Small-scale dispersal of a biological control agent – Implications for more effective releases

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ABSTRACT

_Eichhornia crassipes_ (Martius) Solms Laubach (Liliales: Pontederiaceae) was introduced to Florida in the 1880s as an ornamental and it once infested thousands of square kilometers across the state. _Megamolus scutellaris_ Berg (Hemiptera: Delphacidae) was developed as a classical biological control agent for this plant primarily because its free-living life stages allow it to better integrate with herbicides, which are currently used as the main control method for _E. crassipes_ in Florida. Mass rearing and distribution programs can accelerate the benefits of biological control by augmenting natural dispersal, but an optimal release strategy must consider the entire system including the agent, the target weed, and the habitat. The effectiveness of various release strategies was evaluated using a tank experiment where single and multiple releases of either adult _M. scutellaris_ only or _E. crassipes_ infested with _M. scutellaris_ eggs were compared to control treatments. The post-release dispersal capability of brachypterous _M. scutellaris_ was evaluated using a linear transect of _E. crassipes_. Two density release treatments were tested and emerging nymphs were used as a proxy for female dispersal distances. All release treatments resulted in successful _M. scutellaris_ population establishment and levels of _M. scutellaris_ were not significantly different among them. The dispersal experiment indicated that adult females oviposited near the release point before dispersing. While the release experiment indicated that all treatments were similar, the continually fluctuating populations of _E. crassipes_ makes establishment of populations difficult in the field. By releasing both adults and infested plants, additional propagule pressure can be attained from a single release event which can counter the tendency of adult _M. scutellaris_ to disperse rapidly following release.

1. Introduction

Waterhyacinth (_Eichhornia crassipes_) (Martius) Solms Laubach (Liliales: Pontederiaceae) is a free-floating aquatic plant that has invaded fresh water bodies across the world, altering native habitats and outgrowing native vegetation (Little, 1965; Gopal, 1987; Schmitz et al., 1993; Center, 1994). This species was introduced to Florida in the 1880s as an ornamental (Klorer, 1909) where, because of the warm climate and nutrient rich waters, it once infested thousands of square kilometers across the state (Lugo et al., 1978; Reddy and Debuk, 1984). Since the advent of synthetic herbicides, _E. crassipes_ can now be effectively managed, but relying solely on herbicides requires repeated applications (Schmitz et al., 1993). This has been the experience in Florida, where _E. crassipes_ is managed continually via herbicides by federal, state, and local agencies and costs can run into the hundreds of thousands of dollars annually (Gettys et al., 2014a).

Classical biological control programs in the U.S. utilizing monophagous insect herbivores have developed and deployed four species to increase suppression of this plant (Perkins, 1973; Center and Durden, 1981; Tipping et al., 2014b). The most numerous agent in Florida is _Neochetina eichhorniae_ Warner (Coleoptera: Curculionidae), which is known to reduce the growth and reproduction of _E. crassipes_, but not significantly reduce coverage (Tipping et al., 2014a). It is difficult for biological control agents to build up to damming densities because frequent herbicide applications can cause large fluctuations in _E. crassipes_ populations over wide areas (Center et al., 1999). Despite these challenges, herbivory by biocontrol agents increases the effectiveness of herbicide treatments by allowing for reduced dosages without any loss.
of efficacy, plus retarding the rate of regrowth following applications and has thereby reduced the impact of this plant in Florida (Center et al., 1999; Gettys et al., 2014b; Tipping et al., 2014a; Tipping et al., 2017). While herbicide-managed areas tend to have less E. crassipes coverage, areas where the biological control agent populations are unperturbed by the constant boom and bust cycling of E. crassipes contain smaller plants that are physiologically stressed by the insects (Center et al., 1999).

