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Spatially Targeted Biological Control of Mile-a-Minute Weed Using *Rhinoncomimus latipes* (Coleoptera: Curculionidae) and an Unmanned Aircraft System

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Abstract

Rhinoncomimus latipes Korotyaev is a specialist biocontrol agent of mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross (Caryophyllales: Polygonaceae). Currently, *R. latipes* is released by hand where the presence of the weed is readily detected. However, the hand-release method is not applicable to weed patches spread in hard-to-access areas. This study was conducted to develop a spatially targeted biocontrol strategy by using an unmanned aircraft system (UAS, a.k.a. drone) for the detection of *P. perfoliata* and aerial release of *R. latipes*. A ground survey was performed to locate *P. perfoliata* patches and then a rotary-wing UAS was flown at 15 different altitudes to determine the detectability of *P. perfoliata* patches. We developed an insect-release system including a pod that housed *R. latipes* for aerial release. The pod was 3D printed with biodegradable polyvinyl alcohol (PVA), and field tests were conducted to determine the ability of *R. latipes* to escape the pod and assess their post-release mortality and feeding ability. The results of this study showed that *P. perfoliata* patches were readily detectable on the aerial images taken at ≤15 m above the ground. More than 98% of *R. latipes* (n = 118) successfully escaped from the pod within 24 h after aerial deployment. There were no significant (P > 0.05) effects of PVA exposure on the mortality and feeding ability of *R. latipes*. These results indicate that aerial detection of *P. perfoliata* and deployment of *R. latipes* for spatially targeted biological control in hard-to-access areas can be accomplished using a rotary-wing UAS.

Key words: drone, invasive weed, *Persicaria perfoliata*, release strategy, UAS

As many insect pests are spatially aggregated in the agricultural field (Amtmann et al. 2008, Rasmussen et al. 2013, Sétamou and Bartels 2015, Fernández-Quintanilla et al. 2018, Nguyen and Nansen 2018), site-specific control of pest hotspots can play a significant role in precision pest management. Park et al. (2007) state that spatially targeted insect pest management consists of two parts: technologies to characterize and generate spatial distribution of insect pests and to apply control measures site-specifically by utilizing variable rate technology or unmanned aircrafts. Application of Unmanned Aircraft Systems (UAS; a.k.a. drone) has become a key tool for precision pest management in agriculture and forestry. Many previous studies have shown that the use of UAS could be cost-effective, environmentally friendly, and advantageous compared with conventional platforms of remote sensing, such as ground-based (e.g., handheld or tractor-attached spectral radiometers), manned aerial methods (e.g., airplanes and helicopters), and orbital (e.g., satellite imagery) (Filho et al. 2020).

Mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross (Polygonaceae), is a fast-growing invasive weed widely dispersed in the northeastern and mid-Atlantic United States (Kumar and

DiTommaso 2005, Hough-Goldstein et al. 2012, Hough-Goldstein et al. 2015). *Persicaria perfoliata* was first introduced to the Northeastern United States accidentally in a nursery in York County, Pennsylvania, and is currently distributed over 15 states ranging from New Hampshire to North Carolina (Moul 1948, Hough-Goldstein et al. 2009, Miller et al. 2018, Cheah and Ellis 2019, EDDMaps 2020). This species is found in riparian sites within its native region, including China, Korea, Japan, and the Philippines (Wu et al. 2002, Hyatt and Araki 2006). In contrast, *P. perfoliata* usually invades open fields, forest edges, and disturbed areas (i.e., construction site, roadsides, and utility rights-of-way) in the United States, forming infestations composed of patches with variable connectedness, size, and shape (Cusick and Ortt 1987, Hough-Goldstein et al. 2015). The impacts of *P. perfoliata* include reducing native plant diversity, interfering with recreational use of natural areas, and undermining reforestation and natural forest recovery by overtopping tree seedlings (Wu et al. 2002, Hough-Goldstein et al. 2012). Current methods of *P. perfoliata* management include biological control, physical or mechanical removal, and pre- and postemergent herbicides (Mountain 1989, Colpetzer et al. 2004a, Gover et al. 2008). However, its rapid growth rate and the viability of seeds for 6-yr in the seed bank (Van Clef and Stiles 2001, Hough-Goldstein et al. 2015) make these control techniques time-consuming, expensive, and inefficient. In contrast to its native region, *P. perfoliata* in the United States is not suppressed by native herbivores or pathogens that cause severe damage or form specialized associations with *P. perfoliata* (Fredericks 2001, Ding et al. 2004).

