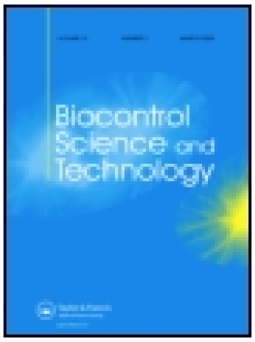

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RESEARCH ARTICLE

First drone releases of the biological control agent *Neomusotima conspurcatalis* on Old World climbing fern

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ABSTRACT

Biological control is a sustainable method of weed management because agents can establish persistent and self-dispersing populations on a landscape scale. Strategic releases are often conducted to accelerate the widespread dispersal and establishment of agents. Drones (unmanned aerial systems, UAS) could facilitate these releases, particularly in remote areas that are difficult or dangerous for humans to access. *Lygodium microphyllum* (Lygodiaceae), Old World climbing fern, is a damaging weed that outcompetes native plants in many habitat types in Florida. This weed often occurs in remote conservation areas accessible only by helicopter, airboat, or swamp buggy. Although the biological control agent *Neomusotima conspurcatalis* (Lepidoptera: Crambidae) is established and dispersing in Florida, populations are patchy and additional releases are needed, particularly in conservation areas where human access is limited. We conducted the first releases of *N. conspurcatalis* using a drone to test agent survival and larval transfer to field populations of *L. microphyllum*. In the survival experiment, 85% of individuals were recovered from the flight, flight with impact, and control treatments with no treatment differences. In the larval transfer experiment, significantly more larvae and pupae were recovered four days post-release from the drone releases than hand releases. Drone releases of *N. conspurcatalis* could improve the establishment and potential impact of this agent on a landscape scale. In general, drone releases in *L. microphyllum* and other biological control systems could help to mitigate limits of agent dispersal.

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Introduction

Biological control is considered more sustainable than other weed management techniques because of the ability of agents to establish persistent and self-dispersing populations on a landscape scale (Van Driesche, 2012). Releases in strategically chosen

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locations can facilitate agent dispersal and achieve widespread establishment (Paynter & Bellgard, 2011; Pratt et al., 2003) by overcoming the limited dispersal ability of an agent (Paynter & Bellgard, 2011) or a fragmented distribution of the target weed (Balentine et al., 2009; Pratt et al., 2003). However, it is not always possible to access these infestations or efficiently release agents within them.

Drones (unmanned aerial systems, UAS) may help solve these logistical challenges and overcome the dispersal limitations of agents. While most drone use in invasion biology and management focuses on applying the imaging capabilities of drones to monitor invasive plant species (Dash et al., 2019), researchers have begun to use drones to release biological control agents (e.g. Park et al., 2018; Teske et al., 2019). Several researchers have conducted drone releases of biological control agents such as mites and parasitoid wasps that target arthropod pests (Iost Filho et al., 2020; Martel et al., 2018; Teske et al., 2019). In contrast, research on drone releases of weed biological control agents is lacking, as only one study has used this technology (Park et al., 2018). For drone release of any biological control agent to be successful, the methods must ensure that agents are not injured during transport or release (Iost Filho et al., 2020). It is not yet clear how effective these new drone-based techniques are at delivering agents compared to traditional release methods (Iost Filho et al., 2020; Martel et al., 2018).

Drone releases of weed biological control agents could be particularly beneficial in conservation lands and areas that are difficult or dangerous for humans to access. Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae), is an ideal target for such releases. This damaging weed often grows in remote conservation areas in Florida that are accessible only by helicopter, swamp buggy, or airboat (EDDMaps, 2020; Hutchinson et al., 2006; Rodgers et al., 2018). Within invaded sites, accessing populations of the weed can require wading through deep water, navigating through sawgrass and other dense vegetation, and avoiding dangerous wildlife (e.g. American alligators, cottonmouth snakes, Burmese pythons) (Rodgers et al., 2018; Lake & David, personal observation). Development of effective, efficient methods to release biological control agents via drones will help minimise gaps in agent establishment across the landscape.

Here, we conducted the first drone releases of *Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae), a defoliating agent of *L. microphyllum* that is actively mass-reared and released across Florida. Our objectives were to assess the impact of drone flight and release on agent survival and subsequent development to adults, and to confirm larval transfer from drone releases to field populations of the weed.

