Evidence of Establishment of Bagous hydrillae (Coleoptera: Curculionidae), a Biological Control Agent of Hydrilla verticillata (Hydrocharitales: Hydrocharitaceae) in North America?

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EVIDENCE OF ESTABLISHMENT OF *BAGOUS HYDRILLAE* (COLEOPTERA: CURCULIONIDAE), A BIOLOGICAL CONTROL AGENT OF *HYDRILLA VERTICILLATA* (HYDROCHARITALES: HYDROCHARITACEAE) IN NORTH AMERICA?

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Abstract

The semi-aquatic weevil *Bagous hydrillae* was released during 1991-1996 at 19 sites in 4 states in attempts to control the aquatic weed hydrilla, *Hydrilla verticillata*. Fourteen of the sites were in Florida, 2 each in Texas and Georgia and one site in Alabama. Over 320,000 adult weevils were included in these releases. Despite the fact that a few adults were recovered as late as 4.5 yr post-release, presence of permanent, self-perpetuating populations was never confirmed. Then, during 2009 adult *B. hydrillae* were collected in southern Louisiana, at least 580 km from the nearest release site and 13 yr after attempts to establish this insect had terminated. This suggests that earlier recoveries were indicative of successful establishment and that this weevil species has persisted and dispersed widely in the southeastern USA. Nonetheless, there is no evidence that *B. hydrillae* has had a suppressive effect on hydrilla.

Key Words: aquatic weeds, *Bagous restrictus*, biocontrol agent release, biocontrol agent establishment, herbivory, phytophagous insects

Resumen

El gorgojo semi-acuático, *Bagous hydrillae,* fue liberado durante 1991-1996 en 19 sitios en 4 Estados en un intento para controlar la maleza acuática, *Hydrilla verticillata*. Catorce de los sitios estaban en Florida, 2 en cada uno de los estados de Texas y Georgia y un sitio en Alabama. Se liberaron más de 320,000 gorgojos adultos. A pesar del hecho de que pocos adultos fueron recuperados tan tarde como 4.5 años después de la liberación, la presencia de poblaciones permanentes, auto perpetúas nunca fue confirmado. Luego, durante el año 2009 se recolectaron adultos de *B. hydrillae* en el sur de Louisiana, por lo menos 580 km del lugar más cercano de donde fueron liberados y 13 años después de haber terminado los intentos de establecer este insecto en los Estados Unidos. Esto sugiere que las recuperaciones anteriores son indicadores del establecimiento exitoso y que esta especie de gorgojo ha persistido y se dispersaron ampliamente en el sudeste de los EE.UU. Sin embargo, no hay evidencia de que el gorgojo ha tenido un efecto supresor sobre la maleza.

Palabras Clave: control biológico de malezas, *Bagous restrictus*, liberación de agentes biocontrol, agente de biocontrol, establecimiento, herbívoros, insectos fitófagos

Hydrilla, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitales: Hydrocharitaceae), a submersed, leafy-stemmed vascular hydrophyte, roots in the soil of water bodies and grows upwards producing thick floating mats at the water's surface. It is widely distributed in the Old World, but was introduced into the United States through the aquarium trade during the early 1950s (Schmitz et al. 1991). Infestations of hydrilla constitute the most severe aquatic plant problem in the southern United States. Hydrilla infested 22,000 ha in Florida by 1988 when the state was spending about $7 million annually in attempts to control about 6000 ha (Schmitz et al. 1991). During this same period it rapidly expanded its range and now occurs coast to coast (Sonder 1979) and as far north as New England (Les et al. 1997; Les & Mehrhoff 1999) and Washington state (Madeira

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et al. 2000). Southern populations tend to represent the dioecious biotype originally introduced into Florida; northern populations tend to be a monoecious biotype from a separate introduction in the Potomac River near Washington, D.C. (Steward et al. 1984; Madeira et al. 2000).

