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Source: Florida Entomologist, 103(1) : 132-135

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.103.0423>

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# Persistence of biological control agents in waterhyacinth following herbicide application

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Waterhyacinth (*Pontederia [Eichhornia] crassipes*) (Martius) Solms Laubach (Pontederiaceae) is an invasive, floating plant that causes environmental and economic damage outside its native range (Center 1987). The infestation in Florida began in the late 1800s, and it still requires diligent management to prevent it from overtaking waterways. In 2017, nearly half of the water bodies in Florida had *P. crassipes* populations, resulting in almost 25,000 acres (10,117.1 ha) being treated with herbicides (FFWCC 2017). Herbicides remain the principal management tactic (Schmitz et al. 1993; Gettys et al. 2014a, b) despite the establishment of 4 biological control agents (Perkins 1973; Center & Durden 1981; Center 1994; Center et al. 2002; Tipping et al. 2011, 2014b).

Tipping et al. (2014a) reported that although these biological control agents, specifically *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), reduced *P. crassipes* biomass and the number of inflorescences by 58.2% and 97.3%, respectively, total surface area was reduced by only 16.8%. As surface area coverage is a primary concern of land managers (Tipping et al. 2014a), areas with biological control agents are still treated with herbicides to maintain plant density below a level that interferes with native habitats or flood control structures (Gettys et al. 2014a).

Biocontrol agents have been shown to increase the efficacy of herbicide treatments by weakening *P. crassipes* plants (Center et al. 1999), thereby permitting reduced application rates and frequency (Gettys et al. 2014b; Tipping et al. 2017). However, herbicide treatments can be an obstacle for biological control agent populations because they cause rapid reductions in plant abundance (Schmitz et al. 1993), thereby limiting the growth of insect populations by eliminating the sessile stages of most of the biological control agents. As a result, biological control agent densities are low, and the regrowing mats subsequently experience reduced herbivory pressure (Center et al. 1999). Integrating the 2 control strategies is difficult because of the coordination and patience necessary; biological control agents require time to build up their population size, during which plant populations grow rapidly with minimal suppression from herbivory. Several experiments have identified the utility of keeping a population of untreated plants near herbicide-treated areas to act as a reservoir or refuge for biological control agent populations. This way, biological control agents could continue to live and reproduce during the decline and ensuing regrowth of the treated mat, and recover rapidly once sufficient plant material regrows

(Haag 1986; Center et al. 1999; Tipping et al. 2017). The objectives of this experiment were to quantify the impact of insect refuges, using groups of untreated *P. crassipes* within treated mats, on the regrowth of the new mat, and the ability of biological control agents to persist following an herbicide treatment.

This research was conducted from Apr through Nov 2018 (JD 92-330) at the USDA-ARS Invasive Plant Research Laboratory in Ft. Lauderdale, Florida, USA, in 20 outdoor, above-ground tanks measuring 2.18 × 0.76 × 0.62 m filled with 0.78 m<sup>3</sup> of water. The experiment was a 2 × 2 factorial arranged in a completely randomized design, with 5 replicates of each treatment (Treatment 1: label rate of penoxsulam herbicide [Galleon SC, SePRO Corporation, Carmel, Indiana, USA] without biological control agents; Treatment 2: half-label rate of penoxsulam without biological control agents; Treatment 3: label rate of penoxsulam with biological control agents; Treatment 4: half-label rate penoxsulam with biological control agents). All tanks were stocked with 10 *P. crassipes* plants and fertilized at the beginning of the experiment with Osmocote Plus 15-9-12 (ICL Fertilizers, Dublin, Ohio, USA; 0.31 g per liter) and chelated iron (Sequestrene 330 Fe, BASF Corporation, Research Triangle Park, North Carolina, USA; 0.02 g per liter). Aquashade (Arch Chemicals, Inc., Germantown, Wisconsin, USA) was added at the label rate to reduce algal growth. *Pontederia crassipes* plants for the treatments without biological control agents (Treatments 1 and 2) were grown with no herbivory prior to the experiment, and were treated with an insecticide, Bifenthrin (Bifen I/T, Control Solutions, Inc., Pasadena, Texas, USA), every 4 to 6 wk to maintain herbivore exclusion. Plants in treatments with biological control agents (Treatments 3 and 4) were sprayed with water following the same application schedule. The biological control agent treatments were stocked with plants already infested with biological control agents: 8 *P. crassipes* plants grown outdoors with natural levels of *Neochetina* spp. (populations of *N. eichhorniae* and *Neochetina bruchi* Hustache [Coleoptera: Curculionidae]; about 3 *Neochetina* spp. per plant) and 2 plants that were exposed to 150 adult *M. scutellaris* (about 1:1 males:females) each for 1 wk prior to the start of the experiment. This technique is used during mass rearing efforts by the USDA-ARS Invasive Plant Research Laboratory, and reliably produces a total of 1,500 to 6,000 F<sub>1</sub> *M. scutellaris* (Goode et al. 2019). One mo after the start of the experiment, additional *Neochetina* spp. (5 adults) and *M. scutellaris* (25 adults) were added to all biological control agent treatment tanks.

