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INVESTIGATION OF TWO INSECT SPECIES FOR CONTROL OF EURASIAN WAT--ETC(U)
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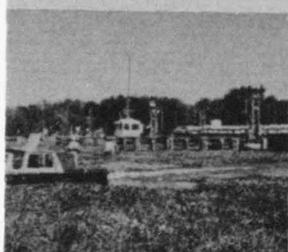

MISCELLANEOUS PAPER A-79-4

INVESTIGATION OF TWO INSECT SPECIES FOR CONTROL OF EURASIAN WATERMILFOIL

By Gary R. Buckingham, Chris A. Bennett,
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Gainesville, Fla. 32601

September 1979

Annual Report for FY 1978

Approved For Public Release; Distribution Unlimited

Prepared for Office, Chief of Engineers, U. S. Army
Washington, D. C. 20314

Monitored by Environmental Laboratory
U. S. Army Engineer Waterways Experiment Station
P. O. Box 631, Vicksburg, Miss. 39180

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Miscellaneous Paper A-79-4	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER (9)
4. TITLE (and Subtitle) (6) INVESTIGATION OF TWO INSECT SPECIES FOR CONTROL OF EURASIAN WATERMILFOIL.		5. TYPE OF REPORT & PERIOD COVERED Annual report for FY 1978 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) (10) Gary R. Buckingham, Chris A. Bennett, Bonnie M. Ross		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U. S. Department of Agriculture Biocontrol Laboratory Gainesville, Fla. 32601		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Office, Chief of Engineers, U. S. Army Washington, D. C. 20314		12. REPORT DATE (11) September 1979 13. NUMBER OF PAGES 20
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) U. S. Army Engineer Waterways Experiment Station Environmental Laboratory P. O. Box 631, Vicksburg, Miss. 39180		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited. (12) 24

(14) WES-MP-A-79-4

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Biological control
Insects
Water milfoil

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report describes the initial work involved with the evaluation of two species of insects for biological control of *Myriophyllum spicatum* L. (Eurasian watermilfoil). It is believed that both insect species were accidentally introduced from Europe. They are *Litodactylus leucogaster* (Marsh.) and *Acentropus niveus* (Olivier). *L. leucogaster* appears to be relatively host-specific. Researchers have been unable to establish a viable colony of *A. niveus*. All results are very preliminary at this writing.

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Preface

This report presents results of a biological control program being conducted for the Aquatic Plant Control Research Program (APCRP) by the United States Department of Agriculture (USDA), Science and Education Administration, Biocontrol Laboratory, Gainesville, Fla. The purpose of this program is to evaluate insects to determine their potential for use in aquatic plant control. This particular project in the overall program involved the evaluation of two insect species for use against Eurasian watermilfoil. Funds for this effort are provided by the Office, Chief of Engineers, under appropriation number 96X3122, Construction General, through the APCRP at the U. S. Army Engineer Waterways Experiment Station (WES).

The principal investigator for the work was Dr. Gary R. Buckingham, USDA, who prepared this report. He was assisted in the conduct of the work and preparation of the report by Mmes. Chris A. Bennett and Bonnie M. Ross.

The work was monitored at WES by Messrs. W. N. Rushing and R. F. Theriot of the Aquatic Plant Research Branch (APRB), under the general supervision of Mr. W. G. Shockley, Chief of the Mobility and Environmental Systems Laboratory, and Mr. B. O. Benn, Chief of the Environmental Systems Division, and under the direct supervision of Mr. J. L. Decell, Chief of the APRB. As a result of reorganization at WES, Mr. Decell is now manager of the APCRP, which is a part of the Environmental Laboratory of which Dr. John Harrison is Chief.

The Commander and Director of WES during this period was COL John L. Cannon, CE. Technical Director was Mr. F. R. Brown.

Contents

INVESTIGATION OF TWO INSECT SPECIES FOR CONTROL OF EURASIAN WATERMILFOIL

Introduction

1. Control of aquatic weeds is one of the most serious problems facing those who must manage our waterways. Rising herbicide costs and increasing environmental concerns are decreasing the reliance on the traditional chemical approach to control and are stimulating increased interest in alternate control methods. One of these methods, biological control, has apparently been successful in the control of alligatorweed, *Alternanthera philoxeroides* (Mart.) Griseb., an aquatic plant introduced from South America.

