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Biological and Host Range Studies with *Bagous affinis*, An Indian Weevil that Destroys Hydrilla Tubers

by *Gary R. Buckingham*
Agricultural Research Service

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WES

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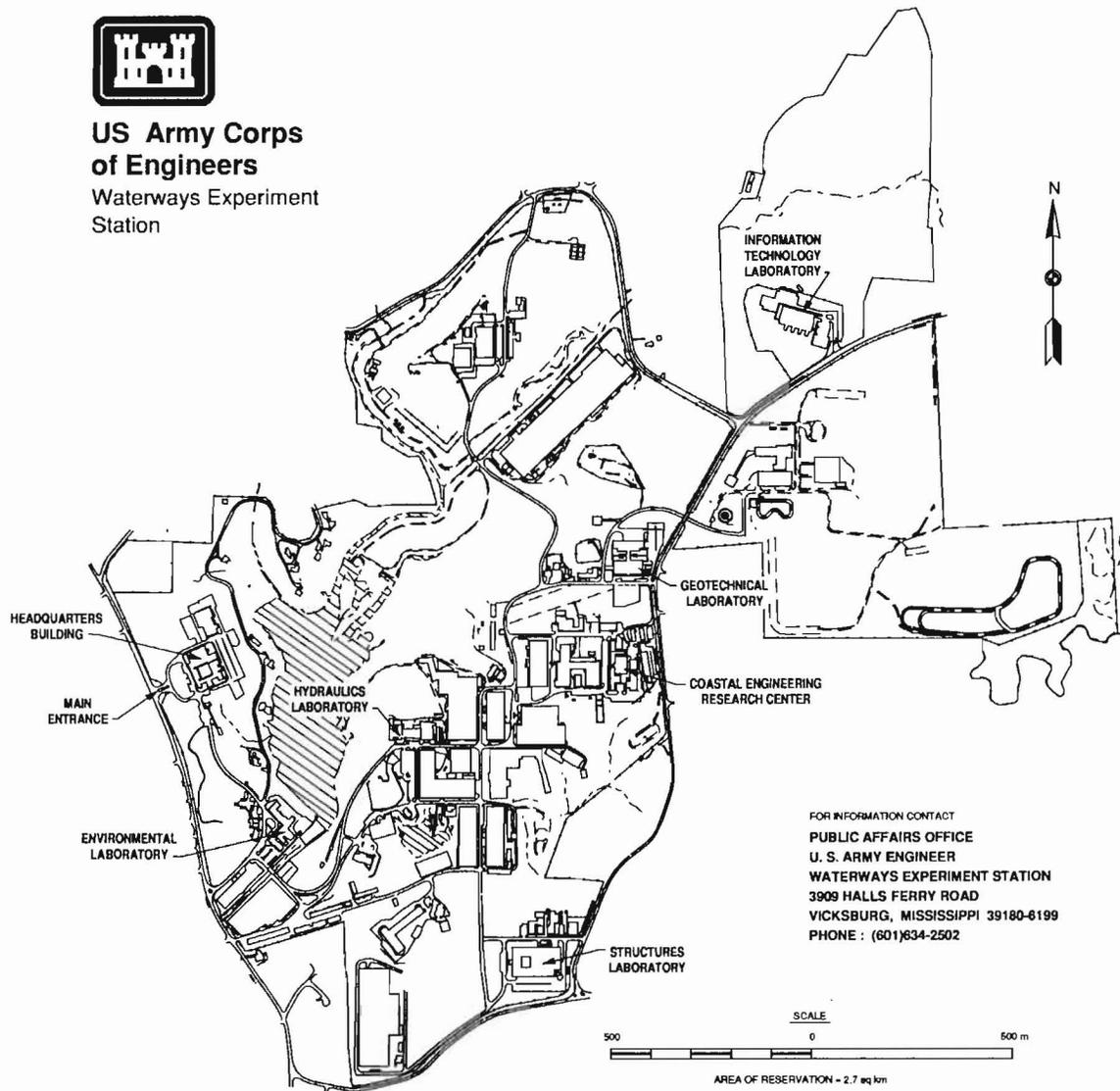
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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 31799. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J. L. Decell, Manager. Mr. Robert C. Gunkel was Assistant Manager, ERRAP, for the APCRP. Technical Monitor during this study was Ms. Denise White, HQUSACE.

The Principal Investigator for the work was Dr. Gary R. Buckingham, U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS). Dr. Buckingham and Ms. Christine A. Bennett, University of Florida, Gainesville, prepared this report. Special thanks goes to Dr. J. Balciunas and Mr. M. Minno, University of Florida, for providing initial shipments to the quarantine facility, and Mr. T. Sankaran and the staff of the Commonwealth Institute of Biological Control, Bangalore, India, for shipments of weevils and for their invaluable assistance during our collecting trip. The authors also wish to thank the following personnel for their assistance with various aspects of identification: Dr. C. W. O'Brien, Florida A&M University, Tallahassee, FL, for identifying the weevils, Dr. H. A. Denmark, Division of Plant Industry (DPI), Florida Department of Agriculture and Consumer Services (FDACS), Gainesville, FL, for identifying the mites, Messrs. K. Langdon and C. Artaud, DPI, FDACS, for identifying the plants, and Dr. A. H. Undeen, USDA-ARS, for checking the colony for disease before being released; Ms. Elizabeth Campfield, Ms. Mary Fredrick, Mr. Timothy Mullin, Ms. May Buckingham, Mr. Charles Bolton, Mr. Emmanuel Okrah, and Dr. Maude Christian-Meier for field or laboratory assistance; Ms. Sandra Davis for providing translations of foreign references, and the Aquatic Plant Center, University of Florida, for the loan of tube-collecting equipment. Funds and arrangements for travel and collection of live material in India and Pakistan were provided by the International Research Division, Washington, DC, and the Far Eastern Regional Research Office, New Delhi, India, both of the USDA's Office of International Cooperation and Development. Facilities and support were provided by the DPI, FDACS, Gainesville, FL.

