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Rearing and Release of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) for Biological Control of Waterhyacinth in 2015

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PURPOSE: Waterhyacinth biological control research at the Engineer Research and Development Center (ERDC), Vicksburg, Mississippi is currently focused on the rearing and release of the delphacid planthopper, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), which has been in culture at the ERDC since 2010. Past failures to establish *M. scutellaris* were attributed to extreme summer temperatures at field sites; therefore, a putatively temperature-tolerant strain of this insect was obtained from field-established stock in Florida. This report details the performance of original and new strains of *M. scutellaris* in greenhouse rearing and field released colonies in 2015.

INTRODUCTION: Waterhyacinth **(***Eichhornia crassipes* (Mart.) Solms-Laub) is considered to be one of the world's worst weeds (Holm et al. 1977). It is an erect, free-floating herbaceous plant native to tropical South America (Gopal 1987). Waterhyacinth can grow rapidly and invade lakes, ponds, and rivers, creating impenetrable barriers and floating mats that impede water traffic and degrade aquatic ecosystems (Center et al. 2002). Herbicide management of waterhyacinth can be very costly. Since 1975, over $100 million has been spent on herbicide control of waterhyacinth in Louisiana alone[1]. An alternative to using herbicides for waterhyacinth management has been the use of host-specific biological control agents.

Three insects were released in the United States for biological control of waterhyacinth during the 1970s; two weevils, *Neochetina eichhorniae* (Warner), and *N. bruchi* (Hustache), and the moth, *Niphograpta* (*Sameodes*) *albigutallis* (Warren). The two weevils are widely established in the southeastern U.S., and have reduced waterhyacinth in the Gulf Coast states to one-third of its former abundance (Center 2004). The effects of the moth have been more difficult to quantify. It targets new, tender plants and their damage is unlikely to control serious infestations of waterhyacinth (Julien 2001). Tipping et al. (2014) found that these agents noticeably reduced the growth and reproduction of waterhyacinth but plant coverage was not markedly reduced. Since the public and aquatic resource managers generally focus solely on reduction in coverage as a metric of success, additional agents were investigated (Tipping et al. 2014).

Ideal agents for waterhyacinth were thought to be mobile, with shorter life cycles and greater reproductive fitness than the early agents (Tipping et al. 2008). From surveys in South America, *Megamelus scutellaris* was identified and selected for further investigation. This insect is associated

[1] Wainger, L., A. McMurray, N. Harms, A. Cofrancesco. In Prep. A framework for assessing economic benefits of invasive plant management in Louisiana).

with waterhyacinth in the majority of the plant's native range (Sosa et al. 2004). In 2008, *M. scutellaris* was brought into quarantine at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Invasive Plant Research Lab (IPRL) in Fort Lauderdale, Florida where it was evaluated and tested. In quarantine, *M. scutellaris* heavily damaged waterhyacinth plants causing them to wilt and die (Tipping et al. 2008). The insects were released from the quarantine facility in 2010 and initial field introductions were made in Florida followed by releases in Louisiana and California (Grodowitz et al. 2014).

Since 2010, *M. scutellaris* has been reared in ERDC greenhouse facilities and in controlled-environment chambers. Despite releases of more than 24,000 individuals at seven sites in Louisiana from 2010 through 2013, establishment has not been confirmed at any of the original release sites (Grodowitz et al. 2014). Lack of establishment was attributed primarily to high ambient temperatures, as the original strain of *M. scutellaris* brought to the U.S. was thought to be impacted by high summer temperatures common to the southern region[1].

Other factors that may limit the establishment success of *M. scutellaris*, but have not been previously considered, are plant quality (i.e., nutritional value, Nitrogen (N) content) and competitive interactions with other biological control agents (e.g., *Neochetina* spp.), though their consideration in other biological control programs is well-documented (e.g., Center and Dray 2010; Heard and Winterton 2000). Plant nutritional quality is crucial to herbivore fecundity (Awmack and Leather 2002), and some biocontrol programs have even promoted agent establishment by increasing plant quality at sites through fertilization (Room and Thomas 1985). Because plant nutritional quality (N) may vary spatially and temporally, and competition between herbivore species may affect establishment, it is important to address each and consider them when choosing release sites.

This technical note documents the culture of both the original strain (OS) and the newly acquired strain (NS) of *M. scutellaris* at the ERDC. Additionally, this technical note will address the performance of both the OS and NS of *M. scutellaris* populations in both greenhouse and field release settings. To detect potential differences in the population growth between the two strains, they were cultured in greenhouse colonies maintained under similar temperature and nutrient regimes. Additionally, both strains were released and monitored at field sites, the results of which are examined in the context of differences in plant nutritional quality and potential competition due to the presence of other biological control agents.

