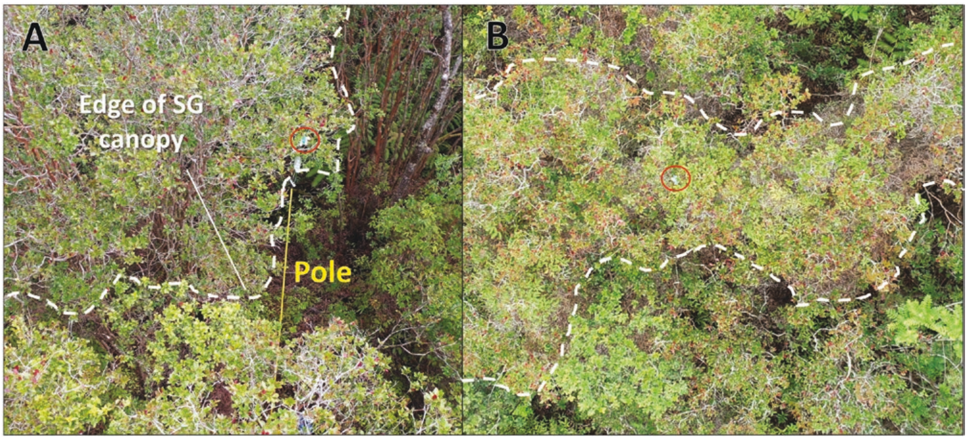

Fig. 7. (A) Example of pole method bola placement from oblique drone video footage collected during the deployment operation. Bolas (circled in red) are typically placed on the very edge of the canopy, potentially limiting the chances of galling by *T. ovatus*. (B) Example of drone method bola placement in center of canopy.

Fig. 8. Maps of *T. ovatus* bolas locations following aerial deployment trials along a straight-line flight path to determine variability in placement relative to a central transect target line for the 54-unit remotely-controlled Kūhualūlū drone system (top) and manual hand throws from a conventional helicopter (bottom).

To compare the economic costs of drone and helicopter deployment operations over the ~17,000 ha Watershed Reserve Section of the Hilo Forest Reserve, we modeled the combined operation time, operating costs, capital costs, and total costs for operations at the 3 different grid spacings (Supplementary Table A1). Drone operations were modeled with and without a Certificate of Authorization from the Federal Aviation Administration for operating beyond visual line of sight (BVLOS) and with a maximum working radio distance of 3,000 m. Under the modeled parameters, helicopter operations were the most economical option for all grid spacings.

Discussion

Monitoring Spread from Established Inoculated Site(s)

Aerial detection of *T. ovatus* galls using sub-cm resolution imagery collected from low-altitude drones is an efficient and practical way of assessing levels of galling in a localized area. Although manual scanning of individual photos is tedious, taking one to 3 min per photo, compared to ground-based detection via binoculars, the coverage and speed drones provide for detecting galled leaves are transformative. In addition, the images of galled leaves analyzed for this project can form the basis of a training dataset for developing an artificial intelligence detection algorithm. Seven years after the initial deployment of *T. ovatus* in the study site, 100% of strawberry guava trees imaged within 200 m of the central release zone displayed galls (Fig. 5). Trees at distances > 200 m, particularly southwest of the release zone, also likely contain galls, though they were not included in this study. Longer distances of spread—averaging 400 m per year—have been documented (Johnson, unpublished data).

Olaʻa T. ovatus Deployment Trials

For local deployment of *T. ovatus*, we demonstrated that a 4-unit drone is nearly 5 times faster than a ground-based pole approach in a heavily invaded lowland tropical forest setting. This improved efficiency would increase even more with a higher capacity drone system like Kūhualūlū, although the logistics of operating a larger drone with a pole attachment require a larger area for takeoff and landing and increased demands on the drone pilot. The demonstration trial described here was also limited in scope, with the furthest trees involved in the study being 41 m from the central road. Line of sight issues also arise with more distant deployments, though solutions exist for meeting these challenges (Hartley et al. 2022, Loffi et al. 2022).

