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Host range of *Lepidelphax pistiae* (Hemiptera: Delphacidae) and its potential impact on *Pistia stratiotes* L. (Araceae)

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ABSTRACT

Pistia stratiotes L. (Araceae) is a floating aquatic plant that has become invasive in Florida. It is primarily controlled with herbicides, but two biocontrol agents have previously been released to assist in management of this species. A new potential biocontrol agent from Argentina, *Lepidelphax pistiae* Remes Lenicov (Hemiptera: Delphacidae), has been evaluated comprehensively for specificity after initial host range studies done in its native range indicated that it is likely specific to *P. stratiotes*. Host range studies indicated that this insect is specific to *P. stratiotes*, with no survival or reproduction occurring on any of the 42 other plant species tested. Impact studies indicated that this insect can significantly damage *P. stratiotes* at medium and high population densities, which were comparable to those seen in its native range. *Lepidelphax pistiae* is sufficiently specific enough to warrant release and has a high probability of aiding management of *P. stratiotes* populations in Florida.

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Waterlettuce (*Pistia stratiotes* L. [Araceae]) is an invasive aquatic plant that has been introduced to many countries (Holm, Plucknett, Pancho, & Herberger, 1977; Neuenschwander, Julien, Center, & Hill, 2009). It threatens native habitats because it grows quickly, alters water chemistry, blocks sunlight (which affects submerged vegetation, fish, and aquatic invertebrates), impedes navigation, and clogs flood control structures (Neuenschwander et al., 2009). In Florida, it also harbours mosquito larvae (Lounibos & Escher, 1985; Neuenschwander et al., 2009). It is on the Florida Prohibited Aquatic Plants List (Florida Administrative Code, 2008) and is a Category I Plant on the Florida Exotic Pest Plant Council's (FLEPPC) List of Invasive Plant Species (FLEPPC, 2017). To attempt to control this species, the biocontrol agents *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae; Dray et al., 1990; Dray & Center, 1992) and *Spodoptera pectinicornis* Hampson (Lepidoptera: Noctuidae; Dray, Center, & Wheeler, 2001; Grodowitz, Johnson, & Nelson, 1992) were released in Florida in 1987 and 1990, respectively. While *N. affinis* is confirmed as established, *S. pectinicornis* did not establish (Dray et al., 2001). *Neohydronomus affinis* has been credited in controlling *P. stratiotes* in Australia (Harley, Kassulke, Sands, & Day, 1990) and parts of Africa (Ajuonu & Neuenschwander, 2003; Chikwenhere, 1994; Hill & Moore, 2012). Lab experiments suggest that a single pair of weevils per plant can effectively control *P. stratiotes* (Diop, Coetzee, & Hill, 2010). Past evaluations in Florida indicated that it had established populations (Dray & Center, 1992); however, no recent evaluations of *N. affinis* have been conducted and so its population and impact is currently unknown and *P. stratiotes* is still considered a major ecological issue in the state (FLEPPC, 2017).

Herbicide application is the preferred management strategy for keeping floating aquatic vegetation (mainly *P. stratiotes* and *Pontederia* [*Eichhornia*] *crassipes* [Martius] Solms. [Pontederiaceae]) at maintenance levels in Florida. Because this is a management strategy, herbicide application must be done regularly and in perpetuity to maintain population control, costing millions of dollars annually (Netherland, Getsinger, & Stubbs, 2005). In order to improve the suppression of this plant, a new potential biocontrol agent, *Lepidelphax pistiae* de Remes Lenicov (Hemiptera: Delphacidae) was collected from Argentina for evaluation.

Lepidelphax pistiae was described from the Paraná/Uruguay basin in Argentina and is the only species within the *Lepidelphax* genus (de Remes Lenicov & Walsh, 2013). It has been observed feeding and reproducing only on *P. stratiotes* both in the field and the laboratory (Cabrera Walsh, Maestro, Sosa, & Tipping, 2014; de Remes Lenicov & Walsh, 2013; de Remes Lenicov, Defea, Rusconi, & Cabrera Walsh, 2017). *Lepidelphax pistiae* by itself can significantly impact growth of *P. stratiotes*, reducing final biomass by approximately one third and resulting in ~20% fewer rosettes compared to controls (Cabrera Walsh & Maestro, 2014). Other studies on this species show that *L. pistiae*, together with *Neohydronomous affinis*, reduce *P. stratiotes* biomass and coverage in field settings (Cabrera Walsh & Maestro, 2016).