Materials and methods

Study system

Lygodium microphyllum is native to parts of tropical and subtropical Asia, Australia, and Africa (Pemberton, 1998). It was first reported as naturalised in Florida in 1965, where it outcompetes native plants via individual rachises (long stems originating from the rhizome) that can reach 30 m long and smother native vegetation as they climb vertically or trail horizontally (Beckner, 1968; Hutchinson et al., 2006). Additionally, the weed invades multiple habitat types, can collapse tree canopies and alter fire regimes (Foxcroft et al., 2013; Hutchinson et al., 2006; Pemberton & Ferriter, 1998).

Neomusotima conspurcatalis was approved for release as a biological control agent for *L. microphyllum* in 2007 by the United States Department of Agriculture (USDA) Animal Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) (Boughton & Pemberton, 2009). Field releases began in Florida the following year and mass-rearing of this agent commenced in 2014 via the Comprehensive Everglades Restoration Plan (CERP) (Boughton & Pemberton, 2009; EvergladesRestoration.gov, 2020). *Neomusotima conspurcatalis* is a small moth with a wingspan of 11 mm; adults oviposit on *L. microphyllum* subleaflets. Larvae are 2-11 mm long, feed on the subleaflets while completing four or five instars, and pupate attached to the plant (Boughton & Pemberton, 2012). The life cycle takes approximately 30 days, depending on temperature, and the moth is active year round in *L. microphyllum* infestations in Florida (Boughton & Pemberton, 2009). *Neomusotima conspurcatalis* has a patchy distribution in Florida and is currently mass-reared and released throughout the southern and central regions of the state (Smith et al., 2014; Lake & David, unpublished data).

Insect colony

The *N. conspurcatalis* larvae used in this experiment were sourced from the USDA Agricultural Research Service (ARS) Invasive Plant Research Laboratory (IPRL) rearing colonies. These colonies consist of individuals field-collected from established populations in Florida. Colonies are replenished or replaced with new individuals every 4–6 mo and are fed field-collected *L. microphyllum*. Large clumps of intertwined *L. microphyllum* foliage (roughly basketball-sized) were cut from plants grown in a shade house at the USDA ARS IPRL. The cut foliage was placed in 30 11.4 L containers (Cambro Manufacturing, Huntington Beach, CA, USA) with 11 × 11 cm mesh vents installed in the lids. We transferred 100 *N. conspurcatalis* larvae (second and third instars) to the cut foliage within each container 24–36 h prior to use. This time period allowed larvae to move from the small subleaflets used to transfer larvae from the rearing operation to the containers and begin feeding, ensuring they were attached to the large mass of *L. microphyllum* foliage at the time of flight. The containers were randomly assigned to the two experiments detailed below.

Drone design modifications

A Phantom 4 Pro quadcopter drone (DJI, Shenzhen, China) was modified by attaching a custom-designed 3D printed bracket with an Arduino Pro Mini controller (SparkFun Electronics, Niwot, CO, USA) with a HS-65MG servo release mechanism (Hitec RCD USA, San Diego, CA, USA) to the landing skids (Figure 1). A piece of monofilament approximately 0.5 m long was attached to the bracket in two locations and released via the servo actuator. The logic was programmed into the mini controller to control a servo to open when the front-mounted LED light was turned on and close when the light was turned off. This light was controlled with the drone transmitter/controller and powered independently via a 9 V battery. One end of the monofilament was inserted through a mass of *L. microphyllum*. Neither the bracket mechanism nor the *L. microphyllum* obstructed the view of the onboard camera, which was used to confirm alignment over target areas for *L. microphyllum* release. The platform with a

Figure 1. Drone used for release of *Neomusotima conspurcatalis* larvae against *Lygodium microphyllum*. *Upper left*: Modified Phantom 4 Pro quadcopter drone fitted with a custom-designed 3D printed bracket. *Bottom left*: Close-up of the HS-65MG servo release mechanism. *Right*: Drone carrying *L. microphyllum* infested with *N. conspurcatalis* larvae during field experiments. Photo credit: Aaron S. David.