Following host range testing, a permit was issued by the U.S. Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine for field release of *Rhinoncomimus latipes* Korotyaev in 2004 (Price et al. 2003, Colpetzer et al. 2004b, Hough-Goldstein et al. 2015). *Rhinoncomimus latipes* showed a strong host preference and survivorship on *P. perfoliata* over other related Polygonaceae species and economically important plants (e.g., nontarget plants) in no-choice tests (Colpetzer et al. 2004b) and open field tests (Frye et al. 2010). Female weevils feed and lay eggs preferentially on capitula (compact flowering head) and less so on other parts of *P. perfoliata*, and this preference increases the larval survivorship because the apical portion including capitula is tenderer to the *R. latipes* larvae to bore into (Colpetzer et al. 2004a). It takes 3–5 d for eggs to hatch and newly hatched larvae bore into the stem. The feeding damage by *R. latipes* adults and stem-boring larvae reduces substantially the growth and reproductive potential of *P. perfoliata* (Smith and Hough-Goldstein 2014).

Rhinoncomimus latipes is known to disperse at an average rate of 1.5–2.9 m/wk from a release point within a *P. perfoliata* patch (Lake et al. 2011) and 4.3 km/yr in long-term monitoring (Hough-Goldstein et al. 2009). Although this is a respectable dispersal rate for a small insect, at this rate it would take several decades to reach the current invasion front of *P. perfoliata* (Hough-Goldstein et al. 2012). Consequently, this indicates that releasing *R. latipes* should be conducted continuously in a site-specific manner at the invasion front primarily using mass-reared weevils. Although there is no recommendation of the number of *R. latipes* release on a per-area basis yet, 200 adults are typically released in a single release (Hough-Goldstein et al. 2015). In addition, *P. perfoliata* populations have a patchy distribution in the landscape and often spreads into hard-to-access areas. The need for multiple weevil releases over time into a patchy, inaccessible invaded landscape makes the release of *R. latipes* challenging with the current hand-release method. Therefore, spatially targeting and precision release of *R. latipes* could be a solution for more effective biocontrol of *P. perfoliata* on a landscape scale.

Aerial deployment of biocontrol agents can be beneficial for large-scale or site-specific pest management. Park et al. (2018) developed an aerial-release system for biocontrol agents of *P. perfoliata*, consisting of a pod that housed *R. latipes*, a pod dispenser, and UAS. Although the aerial-release system performed successfully, three drawbacks needed to be developed and improved upon. First, the primary materials used for making the pods were cardboard for the frame, clay for the end caps, and plastic straws for housing *R. latipes*. Cardboard and clay can be degraded in the field, but plastic straws take up to 450 yr to decompose, resulting in environmental contamination (Kreiger et al. 2013). Because the aerial release system aims to control *P. perfoliata* in hard-to-access area, the pod should be biodegradable. Second, loading *R. latipes* into the pod was time-consuming and required unreasonable precision. The straws used in Park et al. (2018) were 5 mm in diameter and *R. latipes* needed to be loaded one by one into the pod. Third, to release *R. latipes* from the pod after aerial deployment by UAS, the end cap made of clay had to be broken by the ground impact. Because *P. perfoliata* generally makes a thick canopy in a patch, the necessary hard impact required may not occur in many sites. Therefore, the aerial release system for *R. latipes* needed to be modified and improved for successful spatially targeted pest management.