The research was monitored at WES by Dr. Alfred F. Cofrancesco, Jr., Aquatic Ecology Branch (AEB), EL. The study was conducted under the general supervision of Dr. John Harrison, Director, EL; Dr. Conrad J. Kirby, Chief, Environmental Resources Division, EL; and the direct supervision of Dr. Edwin Theriot, Chief, AEB.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) imported into the United States as an aquarium plant and naturalized at least twice, is presently found from Florida to Delaware and westward to Texas and California (Steward and Van 1987). Severe infestations clog irrigation and drainage canals and interfere with navigation, fishing, boating, and other recreational activities in rivers and lakes. Large, fast-growing mats of hydrilla destroy populations of native plants by out-competing them for light and nutrients. Small potato-like tubers are produced in the hydrosol by hydrilla. These tubers survive when the hydrosol is exposed and the hydrilla dries (Figure 1). The methods of control used most commonly for hydrilla are herbicides and mechanical means. Because both are expensive and require multiple treatments, hydrilla has been a biological control target weed for at least 15 years. Fish have been developed as biocontrol agents (Sutton and Vandiver 1986), but there are many limitations to use of these generalist feeders.

Several foreign surveys for hydrilla-feeding insects have been conducted during the last 25 years both by contracted foreign scientists and by United States scientists. These surveys have covered most areas of the world where hydrilla occurs excluding temperate Asia. In 1982, four species of weevils were brought from India to the United States to be evaluated in a quarantine facility for evaluation of their potential as biological control agents against hydrilla (Buckingham 1988). Two species, *Bagous dilgiri* Vazirani and *Bagous vicinus* Hustache, were dropped from consideration after preliminary testing indicated that the former also developed upon *Potamogeton illinoensis* Morong and *Najas guadalupensis* (Spreng.) Magnus and that the latter only damaged stems stranded out of water (unpublished data).

The third species was undescribed when this study began but has subsequently been named *Bagous laevigatus* O'Brien and Pajni (O'Brien and Pajni 1989). The biology of this species is similar to that of the fourth species. In addition to developing on hydrilla tubers, however, larvae developed readily in the laboratory on tubers of sago pondweed, *Potamogeton pectinatus* L. This species was not released.

A fourth species was the hydrilla tuber weevil, *Bagous affinis* Hustache, studied briefly in Pakistan as *Bagous* sp. (Baloch, Sana-Ullah, and Ghani 1980). Adults fed on all parts of hydrilla. Larvae developed in tubers (also



Figure 1. Hydrilla tubers in the hydrosol during a lake drawdown

called subterranean turions) at the margins of waterways as the water receded during dry periods. They did not attack submersed tubers. Baloch, Sana-Ullah, and Ghani (1980) reported that *B. affinis* can be an effective destroyer of hydrilla tubers in Pakistan.

Bagous affinis was released in Florida in 1987 for hydrilla control after extensive host range testing (Buckingham 1988). As of July 1990, there was no evidence of permanent establishment at any of the release sites mentioned by Center (1989). Temporary establishment was obtained in 1989 during a drawdown in north-central Florida (Buckingham, Bennett, and Okrah, In Preparation), but immatures would have been destroyed when the water returned. Adults may have lived until the next year, but without another drawdown, they most certainly perished.

This report has been prepared to consolidate information on the biology and host range testing of *Bagous affinis*. It will provide a convenient reference source to aquatic plant managers and others who wish to utilize this weevil for biocontrol of hydrilla. Most of the information has been taken verbatim from Bennett and Buckingham (In Preparation) and from Buckingham and Bennett (In Preparation).

2 Materials and Methods

Rearing

Adults of *Bagous affinis* were collected near Bangalore, India, and shipped or hand carried to the quarantine facility at the Florida Biological Control Laboratory, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, where this study was conducted. Thirteen shipments were received between June 1982 and June 1985. Total adults received were 265. Most adults were collected from hydrilla stranded along shore, but some were collected from submersed hydrilla or at black lights placed along shore or in dry lake beds.

Hydrilla tubers and waterlogged wood were periodically collected from submersed soil at Rodman Reservoir, Putnam County, FL. The sandy soil was also collected and used for larval rearing and various experiments. Tubers were stored in tap water in a temperature cabinet at 7 to 10 °C until needed. All rearing and experiments except those indicated were conducted in temperature cabinets at constant 27 ± 2 °C and 16-hr photophase. These studies were conducted between July 1983 and March 1989.

Adult colonies were maintained in large (3,840-ml) and small (1,440-ml) clear plastic bowls capped with opaque plastic lids that had the center portions replaced with plastic screen (32 by 32 strands per inch). The bowls contained about 5 to 6 cm of dry, sterilized wellpoint sand. A clay flowerpot saucer containing sand, tubers, and sprigs of hydrilla in approximately 6 mm of water was pressed into the sand. Small pieces of soft, waterlogged wood and tubers were placed alongside the saucer and covered with hydrilla sprigs and crumpled moist paper towels, which helped preserve moisture. The wood, which was used as an oviposition substrate, was changed routinely to collect eggs for rearing and experiments. Prior to the discovery that females preferred to oviposit in wood, folded moist paper towels were used for oviposition substrates.

Larvae were mass reared in 15.2-cm or in 21.6-cm ID clay flowerpots, filled about three-fourths with soil. The soil was steam sterilized, dried, and moistened before use with a solution of the fungicide Benomyl prepared according to label directions. Tubers and egg-infested wood were mixed with the soil in the pots. A mass of hydrilla sprigs was placed on the soil surface,

and the pot was capped with nylon organdy. The hydrilla sprigs dried slowly, providing insulation for the soil and allowing it to dry gradually, as in the field. In addition, the pots were slightly moistened with Benomyl solution three times a week. After 20 days, adults were removed from the soil by sifting using United States standard testing sieves No. 6 and 7. This larval rearing technique was developed after weevil-infested hydrilla sites were observed in India. Prior to that time, larvae were reared in vermiculite with less success.