L. microphyllum payload was able to fly in 16–24 kph winds without flight performance degradation. However, the monofilament did occasionally twist around itself in the wind, preventing it from releasing the *L. microphyllum*. A thick grass stem was attached to the monofilament in two places above the *L. microphyllum* and acted as a stabiliser preventing twisting. At the time of testing, the Phantom 4 Pro was an approved system to be operated by the Department of Defense with the following caveat: the onboard operating firmware on the drone had to be modified to meet cyber security requirements.

Survival experiment

We assessed the effect of flight and impact after drone drop on survival of *N. conspurcatalis* larvae and subsequent development to adults. Containers with *L. microphyllum* foliage and *N. conspurcatalis* were randomly assigned to one of three treatments (6 replicates each): control, flight, or flight with impact. *Control* foliage was transported to the field in its container but was not utilised. *Flight* foliage was removed from its container and attached to the drone in the field, flown a horizontal distance of approximately 150 m, and immediately returned to its container. A flight of 150 m is a reasonable distance to transport larvae to a release site in the field and thus represents the potential for larvae to be lost in flight. *Flight with impact* foliage was similarly flown approximately 150 m, dropped from a height of 2–3 m into a patch of *L. microphyllum* plants in the field and then returned to its container. All containers were then returned to the laboratory and maintained at approximately 24°C and 47% relative humidity with a photoperiod of 10.5:13.5 h light: dark. Over the next six days, the *L. microphyllum* foliage was visually examined and the number of *N. conspurcatalis* larvae and pupae was recorded. To assess the ability of

N. conspurcatalis to complete development, we continued to monitor containers for adult emergence for the next 2 weeks. The containers were checked several times per week, *L. microphyllum* foliage was added to provision larvae as needed, and moths were counted and removed as they emerged.

Larval transfer experiment

The larval transfer experiment tested whether drone dropped larvae transferred to field populations of *L. microphyllum*. Containers were randomly assigned to either a hand transfer or a drone drop treatment (6 replicates each). The hand transfer method, which is the typical method for releasing *N. conspurcatalis*, consisted of tucking clumps of cut *L. microphyllum* infested with larvae into field populations of the weed, while the drone drop method consisted of dropping the infested *L. microphyllum* foliage on top of the plants. For all containers, we flew foliage containing 100 larvae a horizontal distance of approximately 150 m to the release site. For the hand transfer treatment, we manually removed foliage from the drone, and hand transferred each mass into *L. microphyllum* as described above. For the drone drop treatment, we dropped the foliage from a height of approximately 2-3 m. One drop landed on *L. microphyllum* where *N. conspurcatalis* larvae were already present and another landed on bare ground; in both cases, the dropped foliage was carefully moved by hand to a nearby unoccupied patch of *L. microphyllum*. The drone dropped material was secured to field populations of *L. microphyllum* using plastic-coated wire ties because of a forecast for 32–48 kph winds later that day. All release points were marked with PVC pipes and were spaced at least 2 m apart.

Drone flights for both experiments were conducted on the roadside edge of a large population of *L. microphyllum* located in Miramar, Florida (25° 57′ 28″ N, 80° 20′ 31″ W) on 6 February 2020. Line of site was maintained with the drone at all times. The temperature dropped to approximately 12°C that night, and it rained heavily the following morning. Four days post-release, the desiccated release foliage was removed from the field population of *L. microphyllum*, and the surrounding live foliage at each release point was searched for 10 min for *N. conspurcatalis* larvae and pupae.

Statistical analysis

For the Survival Experiment, we analysed the proportion of larvae recovered and the proportion of recovered larvae that completed development to adults. For the Larval Transfer Experiment, we analysed the proportion of larvae recovered from each drop method. Response variables from both experiments were analysed using generalised linear models with a beta distribution and logit link (PROC GLIMMIX). All analyses were performed in SAS Version 9.4 (SAS Institute, 2018).