This study was conducted to improve the aerial release system for spatially targeted management of *P. perfoliata* using *R. latipes* (Fig. 1). The objectives of this study were to develop a biodegradable pod for delivery of *R. latipes* (a.k.a. bug pod), to test the effects of the material to craft the bug pod on *R. latipes*, and to determine the ability of UAS to identify populations of *P. perfoliata* to target for aerial releases.

Materials and Methods

Study Insect

Rhinocomimus latipes used in the study were collected in Waynesburg, PA, and were maintained in a rearing chamber in the Entomology Laboratory at West Virginia University, Morgantown, WV. The rearing chamber was kept as photoperiod of 16:8 photoperiod (L:D) regime under 23°C and 40–60% R.H. To feed *R. latipes*, *P. perfoliata* was grown in the greenhouse. *Persicaria perfoliata* achenes were collected directly from the soil under *P. perfoliata* patches and achenes were cold-moist stratified in a seedling tray with a wet potting soil mix (Pro-Mix BX;

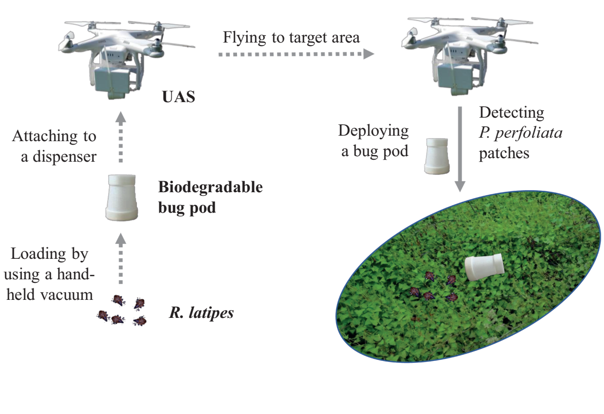

Fig. 1. Schematic diagram of aerial-release systems and an operational protocol with UAS. *Rhinoncomimus latipes* adults are loaded into a bug pod by using a vacuum, and the pod is attached to a UAS dispenser. The UAS is flown to detect a *Persicaria perfoliata* patch and deploys the bug pod on the patch.

Premier Horticulture, Quebec, Canada) at 6.6°C. After stratification, the tray was moved to a greenhouse growing room to germinate the seed. Germinants (cotyledon stage) were transplanted and grown under the same greenhouse conditions in round pots (15 × 14.5 cm), with two or three plants per pot. These plants were maintained to keep *R. latipes* fed until they were senesced in October. The stems were cut to increase branching and capitula material.

Study Site

Experiments for aerial release of bug pod and detection of *P. perfoliata* were conducted on privately owned land in Waynesburg, PA (39° 55′ 3.88″ N, 80° 2′ 18.82″ W). The site was dominated by *P. perfoliata*, and Japanese stiltgrass (*Microstegium vimineum* (Trin.) A. Camus) was commonly observed at the site. The *P. perfoliata* patches were located in an open-cut area among the forest. *Persicaria perfoliata* was first observed in the area in the mid-1990s. Chemical control with a broad-spectrum herbicide (Roundup) was applied on the *P. perfoliata* once in 2010, and 3,000 *R. latipes* were released once in 2018 at the site. Neither a one-time herbicide application nor a release with limited time for weevils to build up larger populations to impact the weed with a 6-yr seed bank would be expected to succeed.

The design goals of the bug pod were: 1) easy loading of *R. latipes*, 2) easy escape of *R. latipes* from the bug pod after aerial release, and 3) environmentally friendly. The design of the bug pod was drawn with a computer-aided design (CAD) tool (Solidworks; Dassault Systems, Vélizy-Villacoublay, France; Fig. 2). For the precise design of the bug pod, the average width and length of 88 *R. latipes* adults were measured using a digital microscope (AM7915MZT Dino-Lite Edge; AnMo Electronics Corp., Taipei, Taiwan). Based upon the width and length of *R. latipes* (Fig. 3), the size and design of the bug pod were determined. The bug pod was printed by using a 3D printer (Ultimaker 3 Extended, Ultimaker B.V., Cambridge, MA). Polyvinyl alcohol (PVA) filament (Ultimaker Natural PVA Filament, Ultimaker B.V.) was used for printing the bug pod. This filament was originally developed as a supporting material in fabrication of complex structures in 3D printing. In this study, we used the filament as the main printing material to utilize its unique property of being soluble in water and biodegradable.

