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Australian Insects as Biological Control Agents for the Submersed Aquatic Weed, *Hydrilla verticillata*, in the USA

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In the USA, the submersed aquatic weed, *Hydrilla verticillata*, has become a noxious aquatic weed after being introduced about 40 yrs ago. Since 1985, extensive field surveys in Australia have found several insect herbivores feeding on hydrilla. The most promising of these insects, the hydrilla stem-boring weevil, *Bagous hydrillae*, and the leaf-mining fly, *Hydrellia balciunasi*, were studied intensively, proven to be relatively host-specific, then shipped to quarantine in Florida, USA. There, further tests confirmed their safety, and both insects have been released in the USA. Three stream-dwelling moths; *Aulacodes siennata, Nymphula eromenalis* and *Strepsinoma repititalis*, were also found damaging hydrilla in Australia. However, after considerable effort they proved difficult to colonize, and their host range remains questionable. These moths may be considered in the future if the existing biological control agents in the USA prove to be ineffective against hydrilla in flowing stream and river sites.

Introduction

Hydrilla verticillata (L.f.) Royle (Hydrocharitaceae) , is a submersed aquatic weed, native to Australia (Swarbrick *et al.* 1982), and to Asia and Central Africa (Cook and Luond 1982). In the USA, where it was first introduced into Florida in the early 1950s (Schmitz 1990), both monoecious and dioecious forms are present (Steward *et al.* 1984), apparently following separate introductions. Hydrilla can now be found from Florida westward across the southern states to California, and northwards to Delaware (Steward and Van 1987). The potential range of hydrilla in North America covers the whole of the USA and the southern part of Canada (Steward and Van 1987). It has the ability to form large masses of vegetation near the water surface (Swarbrick *et al.* 1982) and severe infestations can clog irrigation and drainage canals, and interfere with navigation, fishing, boating, and other recreational activities in rivers and lakes (Bennett and Buckingham 1991).

Because both herbicidal and mechanical control of this weed are expensive, hydrilla has been a target weed for biological control (Bennett and Buckingham 1991). Between the late 1960s and early 1980s, preliminary surveys to locate biological control agents for hydrilla, within its native range, were conducted in India (Rao 1969, Rao and Sankaran 1974), Pakistan (Baloch *et al.* 1980), Malaysia (Varghese and Singh 1976), Africa (Pemberton 1980, Markham

1986) and Panama (Balciunas and Center 1981). Several possible insect agents for hydrilla were discovered during these surveys. Two of these insects collected in India, the tuber-feeding weevil, *Bagous affinis* Hustache (Coleoptera: Curculionidae), and leaf-mining fly, *Hydrellia pakistanae* Deonier (Diptera: Ephydridae), have been tested and released in Florida (Buckingham 1988). After probable introduction through the aquarium trade, the aquatic moth, *Parapoynx diminutalis* Snellen (Lepidoptera: Pyralidae), which is native to tropical Asia, was discovered feeding on hydrilla in Florida in the mid-1970s (Del Fosse *et al.* 1976). Extensive surveys in the USA, during 1978-80, demonstrated the presence of other, native USA herbivores on hydrilla (Balciunas and Minno 1985). Although *P. diminutalis* occasionally reduced hydrilla mats in Florida (Balciunas and Habeck 1981), none of these insects had potential for controlling hydrilla. Accordingly, further overseas surveys were conducted by the senior author from 1981-3 in India, Sri Lanka, Burma, Thailand, Malaysia, Indonesia, the Philippines, Australia and New Guinea (Balciunas 1985). About 45 insect species that would feed on hydrilla were found in these surveys, predominantly nymphuline moths, ephydrid flies and bagoine weevils (Balciunas 1985).

In 1985, a joint University of Florida/United States Department of Agriculture (USDA) project was initiated to survey the natural enemies of hydrilla in Australia. Australia was chosen as it was economically favorable, had climatic zones similar to those in the USA where hydrilla was then particularly noxious, and because all 3 predominant groups (bagoine weevils, ephydrid flies and nymphuline moths) of potential biological control agents, associated with hydrilla, were present (Center *et al.* 1990). A laboratory was established at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Davies Laboratory in Townsville, north Queensland (NQ), and later shifted to nearby James Cook University. It is now in the Kevin Stark Research Building on the main campus of the university. A second, smaller, satellite laboratory was also established at the CSIRO Long Pocket Laboratories in Brisbane, southeast Queensland (SQ), in mid-1985. Since that time we have conducted extensive surveys of hydrilla and other aquatic weeds, and have investigated several promising insects that feed on hydrilla.