Currently, control of hydrilla is achieved primarily with herbicides or with exotic herbivorous fish (Sutton & Vandiver 1986; Langeland 1996), but both methods have limitations. Recently, hydrilla has become resistant to the most widely used and selective herbicide, fluridone (Michel et al. 2004), so alternatives are needed. One possible alternative involves the introduction of host-specific plant-feeding insects from the native range of hydrilla. Faunal studies aimed at finding potential biological control agents of hydrilla began during the 1970s in India and Pakistan and resumed during the 1980s in Africa, Asia, and Australia (Sankaran & Rao 1972; Baloch & Sana-Ullah 1974; Baloch et al. 1980; Pemberton 1980; Center et al. 1990; Zhang et al. 2010). These studies revealed the presence of several biological control candidates, 4 of which were ultimately released. One was the semi-aquatic weevil, *Bagous hydrillae* O'Brien (O'Brien & Askevold 1992).

Bagous hydrillae larvae mine hydrilla stems. It is native to Australia where it is easily extracted from hydrilla fragments found stranded on shorelines. Adults are not good swimmers but readily clamber about on submerged hydrilla feeding on the leaves creating distinctive "pepper shot" holes (Balciunas & Purcell 1991). Their feeding often notches the stems, thereby weakening them and causing them to fragment. Balciunas et al. (2002) reported that in Australia this putative "mowing" effect sometimes removed nearly all of the "topped-out" hydrilla in the upper portion of the water column. The female weevil inserts eggs singly into stem punctures usually near a leaf node (Balciunas & Purcell 1991). Larvae burrow within the stems further weakening the upper portions of the plant. Wind and wave action cause the hydrilla beds to break up stranding the fragments in windrows on exposed shorelines (Balciunas & Purcell 1991). After becoming fully grown, the third instar emerges from the stem to search for a pupation site. The larvae reportedly pupate within the stranded plant material or in the underlying soil. They probably also pupate in dewatered hydrosoil during the dry season after water levels recede or when temporary water bodies dry out. Adults fly at dusk and live about 5 wk (Balciunas & Purcell 1991).

Laboratory and field studies done in Australia (Balciunas et al. 1996) and in a Gainesville, Florida, quarantine facility (Buckingham 1994) verified the narrow host range of *B. hydrillae*. Accordingly, Buckingham petitioned the USDA Animal and Plant Health Inspection Service - Plant Protection and Quarantine (USDA-APHIS-PPQ) during Oct 1989 seeking permission to release this species (unpublished report). Permission was obtained after review by the Technical Advisory Group on Biological Control of Weeds (Coulson 1992), and this insect was released at hydrilla-infested field sites from 1991 to 1996 (Center & Grodowitz, unpublished data). However, it was widely believed that self-perpetuating populations had not established (Balciunas et al. 2002). Herein, we document these early releases, tentative evidence of establishment during that period, and a recent recovery of this species in southern Louisiana.

Methods

Releases

Bagous hydrillae was first released on 8 Mar 1991 at Lake Osborne in Palm Beach Co., Florida. Additional releases were made at sites in Florida, Georgia, and Texas (Fig. 1, Table 1) until 1996. Over 320,000 adult weevils were released at 19 sites, all but 3 of which were in Florida. Adults were generally released directly onto exposed hydrilla but, in some cases, they were placed on fresh hydrilla several days prior to release, and then transported to sites on these sprigs. This infested material likely contained eggs and possibly young larvae. This plant material along with the adult weevils was placed amongst the resident hydrilla beds, sometimes inside cages to restrict dispersal. Evaluations were generally conducted by extracting the adults and larvae from samples of hydrilla using Berlese funnels. Voucher specimens were retained and identifications verified by a taxonomist (C. W. O'Brien). Later determinations were made by a project scientist (GW) after being trained by Dr. O'Brien. Details are provided in Grodowitz et al. (1994).

Louisiana Recoveries

Insects were sampled in mats of *Salvinia minima* Baker (Salviniaceae) in southern Louisiana as part of a different study during the summer of 2009 using floating pitfall traps as described by Parys & Johnson (2011). These traps consisted of a cylindrical glass container mounted into foam, and partially filled with preservative (ethylene glycol). A lid was mounted on plastic struts above the mouth of the cylinder to protect it from rain. The trap floated within the *S. minima* mat so that the mouth of the container was positioned at the same level as the surrounding plant material, and crawling invertebrates were passively trapped as they fell into the preservative. Each trap was tagged with a unique number denoting the location within the site. Insects collected in the traps were identified and sent to specialists for confirmation or further identification. C. W.