Biology

Measurements

Measurements of eggs and pupae were made with living specimens, but measurements of larvae and adults were made with specimens preserved in 75-percent isopropyl alcohol, which was used throughout this study. Measurements were made at either 25X or 50X with a stereomicroscope. Measurements of laboratory-reared and field-collected adults were compared and statistically analyzed using a t-test (SAS Institute Inc. 1985). Measurements of adult length included the head. All means are reported with standard deviation (number and range). Voucher specimens of all stages were deposited in the Florida State Collection of Arthropods, Gainesville, the United States National Museum of Natural History, Washington DC, the Canadian National Collection, Ottawa, and the collection of Charles W. O'Brien, Florida A&M University, Tallahassee, who identified all four species of weevils.

Adult feeding preference: tubers versus stems

A test of adult feeding preference was conducted in a plastic, 9-cm-diam petri dish with a moistened filter paper. Three treatments each with three pairs of weevils were replicated three times with newly emerged adults and three times with adults at least a month old. Treatments were (a) four hydrilla sprigs, (b) four hydrilla tubers, and (c) two sprigs and two tubers. An additional treatment with no food was included for flight muscle studies. The filter paper was divided into four quarters, one sprig or tuber per quarter. In (c), sprigs and tubers were alternated. The duration of the test was 3 days. An additional nine pairs of weevils of each age category were killed in alcohol at the start of the experiment as a baseline control for flight muscle studies. All weevils were killed at the end of the experiment in 75-percent alcohol and later dissected.

Flight muscle dissections

Adults were preserved in alcohol at various times throughout the studies for later dissection to examine flight muscles and ovaries. In addition, a test was

conducted to determine if feeding on nonhost plants stimulated the development of flight muscles. Newly emerged females were held in a plastic bowl with moist paper towels and no food. Three females were immediately preserved in alcohol, and three females were preserved after 3 days. The remaining 81 females were divided into three groups of nine; each replicated three times. One group was fed sprigs and tubers of hydrilla, one was fed sprigs of *Egeria densa* Planch. and crowns of *Vallisneria americana* Michx, and the third was starved. Our host range studies had demonstrated that adults would eat both of these submersed species. Three females from each replicate in each group were preserved after 6 days and the remaining females after 13 days. All weevils in this study were dissected and the muscles categorized as described in Buckingham and Passoa (1986).

Soil oviposition tests

Two tests were conducted to determine if females would oviposit in soil. In the first test, two 30-ml (1-oz) plastic cups with translucent plastic lids were each set up with two females and one male, three or four tubers, several sprigs of hydrilla, and moist soil. Each lid had a cotton-plugged hole, ca. 15-mm-diam, to reduce moisture. After 3 days, adults were removed and the cups filled with a dilute water-soluble glue solution. When the glue dried, the soil, which was lightly glued together, was carefully broken apart under a stereomicroscope and examined for eggs. In the second test, hydrilla tubers were buried in moist soil in a large, adult colony bowl. The soil was covered with nylon organdy mesh that was covered by more soil. Mixed adults along with hydrilla sprigs and tubers were then added. The mesh prevented the adults but allowed neonate larvae to reach the buried tubers. After 2 days, the surface hydrilla was replaced; after 3 days, a piece of soft, waterlogged wood was placed on the surface to stimulate increased oviposition. Every 2 days thereafter, the tubers, sprigs, and wood were removed from the surface before any eggs could hatch and were examined to confirm that females were still ovipositing. The experiment was terminated after 7 days, when the buried tubers were examined for larval attack.

Fecundity and adult longevity

A fecundity and adult longevity test was conducted with newly emerged adults. Adults were held initially with moist paper towels, hydrilla tubers, and pieces of moist wood until eggs were observed in the wood. Eighteen pairs were then separated and held in 30-ml cups with small pieces of moist paper toweling, a hydrilla sprig, a tuber, and piece of soft, moist wood. The cups were checked every 2 to 7 days for eggs and dead adults. Eggs were counted, and unhatched eggs were placed in a cup with moist cotton to determine viability. Dead males were replaced with others from the colony.

Adult submergence tests

A test to detect underwater feeding and oviposition was conducted in a large adult colony bowl with mixed adults using the same techniques as for rearing except that the bowl was filled with water. In another test, seven females and five males were placed singly in 185-ml snap-cap plastic vials, filled three-fourths with water, with stem turions and sprigs of hydrilla. A piece of stiff nylon mesh was included in each vial to provide footholds both underwater and above water. Observations were made on the presence of feeding, number of eggs, and weevil activity in each test.

To determine how long adults could survive submergence, 4-day-old adults and pieces of mesh were placed in the snap-cap vials submerged in tap water in a plastic dishpan. The vial was capped underwater to ensure that all air bubbles were excluded. Two vials, one with 10 females and one with 10 males, were removed each 24 hr for 4 days. One vial of 11 extra males was removed after 5 days. Moribund adults were held on moist filter paper in petri dishes for 24 hr to confirm they were dead. Infested tubers were submerged in the same manner for 24, 48, 52.5, 66, 96, 114, 161, 192, 193, and 260 hr. After submergence, the moribund larvae and teneral adults were removed from the tubers and held as above. Pupae were placed on moist cotton in 30-ml cups and held for 4 to 5 days for adult emergence.

Associated mites

Predation by mites associated with the colonies was tested by removing 20 newly laid eggs from wood and placing them on moist cotton in a 30-ml cup capped as previously mentioned. Four *Blattisocius* sp. adults (Asciadae: Acari) were removed from the wood in the colony rearing bowls and placed in the cups with eggs. Each cup was covered with aluminum foil to exclude light and held in the laboratory at ca. 25 °C. Mites were observed periodically for a feeding response.