The system for loading *R. latipes* into the bug pod was selected based upon the shape, size, and weight of the bug pod. Considering that a vacuum aspirator has been used for collecting small mites and insects in entomological studies (Singer 1964), a hand-held vacuum (Heavy Duty Hand-held DC Vac/Aspirator; BioQuip Inc., Rancho Dominguez, CA) was selected as a tool for loading *R. latipes* into the bug pod quickly and safely.

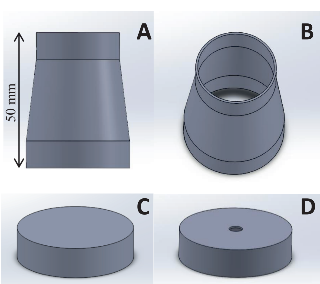

Fig. 2. Schematic design of the bug pod using Solidworks. (A) side view; (B) perspective view; (C) bottom lid for loading the weevils; and (D) top lid with a 3-mm hole for *Rhinoncomimus latipes* to escape after aerial release.

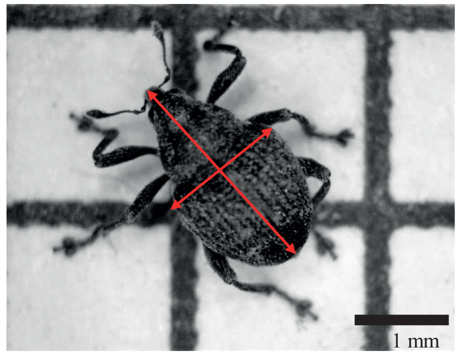

Fig. 3. Estimation of the body size of *Rhinoncomimus latipes* for precise design of bug pod.

Effect of PVA on *R. latipes*

PVA is a synthetic polymer that is widely used in industrial, commercial, medical, and food applications because of its biodegradability and biocompatibility (DeMerlis and Schoneker 2003, Baker et al. 2012, Marin et al. 2014). This polymer can be easily degraded with water contact (e.g., rain, morning dew, and high humidity) and numerous studies reported that microorganisms with oxidases/hydrolases can degrade the PVA (Kawai 1999, Kim et al. 2003, Nogi et al. 2014, Ohtsubo et al. 2015). The safety of PVA was proven in toxicity studies with mammalians including rats and rabbits (DeMerlis and Schoneker 2003), but there is a chance that PVA can negatively affect *R. latipes*. Therefore, a study was conducted to test the effect of PVA on the feeding ability and mortality of *R. latipes*. Two separate trials (10 replications per trial) were made to test the null hypothesis of no difference between control and treatment: *R. latipes* that were kept in a Petri dish (50 mm in diameter × 20 mm in height) with filter paper dipped in deionized water (control) and *R. latipes* that were kept in the Petri dish with a filter paper dipped in 3% PVA-dissolved solution (treatment) which is the optimum concentration allowing the solution to be absorbed into the filter paper (Patton and Robinson 1975). In each replication, four *R. latipes* were randomly selected from the colony and gently placed into the Petri dish. After a 24-h exposure period, *R. latipes* were transferred to a clean Petri dish with a *P. perfoliata* leaf and a wet dental wick. The leaves were replaced daily and each leaf was scanned using a scanner (Brother DCP-7065DN; Brother Industries, Ltd., Nagoya, Aichi, Japan) before and after feeding to measure the daily feeding amount. To quantify the amount of leaf consumed, image analysis was performed to measure changes in the leaf area by using Adobe Photoshop CS4 (Adobe Inc., San Jose, CA). The mortality and feeding amount of *R. latipes* was monitored daily for a week under a photoperiod of 16:8 (L:D) regime and under 23.7 ± 0.01°C and 48.0 ± 0.05 RH. Repeated measures ANOVA was performed on collected data using JMP (SAS 2015) and SAS (SAS 2002) software at $\alpha = 0.05$.

