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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. *Megamelus 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 oviposit 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 Debusk, 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 damaging 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

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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 (flighted) and brachypterous (non-flighted) 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 macropters' 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 I/T, 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 m^2 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 chelated 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.

Table 1
Release method treatments. Treatments were designed to incorporate a "no biological control" scenario (Treatment 1) and an "only *N. eichhorniae*" scenario (Treatment 2), which represents the most common situation in Florida.

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

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 (Synclita) obliteralis* Walker (Lepidoptera: Crambidae), a native moth commonly found on *E. crassipes* in Florida (Habeck et al., 1986), and *Kalopolynema ema* Schauff & Grissell (Hymenoptera: Mymaridae), a native egg parasitoid that utilizes *M. scutellaris* (Minteer et al., 2016), as well as two mite species (the introduced *Orthogalumna terebrantis* Wallwork [Acari: Galumnidae] and the native *Tetranychus tumidus* Banks [Arachanida: 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 weighed 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

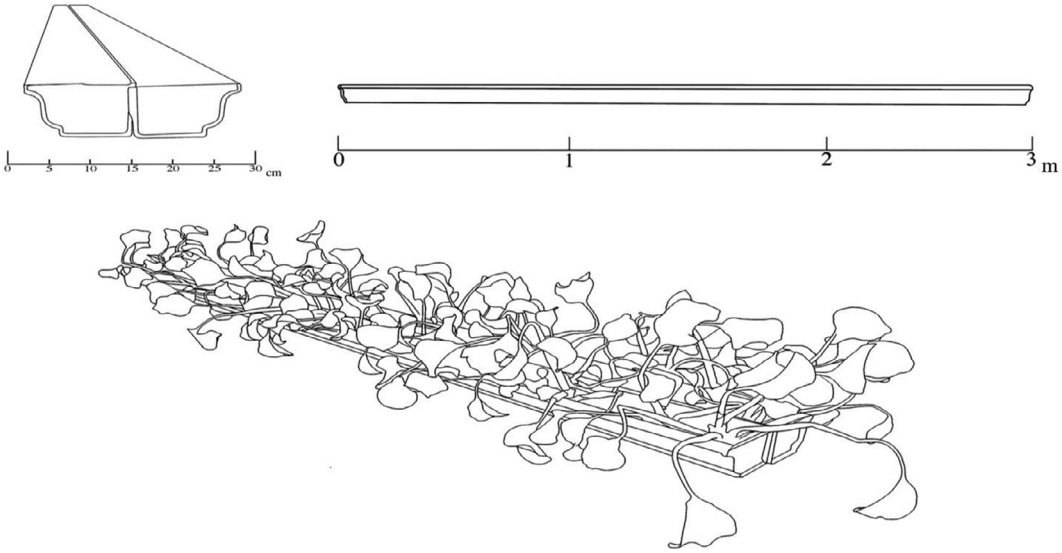

Fig. 1. Schematic of dispersal transect. Transect is 3 m long and 26 cm wide, water depth is ~10 cm. *Eichhornia crassipes* are placed single-file in each gutter, with the two gutters side-by-side. Transects are placed up off the ground, on top of concrete tanks which are 62 cm tall.

habitat, 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. obliteralis* 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/m^2). 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 *Kalopolynema ema* were recovered from Berlese funnel samples. Mites included *Orthogalumna terebrantis* and *Tetranychus tumidus*. Asterisks indicate significance at $\alpha < 0.05$.

	2015			2016		
Variable	df	*F*	*p*-value	df	*F*	*p*-value
Initial Fresh Weight	5, 24	2.34	0.07	5, 24	0.23	0.94
Initial Dry Weight	5, 24	2.34	0.07	5, 24	0.23	0.94
Mean MS Adults	5, 24	2.68	0.05*	5, 24	0.78	0.57
Mean MS Nymphs	5, 24	1.29	0.30	5, 24	0.86	0.53
MS/m^2	5, 24	2.03	0.11	5, 24	0.77	0.58
Mean % Defoliation	5, 24	9.59	< 0.0001*	5, 24	2.67	0.05*
Final Fresh Weight	5, 24	16.35	< 0.0001*	5, 24	4.04	0.008*
Final Dry Weight	5, 24	6.88	0.0004*	5, 24	0.66	0.65
MRGR	5, 24	7.003	0.0004*	5, 24	0.68	0.65
MS Berlese	5, 24	1.003	0.4371	5, 24	1.80	0.15
Neochetina Adults	5, 24	4.00	0.009*	5, 24	0.63	0.68
Neochetina larvae	5, 24	4.15	0.007*	5, 24	1.45	0.24
Mites	5, 24	3.81	0.01*	5, 24	5.92	0.001*
Elophila (Synclita) obliteralis	5, 24	1.13	0.37	5, 24	3.21	0.02*
Kalopolynema ema	5, 24	2.74	0.04*	5, 24	2.28	0.08
Dry Weight of Litter	5, 24	2.10	0.1			
Flowers	5, 24	7.60	0.0002*	5, 24	3.79	0.01*