Development times of Immatures

For developmental studies, eggs were removed from oviposition substrates and held until hatched in cups on moist cotton as in the fecundity tests. Larval and pupal development times were determined by individually rearing larvae in 30-ml cups capped as in the fecundity tests. A mature egg (one that contained a fully formed, often moving, larva) was placed on a tuber in vermiculite moistened with Benomyl solution, and then the tuber was covered with more vermiculite. The cups were covered with aluminum foil to exclude light. Larvae were collected daily until pupation and were preserved in alcohol after being killed in boiled water. Head capsules were measured with an ocular micrometer in a stereomicroscope to determine number of instars. Newly formed pupae were removed from the tubers and transferred to new cups with

moist cotton until adult emergence. Pupae for measurements were removed from larval rearing pots.

Larval tuber Interactions

Mean number of larvae per tuber was determined by dissecting 20 tubers removed from a rearing pot. To determine if larvae would exit one tuber and reenter another, tubers infested with multiple larvae were placed in the 30-ml cups on moist cotton with fresh tubers. They were held for 7 days.

To determine the distance larvae crawl to find them, 10 tubers were buried in moist sandy soil in 11-cm-deep (355-ml) styrofoam cups at depths of 4.5, 7.5, and 11.0 cm and in plastic boxes, 11 by 11 by 9 cm deep at depths of 3.0, 5.5, and 9.0 cm. Ten mature eggs on a small piece of moist cotton were placed on the soil surface of each container and lightly covered with soil. Moist blotter paper was then placed on the soil surface to reduce evaporation. The containers were capped with nylon organdy mesh and held for 10 to 15 days. In addition, 50 tubers and 75 eggs were buried as above in Plexiglas cylinders, 12.5 cm ID by 40 cm in height, at depths of 15, 20, and 30 cm. Hydrilla sprigs were placed on top to reduce evaporation, and the cylinders were held upright for 7 to 8 days. In a horizontal experiment, moist soil was placed to a depth of 5 cm on an aluminum foil-covered board, 80 by 10 cm. The margins of the aluminum foil were bent upwards to form a shallow pan. Ten tubers were placed across the center and five tubers were placed across the board every 5 cm in both directions for a total of 90 tubers. The tubers were covered with 5 cm of soil. A piece of moist cotton with 170 eggs was buried at the center of the board. Sprigs of hydrilla were placed on the soil surface to reduce evaporation. The tubers were examined after 7 days.

Attack on stem turions was tested by burying 16 stem turions in a rearing pot as done with tubers. Fifty-three eggs on moist cotton were covered with soil, and the pots handled as were the larval rearing pots.

To determine if pupation in the soil instead of in the tuber was due to multiple infestation, a low-density rearing pot was set up as previously mentioned with 99 tubers and 55 eggs on moist cotton. The cotton was covered with soil. After 12 to 15 days, both the tubers and soil were examined for pupae.

Host Range Studies

Larval tests

Most no-choice larval feeding tests were conducted using the 30-ml plastic cups with plastic lids described earlier. Moist cotton or moist vermiculite was placed in the cups with the test plant material. Large plant structures were tested in quart jars or in plastic containers with vermiculite or sand. Mature eggs were placed either on the plant material or on the substrate. The

substrate was generally moistened with a Benomyl solution prepared according to the label recommendation. Most tests were conducted in the temperature cabinets, although some experiments were conducted in a controlled temperature greenhouse.

The plant material was usually checked for attack after 6 to 7 days and then held for larval development. The containers were checked for adult emergence after about 20 days. Plant species that had crowns, roots, bulbs, tubers, or turions of sufficient size to permit larval development were chosen for testing. The stems of *E. densa* were tested even though they would not be exposed to these subterranean larva under natural conditions, because of *E. densa's* close relationship with hydrilla. Hydrilla tubers were included as controls in most series of tests. In most tests, a sample of eggs was held on moist cotton in the 30-ml plastic cups to observe hatching success. Additional no-choice and multichoice larval tests were conducted using the clay vases described in the rearing section. The vases were held in the same temperature cabinets and greenhouse and checked for attack as described for the preceding tests. The test plant parts and the hydrilla tubers exposed together in the multiple-choice tests were held in separate vases after the initial check for attack. In these tests, each replicate consisted of a vase with hydrilla tubers, a vase with test plant parts, and a vase with both. The soil was sifted and then placed in water at the termination of the tests to recover adults and other mature stages through flotation.

Techniques for the larval tests are summarized in Table 1.

Adult feeding tests

Adult feeding tests were conducted in various-size plastic and glass containers containing plant material with either water, moist vermiculite, moist filter paper, or paper towels and moist sand. Both no-choice and multichoice tests, with and without hydrilla, were conducted. Some plant material was tested by exposing it to the colonies in the rearing containers. A few potted plants were tested. These techniques are summarized in Table 2.

In the no-choice tests, each replicate generally included one or more test plant species and a hydrilla control. Some multichoice tests did not have companion hydrilla controls. The control cages generally included both hydrilla sprigs and tubers. Various parts of test plants were tested as indicated in the tables. Feeding was generally estimated in square millimeters or cubic millimeters with the aid of a ballpoint pen point ca. 1 mm in diameter and 1 mm long. Estimates in square millimeters were transformed to cubic millimeters for comparison by multiplying by 0.1 mm, which was a rough estimate of the thickness of various test plant leaves that were eaten and the depth of leaf surface feeding on others. This transformation was necessary because test plant feeding was often on leaves, but hydrilla feeding was often deep excavations in stems and turions. The excavations were estimated in cubic

Table 1
Explanation of Test Symbols for Feeding Tests with *Bagous affinis* Larvae

A = 1-oz (40-ml) plastic cups with vermiculite moistened with benomyl. One egg and one plant part per cup. Egg placed on plant part.

B = One-quart (0.95-l) jars with vermiculite moistened with benomyl. Eighteen eggs per jar.

C = Same as A except five eggs per cup.

D = None.

E = Plastic box, 1.5 to 2 pt (0.71 to 0.95 l) (9 cm by 9 by 9 cm) filled with 1 pt (0.47 l) vermiculite moistened with benomyl. Only test plant present. Fifty eggs per box.

F = 39-ml test tubes with hydrilla tuber in vermiculite moistened with benomyl.