The most recently released agent, Megamelus scutellaris Berg (Hemiptera: Delphacidae), was selected primarily because its free-living juvenile and adult life stages allow it to better integrate with herbicides (Tipping et al., 2011). Eggs are laid inside the petiole and lamina of E. crassipes. Once they emerge, M. scutellaris goes through five nymphal instars (Tipping et al., 2011). Generation time is ~25 days outdoors in southern Florida. This species is multivoltine and multiple overlapping generations are observed in the laboratory and at established sites in Florida (Tipping et al., 2014a). While M. scutellaris can be very damaging to E. crassipes (Tipping et al., 2011; Sosa et al., 2007), to date it also has not substantially reduced surface coverage of the plant, which is the primary decision metric used by land managers (Tipping et al., 2014a).

*Megamelus scutellaris* occurs in both macropterous (flewght) and brachypterous (non-flewght) forms (Sosa et al., 2004), with the majority of insects produced for release being brachypterous. The dimorphism is likely density-dependent (Denno, 1994), but the exact mechanism triggering this phenomenon requires further study (Fitzgerald and Tipping, 2013). Other planthopper species that are wing-dimorphic are known for their macropaters’ long-distance migrations (e.g., *N. lugens*, Denno and Peterson, 1995), while it is generally thought that brachypterous individuals do not disperse over longer distances (Kennedy, 1961; Denno, 1976). However, such smaller-scale dispersal may play an important role in the re-colonization of herbicide treated areas, as insects move from pockets of E. crassipes that escaped treatment into the expanding mat (Center et al., 1999).

Although there can be significant initial costs considering the long process before agent deployment, benefit-cost ratios of biological control tend to be high (Harris, 1991; Hill and Greathead, 2000; Culliney, 2005). Mass rearing and distribution programs can accelerate the benefits by increasing both the number of insects available for release and the number of release events, increasing propagule pressure and augmenting natural dispersal. A poor release strategy can potentially contribute to unsuccessful establishment of biological control agents (Grevstad, 1999). Therefore, optimizing a release strategy specific to a particular agent is but one way to decrease the time to establishment while increasing the total area covered.

The spread of *M. scutellaris* on the landscape is important because of its potential to integrate with the widespread herbicidal management of *E. crassipes*. By more efficiently building *M. scutellaris* numbers and increasing establishment, this species can be more effective in a shorter time span. The objectives of this study were to (1) evaluate the effectiveness of various release strategies in establishing *M. scutellaris* populations, and (2) determine the dispersal capability of brachypterous adult *M. scutellaris* post-release.

### 2. Materials and methods

#### 2.1. Release methods

In order to determine the most effective release strategy for *M. scutellaris*, two general release strategies were tested: (1) the release of adult brachypterous individuals, and (2) the release of egg-laden (infested) *E. crassipes* plants. Both strategies were tested as a single release or as a series of three releases. Treatments were compared with two controls, one in which *M. scutellaris* was not released, but *N. eichhorniae* was allowed to immigrate freely and one in which insect establishment was prohibited by insecticide treatment (Bifen 1/7, Control Solutions Incorporated, Pasadena, TX USA) at the labeled rate every 3–4 weeks for the duration of the study. Other treatments were sprayed with water every 3–4 weeks. The experiment was conducted in 40 uncaged, concrete mesocosms (1.6 m² surface area, 782 l volume) at the USDA-ARS Invasive Plant Research Laboratory (IPRL) in Davie, FL. It was repeated twice, once in 2015 (started Julian Date [JD] 174) and again in 2016 (started JD 175). Plant populations in individual mesocosms were started with five similar-sized *E. crassipes* plants which were first weighed to obtain fresh weight biomass. All mesocosms were monitored weekly for flowering (an indicator of reproductive output) for the duration of the experiment. Mesocosms were fertilized with Osmocote Plus 15-9-12 (ICL Fertilizers, Dublin, Ohio; 0.31 g per liter) and che -$\cdot$lated iron (Sequestrene 330 Fe, BASF Corporation, Research Triangle Park, North Carolina; 0.02 g per liter) at the beginning of the experiment and mid-way through (ca. 3 months). Aquashade (Arch Chemicals, Inc., Germantown, Wisconsin) was applied at the labeled rate to reduce algal growth.