MATERIALS AND METHODS

Greenhouse colonies. In 2015, both the OS and NS were maintained in greenhouses at the ERDC and were kept separate to prevent genetic mixing. A number of sources have been used to populate OS *M. scutellaris* rearing cultures at the ERDC. In 2013, 1,000 planthoppers were received at ERDC from a colony reared at the Louisiana State University (LSU), Agriculture Center, Baton Rouge, Louisiana. These insects were maintained and used in 2015 for field releases and greenhouse comparisons with the NS.

[1] Grodowitz, M. J., N. E. Harms, and J. E. Freedman. In Prep. The influence of temperature on *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) survival.

In June 2015, a rearing colony of NS was initiated after receipt of approximately 3,600 *M. scutellaris* from the USDA-ARS laboratory in Ft. Lauderdale, Florida. These insects were field collected in Florida but originated from Paraguay. Additionally, the NS are thought to be more heat tolerant than the OS *M. scutellaris* brought from Argentina.

The OS insects were cultured in Nalgene® tanks (91.5 cm [L] x 61 cm [W] x 61 cm [H] = 341 L volume) placed inside fiberglass water baths (155 cm [L] x 94 cm [W] x 91.5 cm [H] = 1200 L volume) with two tanks per water bath (five water baths total). The NS insects were cultured in six tanks of various sizes; Tank 1: 234 cm (L) x 112 cm (W) x 25.4 cm (H) = 666 L volume; Tank 2: 122 cm inner diameter (i.d.) x 76 cm (depth) = 795 L volume; Tank 3: 201 cm i.d. x 86.4 cm (depth) = 2,460 L volume; Tanks 4, 5, and 6: 76 cm (L) x 91.4 cm (W) x 81.3 cm (H) = 568 L volume.

Waterhyacinth plants were maintained in a third ERDC plant culture greenhouse and were used to stock tanks for both colonies prior to introduction of *M. scutellaris*. Additional plants were cultured to replenish the OS and NS colonies as needed. Replenishment was required periodically because severe feeding damage by *M. scutellaris* caused collapse of the plants in the insect tanks. Culture plants were fertilized approximately once per month using a complete water-soluble fertilizer, Scotts Peters Professional® Water Soluble Fertilizer (20-20-20 N-P-K) and a chelated iron (Fe 6%) mixture to obtain 27 parts per million (ppm) N and 12 ppm Fe.

Temperature was monitored in each of the rearing containers. A HOBO (Honest Observer By Onset). pendant temperature data logger (Onset Computer Corp., Bourne, Massachusetts) was attached to a piece of foam board (~ 15 cm x 15 cm) and floated among waterhyacinth plants to measure the temperature in the lower canopy, approximately 5 cm above the water surface[1] where *M. scutellaris* is commonly found. A Honest Observer by Onset (HOBO) pendant was also suspended from the ceiling in each greenhouse 1.5 m above the top of the plant canopy to record ambient air temperature. Temperature was recorded every 30 minutes during the summer.

Colonies were monitored approximately once per week during the summer months. Because *M. scutellaris* insects are highly mobile, accurate counts on a per plant basis were difficult to attain, so a point-count method was used (Dawson and Bull 1975). For one minute the number of observed individuals was counted. This was repeated four times (five one-minute counts per tank) in different locations of the colony (to avoid double-counting individuals) and averaged to obtain an index of relative abundance per tank. In addition, the percentage of adults and immatures was estimated visually for each one minute count. OS colonies were monitored from May until September 2015; NS from June until October 2015. The number of NS insects became too large for accurate counts in August and September 2015 (Figure 1) so estimates were made in lieu of one-minute counts.

Insect relative abundance were recorded as planthoppers counted per minute. The values reported for insect abundance may not be easily compared with other methods or even point counts made under different conditions (e.g., by different observers). However, when standardized by observer and location, point count methods are useful to monitor relative abundance over time.

[1] Grodowitz, M. J., N. E. Harms, and J. E. Freedman. In Prep. The influence of temperature on *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) survival.

Figure 1. Photograph illustrating the abundance of *M. scutellaris* on waterhyacinth leaf surfaces.

Count information was used to estimate a relative growth rate (RGR) for populations in each tank and then averaged to determine overall relative growth rate for each strain. The RGR was determined by:

$$RGR = (\log N(t+1) - \log N(t)) / sampling\,interval \quad (1)$$

where N(t) was insect relative abundance at time t and N (t + 1) was relative abundance at the subsequent sampling date. The sampling interval was calculated as the number of days between observations.

Field releases. For field releases, planthoppers were harvested from rearing tanks with the highest *M. scutellaris* abundance. Infested plants were transported in Nalgene® tanks measuring 91.5 cm (L) x 61 cm (W) x 61 cm (H) and covered with screen that was held in place by large binder clips to prevent insect escape. As plants were placed in the transport container, a visual count was performed to estimate the total number of insects released as well as proportion of total insects as adults or immatures. Harvest and transport of material was done early in the day to prevent overheating of the insects prior to release.