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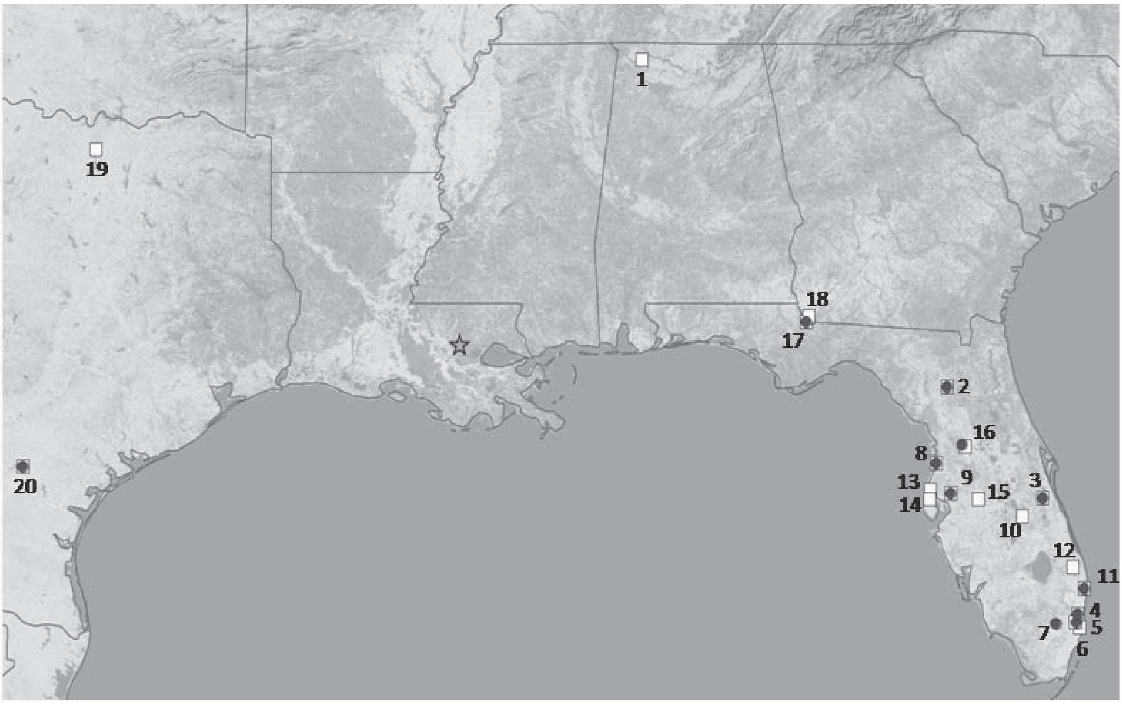

Fig. 1. Map of the southeastern United States showing the original sites where *Bagous hydrillae* was released (open squares), the sites where recoveries were made (solid dots), and the recent recovery in Louisiana (star). See Table 1 for detailed location information.

O'Brien, who originally described and named *B. hydrillae*, identified the aquatic weevils. *Bagous hydrillae* specimens recovered from Louisiana were identified by C. W. O'Brien and independently by R. S. Anderson. These are deposited at the Louisiana State Arthropod Museum (LSAM) collection at Louisiana State University and in the private collection of Dr. O'Brien (CWOB).

Results

Early Releases and Recoveries

During the period of active work on this project (1991-1996), 110 releases were made at 19 sites in 4 states (Table 1). The first evidence that the weevils might have established came from Lake Osborne in Palm Beach Co., Florida. More than 1000 adult weevils along with larval-infested plant material were released there from 26 Mar to 29 Jun 1991 and recoveries were made as late as 6 Nov 1991, 131 days after the final release. However, later efforts to find the weevils at the site were fruitless so it was assumed that the population failed to persist.

Weevils were recovered at several other sites within 2-3 months after the final releases, but these likewise failed to persist. However, a major effort to establish the beetles at Lake Seminole near the Florida-George border in Decatur Co., Georgia, seemed successful. Nearly 44,000 weevils were released between 23 Jun and 5 Sep 1992 near River Junction Landing and recoveries were made as late as 22 Oct 1993, nearly a full yr later.