G = Same as A except cotton moistened with plain water replaced vermiculite and egg placed near, not on, plant part.

H = None.

I = Same as G except five eggs per cup. Hydrilla control had five eggs and five tubers per cup.

J = 100 by 15 mm standard plastic petri dish with vermiculite moistened with benomyl. Ten eggs per dish with one plant part or 15 tubers.

K = None.

L = Quart (0.95 l) jars with moist paper towels and sprigs of test plants. Capped with organdy covered with parafilm, 3-cm hole in center of parafilm. Eggs on pieces of cotton placed on sprigs. Three jars with 235 total eggs.

M = 6-in. ID clay pots, plant parts in soil from dry lake bed, moistened with benomyl, mass of hydrilla sprigs on surface to conserve moisture. Capped with nylon organdy. Moist wood containing uncounted eggs buried near soil surface.

N = Same as M except in glass bell jars (5,000-ml) with 30 adults (20 females, 10 males) in addition to the eggs.

O = Same as M except uncounted eggs on pieces of cotton, not in wood.

P = Same as M except in 8 1/2 in. ID clay pot with one 1 1/2-in. layer of plaster of paris in bottom of pot to retain moisture.

millimeters. The transformation probably overestimated the volume of feeding on many test plant species because their leaves were somewhat less than 0.1 mm thick. For safety considerations, overestimating nonhost feeding was thought to be better than underestimating it. This weevil, which preferred to excavate galleries or pits in crowns, roots, and stems, generally fed only lightly on leaves.

Table 2
Explanation of Test Symbols for Feeding Tests with *Bagous affinis* Adults

A = 1-oz (40-ml) plastic cups, water, and plant material. One pair of weevils per cup. Duration 6 days.

B = Test plants placed in 16-cup (3,840-ml) plastic bowl with colony. (Ca. 100 to 200 unsexed adults. Hydrilla present.

C = 140 by 25 mm plastic petri dishes with moist paper towels and discs or sprigs of test plant. Discs cut with No. 9 cork borer from vegetables and *Nuphar*.

D = 3-cup (720-ml) plastic bowl, vermiculite moistened with benomyl. Twelve females, nine males per replicate. Duration 3 days.

E = 3-cup (720-ml) plastic bowl, vermiculite moistened with benomyl. Choice test with host. Seven females, three males per replication. Duration 4 days.

F = 490-ml plastic container, moist paper towels, test plants in bouquets. Duration 3 to 9 days.

G = Test plants placed in 16-cup (3,840-ml) plastic bowl with colony. Hydrilla not present. Ca. 200 unsexed adults. Duration 2 days.

H = 490-ml and 960-ml plastic containers, moist paper towels, test plants in bouquets. Duration 3 days.

I = Plexiglass cylinder, 14.5 cm ID, 42 cm high. Capped with nylon organdy. One small potted plant per cylinder. Duration 2 days.

J = 5,000-ml glass bell jar with moist paper towel. Hydrilla in 1-qt (0.95-l) jars. Five unsexed adults per jar. Duration 4 days.

K = 1-qt jar (0.95 l) with moist paper towel capped with nylon organdy. Five unsexed adults per jar. Duration 4 days.

L = Same as K except organdy covered with parafilm 3 days after initiation, 3-cm hole in center of parafilm. Duration 4 days.

M = Same as L except parafilm added at initiation. Five unsexed adults per jar. Duration 4 to 7 days.

N = 1-gal (3.8-l) jar, moist paper towels, capped with nylon organdy and covered with parafilm. Hydrilla control in quart (0.95 l) jars. Five unsexed adults per jar. Duration 6 days.

O = Same as I except organdy covered with parafilm, 3-cm hole in center of organdy. Five unsexed adults per cylinder. Duration 4 days.

P = Same as N. Ten females and ten males per jar. Duration 6 days.

Q = 100 by 15 mm standard plastic petri dish with moist filter paper. One weevil pair per dish. Duration 6 days.

R = Same as N except test plant was a potted seedling. Ten pairs adults per jar. Duration 31 days.

S = Same as M except 10 pairs adults per jar. Duration 64 days.

Oogenesis test

Groups of newly emerged females were confined with test plants in various-sized cages. Wood was provided for oviposition. A few females from each cage were dissected at various intervals after eggs were found in companion hydrilla cages. The wood was checked periodically for eggs, which were held for hatching in the 30-ml plastic cups. The remaining females in each test were dissected after oviposition stopped in the hydrilla cages. The *Elodea canadensis* Michx. test was terminated when the supply of the plant was exhausted.

3 Results and Discussion

Biology Studies--Adults

Adults are small, mottled brown weevils, which were recently redescribed by O'Brien and Pajni (1989) (Figure 2). Comparative measurements of laboratory-reared and field-collected adults were made to determine if colonized individuals were smaller and thus perhaps less vigorous. The mean length of colony females was significantly larger than field-collected individuals, 3.8 ± 0.2 mm (10, 3.6-4.0) versus 3.5 ± 0.2 mm (4, 3.2-3.7) ($p = 0.009$). However, the widths were not significantly different, 1.6 ± 0.1 mm (10, 1.5-1.7) versus 1.5 ± 0.1 mm (4, 1.3-1.6) ($p = 0.062$). The mean length of laboratory-reared males was 3.3 ± 0.2 mm (10, 3.0-3.6), which was not significantly different from the length of the field-collected males, 3.4 ± 0.2 mm (10, 3.2-3.7) ($p = 0.16$). The mean width of reared males also did not differ from field-collected ones, 1.4 ± 0.1 mm (10, 1.4-1.6) versus 1.5 ± 0.1 mm (10, 1.4-1.5) ($p = 0.20$). The measurements of male length include the head, whereas those of O'Brien and Pajni (1989) included only the thorax and elytra. The sexes can be separated by the shape of the first abdominal sternite, which is slightly but noticeably concave medially in the male (Figure 3) and convex in the female (Figure 3). The sex ratio was 1:1 ($n = 218$).

