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Progress Report on Field Surveys to Identify Biocontrol Agents of *Hydrilla verticillata* in China during 2011

by Jialiang Zhang¹, Matthew Purcell², Baoliang Tian¹, and Jianqing Ding¹

INTRODUCTION: Native to Asia, Australia, Europe, and Africa, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), or hydrilla, is a submersed aquatic macrophyte (Buckingham and Bennett 1998). Since its introduction in the early 1950s, it has become highly invasive throughout much of North America (Schmitz et al. 1991), where it hinders navigation, impacts water intake and delivery systems, limits recreational uses, out-competes native vegetation, acts as a breeding ground for mosquitoes, and destroys fish and wildlife habitats.

Management of hydrilla is typically attempted with chemical control and introduction of herbivorous fish (Hanlon et al. 2000, Michel et al. 2004). Unfortunately, repetitive chemical applications can lead to the development of herbicide resistance. Resistance to the systemic herbicide, fluridone, has been reported in hydrilla populations found in Florida (Michel et al. 2004). The herbivorous fish grass carp (*Ctenopharyngodon idella*) can remove hydrilla effectively but it also feeds on a wide variety of native plant species. Four insect species were released for the management of hydrilla in the United States, including two weevil species and two leaf-mining flies (Buckingham and Grodowitz 2004). Only two have become established with one species of leaf-mining fly, *Hydrellia pakistanae* Deonier, the most widespread and damaging to hydrilla. Despite its widespread distribution, impact from *H. pakistanae* in the field is limited (Doyle et al. 2002, 2007; Grodowitz et al. 2003; Owens et al. 2006, 2008); therefore, more agents are required. Despite many years of worldwide overseas exploration (Balciunas et al. 2002), regions where the U.S. dioecious hydrilla biotype is native in Southern China and Southeastern Asia have yet to be thoroughly explored for potential biological control agents. The main objective of this project is to continue and expand surveys on hydrilla throughout the entire growing season in un-surveyed regions of southern China.

Previous surveys in China discovered three *Bagous* weevils feeding on hydrilla. *Bagous chinensis* was found on hydrilla in Hunan Province in 2007. Host range testing determined that this weevil could develop on four other aquatic plant species, *Elodea nuttallii*, *Egeria densa* (Planch.) Casp., *Vallisneria spiralis* (Lour.) H.Hara, and *Hydrocharis morsus-ranae* L. (Ding et al 2009). Two *Bagous* species new to science were discovered in Guangxi Province (*Bagous* sp.1) and at Suizhou in Hubei Province (*Bagous* sp.2) in 2009 and 2010, respectively (Ding et al. 2011). *Bagous* sp.1 was not specific and developed on four non-hydrilla hosts: *Lagarosiphon alternifolia* (Roxb.) Druce, *E. nuttallii*, *N. marina*

¹ Invasion Biology and Biocontrol Lab, Wuhan Botanical Institute, Chinese Academy of Sciences (CAS), Wuhan, Hubei Province, 430074 China. Email: ding@wbgcas.cn

² U.S. Department of Agriculture – Agriculture Research Service (USDA-ARS), Australian Biological Control Laboratory (ABCL), 41 Boggo Road, Dutton Park, Queensland, Australia 4102. Tel: 617-38335740; fax: 617-38335504; email: matthew.purcell@csiro.au

L., and *Najas minor* All. (Zhang, unpublished data). Further testing was planned but the colony perished and attempts to recollect this weevil have been unsuccessful. As yet *Bagous* sp.2 has not been cultured in the laboratory and no host range testing has been completed on this species. This document reports field surveys and laboratory tests of insect natural enemies on hydrilla in China and Australia from June through November 2011.

PROJECT UPDATE

Survey areas and sample processing: Field surveys were conducted to collect insect herbivores of hydrilla from June to November 2011 at field sites located in Yunnan, Hubei, Guizhou, and Guangxi Provinces (Figure 1 and Table 1). After collection, hydrilla samples were placed in a cooler/refrigerator, and then transported to the laboratory. Some samples were carefully inspected under a dissecting microscope and insect herbivores and associated damage were noted. Following visual inspections, the samples were placed into Berlese funnels to facilitate further extraction of organisms present on the samples. Immatures were reared to adults when possible to facilitate identification.