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Tanks were monitored every 2 wk for biological control agent density, *P. crassipes* density, and plant surface area coverage. Eighty-eight d into the experiment (JD 180), when most (75%) of the tanks reached 100% surface area coverage, a foliar application of penoxsulam was applied at either the label rate (165.6 mL per 4,046.86 m<sup>2</sup>) or half-label rate (82.75mL per 4,046.86 m<sup>2</sup>) to 90% of the surface area of each tank. Ten percent of the surface area (0.16 m<sup>2</sup>) was protected during treatment to serve as biological control agent “refuges” by covering the plants with an upside-down plastic nursery pot. After herbicide treatment, *P. crassipes* inflorescences were counted in all tanks every wk. All tanks were fertilized 1 mo post herbicide application at levels similar to what is found in Lake Okeechobee (TN = 2.06 mg per L, TP = 511 µg per L [Zhang et al. 2016]; 10.7 g Osmocote, 0.03 g chelated iron). The experiment was harvested when the majority of tanks without biological control agents reached 100% coverage, 150 d (JD 330) after the herbicide treatment.

*Pontederia crassipes* biomass was measured initially, prior to herbicide treatment, and at final harvest. Prior to herbicide treatment and at final harvest, all *P. crassipes* plants in each tank were counted. *Megamelus scutellaris* density was measured by submerging a 0.07 m<sup>2</sup> area of the mat enclosed by a plastic bucket and counting the *M. scutellaris* that climbed out of the water. Five plants were haphazardly selected and weighed for fresh weight, combined, and placed in Berlese funnels for 7 d to capture any *Neochetina* spp. adults and larvae within the plants to determine *Neochetina* spp. density. Material from the Berlese funnels was then placed in a drying oven until it obtained a consistent dry weight (dry weight biomass). At final harvest, defoliation by *Neochetina* spp. also was estimated from the 5 plants prior to the rest of the measurements using the same method as Tipping et al. (2014a). This was done by counting the total number of leaves and the number of damaged leaves on each plant, along with estimating the percentage of the lamina damaged by *Neochetina* spp. herbivory on the oldest and youngest leaves. Estimates were calculated by taking the average of the 2 lamina damage samples and multiplying by the average number of damaged leaves/total leaves per plant to estimate defoliation (Tipping et al. 2014a).

Relative growth rate (RGR) of *P. crassipes* after herbicide application was calculated by:

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

where  $W_1$  and  $W_2$  are the dry weight biomass at the beginning ( $t_1$ , prior to herbicide treatment) and end ( $t_2$ , at final harvest) of the sampling period averaged by treatment, and  $\ln$  is the natural logarithm.