Effects of Aerial Release on *R. latipes*

Park et al. (2018) showed that the aerial release of bug pods at <30 m above the ground did not affect the feeding ability and mortality of *R. latipes*. However, they did not monitor the bug pods after aerial deployment to make sure all *R. latipes* successfully escaped. In this study, we conducted a field test to determine the escaping ability of *R. latipes* from the bug pod after aerial deployment. In total, 120 *R. latipes* were randomly selected from the colony and starved for 24 h in a Petri dish before loading them into six bug pods (20 *R. latipes* per pod) to increase their motivation to chew their way out of the pod. All *R. latipes* were loaded by using the hand-held vacuum loader. Then, a piece of *P. perfoliata* leaf was attached to the lid with a 3-mm hole to keep the *R. latipes* inside but to let them chew out after aerial release. The deployment date was chosen when three consecutive days without rain were forecasted. A bug pod was loaded on a dispenser (FliFli AirDrop Release and Drop Device; Arbity, Incheon, Republic of Korea), then the dispenser was attached to a drone (DJI Phantom 3 Advanced; SZ DJI Technology Co., Ltd., Shenzhen, China). A bug pod was hung by sting on the metal bar of the dispenser and the bar movement was triggered by a remote controller for bug-pod deployment. The experiment was conducted in September 2019. According to Park et al. (2018), the bug pods were deployed aerially at 15 m above the ground onto a *P. perfoliata* patch one at a time. We recovered bug pods 3 d later to count *R. latipes* that escaped from the bug pods. Any *R. latipes* remaining in the bug pods were counted to calculate the rate of a successful escape.

Determination of Aerial Release Sites

For the spatially targeted aerial release of *R. latipes*, we utilized two criteria to detect *P. perfoliata* patches from the aerial photos taken by UAS: 1) the equilateral triangular shape without lobes or indentations of *P. perfoliata* leaves on stem with nodes and 2) the distributional pattern of *P. perfoliata* forming a large monoculture. Although there are several congeners in North America, *P. perfoliata* leaves are readily identifiable by their triangular leaves. Before flying the drone at the site, a ground-truth was performed to mark the centroid of each plot. The aerial images were taken vertically from the centroid points at 15 different altitudes (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, and 100 m above the ground). Each of 15 images was collected from 13 plots (i.e., replications) and examined by using Photoshop to determine the detectability of *P. perforliata* by aerial imagery. The detectability was determined by a unique feature of the triangular leaves on each aerial image; when the margins of the triangular leaf were not straight or distorted, it was considered as nondetectable. In addition, the number of pixels per centimeter on images was counted using Photoshop to set the detection threshold.

Results

Development of Bug Pod Design

We developed a cylindrical-shaped bug pod with a top lid having 1-mm holes (smaller than *R. latipes*, which are 1.23 ± 0.009 mm in width and 2.04 ± 0.036 mm in length; Fig. 4A) and a bottom lid with one 3-mm hole from which the *R. latipes* escape after release (Fig. 4B). The whole parts were fully fabricated from PVA filament. Due to failure to print the top of the lid with precise 1-mm pinholes, we used a 0.8-mm pin to make pinholes, allowing airflow for the vacuum loader when loading *R. latipes* and ventilating to keep *R. latipes* alive inside the bug pod (Fig. 4A). The thickness of the wall of the bug pod was 1 mm considering the balance between sturdiness and readiness to dissolve with water contact. The bug pod tightly fits in the suction pipe of the hand-held vacuum allowing the airflow focused to the 3-mm hole (Fig. 4C).