2. The present study is part of a program to assess the possibility of using biological control against two introduced plants, Eurasian watermilfoil, *Myriophyllum spicatum* L., and hydrilla, *Hydrilla verticillata* Royle. Eurasian watermilfoil occurs along the Eastern seaboard and in scattered areas across the United States. It is especially severe in the Tennessee Valley, and now there is a threat of it moving into the Columbia Valley of Washington via the Okanagan River from British Columbia. The plant is native to Eurasia but the exact area of origin is unknown. Hydrilla is a relatively newly introduced plant which infests most of Florida and has been found in isolated areas in a few other states. It probably has the potential, however, to infest much of the United States. The area of origin is unknown, and there is even disagreement about where it is native. Africa, Asia, and Australia have all been proposed as areas of origin. This confusion over the origins of these two plants means that wide areas must be surveyed for biological agents. Before any agents found in these areas are given final approval for introduction into the United States, they will need to be tested in quarantine. Quarantine testing of the insect candidates will be made by the authors.

3. Hopefully these future foreign surveys will yield potential

biological control agents. However, until that time, insects already present in the United States, both natives and accidentally introduced exotics, will be evaluated for their potential. Those which are sufficiently specific might be useful in an inundatory release program or in a program involving merely distributing them more widely.

4. The two insects which we are presently evaluating may have been accidentally introduced from Europe, although some taxonomists have considered them natives with holarctic distributions. Both of them attack Eurasian watermilfoil. *Litodactylus leucogaster* (Marsh.) (Coleoptera: Curculionidae: Ceutorhynchinae) is a small weevil whose biology has not been well studied. The American population was originally described as *Phytobius griseomicans* Schwarz but has now been synonymized with the European *L. Leucogaster* (Dieckmann 1972). Lekic and Mihajlovic (1970) gave a one paragraph description of the biology in Yugoslavia. This species is of interest because both larvae and adults feed upon the developing flowers thereby destroying the seeds. Although the importance of seed production in the population dynamics and spread of the weed has not been determined, it is believed that seeds do play a role. The reservoir of seeds in the bottom sediments might be of great importance in determining the rate of reinfestation after a control program.

5. *Acentropus niveus* (Olivier) (Lepidoptera: Pyralidae: Schoenobiinae) is a small aquatic moth which is well known in Europe. Many studies have been made of its biology, but the most complete is by Berg (1942). Berg's paper is extremely well done and also includes much morphological information. Batra (1977) studied a New York population, from which our laboratory stock originated, both in the field and in the laboratory. Her studies indicated that *A. niveus* might also have potential for use against hydrilla. The larvae feed on the stems and leaves of their submersed host plants.

Studies With *Litodactylus leucogaster*

Field collection

6. Adults were purchased from Mr. Robert W. Pemberton who collected

them at Lake Pilarcitos, San Mateo County, California, which is south of San Francisco. This location had been suggested by Dr. Charles Obrien, Florida State University, who is one of the foremost weevil taxonomists. Lake Pilarcitos is a small reservoir which is closed to the public. The overwintering weevils were collected from the milfoil debris which collects along the shore of the lake. The first collection was made on 17 April 1978 and the last on 6 June 1978. According to Mr. Pemberton, the early collections were made from debris above the high-water line, but by June the then active beetles had moved down to the fresh plant material at the water edge. The following four shipments were received in Gainesville:

a. FBCL-78-5, approximately 175 adults, 10 dead on arrival.

b. FBCL-78-6, approximately 167 adults, 21 dead.

c. FBCL-78-7, approximately 2400 adults, 10 dead.

d. FBCL-78-8, approximately 608 adults, 13 dead.

A small number of weakened adults died within a few days of each shipment. A sample of the dead beetles plus some freshly killed beetles were examined for pathogens by Mr. Bud Thomas, University of California Diagnostic Laboratory, but none were present. The weevils had been shipped via air freight in mailing tubes containing wood excelsior, which had been soaked in water and then allowed to dry until damp. No plant material was included. This method was apparently highly satisfactory, at least for the 1 or 2 days of shipping time. The total cost of the weevils, including shipping costs, was $584.

Methods and materials
of laboratory studies

7. Initially, a 50-dram plastic shell vial with a snap-on plastic lid was approximately three-fourths filled with tap water, and the host or test plant was floated in it. Since there was high adult mortality and because the beetles were usually above the water surface, indicating that they were not as aquatic as originally believed, an attempt was made to decrease the humidity by making an approximately 38-mm nylon organdy covered hole in the lid. A 38-mm-diam Styrofoam disc was floated on the water surface for support of the plant and to provide a surface

for the beetles to crawl upon. The 17-mm-thick Styrofoam disc was notched on one side, and the test plant was wedged into the notch so there was both a submersed and an emersed portion of the plant. This cage worked well for adults but appeared to be too humid for larval development. In order to decrease the humidity, a hole was made in the bottom of a vial, and the vial was taped to a second vial containing tap water. A milfoil flower spike was then wrapped with cotton just above the leaves and wedged into the hole so that the leaves were submersed in the bottom vial and the spike emersed in the upper vial. Eggs and medium-sized larvae were obtained in this way, but no larvae matured. Mature larvae and pupae were only obtained in nylon organdy covered gallon jars, aquaria, a large greenhouse cage, and open, noncovered vials. Apparently larvae can not tolerate the high humidity in the smaller, closed containers. The vials were kept in a windowless rearing room at about 24°C and 76 percent room humidity. Illumination was by fluorescent Grolux lights on a 12-hour light cycle.