Previously, host range testing of *L. pistiae* was conducted at Fundación para el Estudio de Especies Invasivas (FUEDEI) in Hurlingham, Buenos Aires, Argentina and at the USDA-ARS Invasive Plant Research Laboratory (IPRL) in Ft. Lauderdale, FL, USA. This testing involved no-choice specificity tests on 25 species of Araceae and four additional wetland plant species with 100% adult mortality and no progeny produced on any plant other than *P. stratiotes* (Cabrera Walsh et al., 2014). Herein, *L. pistiae* was

Table 1. List of plant species used in the *Lepidelphax pistiae* host range test with mean survival ± SE of P_1 *L. pistiae* and mean number ± SE of F_1 progeny recovered from each.

Order	Family	Scientific Name	Common name	NA Status	% P_1 Survival Mean ± SE	# F_1 Progeny Mean ± SE
Acorales	Acoraceae	*Acorus calamus* L.	sweet flag, calamus	Exotic	0	0
Alismatales	Alismataceae	*Echinodorus bleheri* Rataj	Amazon sword plant	Exotic	0	0
		Echinodorus cordifolius Engelmann	spade-leaf sword	Native	0	0
		Hydrocleys nymphoides Humb. & Bonpl. ex Willd.	water poppy	Exotic	0	0
		Sagittaria japonica L.	double-flowing arrowhead	Exotic	0	0
		Sagittaria lancifolia L.	duck potato	Native	0	0
		Sagittaria montevidensis Cham. & Schltdl.	giant arrowhead	Native	0	0
		Sagittaria subulata L.	awl-leaf arrowhead	Native	0	0
	Araceae	*Alocasia cucullata* L.	Chinese Taro	Exotic	0	0
		Anthurium andraeanum Linden ex André	anthurium	Exotic	0	0
		Anthurium scandens (Aubl.) Engl.	pearl laceleaf	Exotic	0	0
		Arisaema triphyllum L.	jack-in-the-pulpit	Native	0	0
		Caladium bicolor (Aiton) Vent.	wild cocoyam	Exotic	0	0
		Cryptocoryne wendtii de Wit		Exotic	0	0
		Orontium aquaticum L.	golden-club, floating arum	Native	0	0
		Peltandra virginica L.	green arrow arum, tuckahoe	Native	0	0
		Pistia stratiotes L.	waterlettuce	Exotic	63 ± 4.7	84.8 ± 9.3
		Symplocarpus foetidus L.	eastern skunk cabbage	Native	0	0
	Butomaceae	*Butomus umbellatus* L.	flowering rush	Exotic	0	0
	Juncaginaceae	*Triglochin maritimum* L.	seaside arrowgrass	Exotic	0	0
	Lemnaceae	*Lemna minor* L.	common (lesser) duckweed	Native	0	0
	Tofieldiaceae	*Triantha racemosa* (Walter) Small	coastal false asphodel	Native	0	0
Asparagales	Amaryllidaceae	*Zephyranthes minuta (grandiflora)* (Kunth) D.Dietr.	pink rain lily	Exotic	0	0
Commelinales	Commelinaceae	*Tradescantia pallida* (Rose) D.R. Hunt	purple queen	Native	0	0
	Haemodoraceae	*Lachnanthes caroliniana* (Lem.)	redroot	Native	0	0
Lamiales	Plantaginaceae	*Plantago major (atropurpurea) L.*	purple leaf plantain	Exotic	0	0
Malvales	Malvaceae	*Abelmoschus esculentus* (L.) Moench	okra	Crop	0	0
Nymphaeales	Nymphaeaceae	*Nymphaea mexicana* Zuccarini	yellow waterlily	Native	0	0
	Nymphaeaceae	*Nymphaea odorata* Ait.	American white waterlily	Native	0	0
Poales	Cyperaceae	*Cladium jamaicense* L.	saw-grass	Native	0	0
	Juncaceae	*Juncus effucus* L.	common rush	Native	0	0
	Poaceae	*Oryza sativa* L.	Asian rice	Crop	0	0
	Poaceae	*Saccharum officinarum* L.	sugarcane	Crop	0	0
	Poaceae			Native	0	0

(*Continued*)

Table 1. Continued.