Field releases of both NS and OS were made in Louisiana during 2015. OS insects were released at Lake St. Joseph and NS insects at the Old River Control Structure (ORCS) and Tew Lake (Table 1). In 2014, OS releases were made at the ORCS. However, prior to the release of the NS at the ORCS, it was determined that the previous releases were unsuccessful. No *M. scutellaris* individuals were observed at the ORCS after several sampling visits in 2014 and prior to release of the NS in 2015.

A total of 2,000 OS and 15,000 NS individuals were released at three sites during 2015 (Table 1). Greenhouse populations of the OS colony did not increase sufficiently to allow releases after early July. In contrast, the newly-established greenhouse colonies of NS produced high numbers of individuals through August (Figure 1). This allowed for multiple releases at the two NS sites (Tew Lake and the ORCS).

Table 1. 2015 *Megamelus scutellaris* release sites, dates, and quantities.				
Waterbody in Louisiana	**Strain***	**GPS Coordinates**	**Date**	**Insects Released (%Adults: Immatures)**
Lake St. Joseph	OS	N31°55'6.127" W91°14'0.558"	05-June-2015	1,000 (50:50)
			19-June-2015	500 (75:25)
			10-July-2015	500 (50:50)
ORCS	NS	N 31° 5' 24.81" W 91° 36' 48.248"	07-August-2015	2,500 (50:50)
			27-August-2015	5,000 (50:50)
Tew Lake	NS	N 31° 41' 47.607" W 91° 49' 19.461"	07-August-2015	2,500 (50:50)
			27-August-2015	5,000 (50:50)

*OS = old strain; NS = new strain

During each site visit, three 0.25 m² quadrats were placed haphazardly at each release site and used to determine plant density (number of plants per m^2). Additionally, plants were collected from quadrats for insect extraction. To determine the presence of potential competitors (i.e., other waterhyacinth biocontrol agents), on average, eight plants were removed from each quadrat and returned to the ERDC where they were placed in Berlese funnels for insect extraction into 70% ethanol. Preserved insect samples were later examined to quantify *Neochetina* spp. (adults and larvae) and monitor for *M. scutellaris* presence. Weevils were not separated by species for the purpose of this survey. Insect densities are reported per waterhyacinth plant.

Chlorophyll readings were taken from 30 leaves per site using an atLeaf+ Handheld Chlorophyll Meter® (2015 FT Green LLC, Wilmington, Delaware). Unitless atLeaf+ values were converted to chlorophyll values using regression equations from Zhu et al. (2012):

$$Predicted\ Chlorophyll\ \left(\mu g/cm^2\right) = \left(atLeaf\ Value - 15.1\right)/52.4 \qquad (2)$$

Chlorophyll values were then converted to nitrogen, based on waterhyacinth-specific work by Grodowitz et al. (2016):

$$Nitrogen\ \left(NH_3 mg/g\right) = 29.115 + 33.079\ *\ Predicted\ Chlorophyll\ \left(\mu g/cm^2\right) \qquad (3)$$

Statistical approach. One-way nested ANOVA was used to compare the RGR between tanks nested in planthopper strain (alpha = 0.05). Statistical analyses were performed using Statistica ver. 12 (Statsoft, Inc., Tulsa OK).

RESULTS AND DISCUSSION

Greenhouse colonies. Greenhouse counts of the OS peaked early May through June (Figure 2a) and then declined to undetectable levels by September. The NS maintained low levels until late July, when counts increased significantly in three of the six colonies (Figure 2b). Additionally, the RGRs were, on average, positive for the NS colonies, while the OS colonies were negative (Figure 3). Although the RGR between individual tanks was not significantly different, the RGR of the NS and OS colonies were different (ANOVA, $F[1,9] = 44.321$, $p < 0.001$); the RGR of NS was larger than the OS (Figure 4).