The experiment was a completely randomized design with six treatments in five replications (Table 1). Infested plants were produced by allowing 50 *M. scutellaris* adults (50:50 sex ratio) to oviposit on a single *E. crassipes* plant for seven days (resulting in 400–500 eggs per plant). Adults were removed before placing the plant in the mesocosm. Infested plant placement and adult insect releases began after *E. crassipes* coverage in tanks reached 100%. Five months following the last plant or insect releases (JD 026 in 2015 and JD 017 in 2016), treatments were sampled for *M. scutellaris* and then evaluated destructively by sampling five haphazardly selected plants per mesocosm to measure *N. eichhorniae* densities, insect damage, and plant biomass. Other insects were also counted from this sample, including *Elophila (Synecilia) obliteralis* Walker (Lepidoptera: Crambidae), a native moth commonly found on *E. crassipes* in Florida (Habeck et al., 1986), and *Kalopolypymena ema* Schaff & Grissell (Hymenoptera: Mymaridae), a native egg parasitoid that utilizes *M. scutellaris* (Minteer et al., 2016), as well as two mite species (the introduced *Orthogalumana terebrantis* Wallwork [Acar: Galumnidae] and the native *Tetranychus tumidus* Banks [Aranhidi: Tetranychidae]; Center, 1987). Remaining plant material was bulked and placed in Berlese funnels for one week, after which collection vials were examined and the numbers of arthropods tallied. At the end of the experiment, all plants were removed and weighed to obtain fresh weight biomass per mesocosm. A single plant from each mesocosm was weighted for fresh weight biomass, then dried to a constant weight in order to calculate dry weight biomass. The mesocosms were then drained in order to dry out the litterfall at the bottom, which was recovered and weighed for dry weight biomass.

### 2.2. Initial dispersal

Post-release dispersal behavior of adults was observed in a controlled experiment conducted at the IPRL lab in January – February 2018. Dispersal rate is often difficult to determine in the field, as *M. scutellaris* is difficult to detect at low densities and quick to flee when disturbed. Since their host plant is free-floating within a dynamic marsh

#### Table 1

| Treatment # | Release Method                           |
|------------|-----------------------------------------|
| 1          | Control — Insecticide treatment         |
| 2          | *N. eichhorniae* only                    |
| 3          | 50 *M. scutellaris* adults released 1x  |
| 4          | 50 *M. scutellaris* adults released 3x  |
| 5          | Egg infested plant released 1x          |
| 6          | Egg infested plant released 3x          |
habitats, locations of populations are not static and are especially hard to track because of anthropogenically controlled water level changes and frequent herbicidal management. To evaluate dispersal of adult M. scutellaris immediately post-release, an arena that mimicked a linear transect through an E. crassipes mat was used. The experiment was conducted in transects that consisted of two 3 m-long, aluminum rain gutters with sealed ends placed parallel to each other. The gutters were placed on top of concrete tanks and spaced > 60 cm apart. Approximately 60 insect-free E. crassipes plants were placed in each transect in order to reach typical E. crassipes field densities of about 60 E. crassipes plants m$^{-2}$ (Center and Spencer, 1981) and permitted to acclimate at least 24 h before beginning the experiment (Fig. 1). Plants were fertilized with Osmocote Plus 15-9-12 (0.31 g per liter) and chelated iron (0.02 g per liter) at the beginning of the experiment. The experiment was a completely randomized design with two treatments of nine replications each. The treatments were a high density release treatment (150 adult M. scutellaris) and a low density release treatment (50 adult M. scutellaris). All test insects were 1 to 2 weeks old brachypterous M. scutellaris (∼50:50 sex ratio) that were collected from a laboratory colony at IPRL and anesthetized with CO$_2$ immediately prior to being released in a shaded weigh boat that was floating between two E. crassipes plants in the end of each transect. Adults were monitored up to one hour to quantify their survival following anesthesia and placement. Emigration from the transects was recorded by placing yellow sticky trap cards (Olson Products, Medina, Ohio) at 25-cm intervals along 10 randomly selected transects. The adhesive on the cards was effective in trapping M. scutellaris and the cards were monitored for 48 h post-release for captured adults.