Methods and Materials

From 1985-9, we regularly sampled herbivorous insects on hydrilla, and other aquatic plants, mainly in 2 regions of eastern Australia: from the Daintree River to Townsville in NQ, and from Gympie in SQ, to Coffs Harbour on the northern coast of New South Wales (NSW). Occasionally, we also sampled near Darwin, and at Kakadu National Park in the Northern Territory (NT); at Mount Isa, Mackay, and Rockhampton in Queensland; and in Sydney, Taree and Kempsey in NSW. The project wound down at the end of 1989, and since then, we have collected infrequently, mainly to rear more lepidopteran specimens, monitor populations of biological control candidates, and to provide shipments of these insects to quarantine facilities in the USA

Aquatic plants were usually collected by hand while wading, or in inaccessible locations, by a rake head attached to a rope. In deeper water, a surf ski or a rented boat was used to reach the plants. The samples were briefly drained of water, and then placed into labeled plastic bags for transportation, inside an insulated container, to the laboratory. The sample was weighed in the laboratory, and initially a portion (usually 500 g) was examined manually using a dissecting microscope when necessary, while the remainder was processed in a Berlese funnel. Berlese funnels were the most efficient method of extracting herbivores, especially internal plant-feeders. Between 1-8 Berleses, each with 1-4, 25-watt (occasionally 40-watt) bulbs, were used for each collection, depending on the size of the sample and of the Berlese funnel. In SQ, larger (50 cm diam.) funnels were used, which processed 3-5 times the material of those (20.5 cm diam.) used in NQ. Samples were dried slowly over 4-6 d, the most efficient period for extracting herbivores, and their dry weights were recorded after 1 wk. Most immature herbivores were reared to the adult stage in small plastic cups containing water and a portion of aquatic plant tissue. Adult herbivores were preserved, or used to establish laboratory colonies of priority biological control candidates. Insects from these colonies were mainly used in feeding and oviposition host-tests, and for life history studies. All non-insects and non-herbivorous insects were immediately preserved. The quantitative collections were supplemented by blacklight collections using a 15-watt fluorescent tube. The blacklight was used to collect large numbers of a particular insect species, or to detect the presence of certain desirable insect species at given sites.

Results and Discussion

A total of 1,528 quantitative (Berlese sorted) and 186 qualitative (manually sorted) samples have been processed during this project through the end of 1991. Of these, 546 Berlese and 140 manual samples were of hydrilla. The remaining 982 Berlese and 46 manual samples were of 48 aquatic plant species, from 27 families. A listing of the number of collections for all plant species, in each of the 4 main collecting regions, is presented in Table 1. The author and family names are also provided for each plant species.

A total of 974 Berlese and 55 manual samples were processed by the NQ laboratory, while the SQ laboratory processed 554 Berlese and 131 manual samples. However, due to the larger Berleses used in SQ, a greater wet weight of plant material (736 *vs.* 687 kg for Berlese samples) was processed there. Manually sorted samples yielded very few insects when compared with Berlese sorted samples, thus this processing method was abandoned within 14 months.

Of all the insect herbivores collected from these samples, a stem-boring weevil, a leaf-mining fly, and several moth species were the most promising candidates from Australia for the biological control of hydrilla. These insects are discussed below.