Order	Family	Scientific Name	Common name	NA Status	% P_1 Survival Mean ± SE	# F_1 Progeny Mean ± SE
		Spartina patens (Aiton) Muhl.	saltmeadow cordgrass			
	Poaceae	*Stenotaphrum secundatum* (Walter) Kuntze	St. Augustine grass	Native	0	0
	Poaceae	*Zea mays* L.	corn	Crop	0	0
Zingiberales	Cannaceae	*Canna americanallis variegata*	bengal tiger canna	Exotic	0	0
	Costaceae	*Costus woodsonii* Maas.	red button ginger	Exotic	0	0

tested on an additional 37 plant species including common landscaping plants and economically important crops, along with additional members of the Araceae family, both native and exotic to Florida. The effects of *L. pistiae* were also tested to determine the damage this species could inflict directly on *P. stratiotes*.

Methods and materials

Host range and oviposition testing

No-choice specificity tests were conducted on non-target plants (Table 1) in a quarantine greenhouse at IPRL under natural light conditions, 25–27°C, and 70–85% RH. Test plants were selected based on their relatedness to *P. stratiotes*, use of similar habitats, and economic importance. Testing was done using whole plants because *L. pistiae* feeds on the plant's fluids. Damage from this insect is seen primarily as 'hopper burn' (chlorosis) on leaves and mean growth rate reduction (de Remes Lenicov & Walsh, 2013). Feeding from an individual insect is difficult to detect, so P_1 survival and F_1 nymph emergence were used as a sign of host plant suitability. Caging appropriate for each plant type and size was used and included sleeved cages and 40 L aquaria or 5 L plastic jars with screened lids.

A randomised, complete block design with five replications of each test plant species was used throughout and each trial contained three to five plant species at a time, including a *P. stratiotes* control. Each test plant was enclosed separately and five (3 ♀ / 2 ♂) 1–2 week old *L. pistiae* adults were placed on each plant for seven days; they were then removed and the P_1 mortality was recorded. Test plants and *P. stratiotes* control plants were then monitored for appearance of F_1 adults. Monitoring was discontinued after F_1 adults were found on *P. stratiotes* control plants.

Insects were also tested for longevity without access to *P. stratiotes* or water to simulate what would occur if all of their host plant died off or they were accidentally transported away from their host plant. A randomised design with ten replicates of each treatment was used and the experiment was repeated three times with equal numbers of male and female *L. pistiae*. Each replicate consisted of a single *L. pistiae* placed in a 500 mL plastic container closed with a friction fitting, screened lid that contained either one *P. stratiotes* rosette in fertilised water (water soluble fertiliser [Peters Professional 24–8–16 Fertilizer, Everris, Geldermalsen, Netherlands] at a rate of 1 g / 5 L deionised water), one filter paper sheet moistened with DI water (water only treatment), or nothing (control). The filter paper was remoistened as necessary during the experiment and treatments were monitored twice daily for insect mortality until 100% mortality occurred in the water and control treatments.

Impact testing

Two experiments were conducted to evaluate the impact of *L. pistiae* on *P. stratiotes:* 1) a two-treatment randomised block design with five blocks and, 2) a four-treatment randomised block design with six blocks. For the two-treatment experiment (repeated four times between February 2017 and March 2018), a low density insect treatment (2 ♀ / 1 ♂ *L. pistiae*) was compared to a no-insect control treatment. For the four-treatment experiment (conducted May through June 2018), three insect densities of *L. pistiae* (low – 2 ♀ / 1 ♂, medium – 10 ♀ / 5 ♂, and high – 20 ♀ / 10 ♂) were compared to a no-insect control treatment. Densities were chosen based on previous experiments done in Argentina, where 30 *L. pistiae* were able to negatively impact caged areas of *P. stratiotes* (Cabrera Walsh & Maestro, 2014). Cabrera Walsh and Maestro (2014) also noted that *L. pistiae* is highly mobile in the field, so the medium and low density treatments in this current experiment were meant to mimic other observed field densities.

For each sample, one *P. stratiotes* rosette was placed in an 11 L plastic tub with a screen lid. Initial fresh weight biomass of each *P. stratiotes* was recorded, as well as the number of leaves within each tub.

Plants were fertilised with the same water-soluble fertiliser and rate used as before, and Aquashade (Arch Chemicals, Inc., Germantown, Wisconsin) was added at the label rate to reduce algal growth. For the insect treatments, 1–2 week old brachypterous adult *L. pistiae* were placed in each insect treatment tub for 1 week and then removed and mortality recorded. Any F_1 nymphs that emerged were permitted to develop into adults, which were counted and removed over the course of 1–2 weeks. Final fresh weight biomass of each *P. stratiotes* was recorded, as well as the final number of rosettes and leaves within each tub.