Adult feeding preference: tubers versus stems

Adults fed on hydrilla leaves, stems, stem turions, and tubers; however, stems were preferred. In the adult-feeding preference test with stems and tubers, newly emerged adults fed almost four times more on the stems (3.8 ± 0.93 mm³ (3.0-4.8)/insect versus 0.7 ± 0.7 mm³ (0-1.4)/insect). Older adults fed only on the stems (2.1 ± 1.3 mm³ (1.2-3.5)/insect). Adults ate small holes in the stem usually above the leaf whorls; heavy feeding often severed the stem. Adults bored into the tubers, feeding both internally and half inside, half outside. It was not uncommon to find two or three adults feeding inside the same tuber (Figure 4). Adults appeared to congregate when feeding, suggesting possible attraction to each other or to damaged tubers. Often, many tubers in a cage would be undamaged even though others were almost completely destroyed. Adults in the colony appeared to feed more on tubers than



Figure 2. *Bagous affinis* adults on a hydrilla tuber

on stems, but this was probably because the hydrilla stems dried quickly in the bowls. The extent of adult feeding on tubers in the field is unknown; however, a pheromone or feeding attractant would be highly beneficial for a species feeding on scattered and difficult-to-reach hosts like the buried tubers.

Flight muscles

In the preceding test of adult feeding preference, 55.3 ± 25.4 percent (3, 33-83) of newly emerged weevils had partially developed flight muscles and 27.7 ± 34.4 percent (3, 0-50) had fully developed muscles. Three days later 98 ± 3.5 percent (3, 94-100) of adults that were fed hydrilla sprigs, tubers, or a combination had fully developed muscles, and 1.8 ± 3.2 percent (3, 0-5.6) had partially developed muscles. All but one of those with no food, 93.8 percent had fully developed muscles, and the other had partially developed muscles. At the start of the experiment, the adults that were at least 30 days old had 61 ± 25.5 percent (3, 33-83) with fully developed muscles and 5.6 ± 9.8 percent (3, 0-17) with partially developed muscles. Two of the three females with undeveloped muscles had eggs. Three days later, after feeding on hydrilla, 90 ± 4.4 percent (3, 85-93.3) of the older adults had fully developed muscles. Older adults without food had somewhat less, 77.7 ± 38.7 percent (3, 33-100) fully developed and 11 ± 19.1 percent (3, 0-33) partially developed.

In the test with nonhost plants, 11 ± 19.1 percent (3, 0-33) of newly emerged adults had fully developed muscles, and 33 ± 0 percent had partially developed muscles. After 3 days on moist towels without food, 89 ± 19.1 percent (3, 67-100) had fully developed muscles, and the remaining $11 \pm$

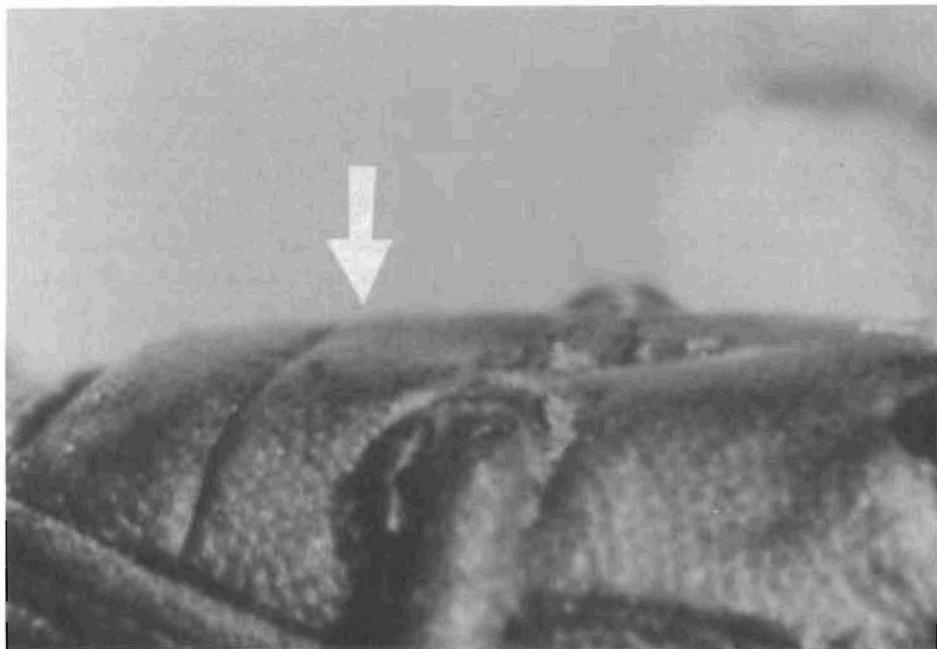
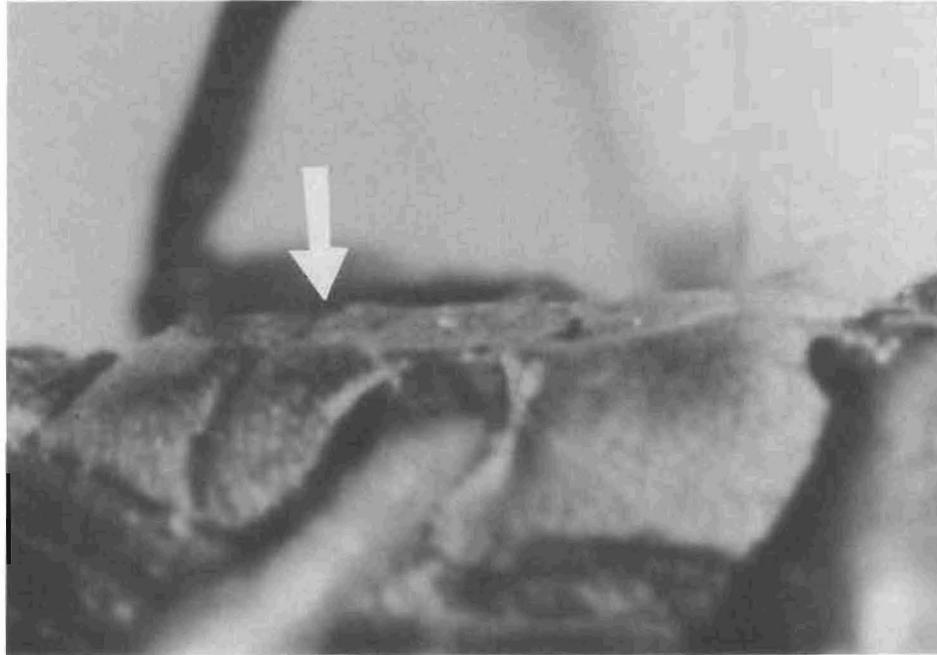