Hydrellia balciunasi *Bock (Diptera: Ephydridae)*

The leaf-mining fly, *H. balciunasi,* was regularly found feeding on hydrilla in NQ, NT, SQ, and northern NSW. Our specimens were initially identified as *H. unigena.* However, Dr. Ian Bock of La Trobe University in Victoria, subsequently determined that our specimens were new and undescribed. In his revision of the Australian members of this genus (Bock 1990), our specimens were named *H. balciunasi.*

Buckingham *et al.* (1991) described the laboratory life history of *H. balciunasi* as follows. Females lay eggs on the leaves or stems of hydrilla at, or mostly above, the water surface. The larvae emerge and wander before entering the leaves, usually through the upper surface. A single larva will mine the leaf completely before moving to another in the same whorl, or on a different whorl. *H. balciunasi* larvae develop through three instars before forming puparia inside leaves at the axils. The development time from newly-laid egg to adult is approximately 23 d, at 27ºC. The adults emerge, and rise in a bubble of air to the water surface.

H. balciunasi adults and larvae were found in >50% of hydrilla collections in NQ and SQ. Over 17,000 *H. balciunasi* adults and larvae were collected on hydrilla, at an average of 28/kg. This fly was also collected from 23 other plant species, but in much lower densities than on hydrilla (nearly 600 adults and larvae at *ca.* 1/kg). On 7 of these other species, only adults were collected, indicating that maybe only pupation occurred on these plants, after larvae had moved from another host. Many of the *H. balciunasi* collected on other plant species may have come from strands of hydrilla contaminating the samples, especially as only low numbers of these flies were extracted. Frequently, field larvae were parasitized by micro- hymenopteran parasites, which contaminated laboratory colonies established at the Brisbane laboratory.

Because our field studies indicated that *H. balciunasi* was sufficiently specific to hydrilla, approval to import this fly into the USA was received in 1987. The first shipment was sent in January 1988, and 5 more consignments have been sent. Flies from both NQ and SQ were included in these shipments. The closely related ephydrid species from India, *H. pakistanae*, had been tested and released in Florida, just before *H. balciunasi* was imported. It was hoped that the flies from Australia, being

Table 1. Number of Berlese collections of herbivores from different aquatic plant species at our four main sampling Regions. [1]

Taxon / Family / Species	QLD	NTR	SQL	NSW	TOTAL
PTERIDOPHYTA					
Azollaceae					
Azolla ?pinnata R. Br.	11		1	1	13
Salvineaceae					
Salvinia molesta D.S. Mitchell	3		1		4
Marsileaceae					
Marsilea ?drummondii A. Br.	133	1	1		35
Parkeriaceae					
Ceratopteris Brongn. sp.	1				1
ALGAE					
Characeae					
Chara L. sp.	10				10
Nitella Ag. sp.	3				3
ANGIOSPERMA					
Monocotyledons					
Aponogetonaceae					
Aponogeton L.f. sp.	1				1
Araceae					
Pistia stratiotes L..	6	1			7
Cyperaceae					
Eleocharis R. Br. sp.	4				4
Cyperus sp.	1				1
Scirpus L. sp.	1				1
Hydrocharitaceae					
Blyxa aubertii Rich.	2				2
B. octandra (Roxb.) Planch. ex Thw.	73				73
Elodea canadensis Rich.	2				2
Egeria densa Planch.			71	44	115
Hydrilla verticillata (L.f.) Royle	312	11	199	24	546
Ottelia alismoides (L.) Pers.	5				5
O. ovalifolia (R. Br.) L.C. Rich	5		10	3	18
Vallisneria gigantea Graebner	4				4
V. spiralis Graebner	3	3			6
Vallisneria ?spiralis (narrow-leaved)	83		69	10	162
Juncaginaceae					
Triglochin procera R. Br.	5				5
Lemnaceace					
Lemna L. sp.	2				2
Najadaceae					
Najas tenuifolia R. Br.	67	1	15	4	87

Table 1. Continued.