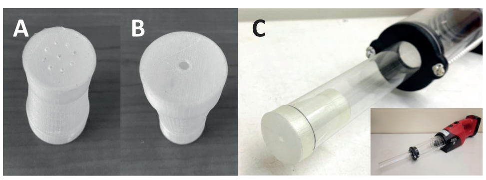

Fig. 4. Example bug pod generated with a 3D printer and PVA: (A) view from the bottom of the bug pod with pin holes for ventilation; (B) view from the top of the bug pod with an exit hole that is designed to be covered with a pieces of *Persicaria perfoliata* leaf; and (C) a vacuum to load *Rhinoncomimus latipes* into the bug pod.

Effect of PVA on *R. latipes*

The average feeding amount of a single *R. latipes* per day was 0.106 ± 0.005 cm^2 for control and 0.104 ± 0.005 cm^2 for treatment in trial 1, and 0.113 ± 0.006 cm^2 for control and 0.093 ± 0.005 cm^2 for treatment (PVA exposure) in trial 2 (Table 1). Although average feeding amounts for both groups decreased gradually during the experiment (63.09% for control and 64.21% for treatment in trial 1 and 58.59% for control and 68.79% for treatment in trial 2), we found no significant differences in feeding ability between control and treatment: $F = 1.28$; df = 6, 98; $P = 0.275$ for trail 1, $F = 1.54$; df = 6, 101; $P = 0.174$ for trial 2, and $F = 0.94$; df = 6, 214; $P = 0.468$ for both trials 1 and 2 combined. No mortality was observed in the controls, and only one and two deaths in the PVA treatments in trial 1 and 2, respectively (Table 1).

The loading 20 *R. latipes* into each bug pod was done instantly with one suction without failure ($n = 6$) and there was no mortality or body damage caused by loading *R. latipes* by using the hand-held vacuum. We found that 98.3% ($n = 118$) of *R. latipes* could chew out and escape successfully from the bug pod within a day after aerial deployment. In the recovered bug pods, two *R. latipes* were remaining 3 d after aerial release: one of which was alive, and the other was deceased.

Determination of Aerial Release Sites

Aerial images were taken at 15 different altitudes to determine the detectability of *P. perfoliata* from other plants in the landscape (Fig. 5; $n = 13$). The size of each pixel on the image was ca. 0.13, 0.25, 0.35, 0.46, 0.60, 0.70, 0.84, 0.95, 1.07, 1.19, 1.39, 1.63, 1.83, 2.08, and 2.27 cm/pixel for 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, and 100 m above the ground, respectively. At the 5, 10, and 15 m above the ground, *P. perfoliata* was readily detectable from the images. Detection of *P. perfoliata* on images taken at 20 m above the ground was successful 46% of the time, and all of the images taken at higher altitudes were too blurred to detect *P. perfoliata* (Fig. 6). These results suggest that *P. perfoliata* is detectable from aerial photos taken at ≤15 m above the ground or with a resolution of ≥0.35 cm/pixel.

Discussion

The utilization of aircraft releasing natural enemies was first suggested by Herren et al. (1987). Since then, there have been several aerial-release systems using manned or unmanned aircraft, including the release of sterile insects for areawide management (Tan and Tan 2013, Rosenthal 2017, M3 Consulting Group 2018) and biocontrol of European corn borer (Carroll et al. 2008). Recent advances in small UAS have created new opportunities to reduce application costs and increase coverage of areas compared to manual distribution (Filho et al. 2020). The UAS equipped with an aerial system we developed in this study presented the potential use of UAS for precision release of biocontrol agent to target weed patches. The bug pod developed in this study possesses three advantages over the one developed by Park et al. (2018). There was less labor involved in making the new bug pod. The former bug pod required multiple steps of cutting, gluing, casting, and

Table 1. Average feeding area (mean ± SEM) of *Rhinoncomimus latipes* in control (n = 40) and PVA-exposed (n = 40) group