Statistical analysis

Analyses were performed using R (version 3.3.2; R Core Team, 2016). For the two-treatment impact experiments, treatments and blocks were compared using ANOVAs to determine differences in initial fresh weight and number of leaves and two sample t-tests were used to compare changes in fresh weight, number of leaves, and number of rosettes. For the four-treatment impact experiment, treatments and blocks were compared using ANOVA. Data were then evaluated post-hoc using Tukey's tests to determine differences among treatments. A significance level of $\alpha < 0.05$ was used for all statistical tests.

Results and discussion

Host range and oviposition

Adult *L. pistiae* did not survive on, nor did any nymphs emerge from, any plants except for *P. stratiotes,* where up to 204 nymphs emerged from each plant (Mean (± SE) number of nymphs = 84.8 ± 9.26; average P_1 survival = 0.63 ± 0.047) (Table 1). Mean survival of adults was 1.38 ± 0.10 days in the water only treatment and 0.63 ± 0.04 days in the control treatment. Average survival of *L. pistiae* under the same ambient conditions in the *P. stratiotes* treatment was 70 ± 8.5% over three days, similar to what was seen in the host range no-choice tests. The no-choice experiments show that in the event that this insect is separated from its host plant, the adults are not able to survive more than a few days without feeding. The specificity of this insect and its inability to survive off of its host plant for any significant period indicate that it would be a safe biological control agent for *P. stratiotes*.

Impact

There was no significant difference in initial fresh weight or initial number of leaves between treatments or blocks in the two-treatment impact experiment. At low density (2 ♀ / 1 ♂) *L. pistiae* did not reduce *P. stratiotes* growth or vegetative reproduction. There was a change in fresh weight ($df = 3$, $F = 26.65$, $p < 0.001$) and a difference in total F_1 *L. pistiae* ($df = 3$, $F = 7.483$, $p = 0.00167$) among treatments in the four-treatment experiment. Further, Tukey's tests indicated that the control and low density treatments had greater increases in fresh weight compared to the medium and high density treatments ($p < 0.001$, Figure 1). The impact experiments found lower insect densities did

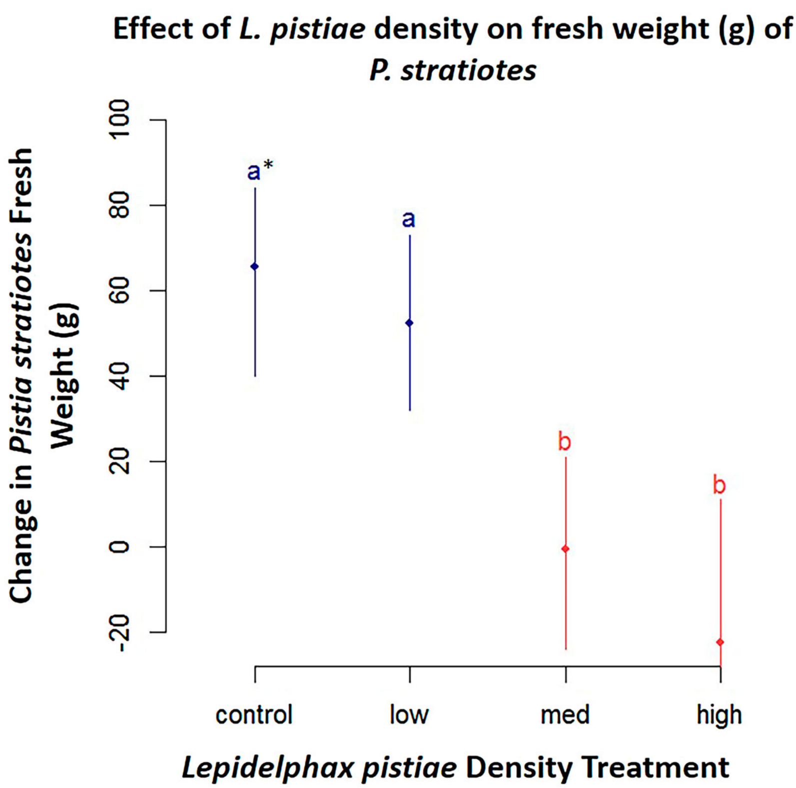

Figure 1. Effect of *L. pistiae* density on fresh weight (g) of *P. stratiotes*. Mean (± SE) change in the biomass of *P. stratiotes* when exposed to a range of densities of *L. pistiae*. *Letters indicate significant differences determined by Tukey's test at $p < 0.05$.