Taxon Family Species	QLD	NTR	SQL	NSW	TOTAL
Poaceae					
Leersia sp.	1				1
Pontederiaceae					
Eichhornia crassipes (Mart.) Solms.	9		1		10
Monochoria cyanea (F. Muell.) F. Muell	7				7
Potamogetonaceae					
Potamogeton crispus L.			16	1	17
P. javanicus Hassk.	13				13
P. ochreatus Raoul.			1	3	4
Potamogeton ?pectinatus L.			1		1
P. perfoliatus L.			13	2	15
P. tricarinatus F. Muell & A. Benn. ex A. Benn. (1982)	27		7		34
Typhaceae					
Typha L. sp.	1				1
Cabombaceae					
Cabomba caroliniana Gray	5		2	1	8
Ceratophyllaceae					
Ceratophyllum demersum L.	106	2	4	4	116
Convulvulaceae					
Ipomoea aquatica Forsk.	12				12
Haloragaceae					
Myriophyllum trachycarpum F. Muell.	20				20
M. verrucosum Lindl.	7	1	5		13
Lentibulariaceae					
Utricularia sp.	17				17
Menyanthaceae					
Nymphoides indica (L.) Kuntze	51	2	18	2	73
Villarsia reniformis R.Br.	1				1
Nelumbonaceae					
Nelumbo ?nucifera Gaertn.		1			1
Nymphaceae					
Nymphaea gigantea Hook.	24	2	2		28
N. mexicana Zucc.			3		3
Onagraceae					
Ludwigia hyssopifolia (G. Don) Exell	2				2
L. peploides (Kunth) Raven.	16		4		20
Philydraceae					
Philydrum lanuginosum Gaertn.	1				1
Polygonaceae					
Polygonum ?decipiens R.Br.	3				3
Total	**949**	**25**	**448**	**106**	**1,528**

from a more temperate environment, would be more cold-hardy than the Indian fly (Buckingham 1988). The experience in rearing and testing *H. pakistanae*, enabled the final evaluation of *H. balciunasi* to be completed rapidly. Laboratory testing showed that the fly from Australia was highly specific, and the first field releases were made in Florida in May, 1989 (Buckingham *et al.* 1991).

Hydrilla Stem Borer (Bagous hydrillae)

In preliminary surveys conducted in NT in 1982 and 1983, the senior author collected a small, undescribed species of *Bagous* (Coleoptera: Curculionidae). These weevils were collected again at NQ and SQ sites soon after the establishment of laboratories in these regions in 1985, and also in northern NSW in 1987. Initially these specimens were identified as *B. australasiae*, one of the 3 *Bagous* weevils known from Australia, all of which were described almost 100 yrs ago by Blackburn (1894*a* and 1894*b*). However, Dr. C.W. O'Brien (Florida Agricultural and Mechanical University, Tallahassee) compared our specimens with the type specimens of *B. australasiae* from the British Museum of Natural History, and determined that our species was new and undescribed. This weevil, as well as more than 20 additional *Bagous* from Australia, has been described as *B. hydrillae* (O'Brien and Askevold 1992), the hydrilla stem borer (HSB).

Our field and laboratory observations on the biology of HSB have been published (Balciunas and Purcell 1991), but can be summarized briefly as follows. Adults fly from hydrilla stranded on the shoreline to submersed hydrilla, topping out at the water surface. They then crawl beneath the surface feeding on both the leaves and stems. Females oviposit eggs singly (rarely 2) into small punctures which they make in the stem, usually near leaf nodes. Larvae tunnel within the stems and develop through 3 instars. The feeding by both adults and larvae causes fragmentation of the hydrilla. Larvae float in these fragments to the leeward shore where prepupae exit the stems, and pupate in the moist soil and stranded hydrilla. Development from newly-laid egg to adult takes 12-14 d. Large numbers of adults can be found in this stranded hydrilla along the shoreline. When adult weevils were required, stranded material was beaten on a white board to dislodge the weevils, which were then readily collected using an aspirator (pooter). The amount of stranded hydrilla is usually a reliable indicator of the size of the HSB population present at some sites. This unique life cycle restricts this weevil mostly to lakes, dams and ponds, where they are often found in high densities, especially in SQ (up to 636/kg). Although HSB was found in some rivers and creeks, densities usually were lower.