A surprising find occurred at 2 sites, one in Texas and one in Florida. Eight releases totaling nearly 41,000 weevils were made at Choke Canyon, Live Oak Co., Texas, from May to Oct 1994 and *B. hydrillae* adults were recovered as late as 13 Dec 1994, 46 days after the final release. Seven releases totaling over 35,000 weevils were made in Bulldozer Canal, Brevard Co., Florida, during 29 Jan to 29 Oct 1993 with hundreds of weevils recovered 60 days later. Strangely, some of the weevils collected by Berlese extraction from Choke Canyon and all from Bulldozer Canal were identified by C. W. O'Brien as *B. restrictus* LeConte, a native species not known to utilize hydrilla and previously unrecorded from Florida (Center 1995).

The longest duration between release and recovery of *B. hydrillae* occurred at Wysong Dam on the Withlacoochee River in Sumter Co., Florida. Weevils were never actually released there, but they were released nearby at a site on Lake Panosofkee, less than 10 km away. They were never recovered at the release site but 2 *B. hydrillae* adults were found in a sample of hydrilla collected near the dam on 13 Aug 1996, more than 4.5 yr later. The presence of only 2 weevils after such

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TABLE 1. DATA ON *BAGOUS HYDRILLAE* RELEASES AND RECOVERIES MADE DURING 1991-1996.

Map ID	Latitude (°N)	Longitude (°W)	State	County	Site	Release dates	Releases (no.)	Adults (no.)	Persistence (days)
1	34.744968	87.667486	AL	Colbert	Muscle Shoals	5 xi 93	1	5,000	—
2	29.692070	82.389351	FL	Alachua	Gainesville Pond	25 viii 93-20 x 93	3	14,400	48
3	27.997525	80.797788	FL	Brevard	Bulldozer Canal	29 i 92-29 x 93	7	35,415	69
4	26.085149	80.215348	FL	Broward	Ferncrest	25 xi 92-13 v 93	5	3,967	703
5	26.204922	80.197905	FL	Broward	Lakeview	11 vi 93-26 viii 93	10	29,000	75
6	26.204922	80.169371	FL	Broward	Orange Brook Golf Club	15 vii 91-17 xii 93	17	44,544	—
7	26.062595	80.569192	FL	Broward	Miami Canal @ New River	never	—	0	Collected 5 x 94
8	28.532469	82.627109	FL	Hernando	WeekiWachee River	30 viii 94-22 ii 95	7	22,614	89
9	28.069370	82.377290	FL	Hillsborough	Hillsborough River	31 iii 94-31 i 95	10	31,693	55
10	27.717162	81.150906	FL	Osceola	Kissimmee River	29 ix 95-15 x i95	5	5,000	—
11	26.597565	80.077822	FL	Palm Beach	Lake Osborne	26 iii 91-28 vi 91	3	1,082	131
12	26.936137	80.278578	FL	Palm Beach	West Jupiter	18 v 92-19 xi 93	6	22,975	—
13	28.121190	82.726622	FL	Pinellas	Lake Tarpon	28 vi 96-13 viii 96	2	1,300	—
14	27.977500	82.738060	FL	Pinellas	Coachman	5 vii 96-26 xi 96	6	5,000	—
15	27.977500	81.903002	FL	Polk	Banana Lake	20 vii 96-26 xi 96	9	8,250	—
16	28.790161	82.130091	FL	Sumter	Lake Panasofkee	25 ix 91-19 xii 91	3	1,578	1699
17	30.713655	84.852799	GA	Decatur	River Junction Landing	23 v i92-5 xi 92	6	43,754	351
18	30.798501	84.810320	GA	Seminole	Reynolds Landing Road	2 v 92	1	1,000	—
19	33.364722	97.051389	TX	Denton	Lake Ray Roberts	13 xi 93	1	3,000	—
20	28.478011	98.297895	TX	Live Oak	Choke Canyon	3v 94-28 x 94	8	40,979	46

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a long time caused us to doubt this record and to suspect that the sample had become contaminated by escapees from a laboratory colony prior to Berlese extraction. This suspicion was reinforced when we recovered a single *B. hydrillae* in a Berlese sample of hydrilla from the Miami Canal in Broward Co., Florida, over 30 km from the nearest release site during Oct 1994 (Table 1). These records were therefore discounted and the lack of subsequent recoveries followed by the untimely termination of the project caused us to conclude that populations had not, in fact, permanently established at any location.