Collections in Hubei Province. *Bagous* sp.2 is of great interest given that, in the laboratory, larvae pupated along the stem in cocoons covered by algae. It is possible that this weevil may pupate underwater. Previously, all other *Bagous* found on hydrilla have pupated on shore either in desiccating hydrilla or in tubers. *Bagous* sp.2 (Figure 2a) was first collected at a site near Hemufan Village in Suizhou City, Hubei Province in 2010 (Ding et al. 2011); therefore, surveys in 2011 were focused at two sites in this general area. Five adults and 63 larvae of *Bagous* sp.2 from hydrilla were collected from site samples in 2011 (Table 2). In addition, 89 *Parapoynx* sp. larvae, 76 Chironomidae larvae, and 4 adults and 28 larvae of *Hydrellia* sp. were also collected.

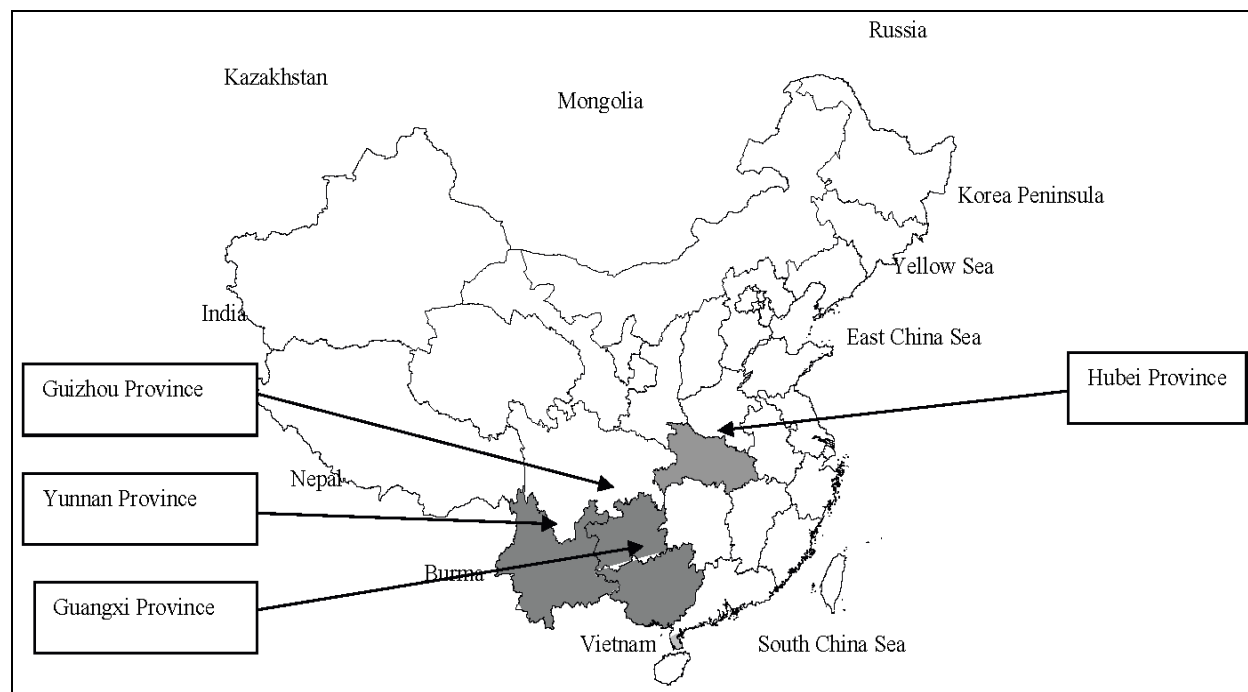


Figure 1. Provinces in southern China where hydrilla was surveyed for insect herbivores.

Table1: Field sites surveyed in Southern China for insect herbivores of hydrilla in 2011.			
Province	Site	GPS	Results
Hubei	Lijiafan Village	N : 31°26' E :113°34'	<i>Bagous</i> sp.3: 3A <i>Parapoynx</i> sp.: 13 L Chironomidae: 16L <i>Hydrellia</i> :3A, 12L
	Hemufan Village	N : 31°46' E :113°07'	See Table 2
	Lishan Town	N : 31°51' E :113°17'	<i>Parapoynx</i> sp.: 5 L Chironomidae: 51L <i>Hydrellia</i> :1A, 8L
	Jiamiao Village	N : 31°45' E :113°10'	<i>Parapoynx</i> sp.: 23 L Chironomidae: 107L, 21A
Guangxi	Yangdi	N : 24°58' E :110°27'	<i>Parapoynx</i> sp.: 8L Chironomidae: 52L
Guizhou	Caohai	N : 26°51' E :104°15'	No herbivores identified
	Yangjie River	N : 26°52' E :104°13'	No herbivores identified
	Huaxi River	N : 26°24' E :106°40'	<i>Parapoynx</i> sp.: 17L Chironomidae: 16L <i>Macrolea japana</i> :13P, 5L