Figure 3. *Bagous affinis* adults. Ventral surface near hind leg. Male (top) female (bottom)



Figure 4. *Bagous affinis* adults feeding inside a hydrilla tuber (photograph by T. McCabe, ARS-USDA Information Staff)

19.1 percent (3, 0-33) had partially developed muscles. After 13 days, however, only 53.3 ± 5.1 percent (3, 50-60) of females on hydrilla, 56.7 ± 5.8 percent (3, 50-60) of females on *E. densa* and *V. americana*, and 49 ± 42.9 percent (3, 0-80) of starved females had fully developed muscles. None had partially developed muscles. The majority of females with undeveloped muscles that fed on the plants had eggs (64.0 ± 26.6 percent (4, 50-89)).

Fully developed muscles and eggs were mutually exclusive. Although weevil numbers were relatively small in these two experiments, the results indicated that flight muscles develop in adults shortly after emergence and become reduced with or without the host plant present; then oviposition begins, at least in many individuals. Muscles then redevelop in many individuals within a month or so. These weevils were unlike waterhyacinth weevils, which did not redevelop muscles within short periods (Buckingham and Passoa 1986). Not surprisingly, both weevil species appear to be well adapted to their respective habitats. *Bagous affinis* undoubtedly flies between sites in different stages of drying. The waterhyacinth weevils live on closely packed plants in relatively stable waterways.

Oviposition

The female probed the oviposition substrate, usually waterlogged wood or paper towels, with her mouthparts and then oviposited into the probed area. The surface of wood that contained many eggs was mushy and full of holes from extensive female probing. Eggs were inserted throughout the towels, but

principally along folds. Eggs were also laid in hydrilla stems, usually near a leaf whorl, and in the rhizome attached to the tuber. In these studies, only once were eggs laid in tubers. These observations conflict with those of Baloch, Sana-Ullah, and Ghani (1980), who reported finding oviposition punctures in field tubers and oviposition in tubers during host range tests. We believe that the punctures they found were larval entrance holes and that oviposition during tests was incorrectly inferred by them because larvae were found in the tubers. This argument is supported by the fact that they only reported counting larvae and pupae during testing, not eggs.

In the field, eggs are probably laid usually in wood and organic material, but in hydrilla stems or possibly soil when the former are not available. Waterlogged wood would seem to be a good oviposition substrate for two reasons: first, it probably retains moisture much longer than does the surrounding soil or plant material during a drying period and, second, tuber density may be high in soil near submersed logs as it often was at our tuber collection sites in Rodman Reservoir. Tubers were even formed inside the soft wood of submersed logs.

Soil oviposition tests

In the first soil oviposition test, eggs were obtained in only one cup. A total of 16 eggs were laid, 10 individually among sand grains and 6 in a short rhizome section attached to a tuber. None were laid in three tubers resting on the soil surface. In the second test with a mesh barrier preventing female access to the tubers, 7 of 40 buried tubers were attacked by larvae, thus confirming that eggs had been laid in the soil. Uncounted eggs were present in the wood and stems removed every 2 days before larvae could hatch. There are no previous reports of *Bagous* ovipositing away from the host plants, although a related Indian weevil, *Echinocnemus* sp., oviposits on soil near its host plant *Marsilea minuta* L. growing terrestrially (Loyal and Kumar 1977).

Fecundity and adult longevity

Fecundity and adult longevity are reported in Table 3 along with mean oviposition and preoviposition and postoviposition time periods. All varied greatly among individuals. Six of the eighteen females had preoviposition periods greater than 100 days. Perhaps those females retained their flight muscles and remained in a migratory state during the preoviposition period. When those females were excluded from the calculations, the preoviposition period was 30.9 ± 22.0 days ($n = 12$, range = 6-61). Male and female longevities were about equal. Egg viability was 99 percent ($n = 4647$). Unmated females laid a few eggs, but none hatched. Most of these unfertilized eggs were laid on the surface of leaves rather than inserted into wood. Unfortunately, there are no detailed studies on other species of *Bagous* for comparison with these data.

Table 3 Life History Data for <i>Bagous affinis</i>			
Developmental Period, Days	$\bar{X} \pm SD$	Range	N
Egg	3.8 ± 0.6	3-5	324
1st instar	--	1-2	48
2nd instar	--	2-6	54
3rd instar	--	4-13	75
Total larval	14.7 ± 1.0	14-17	23
Pupa	5.0 ± 0.6	4-6	7
Total	21.9 ± 1.5	18-29	96
Adult longevity, days			
Males	136.8 ± 46.0	55-211	18
Females	117.0 ± 53.1	55-225	17
Total Mixed	127.5 ± 50.2	55-225	35
Preoviposition, days	65.1 ± 55.0	6-186	18
Oviposition, days	40.4 ± 23.1	10-79	18
Postoviposition days	9.5 ± 12.1	0-48	15
Fecundity (eggs)			
Lows dropped (<42)	287.9 ± 190.3	75-653	16
Total	231.7 ± 203.3	0-653	18
Measurements, mm			
Eggs			
Length	0.52 ± 0.03	0.50-0.60	14
Width	0.35 ± 0.02	0.32-0.38	14
Larval head capsule			
1st instar	0.22 ± 0.03	0.12-0.26	50
2nd instar	0.40 ± 0.03	0.32-0.46	50
3rd instar	0.66 ± 0.04	0.56-0.76	87
Pupa			
Length	3.75 ± 0.55	3.20-4.76	9
Width	1.81 ± 0.28	1.50-2.29	9