Louisiana Recoveries

This conclusion changed when one of us (KP) found bagoine weevils in pitfall samples from *S. minima* mats in southern Louisiana (Gramercy, Ascension Parish 31 Jul-16 Aug 2009, N 30° 09.804' W - 90 48.643›). Dr. O'Brien identified 2 of the specimens as *B. hydrillae*. This collection record was nearly 13 yr after the last known release in Florida and 580 km WSW of the nearest release at Lake Seminole, Georgia, and almost 750 km ENE of the next closest release at Choke Canyon, Texas (Fig. 1). The Gramercy site and nearby areas were revisited during Jun 2010 (by KP, MG, and SJ) but no hydrilla infestations were identified. This was almost a year after the pitfall samples had been collected so conditions could have changed. However, the site was a cypress-tupelo swamp with extensive coverage of floating macrophytes so it did not appear to be suitable habitat for hydrilla. Furthermore, the site had not visibly changed to any great extent during the interim.

Discussion

Early failure to detect persistence of a released weed biological control agent followed by later evidence of population establishment is not unknown. Mo et al. (2000) reported that the rubber vine moth *Euclasta whalleyi* Popescu-Gorj & Constantinescu (Crambidae) had remained undetected for more than 4 yr in Australia after releases ceased, yet it later achieved outbreak densities on its host *Cryptostegia grandiflora* R. Br. (Gentianales: Apocynaceae). The chrysomelid beetle *Zygogramma bicolorata* Pallister disappeared after being introduced in Australia to control *Parthenium hysterophorus* L. (Asterales: Asteraceae) during 1980 but reappeared 10 yr later during periodic outbreaks (Dhileepan et al. 1996). The discovery of populations of biological control agents considerable distances from their release sites is also not unique to this example, as was recently documented (Pratt & Center 2012). Likewise, it isn't unusual for an agent to not be detected at a release site and then be found some distance away, as was the apparent case with the release of *B. hydrillae* at Lake Panosofkee and the recovery at Wysong Dam. We also had this experience when releasing the pyralid moth *Niphograpta albiguttalis* (Warren) on water hyacinth during the late 1970s (Center & Durden 1981) though in both cases recoveries were usually somewhat nearer release points.

The presence of *B. hydrillae* at a location that lacked hydrilla is puzzling. It is possible that hydrilla was present when samples were collected but had disappeared by the time weevil species had been determined and the site was re-inspected. Assuming that hydrilla was not present when the samples were collected, and with the absolute certainty regarding the identification of the beetle, 2 possibilities remain. One is that hydrilla existed somewhere elsewhere within the general area but not found when the site was revisited. The other possibility is that *B. hydrillae* was utilizing a plant other than *H. verticillata* as a host. The submersed aquatic plants bladderwort (*Utricularia* sp.; Lamiales: Lentibulariaceae), coon's tail (*Ceratophyllumdemersum* L; Ceratophyllales: Ceratophyllaceae), and naiad (*Najas* sp.; Hydrocharitaceae) were observed at and around the collection site. Buckingham & Balciunas (1994) noted that *Najas guadalupensis* (Sprengel) Magnus and *N. tenuifolia* R. Br. did produce a few adults during laboratory host range tests in Florida and Australia, respectively. So naiad, another serious aquatic weed, may have been supporting a marginal population of *B. hydrillae* at the site. This possibility merits further investigation.

The unexpected occurrence of *B. restrictus* on hydrilla at very distant *B. hydrillae* release sites remains a mystery. Only adults were found in both cases so there was no field evidence of its use of hydrilla as a developmental host, even though Wheeler & Center (2007) showed that it could develop in hydrilla stems. But dozens were extracted from the Bulldozer Canal, Florida samples, so it was not an incidental occurrence. The host plant of this native species is not known and no other potential host plants were noted at either location so further study is warranted.

The recent discovery of *B. hydrillae* in Louisiana suggests that earlier recoveries, such as that at Wysong Dam, represented small but persistent populations of the weevil. The most likely source for the Louisiana specimens was Lake Seminole on the Florida-Georgia border. Weevils were recovered at this site nearly a year after the releases were made which allowed time for them to disperse into other areas. Furthermore, this is the location nearest to the Louisiana site. Nonetheless, it now seems reasonable to conclude that *B. hydrillae* populations have established at a low level, although evidence of a corresponding reduction in hydrilla mats is lacking.

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