At the same time adults were released onto the transects, 10 males and 20 females were released into four screened rearing containers (square plastic 20 L containers) with 2 to 4 E. crassipes plants that were fertilized with Osmocote and chelated iron at the same rate as in the transects. These containers were placed outside near the transects (partially under shadecloth) and were used to estimate the dates for first emergence of nymphs in transects. Transects were not monitored or disturbed other than for watering (which was done at the opposite end of each transect from release) until the control container nymphs emerged (JD 033) in order to avoid confounding insect movement via disturbance.

Once emergence occurred in the control containers, the transects were surveyed daily only for first and second instars because they were considered less likely to move significant distances. Nymphs were counted and removed and their distance from the release point was recorded. The positions of any adults seen during monitoring were recorded as well. It was assumed that the locations with nymphs corresponded closely with oviposition sites from adults.

2.3. Statistical analysis

In the release methods experiment, mean relative growth rate of E. crassipes was calculated by the equation: (final dry weight-initial dry weight [g])/duration (days). Analyses were performed in R (version 3.3.2, R Core Team, 2014). An ANOVA was used to determine differences among the six release methods treatments because this test is robust to deviations from normality as long as the other assumptions hold (homogeneity of variance and independence) (Schmider et al., 2010). These data exhibited a near Poisson distribution with homogenous variances. Data were also evaluated post-hoc using Tukey tests.

For the dispersal experiment, two sample t-tests were used to compare high vs. low density release treatment mortality, total nymphs recovered per day, and farthest distance per day post-release. An ANOVA was used to determine if sticky traps or insect treatment affected mortality.

3. Results

3.1. Release methods experiment

There were significant differences ($F_{1,50} = 27.78, p < 0.0001$) in initial fresh weight between 2015 and 2016, so each year was analyzed separately.

The ANOVA of the 2015 data showed differences among treatments in average adult M. scutellaris found, average percent defoliation by N. eichhorniae, final fresh weight of E. crassipes, mean relative growth rate of E. crassipes, N. eichhorniae adults recovered, N. eichhorniae larvae recovered, mites (both species, combined) recovered, K. ema recovered, and the number of flowers produced (Table 2). Post-hoc Tukey tests indicated that the no-insect control differed from all other treatments for some variables. None of the Tukey tests indicated significant differences among the insect release treatments (Table 3).

The ANOVA of the 2016 data showed differences among treatments in average percent defoliation by N. eichhorniae, final fresh weight of E. crassipes, mites (both species, combined) found, E. oblitralls found, and the number of flowers produced (Table 2). Post-hoc Tukey tests
Table 2
ANOVA results from the release methods experiment. Mean numbers of adult and nymph M. scutellaris were calculated from two samples taken from each mesocosm and were then used to calculate M. scutellaris population density (MS/m²). MRGR, mean relative growth rate of E. crassipes, was calculated from the difference of final fresh weight and initial fresh weight divided by the duration of each year’s experiment (217 days in 2015, 208 days in 2016). Numbers of Megamelus scutellaris (MS Berlese), Neochetina adults and larvae, mites, Elophila (Synclita) obliteralis, and Kalopulmena ema were recovered from Berlese funnel samples. Mites included Orthogalumna terebrantis and Tetranychus tumidus. Asterisks indicate significance at α < 0.05.