Results

In the Survival Experiment, 85% of individuals were recovered across all treatments, and recovery did not differ by treatment ($F_{2,15} = 0.20$, $P = 0.8193$; Figure 2). Approximately

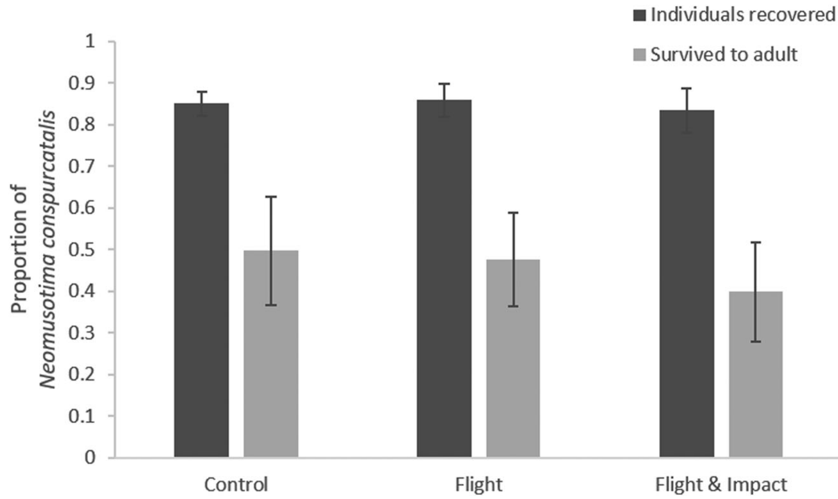

Figure 2. Proportion (mean ± standard error) of *Neomusotima conspurcatalis* individuals recovered (larvae or pupae) and survival of recovered individuals to adult from control, flight, and flight and impact treatments. Neither response variable significantly differed among treatments.

45% of the recovered larvae completed development to adults and survival to adult did not differ by treatment ($F_{2,15} = 0.18$, $P = 0.84$; Figure 2).

In the Larval Transfer Experiment, significantly more larvae and pupae were recovered four days post-release from the drone releases (38 ± 10%) compared to the hand transfers (18 ± 11%) ($F_{1,10} = 9.92$, $P = 0.0103$; Figure 3).

Discussion

The success of a weed biological control programme may depend on landscape-scale dispersal and establishment of agents. Drone release of agents may facilitate dispersal and establishment, particularly in areas that are unlikely to be colonised by dispersing agents or are difficult for humans to access. Here, we conducted the first drone releases of the biological control agent *N. conspurcatalis* and evaluated agent survival and larval transfer to field populations of the target weed *L. microphyllum*. Drone flights and releases did not result in loss of *N. conspurcatalis* larvae or reduced development to adults, and larvae successfully transferred from dropped, infested foliage to *L. microphyllum* in the field. Our findings support the use of drones for larger scale field releases of *N. conspurcatalis* and demonstrate the utility of this technology for releases of agents targeting other weed species.

Drones can be an effective tool for distributing biological control agents provided there is minimal harm of flight, release, and impact on the agents. A similar study that conducted drone releases of the weevil *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), a biological control agent for mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross (Polygonaceae), documented no deleterious effects on the agents (Park et al., 2018). However, Park et al. (2018) released adult weevils while we used larvae, which is the preferred life stage for release of *N. conspurcatalis*. Despite a potential 'cushioning' effect of the cut *L. microphyllum* foliage, we were concerned that soft-bodied

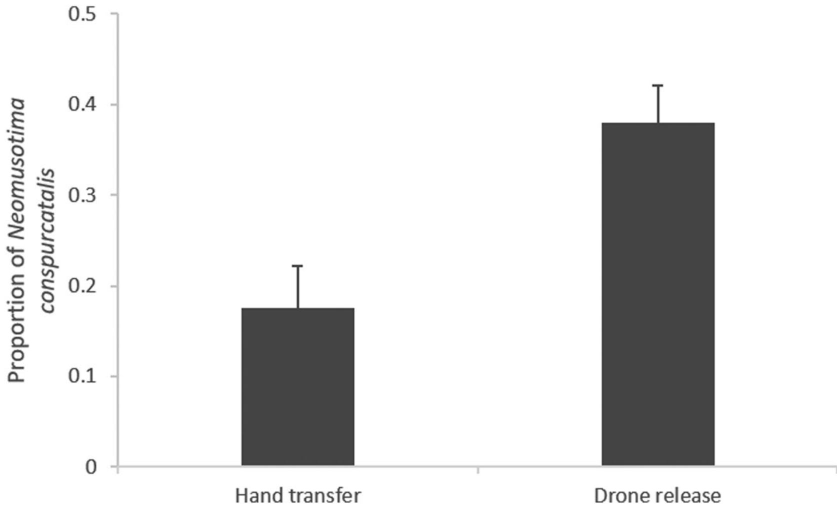

Figure 3. Proportion (mean ± standard error) of *Neomusotima conspurcatalis* larvae and pupae recovered in the field four days after hand transfer and drone releases.

larvae could be vulnerable to injury during flight, and particularly, impact when dropped. *N. conspurcatalis* larvae survived the flight and impact of the drone drop and completed development at rates no different from the controls, indicating this release method does not cause larvae to fall off the foliage or harm the agents.