*: A=adult, L=larva, P=pupa

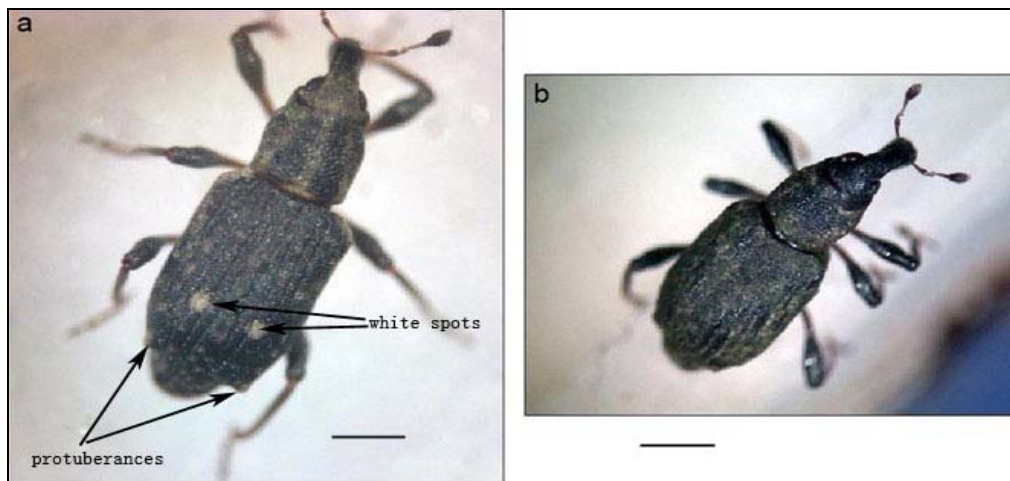


Figure 2. Two *Bagous* sp. collected from Hubei Province during 2011: a) *Bagous* sp.2 from Hemufan village, and b) *Bagous* sp.3 from Lijiafan Village. (a, b scales = 1 mm).

Another weevil species (designated *Bagous* sp.3 in this report, Figure 2b) was collected in a pond near Lijiafan Village, along the Fuhe River (Figure 3). These weevils were collected from hydrilla in isolated pools along the river following drawdowns in the water level.

Three characters apparently differentiate *Bagous* sp.2 from *Bagous* sp.3 (Figure 2). *Bagous* sp.2 has two clear white spots and two obvious protuberances on the posterior of the elytra as well as two clear black stripes on the pronotum; all absent on *Bagous* sp.3. Generally, adults of *Bagous* sp.2 are larger than *Bagous* sp.3. Specimens will be sent to Dr. Charles O'Brien, Green Valley, AZ, for identification.

Table 2. Insects identified at Hemufan Village, Suizhou, Hubei Province.¹	
Collection date	Collection results
3-4 th June, 2011	<i>Bagous</i> sp.2: 2A, 48L <i>Parapoynx</i> sp.: 23 L Chironomidae: 16L <i>Hydrellia</i> :4A,15L
19-20 th June, 2011	<i>Bagous</i> sp.2: 7L <i>Parapoynx</i> sp.: 17 L Chironomidae: 5L
9-10 th August, 2011	<i>Bagous</i> sp.2: 2A,5L <i>Parapoynx</i> sp.: 23 L Chironomidae: 20L <i>Hydrellia</i> :6L
10-11 th October, 2011	<i>Bagous</i> sp.2: 1A, 3L <i>Parapoynx</i> sp.: 26 L Chironomidae:36L <i>Hydrellia</i> :7L

¹: A=adult, L=larva



Figure 3. *Bagous* sp.3 were collected from Lijiafan Village along the Fuhe River.