Data was analyzed in R (R Core Team 2019) and was initially tested for normality and equality of variances using the Shapiro-Wilk test and Levene’s test. Data was then analyzed using ANCOVAs with the initial calculated *P. crassipes* dry weight biomass as the covariate. There were significant differences in final *P. crassipes* density, dry weight biomass, and relative growth rate, *Neochetina* spp. damage (defoliation and percent of damaged leaves per plant), and total inflorescences among treatments (Table 1). Post-hoc Tukey tests indicated that plants in Treatment 2 grew more densely, had greater dry weight biomass, higher relative growth rate, and produced more inflorescences than the plants in treatments with biological control agents (Treatments 3 and 4; Table 1). Plants in Treatment 2 were under the lowest control pressure, with only half-label rate herbicide applied and no biological control agents, allowing the *P. crassipes* to grow with fewer restrictions. Treatment 4 (half-label herbicide and biological control agents) performed as well as both Treatments 3 and 1, both with label rate herbicide, with and without biological control agents, respectively, in lowering *P. crassipes* density, final dry weight biomass, and relative growth rate. This corroborates other studies showing how biological control agents increase the control of *P. crassipes* by herbicides (Gettys et al. 2014b; Tipping et al. 2017) and how this interaction is an important, albeit largely unappreciated, aspect of the integrated management of this plant in Florida. Plants in Treatment 4 experienced the most *Neochetina* spp. damage while defoliation in Treatment 3 was not statistically different from the treatments without biological control agents. This indicates that the label rate herbicidal treatments (Treatments 1 and 3) have a significant effect on *Neochetina* spp. populations. This has been noted before in other studies (Haag 1986; Center et al. 1999), where *Neochetina* spp. populations decline post-herbicide treatment because of the loss of the juvenile forms inside the plant tissue. There were significantly more inflorescences on treatments without biological control agents, confirming earlier studies that biological control agents significantly limit sexual reproduction in *P. crassipes* (Center et al. 1999; Tipping et al. 2017).

*Megamelus scutellaris* density and *Neochetina* spp. density were not normally distributed and could not be transformed successfully due to the prevalence of zeros in the data. Non-parametric Kruskal-Wallis tests were used to determine differences among treatments. At final harvest, *M. scutellaris* densities were not significantly different among treatments ( $\chi^2 = 4.7778$ ;  $df = 3$ ;  $P = 0.1888$ ), unlike *Neo-*

**Table 1.** ANCOVA results and means (± SE) of experimental plant metrics including *Pontederia crassipes* (PC) dry weight biomass (DW), density, relative growth rate (RGR), % defoliation, % damaged leaves, and total inflorescences produced. Biological control agent metrics means (± SE) included *Megamelus scutellaris* (MS) and *Neochetina* spp. (NEO) densities. Treatment 1: label rate penoxsulam without biological control agents; Treatment 2: half-label rate penoxsulam without biological control agents; Treatment 3: label rate penoxsulam with biological control agents; Treatment 4: half-label rate penoxsulam with biological control agents.

Variable	ANCOVA			Treatment			
	df	F	P	1	2	3	4
Pre-treatment							
MS/m <sup>2</sup>				2.9 ± 2.6	8.6 ± 5.1	305.7 ± 217.5	422.9 ± 266.7
NEO/m <sup>2</sup>				0 ± 0	11.4 ± 10.2	162.9 ± 49.2	105.7 ± 40.9
PC/m <sup>2</sup>	3,16	2.025	0.151	39.2 ± 4.0	37.6 ± 1.8	27.2 ± 3.5	33.6 ± 4.6
Final Harvest							
DW biomass	3,16	6.558	0.00424*	13.6 ± 1.8 (ab) <sup>1</sup>	25.2 ± 4.0 (a)	8.4 ± 3.3 (b)	10.4 ± 0.5 (b)
MS/m <sup>2</sup>				0 ± 0	0 ± 0	17.9 ± 8.0	8.6 ± 5.1
NEO/m <sup>2</sup>				0 ± 0	0 ± 0	71.4 ± 30.8	105.7 ± 51.7
PC/m <sup>2</sup>	3,16	6.314	0.00497*	66.2 ± 14.0 (b)	124.8 ± 9.9 (a)	36.2 ± 15.1 (b)	60.5 ± 14.0 (b)
RGR	3,16	4.383	0.0197*	0.007 ± 0.002 (b)	0.02 ± 0.001 (a)	0.005 ± 0.002 (b)	0.006 ± 0.002 (b)
% Defoliation	3,16	4.331	0.0205*	0.08 ± 0.06 (b)	0.05 ± 0.03 (b)	10.0 ± 4.2 (ab)	12.2 ± 3.6 (a)
% Damaged Leaves	3,16	7.284	0.00269*	0.12 ± 0.04 (bc)	0.05 ± 0.03 (c)	0.55 ± 0.2 (ab)	0.80 ± 0.1 (a)
Total Flowers	3,16	11.68	0.000264*	19 ± 9.8 (ab)	58.8 ± 15.1 (a)	2.2 ± 1.2 (b)	1.4 ± 0.6 (b)

<sup>1</sup>Means within rows across treatments followed by different lower case letters are significantly different at  $P = 0.05$  (Tukey’s HSD). \* $P = 0.05$ .