Trial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Daily average
Trial 1								
Control	0.164 ± 0.021	0.104 ± 0.023	0.126 ± 0.029	0.086 ± 0.012	0.108 ± 0.023	0.092 ± 0.024	0.061 ± 0.017	0.106 ± 0.005
PVA	0.154 ± 0.030	0.107 ± 0.019	0.163 ± 0.029	0.113 ± 0.015	0.101 ± 0.021	0.095 ± 0.014	0.055 ± 0.011	0.104 ± 0.005
Trial 2								
Control	0.152 ± 0.018	0.129 ± 0.026	0.109 ± 0.023	0.102 ± 0.017	0.080 ± 0.011	0.096 ± 0.019	0.063 ± 0.012	0.113 ± 0.006
PVA	0.143 ± 0.022	0.128 ± 0.017	0.104 ± 0.011	0.069 ± 0.024	0.085 ± 0.015	0.069 ± 0.014	0.044 ± 0.007	0.093 ± 0.005

The unit of measurement was cm^2. No significant differences were found between control and PVA-exposed groups ($P > 0.05$)

delicate handling to complete. The new bug pod was designed for 3D printing, and this can be easily reformed or resized. With the 3D printer used in this study, the bug pod could be printed in ~3 h with an average weight of 6.0 g (n = 10). The amount of PVA used for printing a single bug pod costs approximately $1.23, which is cheap and biodegradable. Moreover, *R. latipes* was loaded quickly and easily into the bug pod by using the insect vacuum loader. Although the bug pod is fully water-soluble, we observed in the field that the bug pod in high humidity became slimy before it fully liquified, which could increase the mortality of *R. latipes*. Therefore, when *R. latipes* is released with the bug pod, at least 1 dry day would be needed for the successful release of *R. latipes* as this study showed that most of *R. latipes* escaped from the bug pod within 24 h.

In addition to the improvement of the bug pod, this study demonstrated the potential of site-specific invasive weed management (see Shaner and Beckie 2014 for review). The results of this study showed that UAS could be used to detect and confirm the presence of *P. perfoliata* patches at ≤15 m above the ground and the same UAS could carry bug pods for the aerial deployment of *R. latipes* on the *P. perfoliata* patches. This new approach can play a key role in integrated pest management for invasive plants that outcompete some native plants due to their high reproductive potential and their lack of natural enemies in their invaded range (Hough-Goldstein et al. 2015). Physical and chemical control may reduce the invasive plant populations immediately, but reemergence after a single treatment of invasive plants is likely due to propagules remaining in the soil as well as dispersal from other invaded areas. Longer-term control, thus, often requires frequent use of herbicides or more expansive removal of vegetation, which can increase negative impacts on nontarget native plants. Mowing, cutting, and hand pulling, and application of broad-spectrum herbicides can immediately decrease *P. perfoliata* cover (Mountain 1989, Gover et al. 2008, Hough-Goldstein et al. 2015). However, these controls are most effective in the earlier stages of invasion, before the seed set has occurred over multiple growing seasons. Otherwise, multiple applications of the treatment are needed. Moreover, the removal of any invasive plants from the site leaves it open for re-invasion by *P. perfoliata* as well as invasion by different exotic plants (Lake 2011). The use of *R. latipes* as a classical biological control agent may enable a slower suppression of *P. perfoliata*, giving any existing or planted native species the upper-hand without additional disturbance and

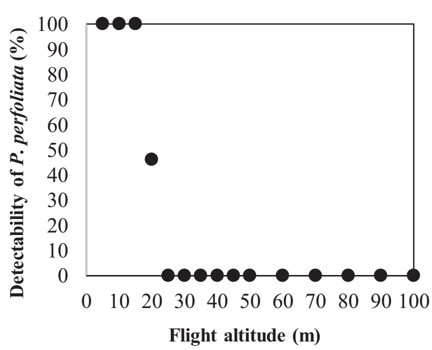

Fig. 5. Detectability of *Persicaria perfoliata* by UAS flown at different altitudes (n = 13 per flight altitude). The size of each pixel on the image was ca. 0.13, 0.25, 0.35, 0.46, 0.60, 0.70, 0.84, 0.95, 1.07, 1.19, 1.39, 1.63, 1.83, 2.08, and 2.27 cm/pixel for 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, and 100 m above the ground, respectively.