Collections in Guizhou Province. At the end of October 2011, surveys were conducted in Guizhou Province, Weining County at Caohai Lake (Figure 4), a nature reserve for birds on the Yunnan-Guizhou Plateau, and the Huaxi River in Guiyang. Caohai Lake is a natural water body

containing a diverse assemblage of native submersed macrophytes including *Ceratophyllum demersum* L., *Myriophyllum* spp., and *Potamogeton* spp. and only minimal hydrilla. No insect herbivores were collected at this site. However, *Parapoynx* sp., chironomids, and the beetle *Macroplea japana* were collected from the Huaxi River (Table 1).



Figure 4. Caohai Lake in Guizhou Province is a natural water body containing a diverse assemblage of aquatic macrophytes.

Collections in Guangxi and Yunnan Provinces. Samples were collected at Yangdi, on the Lijiang River in Guangxi Province in early November 2011. The only potential herbivores observed included both *Parapoynx* sp. and several species of Chironomidae (Table 1).

Samples were also collected at three sites in Yunnan Province, including Mengla and two sites in Menglun. Unfortunately, these samples were shipped to the lab in Wuhan for processing and lost during transport. These sites will be sampled again during 2012.

LABORATORY REARING:

***Bagous* sp.2.** Although as many as 48 larvae of *Bagous* sp.2 were obtained from one collection (Figure 5), it was difficult to collect large numbers of adults in the field. The survival rate of immatures was low: a total of only six adults of *Bagous* sp.2 were reared successfully on hydrilla held in containers on paper toweling and not underwater. Further tests are planned to test if pupation in algal cocoons along the stem can occur underwater. Adults did not oviposit in the laboratory; therefore a colony of this species could not be established. Conditions provided in the laboratory during 2011 were apparently unfavorable for oviposition and development. Various holding conditions, rearing containers, light regimes, etc. will be evaluated in 2012, once weevils become available after winter.

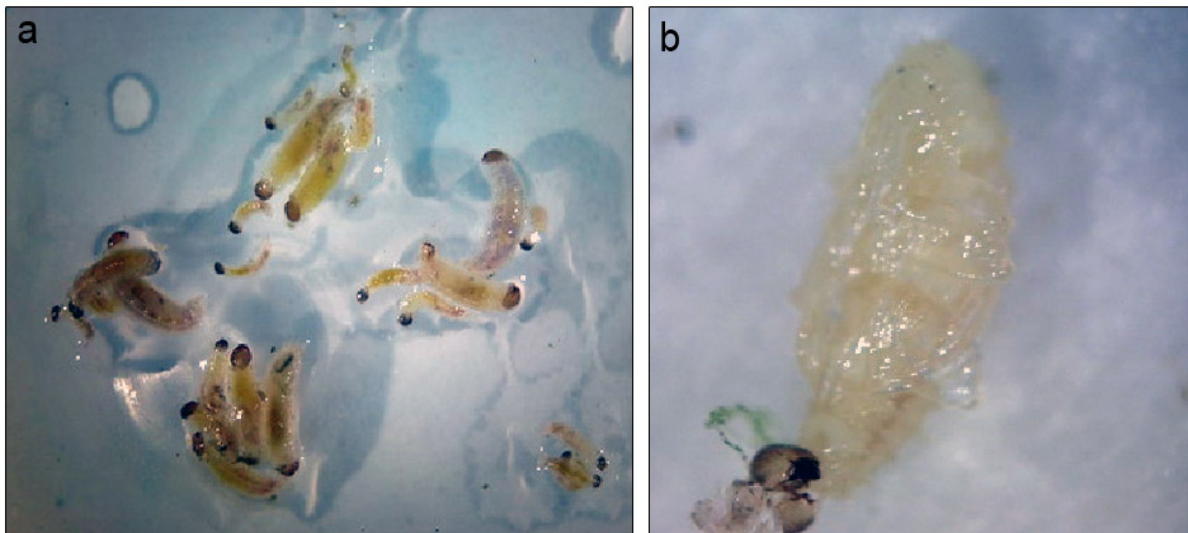


Figure 5. The immature life stages of *Bagous* sp.2: a) larvae, and b) pupa.

***Bagous* sp.3:** *Bagous* sp.3 were collected during four surveying trips to the Lijiafan Village Site. Even though only three adults of *Bagous* sp.3 and no larvae from the first collection were extracted, a colony was established. The colony presently contains between 12 and 20 adults. Six adults were hand carried to the U.S. Department of Agriculture – Agriculture Research Service (USDA-ARS) Australian Biological Control Laboratory (ABCL) in Brisbane, Australia in August 2011. A colony has been established and is currently in its third generation of almost 60 weevils. Host range testing is planned at ABCL when numbers reach 150 adults. Testing will supplement trials conducted in Wuhan, China during 2012.