*chetina* spp. densities ( $\chi^2 = 9.9666$ ;  $df = 3$ ;  $P = 0.01885$ ). *Megamelus scutellaris* and *Neochetina* spp. were never found in non-biological control agent treatments (Treatments 1 and 2), confirming the efficacy of the insecticide treatment. However, *M. scutellaris* densities were very low in the biological control agent treatments and highly variable. Despite their variability, this species was able to persist in most of the mats that had been sprayed and was present on the regrowth. Densities of *M. scutellaris* reported from the field range from  $0.06 \pm 0.04$  to  $8.9 \pm 1.6$  *M. scutellaris* per plant in California, USA (Moran et al. 2016). Average *M. scutellaris* density in the biological control agent treatments was  $15.3 \pm 7.2$  per plant before the herbicide treatment and  $0.61 \pm 0.15$  per plant at final harvest. A paired Wilcoxon signed rank test confirmed a difference in *M. scutellaris* density before and after the herbicidal treatment ( $V = 77$ ;  $P = 0.003204$ ). The low numbers of *M. scutellaris* also may indicate that this species disperses from plants with reduced quality more readily than *Neochetina* spp. *Neochetina* spp. densities were not different between pre-treatment and final samples ( $V = 42$ ;  $P = 0.450$ ), indicating that penoxsulam did not reduce *Neochetina* spp. density within the biological control agent treatment tanks. *Neochetina* spp. densities in the field average 0.7 to 1.7 per plant (Haag 1986; Tipping et al. 2014a); in this experiment the weevil density was  $5.0 \pm 1.5$  weevils per plant prior to herbicide treatment and  $2.0 \pm 0.3$  weevils per plant at final harvest.

Penoxsulam often is applied as a systemic treatment in certain water bodies, so its negative influence on plants in the refuges, while not a surprise, prevented a full examination of the utility of biological control agent refuges. Despite the destruction of these experimental refuges, biological control agents were able to persist without additional agents being released. It remains to be seen if intact refuges result in increased biological control agent densities following herbicide application when using a slower acting herbicide like penoxsulam. In future studies, steps will be taken so that the refuge plants will not be exposed to fatal levels of herbicide. In field settings, water flow would likely dilute and displace any herbicide overspray that reached the water column. In a tank study, this would be accomplished by flushing the tank with fresh water immediately after herbicide application.

This experiment supports previous research on the effects of biological control agents and herbicides (Van 1988; Gettys et al. 2014b; Tipping et al. 2017). It also demonstrated that insect populations were able to persist following applications of penoxsulam. Future research will examine if refuges can preserve a critical density of biological control agents so that regrowing *P. crassipes* will be exposed to greater levels of herbivory earlier, thus preventing a negative feedback cycle leading to more herbicide applications (Center et al. 1999; Tipping et al. 2017). A biological control agent refuge system could be integrated into the current herbicide management regime of *P. crassipes* pending the evaluation of the most efficient temporal or spatial strategies and the acceptance of the concept by land managers.

The authors thank Yuichi Shinno at the Invasive Plant Research Laboratory for his assistance with the experiment; Chris Stauffer, Frangely Tejada, and Alvaro Salgado from the Broward College Environmental Science Program for their help with the set-up and data collection; and Kyle L. Thayer, Ian J. Markovich, and Joseph W. Sigmon at the University of Florida, Ft. Lauderdale Research and Education Center for their assistance with herbicide application. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA).