Beyond the increased deployment efficiency of the drone method, we also observed a significantly higher percentage of *T. ovatus* galling on the drone-treated trees relative to the ground-based pole treatment in our repeat monitoring surveys. This difference, 63% vs. 38% gall success at 12 mo, suggests that there may be something about the aerial-based placement of the bola that contributes to a higher success rate. Based on canopy placement differences between the two methods (Fig. 7), we believe the central and higher placement accomplished via drone likely provides more opportunities for the *T. ovatus* insect to find appropriate new leaves to gall.

Scaling up to Landscape-level Deployments

From a landscape-scale deployment perspective, our experimental trial results show that aerial deployment is at least 5 times faster than ground-based methods along road corridors with relatively easy ground access. Aerial deployment also resulted in better inoculation success. For larger and more remote target areas without roads, aerial methods are the only feasible means of introducing *T. ovatus*.

We were encouraged by the targeting accuracy results for both the Kūhualūlū attachment and the hand-thrown helicopter method. That said, rotor wash from the larger drone and the Hughes 500 helicopter affected drop trajectories and caused a number of bolas to be blown off of apparently successful drops, decreasing the overall

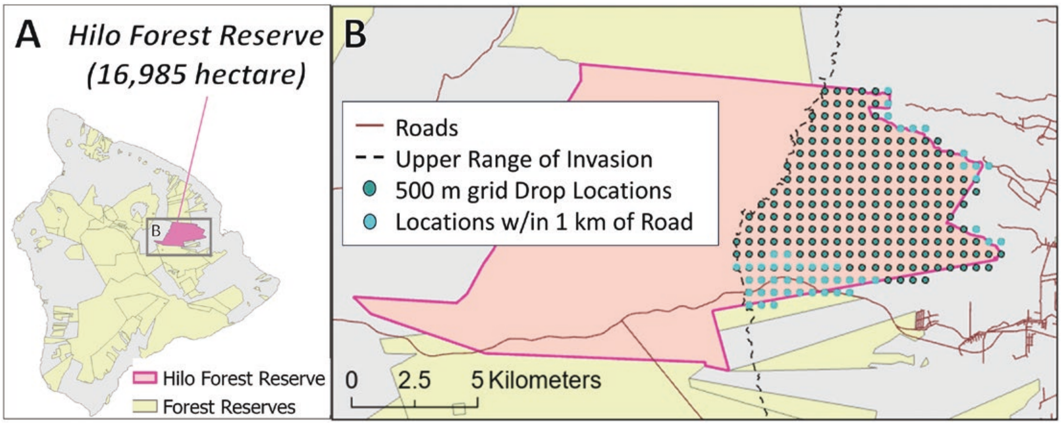

Fig. 9. Candidate location for landscape deployment of *T. ovatus* via aerial methods. (A) Location of Hilo Forest Reserve. (B) 500 m grid spacing option of potential *T. ovatus* drop sites over the portion of the Hilo Forest Reserve invaded by strawberry guava. Blue dots are sites within 1 km of an existing road that could be candidates for drone deployment.

Table 1. Estimates for conventional helicopter operations to cover strawberry guava invaded portion of the ~17,000 ha Hilo Forest Reserve Watershed Section on Hawai'i Island at different grid spacings for the release of *T. ovatus*, an approved biocontrol agent of the invasive tree species *Psidium cattleyanum* Sabine (strawberry guava).

Drop grid spacing (m)	# of drops	Average speed between drops (m/sec)	Distance between drops	Time per drop: transit + 30 s (seconds)	Heli time (Hrs)
100 × 100 m	5,672	10	100	40	63.0
500 × 500 m	250	20	500	55	3.8
1,000 × 1,000 m	65	25	1,000	70	1.3

hang success rate. Rotor wash effects need to be taken into account for future missions using these methods. Of the 3 different bolas storage types tested for use in the helicopter trials, the homemade wooden slat system (Fig. 4D) was the best in terms of ease of use and storage capacity.