In September, discussions were held with Dr. Sonja Scheffer of the USDA-ARS Systematic Entomology Laboratory in Beltsville, Maryland. Dr. Scheffer has agreed to genetically sequence all *Bagous* weevils collected from Asia and Australia in view of assessing relatedness, host associations, and elucidation of cryptic species. Identification of cryptic species may lead to evaluation of untested species for biological control.

HOST RANGE TESTING: *Bagous* sp.1 weevils were not found in hydrilla samples from the Lijiang River during 2011 and a colony could not be reestablished. Previous host range testing found that this weevil could develop on four other aquatic plant species: *L. alternifolia*, *E. nuttallii*, *N. marina*, and *N. minor* (Zhang, unpublished data). Since *Bagous* sp.2 could not be reared (see above), no host range testing was completed on this weevil.

Because the colony size of *Bagous* sp.3 was small, choice tests in three trials were conducted from late August to mid September 2011 (Table 3). Twelve adults were selected randomly and placed in a container with material from two to three test plants (shoots with leaves) and hydrilla for one week, and then all the adults were removed. After feeding was recorded, each individual test plant was placed into a separate container to determine development as a result of oviposition on the test plant species. Larvae or adults were monitored on each test plant daily.

Date	Test plants (with hydrilla)	Feeding	#larvae	# Emerged adults
27/08/11-01/09/11	<i>Elodea nuttallii</i>	+	7	5
	<i>Najas marina</i>	+	2	0
	<i>Lagarosiphon alternifolia</i>	+	1	1
	<i>Myriophyllum verticillatum</i>	minimal	0	0
02/09/11-07/09/11	<i>Vallisneria natans</i>	minimal	0	0
	<i>Ottelia acuminata</i>	minimal	0	0
08/09/11-13/09/11	<i>Marsilea quadrifolia</i>	minimal	0	0
	<i>Brasenia schreberi</i>	minimal	0	0
	<i>Hydrocharis dubia</i>	minimal	0	0

The weevils fed substantially on *N. marina* (Figure 6a), *E. nuttallii* and *L. alternifolia* (Figure 6b) and minimal feeding was observed on *V. natans* (Figure 6c), *M. verticillatum* (Figure 6d), *Ottelia acuminata* (Gagnep.) Dandy, *Marsilea quadrifolia* L., *Brasenia schreberi* J. F. Gmel and *Hydrocharis dubia* (Blume) Backer. Five adults emerged from *E. nuttallii* and one from *L. alternifolia*. These results indicate that *Bagous* sp.3 may not be host-specific to hydrilla. Since these tests were conducted in the laboratory, results may reflect the weevil's physiological host range, rather than ecological host range. Thus, further surveys will be conducted on potential hosts at Lijiafan Village to determine the host range of *Bagous* sp.3 under natural conditions.