## Summary

In Florida, waterhyacinth (*Pontederia [Eichhornia] crassipes*) (Martius) Solms Laubach (Pontederiaceae) is primarily controlled by herbicides, but overall control is enhanced by insect biological control agents that decrease growth and reproduction and slow regrowth. However, herbicide applications often disrupt the biological control agent populations when applied indiscriminately. Previous studies identified the utility of preserving populations of biological control agents in the vicinity of herbicide treated areas by establishing refuges for the insects. The objectives of this experiment were to quantify the impact of insect refuges, using groups of untreated *P. crassipes* within treated mats, on the regrowth of the new mat and the ability of biological control agents to persist following an herbicide treatment. *Pontederia crassipes* mats were grown with and without biological control agents, then treated with 2 concentrations of the herbicide penoxsulam. Plant growth metrics and biological control agent densities were monitored pre- and post-treatment and compared using ANCOVAs and non-parametric Kruskal-Wallis tests. Although the systemic activity of penoxsulam in the water column prevented the establishment of refuges in this study, biological control agent populations persisted following herbicide applications without additional releases and were able to remain at field densities after the decay and loss of *P. crassipes*. The treatment with no biological control agents and only half-label rate herbicide grew more densely, had greater dry weight biomass, higher relative growth rate, and produced more inflorescences than the plants in treatments with biological control agents. The half-label herbicide and biological control agent treatment performed as well as both treatments with label rate herbicide, and with and without biological control agents, respectively, in lowering *P. crassipes* density, final dry weight biomass, and relative growth rate. Although the concept of refuge systems at operational field scales requires further study, demonstrating the ability of biological control agents to persist even on sprayed mats is a necessary first step in determining the temporal and spatial factors that might influence the utility of such refuges.

Keywords: biological control; *Megamelus scutellaris*; *Neochetina eichhorniae*; *Pontederia (Eichhornia) crassipes*; integrated pest management

## Sumario

En Florida, el jacinto de agua (*Pontederia [Eichhornia] crassipes*) (Martius) Solms Laubach (Pontederiaceae) está controlado principalmente por herbicidas, pero el control general se ve reforzado por los agentes de control biológico de insectos que disminuyen el crecimiento y la reproducción y retardan el crecimiento. Sin embargo, las aplicaciones de herbicidas a menudo interrumpen las poblaciones de agentes de control biológico de insectos cuando se aplican indiscriminadamente. Estudios previos identificaron la utilidad de preservar las poblaciones de agentes de control biológico de insectos en las cercanías de áreas tratadas con herbicidas mediante el establecimiento de refugios para los insectos. Los objetivos de este experimento fueron cuantificar el impacto de los refugios de insectos, utilizando grupos de *P. crassipes* no tratados dentro de las esterillas tratadas, sobre el nuevo crecimiento de la nueva esterilla y la capacidad de los agentes de control biológico de insectos de persistir después de un tratamiento con herbicida. Las esteras de *Pontederia crassipes* se cultivaron con y sin agentes de control biológico de insectos y luego se trataron con dos concentraciones del herbicida penoxsulam. Las métricas de crecimiento de las plantas y las densidades de agentes de control biológico de insectos se monitorearon antes y después del tratamiento y se com-



pararon usando ANCOVA y pruebas no paramétricas de Kruskal-Wallis. Aunque la actividad sistémica del penoxsulam en la columna de agua impidió el establecimiento de refugios en este estudio, las poblaciones de agentes de control biológico de insectos persistieron después de la aplicación de herbicidas sin emisiones adicionales y pudieron permanecer en densidades de campo después de la descomposición y pérdida de *P. crassipes*. El tratamiento sin agentes de control biológico de insectos y solo el herbicida de tasa de medio etiquetado creció más densamente, tuvo mayor biomasa tasa de crecimiento relativo, mayor tasa de crecimiento relativo y produjo más inflorescencias que las plantas en tratamientos con agentes de control biológico de insectos. El tratamiento con herbicida de media etiqueta y agentes de control biológico de insectos se realizó tan bien como los dos tratamientos con herbicida con índice de etiqueta y con y sin agentes de control biológico de insectos, respectivamente, para reducir la densidad de *P. crassipes*, la biomasa tasa de crecimiento relativo final y la tasa de crecimiento relativo. Aunque el concepto de sistemas de refugio a escalas de campo operacionales requiere más estudio, demostrar la capacidad de los agentes de control biológico de insectos de persistir incluso en esteras rociadas es un primer paso necesario para determinar los factores espaciales y temporales que pueden influir en la utilidad de dichos refugios.

Palabras Clave: control biológico; *Megamelus scutellaris*; *Neochetina eichhorniae*; *Pontederia (Eichhornia) crassipes*; manejo integrado de plagas

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