8. The large greenhouse cage consists of a basal box, 2.4 m square and 0.27 m deep, sitting on 0.9-m legs. This box was waterproofed inside with multiple coats of liquid fiberglass. Attached to the box was a 1-m-high wooden frame which was covered on the sides with nylon organdy and on the top with a translucent fiberglass panel. Sleeve openings in the center of each side allowed access to the cage. The cage was kept filled with field-collected flowering tips of milfoil.

9. Eggs and pupae were held in 1-oz (29.5-ml) plastic cups with cardboard lids. Tightly pressed cotton, which was moistened until damp but not saturated, filled the bottom third to half of the cup. This technique has been described by Rizza (1977). Measurements of the various stages were made at various magnifications with a Wild dissecting microscope equipped with 10X oculars. A 2X magnifier was also used when the eggs and neonate larvae were measured.

10. Estimation of feeding damage in the host specificity tests was done both by creating subjective feeding categories and by visually estimating square millimetres eaten. Field-collected adults were used in Test 1 and in the miscellaneous tests, but laboratory-reared, newly

emerged adults which had not yet fed were used in Test 2. In Test 1 there were three replications of two females and two males per vial for each plant species, and in Test 2 there were five replications of three females and three males per vial for each plant species. There were usually two females and two males per vial with no replications for the miscellaneous tests. These tests were dependent upon beetle and flower availability. Plants in Test 2 were flowering, but those in Test 1 were not.

Results of biology studies

11. Adults collected in April did not mate until they had been in the laboratory several weeks. Mating was immediate, however, with those collected in late May-early June. Mating pairs were observed sitting on the flower stalks or on the sides of the containers above the water surface.

12. When the adults were threatened by a small object, such as a brush, they often moved around to the other side of the flower stalk so they could not be seen. If the object was larger, such as a hand, they usually feigned death and dropped from the plant. In nature, since the plants are usually quite dense at the water surface by flowering time, the beetles would probably find it easy to crawl away even if they dropped from the plant. If necessary, they can also swim well on the surface. Sometimes when threatened they would fly, and possibly this is the usual behavior in a natural situation, since it is for closely related species.

13. The adults, which apparently have plastron respiration, were able to walk down the stem into the water and to remain there for at least 45 minutes. Their bodies were completely covered by air so they appeared silvery. They fed commonly on the stem at the water line or just beneath the surface, but the majority of the feeding was on the flowers or on the stem among the flowers. (Heavy feeding can completely destroy the flowers and can cut the flower stalk so that it will fall over.) Seeds were also eaten. Adult feeding in a test conducted in the greenhouse occurred about equally during the day and the night. Beetles were active during the daytime in the large greenhouse cage and were

often seen walking and flying on the screen sides, especially in the corner nearest the sun.

14. Eggs were placed mostly in the flowers. The female generally ate the ovary, or a portion of it, and replaced it with an egg. Some eggs were also placed among the anthers, on the stems among the flowers, or in holes eaten into the stems, but this behavior may have been due to the artificial conditions of the laboratory. When the young larva hatched it fed on the flowers and was small enough to enter the buds or ovaries. Later, when the larva grew larger, the body encircled the stem and the head could be seen in the flower. The maturing larva moved down the flower stalk eating the ovaries, seeds, and stem and occasionally severing the stem. If one flower stalk was not sufficient for maturation, the larva crawled along the submersed stems to the next flower stalk. When necessary the larva can swim and they have been observed feeding upon submersed flower stalks, although this is not the usual case. Pupation occurs on the submersed stem. A cavity is eaten into the stem and a hemispherical brown cocoon is formed over the cavity. The pupa lies in the cavity surrounded by air which evidently flows from the broken vessels of the stem into the cocoon. If the stem becomes waterlogged so does the cocoon and the pupa dies. Pupae removed from the stem to the moist egg hatching cups develop normally.