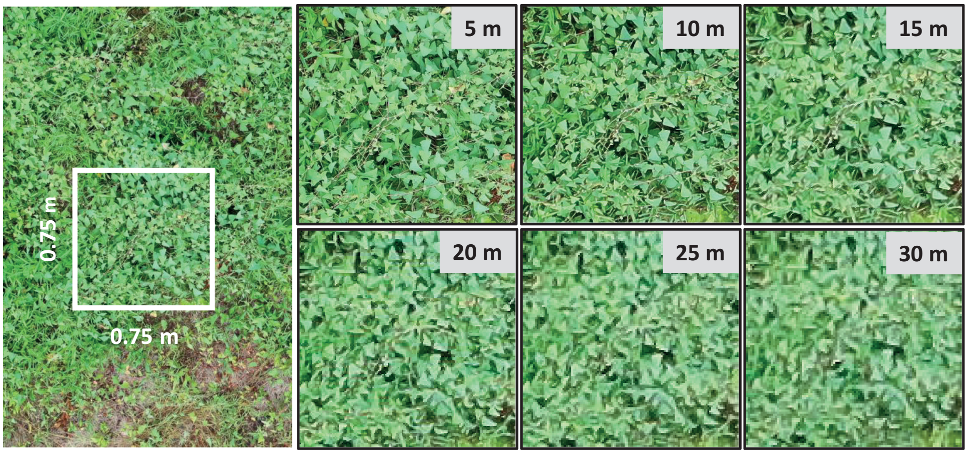

Fig. 6. Detectability of *Persicaria perfoliata* patches based on aerial images acquired from UAS at different flight altitudes. Note that *P. perfoliata* could not be detected from the image taken by UAS at >25 m above the ground.

time to recover or grow (Hough-Goldstein et al. 2009, Hudson et al. 2017). In addition, *R. latipes* has been successfully integrated with herbicide applications and native plantings to control *P. perfoliata* and restore native plant communities (Cutting and Hough-Goldstein 2013, Lake et al. 2014).

This study and a few previous studies (Carroll et al. 2008, Tan and Tan 2013, Park et al. 2018, M3 Consulting Group 2018) demonstrated the potential of the aerial release of insects, but the use of UAS for the aerial deployment of control measures need to comply with the Federal Aviation Administration (FAA) regulations. The FAA set specific rules and regulations for the operation of small UAS in the National Airspace System (NAS). Title 14 of the Code of Federal Regulations (14 CFR) Part 107 specifically addresses not only the classification and certification of UAS but also the operational limitations of UAS flights (FAA 2016). The 14 CFR prohibits careless or reckless operation that endangers life or the property of another and cannot drop an object creating an undue hazard to persons or property (14 CFR §107.23). The UAS system developed in this study drops a relatively light-weight bug pod (6.0 g) and operates in a landscape where humans may not have easy access. So, the possibility of harming persons or property is negligible. Also, the FAA regulates the maximum altitudes for flying UAS: 400 ft or 122 m. The operation of the UAS we developed is performed at 15 m above the ground for aerial deployment and below 20 m above the ground for detection of *P. perfoliata*. This falls well below the maximum allowable altitude for UAS.

In conclusion, this study demonstrated the potential of UAS for precision aerial release of *R. latipes* in hard-to-access areas with a patchy distribution of *P. perfoliata*. The bug pod developed in the study was easy to create, easy to load, and fully fabricated with environmentally-friendly material with no negative effects on *R. latipes* mortality and feeding ability. Our study also showed that patchy populations of *P. perfoliata* for targeted aerial release of *R. latipes* could be successfully identified with UAS flown below 15 m above the ground. These results indicate that aerial detection of *P. perfoliata* and deployment of *R. latipes* for biological control in hard-to-access areas can be accomplished using a rotary-wing UAS. Future studies should be directed at long-term monitoring of *R. latipes* and *P. perfoliata* populations after aerial deployment.

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