| Variable                  | 2015                  | 2016                  | df | F   | p-value | df | F   | p-value |
|---------------------------|-----------------------|-----------------------|----|-----|---------|----|-----|---------|
| Initial Fresh Weight      | 5, 24                 | 2.34                  | 0.07 | 5, 24 | 0.23     | 0.94 | 5, 24 | 0.23     | 0.94 |
| Initial Dry Weight        | 5, 24                 | 2.34                  | 0.07 | 5, 24 | 0.23     | 0.94 | 5, 24 | 0.23     | 0.94 |
| Mean MS Adults            | 5, 24                 | 2.68                  | 0.05* | 5, 24 | 0.78     | 0.57 | 5, 24 | 0.78     | 0.57 |
| MS/m²                     | 5, 24                 | 2.03                  | 0.11 | 5, 24 | 0.77     | 0.58 | 5, 24 | 0.77     | 0.58 |
| Mean % Defoliation        | 5, 24                 | 9.59                  | < 0.0001* | 5, 24 | 2.67     | 0.05* | 5, 24 | 2.67     | 0.05* |
| Final Fresh Weight        | 5, 24                 | 16.35                 | < 0.0001* | 5, 24 | 4.04     | 0.008* | 5, 24 | 4.04     | 0.008* |
| Final Dry Weight          | 5, 24                 | 6.68                  | 0.0004* | 5, 24 | 0.66     | 0.65 | 5, 24 | 0.66     | 0.65 |
| MRGR                      | 5, 24                 | 7.003                 | 0.0004* | 5, 24 | 0.68     | 0.65 | 5, 24 | 0.68     | 0.65 |
| MS Berlese                | 5, 24                 | 1.003                 | 0.4371 | 5, 24 | 1.80     | 0.15 | 5, 24 | 1.80     | 0.15 |
| Neochetina Adults         | 5, 24                 | 4.00                  | 0.009* | 5, 24 | 0.63     | 0.68 | 5, 24 | 0.63     | 0.68 |
| Neochetina Larvae         | 5, 24                 | 4.15                  | 0.007* | 5, 24 | 1.45     | 0.24 | 5, 24 | 1.45     | 0.24 |
| Mites                     | 5, 24                 | 3.81                  | 0.01* | 5, 24 | 5.92     | 0.001* | 5, 24 | 5.92     | 0.001* |
| Elophila (Synclita) obliteralis | 5, 24   | 1.13                  | 0.37 | 5, 24 | 3.21     | 0.02* | 5, 24 | 3.21     | 0.02* |
| Kalopulmena ema           | 5, 24                 | 2.74                  | 0.04* | 5, 24 | 2.28     | 0.08 | 5, 24 | 2.28     | 0.08 |
| Dry Weight of Litter      | 5, 24                 | 2.10                  | 0.1   | 5, 24 |          |      | 5, 24 |          |      |
| Flowers                   | 5, 24                 | 7.60                  | 0.0002* | 5, 24 | 3.79     | 0.01* | 5, 24 | 3.79     | 0.01* |

3.2. Initial dispersal experiment

Analysis indicated differences between the two treatments in mortality, total nymphs on day 7 post-release, total nymphs on day 8 post-release, and total nymphs overall. There was no statistical difference in farthest dispersal between treatments (Table 5). Transects with and without sticky traps had similar mortality (F1,15 = 3.08, p = 0.1), but there was a difference in mortality between density treatments (F1,15 = 19.72, p = 0.0005). The majority of mites were produced within 0.5 m of the release point (Fig. 2). The farthest nymphs recorded emerged from eggs laid on day 8 post-release at 261 cm from release, and the fastest movement by an adult female based on occurrence of nymphs was 137 cm by day 4 post-release.

4. Discussion

Many variables need to be considered when planning a release program for a biological control agent, such as the biological attributes of the agent and the target, the habitat, and the overall management system. Biological control agents are initially costly to develop but have high benefit-cost ratios over the long term (Harris, 1991; Hill and Greathead, 2000; Culliney, 2005). It is important that an effective and efficient release strategy be developed, so that the years of development were not wasted. In the case of M. scutellaris on E. crassipes in Florida, two release strategies were tested as either single releases or in a series of three releases. The lack of differences among insect treatments in the numbers of M. scutellaris adults and mites indicate that they were equally effective release strategies. This was supported by observations of post-release dispersal behavior.

Releasing adults only or releasing egg-laden plants produced similar results. The initial dispersal experiment demonstrates one reason for this, namely that adults tended to lay eggs before dispersing themselves, thus creating egg-laden, infested plants at or near the release site. While the idea that a single release of insects is equivalent to multiple releases is also exhibited here, it is important to note that more insects overall were released in the multiple release treatments. In other studies of propagule pressure, the same numbers of individuals were released as either one single large release or in multiple smaller releases, leading to most concluding that a single large propagule is more likely to establish a population than multiple smaller ones (see Simberloff, 2009 for review). However, many studies also concluded that increased propagule pressure in general (both size and number) increased establishment success (Grevstad, 1999; Memmott et al., 1998). This may explain why both single and multiple release treatments produced similar results in this study.