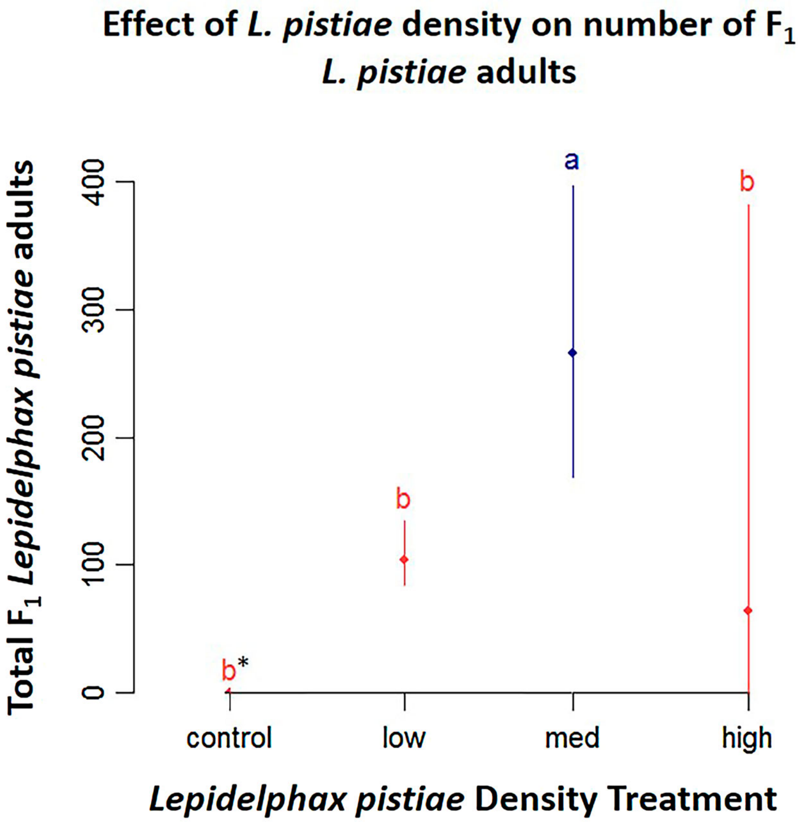

Figure 2. Effect of *L. pistiae* density on the number of F_1 *L. pistiae* adults. Mean (± SE) number of F_1 adults produced under different initial densities of *L. pistiae*. The high density treatment only had one replication with live plant material at the end of the experiment. *Letters indicate significant differences determined by Tukey's test at $p < 0.05$.

not affect the growth rate of *P. stratiotes* while moderate to higher densities did. The high density treatment resulted in the complete destruction of the experimental plant population, thereby causing the insects to starve and the final total F_1 count to be zero for most of the samples. This explains why the medium density treatment had significantly more total F_1 *L. pistiae* than either of the other insect treatments or the control ($p <$ 0.05, Figure 2).

In a previous lab study, *L. pistiae* reached similarly high densities to those in this study (Cabrera Walsh et al., 2014). In field studies in the native range of *L. pistiae*, caged plots containing *P. stratiotes* that were exposed to 30 *L. pistiae* adults (comparable to the high density impact treatment in this study) produced lower biomass and plants with smaller diameters than those in control enclosures (Cabrera Walsh & Maestro, 2014). *Lepidelphax pistiae* also produced high population densities and dispersed across a 7,000 m^2 lake within 40 days of release (Cabrera Walsh & Maestro, 2016). In this study, *L. pistiae* exposures at medium density resulted in a ~36% increase in biomass, compared to *a* > 200% increase on average in the control treatment. These results suggest that, if released, this insect may be able to reach useful densities in the field and cause significant damage to *P. stratiotes*.

Lepidelphax pistiae has been shown to be highly specific to *P. stratiotes* and can be damaging at medium and higher densities, indicating that it would be a suitable biological control agent for this species. However, the question of whether *P. stratiotes* is native to Florida must be considered and requires further research (Evans, 2013).

Disclosure statement

No potential conflict of interest was reported by the authors.

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