Descriptions and development times

15. The following is a description of the general physical characteristics and developmental stages of the *L. leucogaster*:

a. Egg. Ovate, 0.51 mm (r = 0.44 to 0.56 mm, n = 18) by 0.40 mm (0.31 to 0.47 mm), pale yellow covered by a thin net-like exochorion having hexagonal cells, which gives the egg a sculptured appearance. This exochorion breaks apart as the egg develops. Duration was 3 to 4 days at 24°C constant temperature and light and 4 to 5 days at 27°C day and 13°C night with a 16-hour day.

b. Larva. The neonate larva is cream-colored with a light brown head capsule and reddish mandibles. The body is slightly dorsoventrally flattened, and the central portions of the body segments project laterally giving the sides a scalloped appearance when viewed from above. The dorsum is covered with fine setae. Spiracles are present in abdominal segments 1 to 8. The larvae lack both legs and prolegs, but there are two fleshy projections on the last abdominal segment. The neonate larva is about 1 mm long, and the head capsule is about 0.22 mm wide. Older larvae are reddish-yellow with dark brown head capsules and thoracic shields. The body is completely covered with fine setae. The lateral scalloping is even more pronounced. The mature larva is about 4.4 mm long, and the head capsule is about 0.49 mm wide. The duration of the larval stage is 8 to 10 days at 24°C constant temperature and light and 9 to 11 days at 27°C day 13°C night with a 16-hour day.

c. Pupa. Whitish with dark brown eyes and reddish mandibles. Darkens as it develops. Duration is approximately 5 to 8 days.

d. Adult. Black, but covered with gray scales imparting a velvety grayish-black appearance (Figure 1). Small

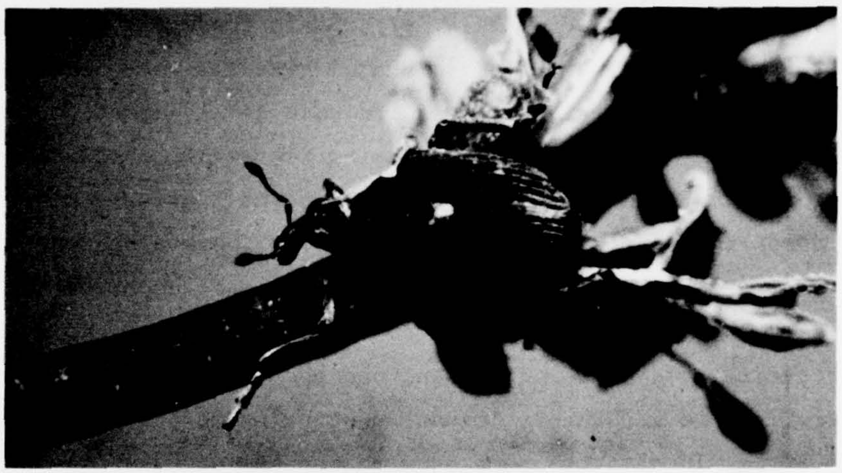

Figure 1. Adult *Litodactylus leucogaster* on a Eurasian watermilfoil flower stalk

yellowish scales cover the snout, the legs, and the thoracic pleurae and are in patches around the eyes. Larger yellowish scales cover the sternum and the venter. The antennae and the legs are reddish-brown. The tarsi and the knees are black. The pronotum has a pair of distinct lateral tubercles. An elongated patch of whitish scales is found along the midline of the elytra. The fifth strial interval is distinct and raised at the base. The first and second ventral abdominal segments of the female are convex, whereas those of the male are slightly flattened and concave along the midline. The pygidium of the female is shorter than the preceding segment and is apically pointed, whereas that of the male is about equal in length to the preceding segment and is apically rounded. The adults are about 2.71 mm in length (r = 2.48 to 3.00 mm, n = 20) and 1.45 mm in maximum width (1.36 to 1.56 mm).

Host specificity studies

16. Test 1. *M. spicatum, M. brasiliense* Camb., *Proserpinaca palustris* L., *P. pectinata* Lam., *Ludwigia palustris* (L) Ell., *Potamogeton* sp. (narrow leaved), *Ceratophyllum demersum* L., *Polygonum punctatum* Ell., and *Nasturtium officinale* R.Br. were tested. No feeding occurred on *P. punctatum* and *L. palustris*. *C. demersum* was dropped after the second change because it deteriorated quickly when exposed to the air and the beetles immediately started dying. There was no feeding on the submersed portion, and because of the deterioration feeding could not be detected on the emersed portion. On *N. officinale* there was only light feeding on the flowers (13 mm^2 total), and all weevils were dead in 2 weeks. One egg was found in a flower. The same small amount of feeding (10 mm^2) was found on *Potamogeton* sp. Feeding was light to moderate on the two *Proserpinaca* with the feeding mostly on the leaves. Some feeding also occurred on the flowers and seeds of *P. palustris* and on the stem of *P. pectinata* at the water line. The first species had a total 404 mm^2 of feeding, two adults alive, and no eggs; the latter species had 289 mm^2 of feeding, one adult alive, and one egg. Feeding on *M. brasiliense* was also light to moderate. It was principally on the leaves, especially the apical leaves, but some occurred on the stems at the water line. The total feeding was 488 mm^2, 9 adults alive, and there were many eggs. Feeding on the control *M. spicatum* was very heavy. A total of 1376 mm^2 was recorded with many eggs.