The occurrence of K. ema could have contributed to the lack of
Significant Tukey test means and groupings of 2016 release methods experiment data. The no insect control had higher final fresh weight of *E. crassipes* than the single infested plant (*p* = 0.01) and multiple release of adults treatments (*p* = 0.005), less average percent defoliation by *N. eichhorniae* from the multiple release of adults treatment (*p* = 0.01), fewer mites than the *N. eichhorniae* only control (*p* = 0.04), the multiple releases of an infested plant (*p* = 0.001), and the single release of adults treatments (*p* = 0.002), fewer *E. obliteralis* found than the multiple releases of an infested plant treatment (*p* = 0.02), and more flowers produced than the single infested plant (*p* = 0.04) and multiple releases of adults treatments (*p* = 0.02).

### Table 4
ANOVA results from the small scale dispersal experiment. Asterisks indicate significance at *p* < 0.05.

| Variable | Mean (SD) | t | df | p-value |
|----------|-----------|---|----|---------|
| **Mortality** | | | | |
| Total Nymphs Day 1 | 1.11 (1.44) | 1.88 (3.14) | -0.64 | 16 | 0.53 |
| Day 2 | 1.33 (2.26) | 2.33 (4.05) | -0.61 | 16 | 0.55 |
| Day 3 | 8.11 (7.00) | 9.56 (18.64) | -0.21 | 16 | 0.84 |
| Day 4 | 22.44 (22.07) | 13.56 (16.45) | 0.91 | 16 | 0.37 |
| Day 5 | 14.22 (17.76) | 4.56 (8.80) | 1.38 | 16 | 0.19 |
| Day 6 | 95.11 (92.36) | 49.56 (57.11) | 1.18 | 16 | 0.25 |
| Day 7 | 246.33 (202.67) | 75.11 (48.55) | 2.32 | 16 | 0.03 |
| Day 8 | 156.11 (74.10) | 47.33 (49.32) | 2.82 | 16 | 0.01 |
| **Overall** | 524.78 (308.69) | 203.89 (162.39) | 2.60 | 16 | 0.01 |
| **Farthest Distance (cm)** | | | | |
| Day 1 | 2.44 (3.75) | 2.67 (4.52) | -0.11 | 16 | 0.91 |
| Day 2 | 4.22 (6.83) | 1.89 (5.00) | 0.76 | 16 | 0.45 |
| Day 3 | 10 (15.76) | 3.33 (5.25) | 1.14 | 16 | 0.27 |
| Day 4 | 16.67 (14.17) | 14.78 (11.75) | 0.29 | 16 | 0.78 |
| Day 5 | 14.77 (9.46) | 23.11 (41.78) | -0.55 | 16 | 0.59 |
| Day 6 | 54.67 (61.10) | 34.78 (38.96) | 0.78 | 16 | 0.45 |
| Day 7 | 51.56 (52.24) | 63.56 (61.02) | -0.42 | 16 | 0.68 |
| Day 8 | 101.22 (83.99) | 47.89 (38.17) | 1.64 | 16 | 0.12 |

### Fig. 2
Approximate locations of adults based on the occurrence of nymphs. Chart is based on data from all replications and both treatments. Points on each bar are the average distance adults were found for that day and bars indicate minimum and maximum distance from release point.
released adults. In this way, persistent populations can be supple-
mented and new populations established.

These experiments provided evidence of how insect behavior in-
fuences the likelihood of establishment in the field and how un-
derstanding this behavior can guide an effective release strategy. Those
agents that can be produced in large numbers and released at multiple
life stages may benefit most from a multipartined approach to increase
propagule pressure to enhance the establishment of sustainable popu-
lations.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://
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