Furthermore, a surprising result was the higher recovery of individuals from the drone release than the hand transfer sites four-days post-release. When hand transferring clumps of *L. microphyllum* with *N. conspurcatali*s larvae to the field, the cut foliage is nestled into field populations and protected from exposure to direct sunlight. Perhaps the rapid desiccation of exposed material from the drone release prompted the larvae to move quickly to the field *L. microphyllum*. Additional work could evaluate the potential for increased mortality if the agents are released from higher altitudes to maintain line of sight contact with the drone, though we emphasise that our results did not suggest any drop-related mortality.

Tethering the drone release material could have artificially increased recovery of drone dropped *N. conspurcatalis* by ensuring access to *L. microphyllum* for larvae to transfer to; however, due to the strong winds forecast following the release this was necessary to ensure we could recover the dropped material. During drone releases, the dropped material may miss its target due to several factors such as wind or pilot error. Fortunately, the consequences of a missed target will likely be minimal because most field releases occur in large monocultures of *L. microphyllum*, minimising the chance that larvae would die before finding their host. Regardless, our findings add to the existing literature highlighting the utility of evaluating different release methods (Dray et al., 2001; Goode et al., 2019).

Releases throughout the landscape are critical, even after initial agent establishment, to overcome limits to agent dispersal, and drone releases can complement efforts conducted via typical hand releases. For example, agent dispersal can vary through a landscape mosaic (Dávalos & Blossey, 2011; Hough-Goldstein et al., 2012a; Pratt et al., 2003) and drones could release agents in areas unlikely to be colonised by dispersing individuals. Additionally, the dispersal rate of biological control agents is highly variable (Paynter & Bellgard, 2011) and may not be sufficient to achieve landscape-scale colonisation or impact of the agent, at least in the short-term. For example, Hough-Goldstein et al. (2012b) determined that without additional releases, it would take the biological control weevil *R. latipes* 100 years to disperse from established populations to the invasion front of mile-a-minute weed (Hough-Goldstein et al., 2009). Furthermore, weed populations colonised by dispersing agents may not, at least initially, be as heavily damaged as plants at release sites (Boag & Eckert, 2013), and it may take an unacceptable amount of time for naturally-dispersing agents to colonise and build up population numbers needed to achieve desired damage levels in some locations (Pratt et al., 2003). Therefore, remote and isolated infestations considered unlikely to be colonised by dispersing individuals or accessed by researchers and land managers should be prioritised for drone releases.

In the *L. microphyllum* system, drones may significantly accelerate the weed biological control programme by releasing large numbers of agents in critical yet difficult to access locations such as Everglades tree islands. Both the effective range (∼1 km when accounting for the need to maintain line of sight contact) and duration (∼22 min flight time per battery) of the Phantom 4 Pro drone used in this study are sufficient for effective field deployment for *L. microphyllum* agent releases. Launching a drone from a stationary airboat to release agents at several invaded tree islands may prove more efficient than visiting islands individually, navigating through dense vegetation on foot and conducting hand releases.

Drones are increasingly being utilised to monitor invasive weeds (Dash et al., 2019), and one attempt has been made to use drones to assess future impacts of a biological control agent (de Sá et al., 2018). Our study adds to the nascent literature demonstrating that drones can be modified to assist in releases of weed biological control agents. The next steps in the *L. microphyllum* system are to use drone-based cameras to monitor the development, spread, and duration of *N. conspurcatalis* outbreaks, which could improve our understanding of conditions that facilitate outbreaks, furthering management goals. Future drone releases of biological control agents targeting *L. microphyllum* and other invasive weeds can be especially helpful in natural areas that are either difficult to access or are inhabited by dangerous wildlife, and we hope that their use is integrated into landscape-scale release strategies.

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Disclosure statement

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