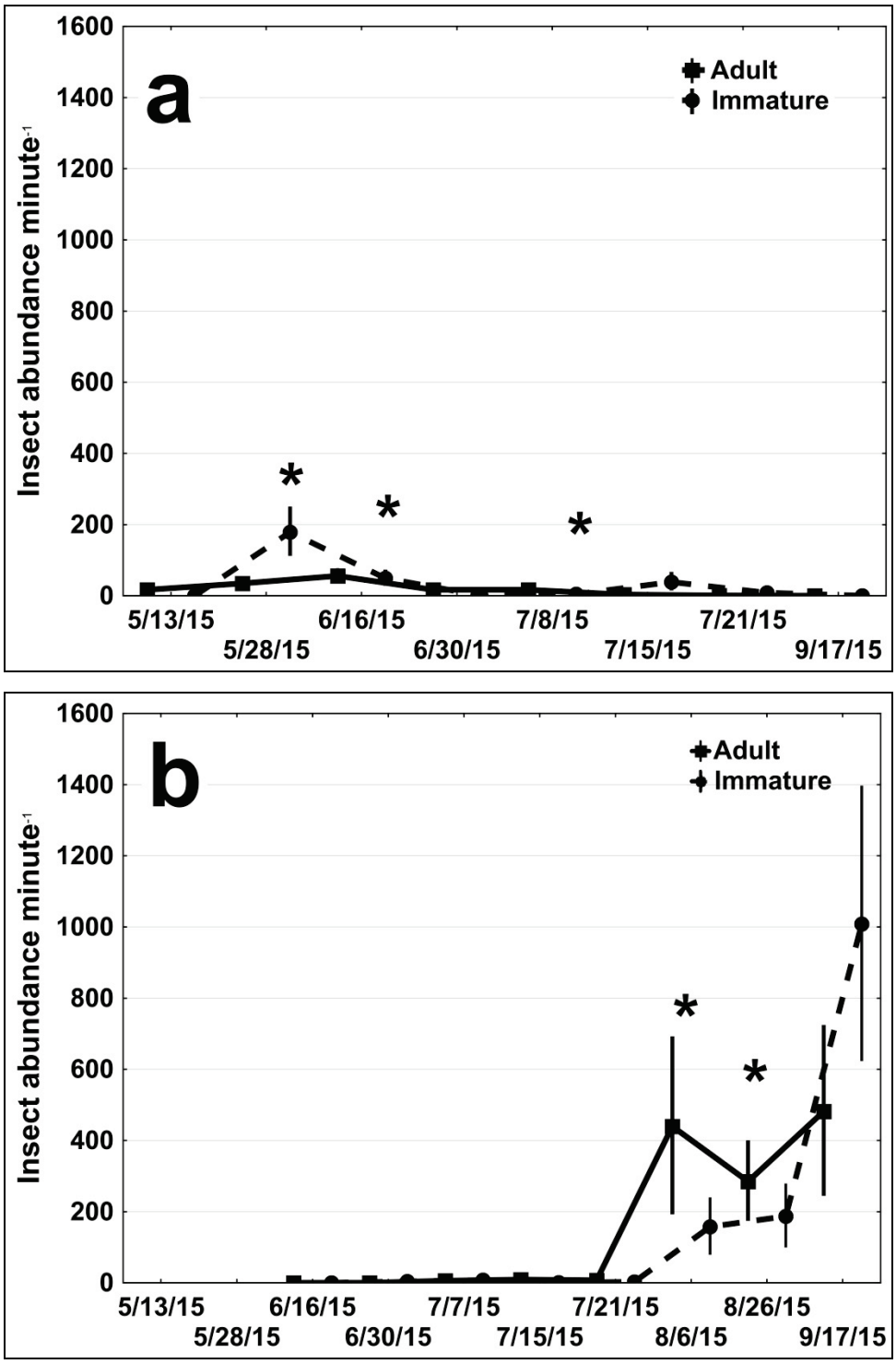

Figure 2. Overall *M. scutellaris* mean abundance in old (a) and new strain (b) colonies. Asterisks denote dates on which planthoppers were harvested from colonies for field release. Harvest dates from old strain colonies were June 5 (~1,000 planthoppers), June 19 (~500), and July 10 (~500). Harvest dates from new strain colonies were August 7 (~5,000 planthoppers) and August 27 (~10,000).