indicated that the no-insect control differed from the *N. eichhorniae* and release method treatments for some variables. The only significant variation among insect treatments was in number of *E. obliteralis* found between multiple releases of an infested plant and single release of adults treatments ($p = 0.04$, Table 4).

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 ($F_{1,15} = 3.08$, $p = 0.1$), but there was a difference in mortality between density treatments ($F_{1,15} = 19.72$, $p = 0.0005$). The majority of nymphs 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 nymphs 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

Table 3
Significant Tukey tests means and groupings of the release methods experiment 2015 data. The no insect control differed from all insect treatments with higher final fresh weight of *E. crassipes* ($p < 0.0002$), higher mean relative growth rate of *E. crassipes* ($p = 0.03$–0.0002), lower average percent defoliation by *N. eichhorniae* ($p = 0.002$–0.0003), and more flowers produced ($p = 0.002$–0.0005). The no-insect control also varied from the multiple release of adults treatment specifically with fewer adult *M. scutellaris* found ($p = 0.04$), fewer mites found ($p = 0.01$), and fewer *K. ema* found ($p = 0.04$), and from the single release of an infested plant treatment with fewer *N. eichhorniae* adults ($p = 0.003$) and mites found ($p = 0.01$).

Treatment	FFW		MRGR		Mean Adult MS		Average % Defol.		FDW	
	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group
1	32128.8	a	12.41	a	0.1	b	0.50	b	2752.95	a
2	16945.6	b	7.45	b	4.1	ab	12.00	a	1687.83	b
3	15188.4	b	6.19	b	11.2	ab	15.20	a	1430.43	b
4	17611.6	b	8.05	b	18.0	ab	13.22	a	1816.44	b
5	18297.0	b	7.74	b	8.9	ab	13.75	a	1746.45	b
6	20681.6	b	8.68	b	26.3	a	11.17	a	1960.94	b
	N. eichhorniae Adults		*N. eichhorniae* Larvae		Mites		Mymarids		Flowers	
	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group
1	0.0	b	0.0	b	27.20	b	0.2	b	385.0	a
2	6.2	ab	27.8	a	596.40	ab	3.8	ab	255.4	b
3	13.6	a	28.6	a	827.15	a	8.8	ab	248.4	b
4	5.2	ab	25.2	ab	315.95	ab	11.0	ab	234.8	b
5	6.2	ab	25.4	a	486.35	ab	5.8	ab	254.2	b
6	9.0	ab	33.0	a	816.50	a	12.8	a	235.6	b

Table 4
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 ($p = 0.02$).

Treatment	FFW		Mean % Defol.		Mites		*E. obliteralis*		Flowers	
	Mean	Group	Mean	Group	Mean	Group	Mean	Group	Mean	Group
1	28569.4	a	0.00	b	8.36	b	0.2	b	187.6	a
2	24399.8	ab	3.80	ab	938.10	a	2.8	ab	144.6	ab
3	23575.0	b	5.04	ab	795.00	ab	0.8	ab	121.4	b
4	24407.2	ab	3.98	ab	1415.10	a	6.4	a	132.2	ab
5	24968.6	ab	4.48	ab	1319.70	a	0.6	b	174.4	ab
6	22919.0	b	8.39	a	594.62	ab	1.0	ab	116.0	b

Table 5
ANOVA results from the small scale dispersal experiment. Asterisks indicate significance at $\alpha < 0.05$.