17. Test 2. The plant species tested were *M. spicatum, Polygonum pennsylvanicum* L., *P. punctatum, Potamogeton illinoensis* Morong., and *P. nodosus* Poir. This test was still continuing at the writing of this report, but at the end of 1 month, 26 of 30 weevils were still alive on *M. spicatum*, 15 were alive on *P. pennsylvanicum*, and none were alive on the other three plant species. Eggs had been produced on *M. spicatum* but none on the other plant species. Feeding on *M. spicatum* had been moderate to heavy. Most of the feeding had been on the flowers and flower

stalk but much had also been on the stem, at or just below the water line. Some leaf fronds had also been clipped off. Feeding had been moderate to heavy on the flower buds and petals of *P. pennsylvanicum* but only sporadic on the leaves. Occasionally leaves below the water line had been eaten moderately to heavily. The seeds had not been eaten. On *P. punctatum* there had generally been no feeding, with only occasional slight stem or bud feeding. Almost no feeding had occurred on the flowers of either species of *Potamogeton* although in rare instances the flower stalk had been eaten, thereby damaging the bud. Feeding on the stems and leaves had been sporadic, with an occasional leaf being moderately to heavily eaten.

18. Miscellaneous adult tests. Flowers of the following aquatic or shoreline plants were tested as they became available: *A. philoxeroides*--no feeding, *Diodia virginiana* (Rubiaceae)--no feeding, *Erigeron* sp. (Compositae)--some feeding on blossom and stem, *Galactia* sp. (Orchidaceae)--feeding on blossom, *Hypericum mutilum* L.--no feeding, *Polygonum hydropiperoides* Michx.--feeding on buds, blossoms, leaves, and stems, *Pontederia cordata* L.--no feeding, *Rhexia* sp.--some feeding on blossom and stem, *Sagittaria* sp.--no feeding on blossoms but possibly some on bracts, *Tradescantia rosa* (Commelinaceae)--no feeding.

19. Larval tests. Attempts were made to rear larvae on the aerial leaf stalks of *M. brasiliense*, but none survived. Eggs were transferred from *M. spicatum* to flowers of *P. pennsylvanicum* and *P. illinoensis*, but again no larvae developed even though the eggs hatched.

Studies with *Acentropus niveus*

Field collection

20. One shipment of larvae which had been collected at Robert Moses State Park, Massena, N. Y., in September 1977, was hand-carried to the laboratory in March by Dr. Suzanne Batra. Only eight living larvae were obtained from this shipment, and from these only two males were reared.

21. On 19 July, Dr. Ted Center, Dr. Batra, and Dr. Buckingham met

at Robert Moses State Park in order to collect more larvae. The water temperature was about 17°C, and most of the milfoil plants had not yet started growing at the Barnhart Boat Marina and the bathing beach collecting sites. Most plants were rooted and standing upright, were approximately 13 to 50 cm tall, and were found in small clusters of plants about 30 to 100 cm apart. Areas between the clusters would have no plants. There were, however, many single plants and also areas with more dense plants, especially among large rocks. Most plants were covered with filamentous algae and other debris. Some plants were lying on the bottom covered with algae, so that they appeared to be only an algal mass. Possibly, these were broken strands which had only recently rooted. A few plants along the shore opposite the marina had started growing so that the tips were bright green, but this was not the norm. Plants were collected from depths of approximately 1 to 3 m but mostly at 1.5 to 2 m. This distribution may have been due mostly to the collecting technique; however, the plants did seem to be more abundant at those depths. The collections were made by snorkling with full wet suits. The plants were placed into plastic bags and when filled handed to Dr. Batra in the trailing boat. At depths less than about 1 m, so much silt was disturbed that visibility was poor; at depths greater than 3 m, there was little time to collect once the bottom was reached. Almost every plant had larval cases, and generally there were multiple cases. In some areas *Ceratophyllum* was abundant, and larval cases were also seen on it. A small amount of *Elodea* was also collected, and larval cases were found on it in later examination. This probably indicates a natural infestation of *Elodea*, but since it had been in the container with milfoil, the larvae might have moved onto it. A total of 4 hours of collecting produced sufficient plant material that it took 2 days of sorting to pull off the larval cases. Both large and small larvae were found; however, the majority appeared to be large. The stem fragments with the larval cases were transported in plastic bags and hand-carried on the plane. The water was aerated by portable air pumps a few hours before the flight.