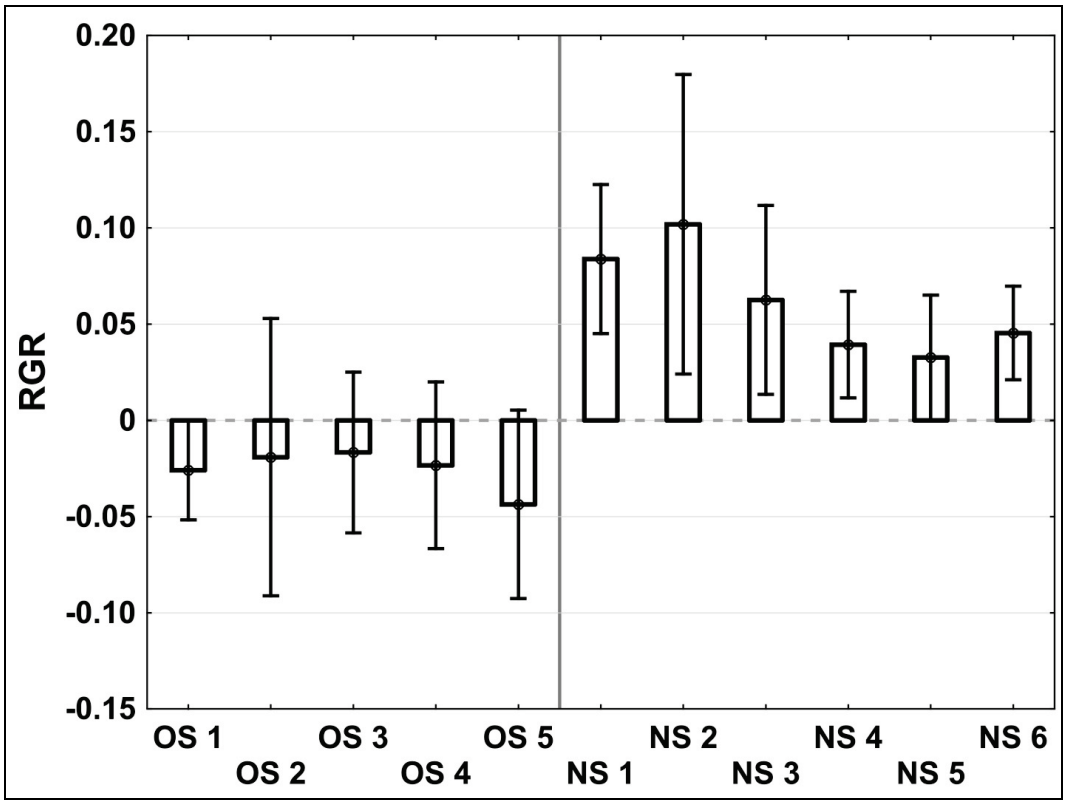

Figure 3. Relative growth rates (RGR; Mean ± SE) for old and new strains of *M. scutellaris* in numbered greenhouse tanks averaged over the entire summer culture period.

The reason for differential performance between strains may be related to differences in thermal tolerance. Air temperatures in both greenhouses were consistently below the threshold (29° C) thought to be lethal to *M. scutellaris* (Figure 5), and the NS performed best (i.e., population increased fastest and had largest overall abundance) during the warmest portion of the season, suggesting that the OS strain was more limited by increased temperatures than the NS. This finding suggests better field performance by the NS could be expected at sites prone to high summer temperatures.

Field releases. During late fall (November, 2015) approximately 12 weeks after the last insect releases, all sites were visited and an assessment of plant hopper presence was conducted. A single adult *M. scutellaris* was observed at the ORCS (NS site), but not at the other sites.

Plant quality (nitrogen as NH_3) was assessed at Tew Lake and ORCS prior to release of the *M. scutellaris* NS. Both sites had plants high in estimated nitrogen (49 ± 0.79 mg N/g at the ORCS and 53 ± 1.14 mg N/g at Tew Lake). Plant quality in the current study was comparable to that in a previous study by [1]in which planthoppers thrived at plant nitrogen levels around 46 mg/g. Similarly, in a study by Heard and Winterton (2000) planthopper populations surged in the high-nutrient treatment (approximately 48 mg N/g). Clearly, neither site should be limiting with regards to plant quality.

[1] Grodowitz, M. J., N. E. Harms, and J. E. Freedman. In Prep. The influence of temperature on *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) survival.

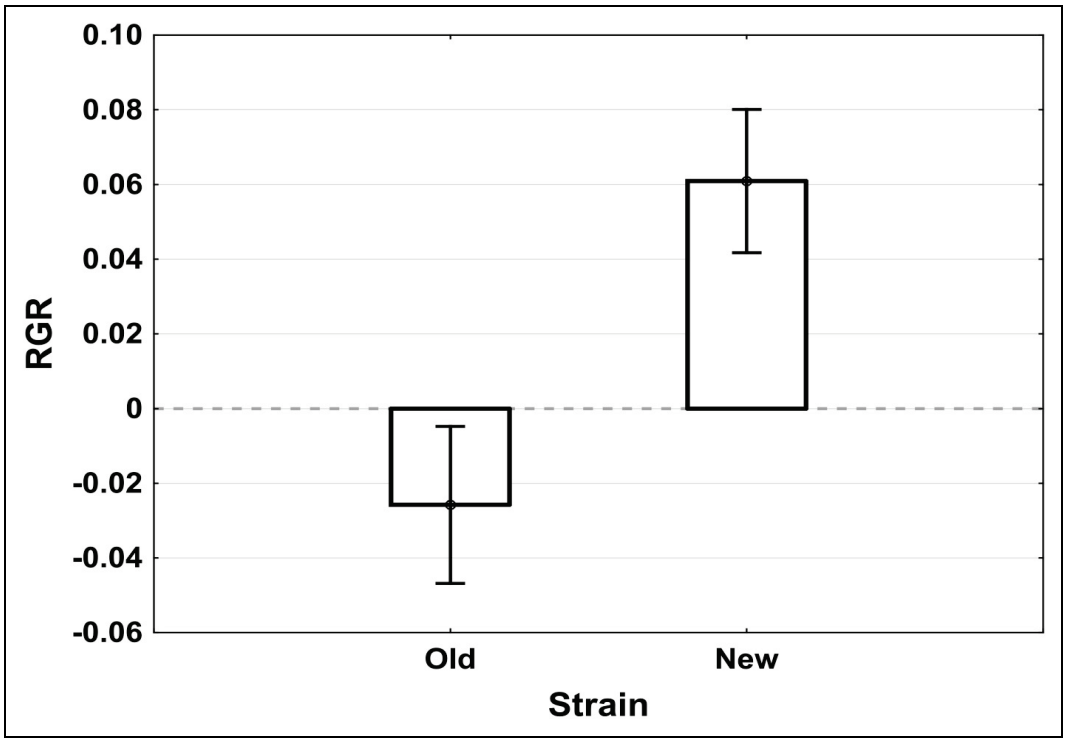

Figure 4. Overall average relative growth rate (RGR) of *M. scutellaris* old and new strains in greenhouse colonies.