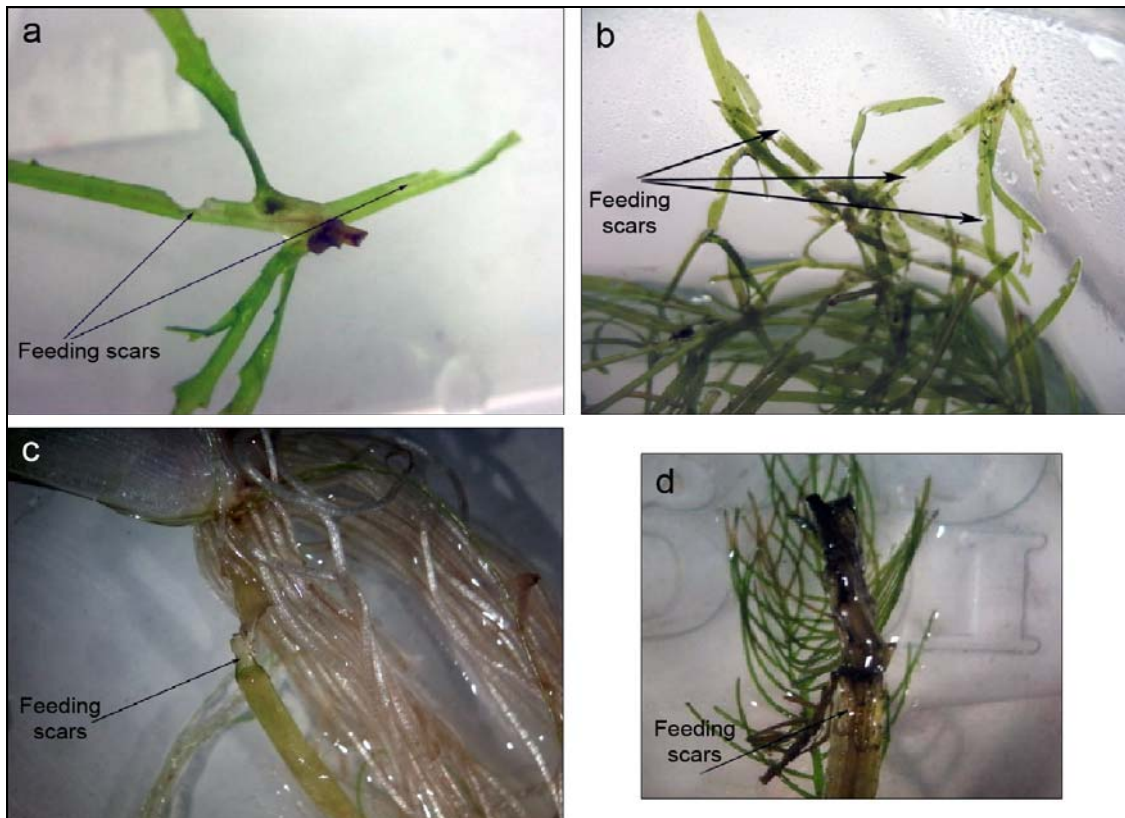


Figure 6. *Bagous* sp.3 feeding scars: a) *Najas marina*, b) *Lagarosiphon alternifolia*, c) *Vallisneria natans*, and d) *Myriophyllum verticillatum*.

FUTURE PLANS: Further study will include the following actions/activities:

1. Continue to rear *Bagous* sp.3 at both the Chinese Academy of Sciences (CAS) and the ABCL in an effort to increase colony size to further document host range.
2. Continue to attempt to establish a colony of *Bagous* sp.2 by modifying conditions in the laboratory to suit its biology. Once colonized, host range testing will be initiated.
3. Survey other potential host plants of *Bagous* sp.2 and *Bagous* sp.3 in Suizhou City to determine the host range of these species in the field.
4. Recollect and colonize *Bagous* sp.1 and complete host range testing.
5. Conduct additional field surveys in Yunnan, Guizhou, and Hubei Provinces to identify other potential biological control agents of hydrilla.
6. *Bagous* specimens will be sent to Dr. Sonja Scheffer, USDA-ARS Systematic Entomology Laboratory, for genetic characterization.

CONCLUSIONS: Surveys to identify insect herbivores of hydrilla were conducted in Yunnan, Guizhou, Guangxi, and Hubei Provinces in southern China from June 2011 through November 2011. One of the more significant findings was the collection of a new aquatic weevil, *Bagous* sp.3 from a site in Suizhou, Hubei Province. This is the second *Bagous* weevil found in Suizhou; *Bagous* sp.2, which pupates in an algal cocoon on hydrilla stems, was initially collected here in 2010. In addition, *Parapoynx* sp., Chironomidae and *Hydrellia* spp. were also commonly collected from most sites surveyed. A colony of *Bagous* sp.3 was successfully established and maintained in the lab in Wuhan and at the USDA-ARS ABCL in Brisbane, Australia, but attempts to colonize *Bagous* sp.2 failed under laboratory conditions. Laboratory choice feeding tests indicated that *Bagous* sp.3 strongly preferred hydrilla over *Myriophyllum verticillatum* L., *N. marina* L., *E. nuttallii*, *L. alternifolia* and *Vallisneria spiralis* (Lour.) Hara. Immatures were able to develop to adulthood on *Elodea nuttallii* and *Lagarosiphon alternifolia*. Since these tests were conducted under laboratory conditions, to fully understand the host range of this weevil under natural conditions, the field host range will be evaluated in 2011/2012.

POINTS OF CONTACT: For additional information, contact Michael Grodowitz (601-634-2972, Michael.J.Grodowitz@usace.army.mil) or the Program Manager of the Aquatic Plant Control Research Program (APCRP), Dr. Linda Nelson (601-634-2656, Linda.S.Nelson@usace.army.mil). This technical note should be cited as follows:

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