22. Another location in Robert Moses State Park was found to have

a population of *Acentropus* which was much more advanced into the season. This was a small inlet behind cabins 14 and 15. This inlet was about 50 m long and 6 m wide. The maximum water depth fluctuated from about 0.3 to 1 m because of nearby locks on the river. These locks also caused a current in the inlet which changed direction about every minute. Small clumps of milfoil were scattered along this inlet. From 2200 to 2400 hours on both 21 and 22 June, males were observed just above the surface of the water. On 22 June the water temperature was 19.4°C and the air temperature was 14°C. The males were rather fast fliers and flew in wide circles, occasionally touching the surface of the water. They do not fly erratically like many nymphulines. They were also observed sitting on emergent vegetation and algal mats. A few times they appeared to exit from the water while we walked around, but when the plants were disturbed with a net handle and observed closely none exited. No females were found during the 2 nights even though the plants were illuminated from below by a submersible light which would have made the wingless females more visible. Males were observed landing momentarily on another male floating on his back with his wings stuck to the surface. Although this may have been merely a visual response by the other males, it could indicate that the male had just recently mated and was coated with female pheromone.

23. The males fly so close to the water surface that they are best collected by submerging a net in the water and raising it a few inches as they fly over. They do not fly out of the open net but rather keep flying along the edge of the net. If a vial or a cup is dipped into the water directly behind them as they are flying, they appear to be sucked into the container along with the water. In this manner they can be handled very easily.

Methods and materials
of laboratory studies

24. Larvae were kept in aquaria of various sizes and in gallon jars. Aeration was by air pumps connected to air stones or sponge water filters. These containers were kept in the rearing room under the same conditions as *L. leucogaster*. A small number of larvae were released

into the large greenhouse cage described in the *Litodactylus* section in paragraph 8. Larval feeding tests were conducted by placing one larva and about a 10-cm section of test plant stem in a 150- by 20-mm culture tube. The tubes were held in a Kim Pak and were covered by a piece of nylon organdy held in place by a Kim Kap. Because of the varying leaf shapes of the test plants, feeding was evaluated by a subjective description.

Results of biology studies

25. As mentioned earlier, detailed biological studies have already been mady by other researchers. For this reason we did not study the biology but merely tried during the course of our study to confirm the observations of the previous studies. The following brief synopsis of the biology has been excerpted from the papers of Berg (1942) and Batra (1977).

26. The adults emerge from late May through September depending upon the habitat. There are two forms of females: a normally winged form and a short-winged, flightless form (Figure 2). Only the flightless form was found by Batra in New York, although normally winged females have been collected by others in Canada. Males (Figure 3) appear to be

Figure 2. Short-winged, flightless *Acentropus niveus* female clinging to a leaf of Eurasian watermilfoil at the water surface in an aquarium