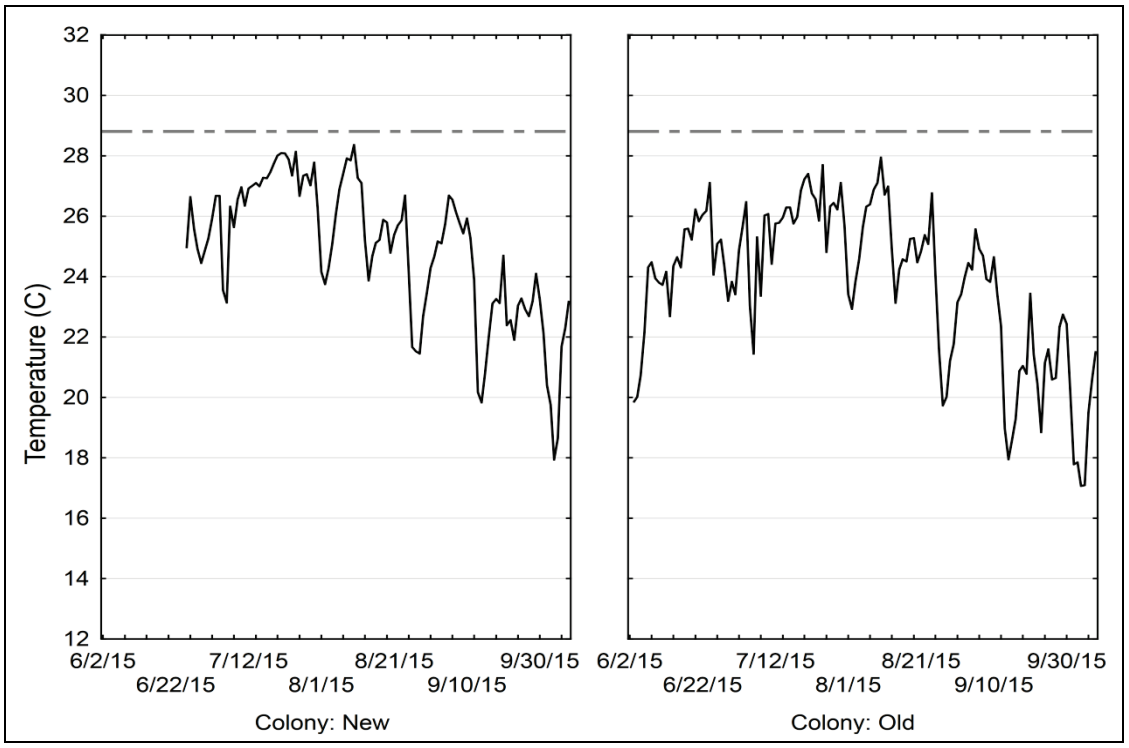

Figure 5. Temperatures recorded in the lower canopy of waterhyacinth plants in both old and new strain colonies. The dashed line represents the temperature thought to be limiting to *M. scutellaris* development and survival (~29° C).

Neochetina spp. were present at all release sites but in low densities (Figure 6). Lake St. Joseph had consistently low weevil populations during the summer, never exceeding one per plant, while populations increased at the ORCS from approximately one to five weevils per plant (approximately 60 weevils/m^2). Other than the ORCS, sites had weevil populations that were negligible compared to published values at sites where infestations declined (approximately 60 weevils/m^2) (Cofrancesco et al. 1985; Goyer and Stark 1984). It seems unlikely that the low numbers of *Neochetina* at release sites would compete strongly with *M. scutellaris* and that it would limit establishment, especially since high quality plants (in terms of Nitrogen content) were found at the sites. In reality, the mechanisms by which *Neochetina* would most likely limit *M. scutellaris* are by damage to oviposition sites of *M. scutellaris* (adult *Neochetina* feed on leaf and petiole lamina, where *M. scutellaris* oviposition occurs) and reduced plant quality due to heavy infestation/feeding by weevils (Heard and Winterton 2000). Neither of these scenarios appears to be occurring at the release sites, and competition between weed biological control agents is apparently rare (Denoth et al. 2002).