Variable	Mean (SD)		*t*	df	*p*-value
	High Density	Low Density			
Mortality	5.41 (2.67)	1.26 (0.86)	4.18	16	0.0007*
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	136.11 (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)	0.78	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

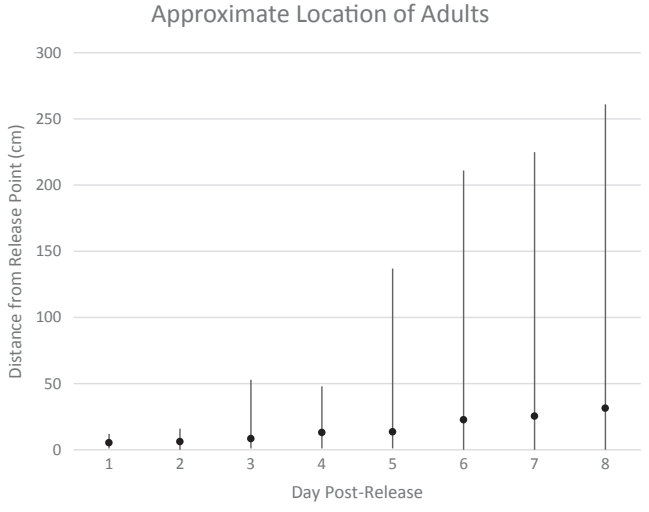

Fig. 2. Approximate locations of adults based on the occurrence of nymphs. Chart is based on data from all replications and both treatments. Points on each bar are the average distance adults were found for that day and bars indicate minimum and maximum distance from release point.

significance in *M. scutellaris* density between insect treatments, as egg parasitism would have decreased the number of nymphs emerging. Minteer et al. (2016) noted that parasitism occurred more often when *M. scutellaris* and *E. crassipes* were at artificially high densities compared to field sites. As this experiment was conducted at the same facility as the rearing tanks in Minteer's study, it is likely that parasitism played some part in the population dynamics of *M. scutellaris*.

In the dispersal experiment, higher mortality in the high density treatment may be related to the way the *M. scutellaris* were released onto the transect with all 150 adults placed in a single weigh boat, an approach that may have increased the number of injuries. However, this mortality was less than what normally occurs in a routine release situation (Goode, unpublished data). Approximately 19% mortality occurs when the adults are kept conscious and in a container with *E. crassipes* plants for 1–7 days prior to release as part of the rear and release program at IPRL (Goode, unpublished data). Increased release density may have also caused increased dispersal away from the transect, although this was not reflected in increased adult captures on sticky traps. Overall, the high density treatment produced twice as many nymphs as the low density treatment despite releasing three times the number of adults. It is unlikely that lack of oviposition locations would have limited reproduction, as many plants within the first meter of the transect had no emergence recorded.

Low release numbers of biological control agents have been linked to failure to establish populations in the field (Memmott et al., 1998). *Megamelus scutellaris* may exhibit a form of negative density dependence, whereby the larger the propagule size, the greater the rate of dispersal, resulting in lower population numbers at the release site and possible Allee effects over the long term.

While studies indicate that a large propagule size is more important than multiple introductions in establishing non-natives (Simberloff, 2009), propagule number may be more important when there is increased environmental variability (Grevstad, 1999; Sinclair and Arnott, 2016). In this system, the periodic disruptions of the host plant from herbicide management operations is a bigger problem because *M. scutellaris* is easily reared and released in large numbers using both direct release of insects and infested plants. Fewer small introductions (propagules) may result in populations unable to sustain themselves during poor environmental conditions or stochastic population disturbances (Simberloff, 2009), thus introducing multiple life stages of *M. scutellaris* (adults, nymphs, and eggs) at the same time may help buffer the dynamism of *E. crassipes* populations that receive periodic and usually unpredictable herbicide applications. Adults can disperse if the area is treated immediately after release and infested plants will sustain emerging nymphs if the area was treated prior to release. The release of both adults and infested plants also creates a continuous flow of propagules, starting with the adults who lay eggs at the release site and then disperse, followed by the nymphs from eggs within the laboratory infested plants, and finally by the F_1 nymphs from the originally

released adults. In this way, persistent populations can be supplemented and new populations established.

These experiments provided evidence of how insect behavior influences the likelihood of establishment in the field and how understanding 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 multipronged approach to increase propagule pressure to enhance the establishment of sustainable populations.

Declaration of interest

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CRediT authorship contribution statement

Ashley B.C. Goode: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Carey R. Minteer:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Philip W. Tipping:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing, Supervision. **Brittany K. Knowles:** Investigation, Writing - review & editing. **Ryann J. Valmonte:** Investigation, Writing - review & editing. **Jeremiah R. Foley:** Conceptualization, Methodology, Investigation, Writing - review & editing, Visualization. **Lyn A. Gettys:** Writing - review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2019.01.016.

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