In the field collections of aquatic plants, this weevil was found on hydrilla at 21 sites, at an average density of 7.5/kg. Although HSB was collected on 10 other aquatic plant species, it never was collected at non-hydrilla sites. We interpret this to indicate that in the field, HSB probably requires the presence of hydrilla. Eighty-five percent of the weevils found on other aquatic plants were collected on *Vallisneria ?spiralis* Graebner (narrow-leaved), another Hydrocharitaceae, at an average density of 9.3/kg. High densities of these weevils were found on *V. ?spiralis* (narrow-leaved) mostly when water levels decreased, and mats of this plant became stranded and exposed to populations of HSB on the shoreline, especially when little, or no other hydrilla was available. In the USA, if HSB attacks the native *V. americana* Michx., only plants which become stranded during droughts should be damaged. These plants probably would be doomed anyway due to desiccation. The biggest threat to *V. americana* in Florida is hydrilla, which has displaced *V. americana* at several locations (Balciunas, personal observation). In Australia, HSB weevils were also collected in low densities on 2 other Hydrocharitaceae, *Egeria densa* (<0.1/kg) and *Ottelia alismoides* (0.4/kg), and 7 non-Hydrocharitaceae: *Ceratophyllum demersum* L. (Ceratophyllaceae) (0.6/kg), *Ludwigia peploides* (Kunth) Raven. (Onagraceae) (<0.1/kg), *Marsilea ?drummondii* A. Br. (Marsileaceae) (<0.1/kg), *Najas tenuifolia* R. Br. (Najadaceae) (3.5/kg), *Nymphoides indica* (L.) Kuntze (Menyanthaceae) (0.11/kg), *Potamogeton crispus* L. (Potamogetonaceae)

(0.52/kg) and *Potamogeton perfoliatus* L. (0.06/kg).

HSB feeding tests were conducted in Townsville in 1985 and 1986. The weevils fed on hydrilla and 16 other plant species from 10 families. Feeding was highest on hydrilla and *Blyxa octandra*, another Hydrocharitaceae. Interestingly, HSB was never collected in 73 field collections of *B. octandra*. Only moderate feeding scores were recorded for *V. ?spiralis* (narrow-leaved), even though HSB was collected in significant numbers from this plant in field surveys (Balciunas and Purcell 1991). Full results of these feeding tests will be published elsewhere.

In late 1985 and through 1986, we conducted laboratory oviposition and survival trials of HSB in NQ and SQ. Other than hydrilla, the weevil oviposited on 8 other Hydrocharitaceae plant species, as well as 3 other aquatic plants, from 3 families. Immatures were reared to the adult stage on all plants on which oviposition occurred. However, hydrilla was the only host on which both oviposition and survival were high, indicating that it is the true host of HSB. Detailed results of these tests will also be published elsewhere.

Although HSB may oviposit on other native plants in the U.S., it is unlikely to complete development on them. It is doubtful that this weevil could fragment aquatic plants which have stems significantly thicker than that of hydrilla. We often found extensive windrows of hydrilla along the shoreline with high densities of HSB, but only traces of other aquatic plant species. Fragmentation of the host plant appears essential to allow this weevil to complete its life cycle. Its ability to use a limited number of other aquatic plants as a temporary food source during unfavorable periods (e.g. no hydrilla present) might be beneficial in sustaining field populations.

HSB was imported into quarantine facilities in Florida in 1987, and after further host-testing, was considered to be sufficiently host-specific to be released in Florida in 1991 (Center, T.D., personal communication, 1992). Because this weevil damages hydrilla, and because it has a short life cycle, we believe this will be the most damaging insect yet released on hydrilla.

Aquatic Moths

We collected four stream-dwelling pyralid moths; *Aulacodes siennata* Warren, *Nymphula eromenalis* Snellen, *Nymphula dicentra* Meyrick and *Strepsinoma repititalis* (Walker); feeding on hydrilla in NQ. *Nymphula dicentra* is soon to be synonymized as *Parapoynx diminutalis,* which is a herbivore of hydrilla in South-East Asia, and is already present in Florida (Del Fosse *et al.* 1976). This moth was therefore not evaluated further.

We attempted to colonize and conduct preliminary host-testing on the remaining three moth species. Establishing colonies was difficult, as we were only able to rear 10% of field-collected larvae to the adult stage. As adults only lived for about 1 wk, mating pairs were hard to obtain, and egg production was low. Hydrilla, *Blyxa octandra* (Roxb.) Planch. ex Thw. (Hydrocharitaceae) and *V. ?spiralis* (narrow-leaved) were always the preferred field hosts of these 3 moth species. Larval choice-feeding tests were conducted using these 3 plant hosts, for each moth species.