Existing Federal Aviation Administration regulations currently preclude extensive drone operations beyond visual line of sight in the United States (14 CFR §107.31), though waivers are being granted (Coldsnow et al. 2023, Schultz et al. 2025). Even with beyond visual line of sight capabilities, radio range limitations of current drone systems still limit any advantages conferred by longer flight time or additional payload capacity, especially when using larger spacings between drops (Supplementary Table A1). Technological improvements in communications and control for extended operational ranges are necessary before additional payload or flight time could make UAS operations more viable.

Given current regulatory and technical constraints and the limited capacity of our existing drone-based application tools, conventional helicopter operations are the best means of deploying *T. ovatus* at a landscape scale. Extending our calculations for deploying *T. ovatus* at a 1000 m grid spacing across 17,000 ha of the Hilo Forest Reserve (Table 1), we estimate that the 475,000 ha of strawberry guava statewide in Hawai'i could be treated by helicopter in under 100 h, not including ferry and refueling time. In the context of annual conservation-related helicopter mission hours that occur across the state (Kawelo et al. 2012, Seitz et al. 2012, Leary et al. 2014), this amount is not unreasonable.

This simple approach assumes strawberry guava trees are evenly distributed and visible from the air across the target area, which we recognize to not be the case. Strawberry guava invasion levels vary with elevation and historic land use (Potter et al. 2023), and in some places are mostly covered by existing overstory vegetation canopy (Gillespie et al. 2008, Asner et al. 2009, Owen et al. 2022, Denslow et al. 2024). A more selectively directed approach, focusing on strawberry guava trees in the mid- and upper forest regions would likely result in faster *T. ovatus* penetration across the leading edge of the strawberry guava invasion front and require less resources. And, unlike other helicopter-based control efforts that require repeated deployments, dead newborn mice containing acetaminophen as an oral toxicant for the brown tree snake in Guam (Siers et al. 2020, Goetz et al. 2021) and in Hawai'i the larvicide *Bacillus Thuringiensis Israelensis* for combating avian malaria (Hawai'i DLNR 2024 Aug 30), there would be no need for additional deployments after successful initial *T. ovatus* releases.

We developed a successful method for aerially deploying and monitoring an approved leaf-galling biocontrol for strawberry guava, an invasive species of high concern across tropical forests around the globe (Tng et al. 2016, Paynter et al. 2018, Koerner et al. 2022). To our knowledge, this is the first use of drones for release of a weed biocontrol agent in the Hawaiian islands. Our results showed that aerial deployment is faster and more effective than a more traditional ground-based method and could be adopted by forest managers wherever control efforts are underway. We found high-resolution aerial imagery to be useful for detecting leaf galls and with repeat monitoring flights can be used to map spatiotemporal spread in the forest. We believe landscape-scale deployment operations are feasible using these methods and look forward to greater distribution of biocontrol of strawberry guava across the Hawaiian Islands. These findings are relevant to others working to deploy and monitor biocontrol in tropical and forested ecosystems worldwide.

Supplementary material

Supplementary material is available at *Journal of Economic Entomology* online.

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Author contributions

Ryan Perroy (Conceptualization [lead], Data curation [equal], Formal analysis [equal], Funding acquisition [lead], Investigation [equal], Methodology [equal], Project administration [lead], Resources [lead], Supervision [lead], Validation [equal], Visualization [equal], Writing – original draft [lead], Writing – review & editing [lead]), Roberto Rodriguez (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Validation [equal], Visualization [equal], Writing – original draft [supporting], Writing – review & editing [supporting]), M. Tracy Johnson (Funding acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Resources [supporting], Validation [equal], Writing – original draft [supporting], Writing – review & editing [supporting]), and Olivia Jarvis (Formal analysis [supporting], Investigation [supporting], Writing – original draft [supporting], Writing – review & editing [supporting])

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