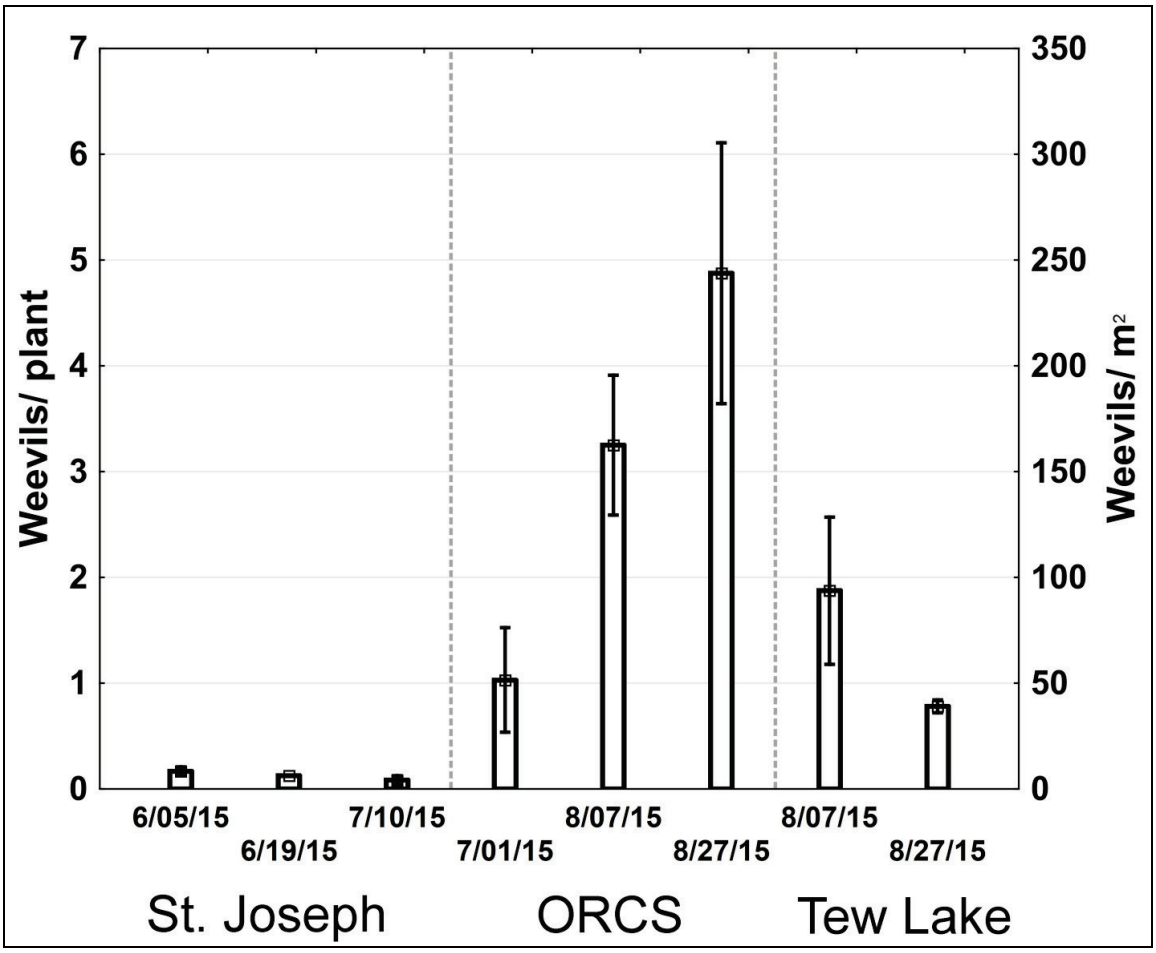

Figure 6. Number of *Neochetina* spp. (larvae and adults combined) per plant and per m^2 at release sites on each sampling date.

Another factor that has not received any attention but may limit *M. scutellaris* establishment and population growth is predation. It has been recognized that predation can limit establishment of biological control agents and thus have a negative effect on biological control. For instance, the

presence of red imported fire ants (*Solenopsis invicta* Buren; Hymenoptera: Formicidae) has been documented to reduce impacts by agents on common salvinia (*Salvinia minima* Baker), water lettuce, (Pistia stratiotes L.), and Azolla spp. (Parys and Johnson 2012; Dray et al 2001; Cuda et al. 2004). Although ants were not observed in abundance during the current work, it may be a productive research direction if efforts at establishment of *M. scutellaris* continue to fail.

FUTURE CONSIDERATIONS: In 2015, receipt of the NS of *M. scutellaris* allowed for an increased production of waterhyacinth biocontrol insects at the ERDC greenhouse rearing facilities. Due to this increase, more insects were available for field releases than in previous years. Whether or not the NS has become established at the Louisiana field sites will be determined in early 2016. Additionally, winter cultures of insects will be maintained to allow for releases of individuals in the spring and early summer of 2016. Releasing a large number of insects at a time when temperatures are mild and waterhyacinth populations have not increased to their maximum will provide the best chance for establishment.