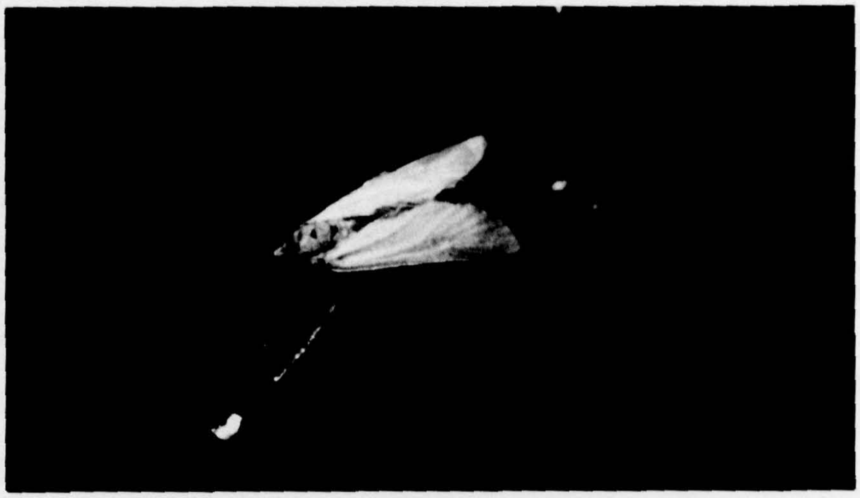

Figure 3. *Acentropus niveus* male sitting on the water surface in an aquarium

always normally winged. Mating occurs immediately, and the females live only 1 night. The eggs are deposited on submerged vegetation. After about 2 weeks the eggs hatch, and the first instar larvae bore into the stems of the host plants. Older larvae build shelters of plant parts and feed externally on the leaves and stems. The shelter usually is stationary and is not carried around with the larva as happens with some other aquatic species. Total feeding time is about 6 weeks, but it is disrupted by a hibernal diapause period. A tightly spun cocoon is made in a depression eaten into the stem. The pupa, which lasts about 3 weeks, obtains oxygen from the stem. Larvae have reportedly been collected from or reared upon a total of seven plant species.

27. One curious behavior of the larvae which has not been reported previously is their willingness to crawl out of the water. Large numbers of mature larvae crawled out of the gallon jars in quarantine and formed loose silken shelters in the angles of the holding cages or in paper toweling on the bottoms of the cages. What stimulus caused this behavior is unknown. None of these larvae pupated, but they did live for several days. Most of the other species in the subfamily Schoenobiinae are not aquatic. The length of time that they can survive in a moist but nonaquatic situation will be investigated.

Host specificity studies

28. Most of the larvae were held on milfoil in an attempt to obtain adults and thereby neonate larvae. A few larvae, however, were used for testing, and the following plant species were tested individually in vials (the number of vials is in parenthesis): *A. philoxeroides* (3), *Cabomba caroliniana* Gray (3), *Eleocharis acicularis* (L) R and S (4), *H. verticillata* (5), *Hydrocotyle umbellata* L. (3), *Limnobium spongia* (Bosc.) Steud. (2), *L. palustris* (3), *M. brasiliense* (2), *M. spicatum* L. (5), *Najas guadalupensis* (Spreng.) *Magnus* (6), *N. officinale* (2), *P. illinoensis* (2), *P. palustris* (2), *P. pectinata* (2), *Salvinia rotundifolia* Willd. (3), *Typha* sp. (1), and *Sagittaria* sp. (narrow leaved) (4). There was no feeding on *A. philoxeroides, E. acicularis, H. umbellata, L. spongia, Sagittaria* sp., or *Typha* sp. There was very little feeding on *L. palustris, C. caroliniana,* or *N. officinale*. The leaves of *S. rotundifolia* were eaten very little, but the roots were always cut off. There was moderate to heavy feeding on the remaining species, with *N. guadalupensis* and *P. illinoensis* being especially heavily eaten. In other tests large larvae fed upon *Ceratophyllum demersum, H. verticillata, N. guadalupensis,* and *P. illinoensis* even in the presence of *M. spicatum*. Since no fertile eggs were obtained, tests could not be conducted with neonate larvae.

Discussion and Recommendations

29. Of what significance are the results obtained thus far? Does either candidate appear to have promise for biological control? What else must be done? These are obvious questions which should be asked at this time. The answers, however, are difficult and certainly not clear.

L. leucogaster

30. The biology studies with *L. leucogaster* have indicated that it is a relatively specialized weevil. The eggs are placed in the ovaries of this special type of flower and the larvae feed on the watermilfoil flowers by encircling the flower stalk. Both of these stages occur in air, not in the water; however, pupation takes place in the water.

Totally submersed plants are thus eliminated as hosts because of the adult and larval habits, and land plants might be eliminated by the pupal habit. Larvae would probably have difficulty crawling on large-diameter flower stalks or on flattened surfaces, which would add to a decreased survival rate. The weevils have been easily reared on milfoil in a large cage, which is a plus if an inundatory type program is ever considered. Apparently, the availability of milfoil flowers would be the greatest difficulty in a rearing program.

31. Thus far the larvae appear to be specific. Adult feeding is not as specific, but it has been relatively predictable, which is good. Feeding occurred on plants closely related to Eurasian watermilfoil (i.e., *M. brasiliense, Prosorpinaca*) and on plants which are hosts or closely related to hosts of related weevils (i.e., *Polygonum* and *Rhexia*). Some unpredictable feeding occurred on two land plants, *Erigeron* and *Galactia,* but this may indicate that the weevils feed slightly on land plants either just before or just after hibernation. This type of minor feeding is apparently common in weevils which diapause as adults and thus live relatively long periods without the host plant being available. Of interest is the lack of feeding on *Ludwigia*, which is in a family closely related to milfoil, and the small amount of feeding on *Potamogeton*, which is a host of closely related weevils.