Seventy-two percent of *A. siennata* larvae were collected from hydrilla, and this host was preferred in larval choice-feeding tests. They were collected in lower numbers from 2 other Hydrocharitaceae and a non-Hydrocharitaceae species. *A. siennata* larvae were found mainly in flowing rivers and creeks in the Cairns-Daintree region. There are numerous museum specimens of this moth from further north, especially around Cooktown, but a lack of a 4-wheel drive vehicle prevented us from collecting in this vicinity.

Eighty-five percent of *N. eromenalis* larvae were collected from hydrilla, and this host was preferred in larval choice-feeding tests. Larvae were found on 2 other Hydrocharitaceae, and a single larva was found on a non-Hydrocharitaceae species. Hydrilla was preferred in the larval no-choice feeding tests. Unlike *A. siennata* and *S. repititalis,* which feed from a silken web between strands of hydrilla, *N. eromenalis* larvae form a portable case from the leaves of hosts plants, from which they feed, and within which they pupate. Low egg production in the laboratory usually caused our colonies to collapse after several generations.

S. repititalis larvae were collected mostly from *B. octandra* (49%) and narrow-leaved *Vallisneria ?spiralis* (36%) in the field. Larvae were also collected from hydrilla, while a single larva was found on each of 3 other plant species from 3 families. Interestingly, in choice-feeding tests, hydrilla was preferred over its 2 main field hosts. As maintenance of moth colonies was time consuming, and because *S. repititalis* was more commonly found on 2 other Hydrocharitaceae, that colony was terminated.

We received permission to export these 3 stream-dwelling moths to quarantine facilities in the USA in 1989. However, hydrilla was scarce at the time in the Daintree-Cairns region, and no larvae could be found. Due to the difficulty in rearing and sustaining colonies of aquatic moths, and their questionable host range, no further attempts have been made to introduce them into quarantine facilities in the USA. The use of herbicides in flowing water is highly restricted in the United States, and if the currently introduced biological control agents for hydrilla prove to be ineffective in flowing water bodies, these moths may be reconsidered. If sufficiently host-specific, their preference for flowing stream sites could make them useful biological control agents in the spring fed rivers of Florida and Texas.

Other Insects

Several other insect herbivores were collected which fed on hydrilla. However, these were not as damaging as our top priority candidates. Due to our limited resources, further investigation of these insects was not warranted. These insects are discussed briefly below.

We collected specimens of the beetle *Donacia* spp. (Coleoptera: Chrysomelidae) associated with hydrilla roots (and other aquatic plants). They were first found in Ingham, but have since been widely collected from several sites. These specimens have been identified as *D. australasiae* Blackburn. In the laboratory, larvae attach to hydrilla stems and remain there for many months, apparently not feeding.

Caddis-fly (Trichoptera) larvae were collected from many sites, in varying case types, most of which were made of plant tissue. Most of the cases were constructed of whole leaves or leaf parts, however, larvae sometimes drilled holes in wooden twigs or parts of plant stems. Feeding occurred on hydrilla, but most larvae failed to feed after collection. Only 20% of larvae placed into rearing cups reached the adult stage, and several species appear to be involved.

Chironomidae (Diptera) larvae frequently were found in hydrilla collections, some of which damaged hydrilla by boring into stems. They were very difficult to rear in the laboratory. Rearing them in cups containing a portion of host plant and some water, was not successful. However some larvae were reared to the adult stage by placing them into aerated water with host plant material.

Summary

After conducting extensive field surveys of hydrilla and other aquatic weeds in Australia, and after performing extensive life history studies and host-testing, there are 5 insects which show considerable potential for use as biological control agents for hydrilla. Two of these insects, the hydrilla stem boring weevil, *Bagous hydrillae*, and the leaf-mining fly, *H. balciunasi,* have been quarantine tested and released in Florida. The remaining candidates, 3 stream dwelling moths; *A. siennata, N. eromenalis* and *S. repititalis*; are difficult to colonize, and appear to have a broad host range. If the biological control agents already introduced against hydrilla prove to be ineffective in flowing water sites, these moths may be considered further. Other insect herbivores did not warrant further investigation.

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