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REFERENCES

Awmack, C. S., and S. R. Leather. 2002. Host plant quality and fecundity. *Annual Reviews of Entomology* 47:817–844.

Center, T. D., M. P. Hill, H. Cordo, and M. H. Julien. 2002. Waterhyacinth. In *Biological Control of Invasive Plants in the Eastern United States*. USDA Forest Service, Publication FHTET-2002-04.

Center, T. D. 2004. Waterhyacinth *Eichornia crasippes*. In *Biological Control of Invasive Plants in the United States*. Editors. E. M. Coombs, J. K. Clark, G. L. Piper, and A. F. Cofrancesco, Jr. Oregon State University Press.

Center, T. D., and F. A. Dray Jr. 2010. Bottom-up control of water hyacinth weevil populations: Do the plants regulate the insects? *Journal of Applied Ecology* 47:329–337.

Cofrancesco, A. F., R. M. Stewart, and D. R. Sanders. 1985. The impact of *Neochetina eichhorniae* (Coleoptera: Curculionidae) on waterhyacinth in Louisiana. In *Proceedings VI International Symposium on the Biological Control of Weeds* 525–535.

Cuda, J. P., A. S. Brammer, R. M. Pereira, and M. Broza. 2004. Interference of natural regulation of the aquatic weed mosquito fern (*Azolla caroliniana*) by the red imported fire ant. *Aquatics* 26:20–26.

Dawson, D. G., and P. C. Bull. 1975. Counting birds in New Zealand forests. *Notornis* 22:101–109.

Denoth, M., L. Frid, and J. H. Myers. 2002. Multiple agents in biological control: Improving the odds? *Biological Control* 24:20–30.

Dray, F. A., T. D. Center, and G. S. Wheeler. 2001. Lessons from unsuccessful attempts to establish *Spodoptera pectinicornis* (Lepidoptera: Noctuidae), a biological control agent of waterlettuce. *Biological Control Science and Technology* 11:301–316.

Gopal, B. 1987. *Water Hyacinth*. Elsevier, New York, NY.

Goyer, R. A., and J. D. Stark. 1984. The impact of *Neochetina eichhorniae* on waterhyacinth in Southern Louisiana. *Journal of Aquatic Plant Management* 22:57–61.

Grodowitz, M. J., N. E. Harms, and J. E. Freedman. (2016). Use of an inexpensive chlorophyll meter to predict nitrogen levels in leaf tissues of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms). *Journal of Aquatic Plant Management* 54:106-108.

Grodowitz, M. J., S. Johnson, and N. E. Harms. 2014. The use of *Megamelus scutellaris* Berg in the southern U.S. as a biocontrol agent of waterhyacinth (*Eichhornia crassipes* (Mart)). ERDC/TN APCRP-BC-33. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Heard, T. A., and S. L. Winterton. 2000. Interactions between nutrient status and weevil herbivory in the biological control of waterhyacinth. *Journal of Applied Ecology* 37:117–127.

Holm, L. G., D. L. Pluckett, J. V. Pancho, and J. P. Herberger. 1977. *The world's worst weeds distribution and biology*. The University Press of Hawaii.

Julien, M. 2001. Biological control of water hyacinth with arthropods: a review to 2000. Editors: Julien, M., M. Hill, T. Center, and Ding, J. In *Proceedings of the Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth*. Beijing, China, 9-12 December 2000. Australian Centre for International Agricultural Research, Canberra, Australia. 8–20.

Parys, K. A., and S. J. Johnson. 2012. Impact of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), on biological control of *Salvinia minima* (Hydropteridales: Salviniaceae) by *Cyrtobagous salviniae* (Coleoptera: Curculionidae). *Florida Entomologist* 95:136–142.

Room, P. M., and P. A. Thomas. 1985. Nitrogen and establishment of a beetles for biological control of the floating weed salvinia in Papua New Guinea. *Journal of Applied Ecology* 22:139–156.

Sosa, A. J., A. M. Marino De Remes Lenicov, R. Mariani, and H. A. Cordo. 2004. Redescription of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), a candidate for biological control of waterhyacinth. *Annals of the Entomological Society of America* 97: 271–275.

Tipping, P. W., T. D. Center, and F. A. Dray, Jr. 2008. Proposed field release of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) for control of waterhyacinth *Eichhornia crassipes* Mart. (Solms) (Pontederiales: Pontederiaceae). USDA, ARS Invasive Plant Research Lab, 3205 College Avenue, Ft. Lauderdale, FL. 33314.

Tipping, P., M. Martin, E. Pokorny, K. Nimmo, and D. Fitzgerald. 2014. Current levels of suppression of waterhyacinth in Florida USA by classical biological control agents. Publications from USDA-ARS/UNL Faculty Paper 1423.

Zhu, J., N. Tremblay, Y. Liang. 2012. Comparing SPAD and atLEAF values for chlorophyll assessment in crop species. *Canadian Journal of Soil Science* 92:645–648.