32. Based upon these preliminary results, it is believed that *L. leucogaster* will be found sufficiently specific for use in a biological control program, especially since it already occurs in the United States. If this is true, then the amount of potential impact upon the plant population will be the deciding factor in whether it is used. Field studies of U. S. populations should indicate what might happen if the beetle is distributed more widely in an innoculative program. If the beetle is indeed native, this type of program would probably be unsuccessful due to natural enemies, which should be found in the field studies. If field studies or possibly outdoor pool studies indicate that large beetle populations can cause extensive damage to the flowers, distribution of the beetles would be warranted in order to provide an eventual complex of natural enemies. An inundatory program or rearing

and mass release would only be of interest in a situation where seed production was of great importance or where there was the possibility of mechanical transmission or aiding the penetration of a pathogen.

33. Further clarification of the host specificity is needed, and this would include adult feeding tests, oviposition and oogenesis tests, and larval survival tests. Although the laboratory biology is understood quite well, a few more studies are needed including larval morphology and diapause studies. The U. S. distribution will need to be determined as precisely as possible from specimen and literature records. Either field observations or pool experiments will need to be made in order to determine potential plant damage.

A. niveus

34. Since we have not yet been able to establish a colony of *A. niveus*, our results are not highly significant. The feeding by the older larvae was more specific than was expected; however, they did feed upon a relatively large number of diverse plant species. The failure to rear this species indicates that host specificity studies will be difficult and possibly prolonged.

35. It is difficult to assess the promise of *A. niveus* for use in biological control. Indeed if it were an exotic, it would never have passed the preliminary trials or possibly not even the literature search, due mostly to the feeding on *Potamogeton*. However, it already occurs in the United States and will presumably expand its range to all the suitable milfoil areas. This expansion will be slow because of its low adult mobility, but it will probably occur eventually. The expansion should be aided by the ability of the larvae to survive for long periods in air, especially since the plant is easily caught on boat propellers and trailers. Thus, the crucial questions are: How much damage to *Potamogeton* can be expected from a large population of *A. niveus* in a biological control program, and how important will this damage be? The amount of expected damage to milfoil is also an unknown. The field populations in the northern United States have not been reported to be controlling the plant, but perhaps in warmer waters and in areas of dense milfoil with few competing host plants the results would be better.

Even if this species is not able to control the plant alone, however, it could be valuable as a member of a complex. Batra (1977) has suggested that it may have potential for use against hydrilla, which may even be due to its lack of narrow host specificity.

36. The establishment of a population is essential to continued studies. It must be determined if a complete generation can be made on *Potamogeton* and some of the additional hosts. The ovipositional and larval preferences should also be determined, although the low mobility of the females decreases the importance of ovipositional preference.

Future plans

37. Based upon the results of this year and upon what still remains to be accomplished, the following program is planned for FY 1979:

a. *L. leucogaster*:

(1) Complete the studies of the biology and larval morphology.

(2) Determine the U. S. distribution as precisely as possible.

(3) Complete the adult feeding, oogenesis, oviposition, and larval survival tests.

(4) Evaluate the results and either drop from the program, observe natural field populations, or apply for permission to perform pool experiments.

b. *A. niveus*:

(1) Establish a colony or drop from the program.

(2) Test a complete generation on *Potamogeton* and other host plants.

(3) Perform oviposition and larval preference tests.

(4) Evaluate the results and either drop from the program or apply for permission to release in the Tennessee Valley.

c. Other: Aid Fort Lauderdale as needed in the foreign exploration program. This might include surveys in Europe.

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Buckingham, Gary R
  Investigation of two insect species for control of Eurasian watermilfoil / by Gary R. Buckingham, Chris A. Bennett, Bonnie M. Ross, U. S. Department of Agriculture, Biocontrol Laboratory, Gainesville, Fla. Vicksburg, Miss. : U. S. Waterways Experiment Station ; Springfield, Va. : available from National Technical Information Service, 1979.
  20 p. : ill. ; 27 cm. (Miscellaneous paper - U. S. Army Engineer Waterways Experiment Station ; A-79-4)
  Prepared for Office, Chief of Engineers, U. S. Army, Washington, D. C.
  References: p. 20.

1. Biological control. 2. Insects. 3. Water milfoil. I. Bennett, Chris A., joint author. II. Ross, Bonnie M., joint author. III. United States. Army. Corps of Engineers. IV. United States. Dept. of Agriculture. Biocontrol Laboratory, Gainesville, Fla. V. Series: United States. Waterways Experiment Station, Vicksburg, Miss. Miscellaneous paper ; A-79-4.
TA7.W34m no.A-79-4