Adult submergence tests

In containers with water, most adults sat above the water surface. They were unable to swim and moved along the water surface by ungainly walking movements. The few observed underwater had crawled down hydrilla stems, and when disturbed, they let go and popped to the surface. They ate small amounts of submersed stems and leaves, but not submersed stem turions or tubers. They laid no eggs underwater. Baloch, Sana-Ullah, and Ghani (1980) reported that adults fed on but did not oviposit on submersed tubers in a laboratory study. Although *B. affinis* was unable to swim, some species of *Bagous* are excellent underwater swimmers (Menier 1970; O'Brien and Marshall 1979)

All females (n = 10) and 90 percent (n = 10) of the males survived 1 day of submergence and 90 percent of both sexes survived 2 days. Female survival was 60 and 50 percent after 3 and 4 days, respectively, and male survival was 40 and 80 percent. However, the survivors in the cages submersed 4 days were weak and barely moved. The extra males (n = 11) submersed for 5 days were all dead. Twenty-seven percent of larvae in tubers submersed 24 hr survived (n = 61); 67 percent survived in tubers submersed 48 hr (n = 151); and 1 percent survived in tubers submersed 52.5 hr (n = 2). All larvae in tubers submersed 66 hr died (n = 16). Pupae in tubers developed normally after 114 hr of submergence (4.8 days) (n = 2), but all pupae were dead after 192 hr (8 days) of submergence (n = 3). Teneral adults in tubers were dead after 193 hr (8.0 day) of submergence (n = 3). Air pockets in the hollowed-out tubers probably increased the submergence survival times and accounted for the variability of the results. Naked larvae or pupae were not tested.

Submersed *Bagous* adults obtain oxygen through plastron respiration. The plastron is a tightly held air layer into which oxygen diffuses from the water. Air is held on *Bagous* adults by "plastron scales" (Hinton 1976) or "mushroom-shaped bristles" (Langer and Messner 1984; Messner and Langer 1984) on the dorsum and on the abdominal venter, which are wetted during submersion. Hydrophobic setae beneath the head and thorax trap a noticeable bubble of air, which was erroneously identified by Ruter (1937) and recently by Van der Velde, Kok, and Van Vorstenbosch (1989) as the major air supply for respiration. Langer and Messner (1984) suggested that the air bubble provides buoyancy and possibly supplementary oxygen to the plastron. Scanning micrographs that these authors made of *B. affinis* revealed that it has scales and setae similar to those illustrated for other species of *Bagous* by Hinton (1976) and by Langer and Messner (1984).

Pathogens

Adults in the colonies of these authors were often killed by the fungus *Beauveria bassiana* (Balsamo), a common disease in laboratory cultures. Some of the adults received in shipments from India had also died from this disease during shipment. Prior to the field release, samples from the colony were examined by a pathologist for disease. Only yeast-like cells and eugregarine oocysts were found and only in a few individuals. Neither of these was thought to pose a problem for release.

Associated mites

Several cosmopolitan species of mites were commonly associated with the weevils in the colonies. These mites were as follows: *Lasioseius subterraneus* Chant, *Proctolaelaps* nr. *longiplis* (Chant), *Laelaps* sp., and *Blattisocius* sp., all predaceous mites, and *Tyrophagus putrescentiae* (Schrank), a mold mite. Only *Blattisocius* sp. was observed feeding and only on one egg of *B. affinis*. However, the mites did bother the adults by walking and riding on them.

Occasionally, they completely covered an adult's body thus restricting its normal activities. Mites were then hand picked from the adult, or the adult was shaken in a vial of water thus dislodging most of the mites. Weevils were individually examined under a stereomicroscope, and all mites were removed before release of the weevils from quarantine. The predaceous mites may have been mostly beneficial, however, because the colony established in Fort Lauderdale after release from quarantine had a much greater problem with the omnipresent *T. putrescentiae* than the colonies with mixed mites.

Egg

The newly laid egg was white to off-white with a transparent chorion. It was elongate in shape (Figure 5a). Eggs were laid singly but closely packed into the small pieces of wood exposed to large numbers of females (Figure 5b). Measurements of mature eggs and duration of the egg stage is reported in Table 1.

Larvae

Larvae were whitish with tan heads (Figure 6). There were three instars. The body of the first instar was slightly more flattened dorsoventrally than the other instars, and setae along the lateral margins were more noticeable. Instars were separated by head capsule measurements, which are reported in Table 1 along with the durations of the instars. The neonate bored into the tuber through a pinpoint-sized hole and often tunneled just under the epidermis. This feeding gallery turned black, revealing the presence of the larva (Figure 7). Other larvae tunneled deeper into the tuber, and their early presence could only be detected by the entry hole.

Larval-tuber Interactions

The mean number of larvae per tuber in one rearing pot was 3.1 ± 2.6 (20, 1-12). The mean for only those tubers with multiple larvae was 3.6 ± 2.7 (16, 2-12). The larva usually matured inside one tuber. When multiple larvae destroyed a tuber (Figure 8), they reentered other tubers or if mature, pupated in the soil.

Neonates crawled at least 30 cm downwards and 40 cm horizontally to find tubers. In the cups, neonates attacked 47 percent of tubers buried 4.5 cm ($n = 30$), and 53 percent buried at both 7.5 ($n = 30$) and 11 cm ($n = 30$). In the boxes, 42 percent of the tubers buried 3 cm ($n = 55$), 58 percent buried 5.5 cm ($n = 60$), and 40 percent buried 8 cm ($n = 60$) were attacked. In the cylinders, 38 percent of tubers buried 15 cm ($n = 50$), 6 percent buried 20 cm ($n = 50$), and 4 percent buried 30 cm ($n = 50$) were attacked. These results indicate that most tubers in the field should be accessible to neonates. Maximum tuber depth at Rodman Reservoir was found to be about 20 cm with

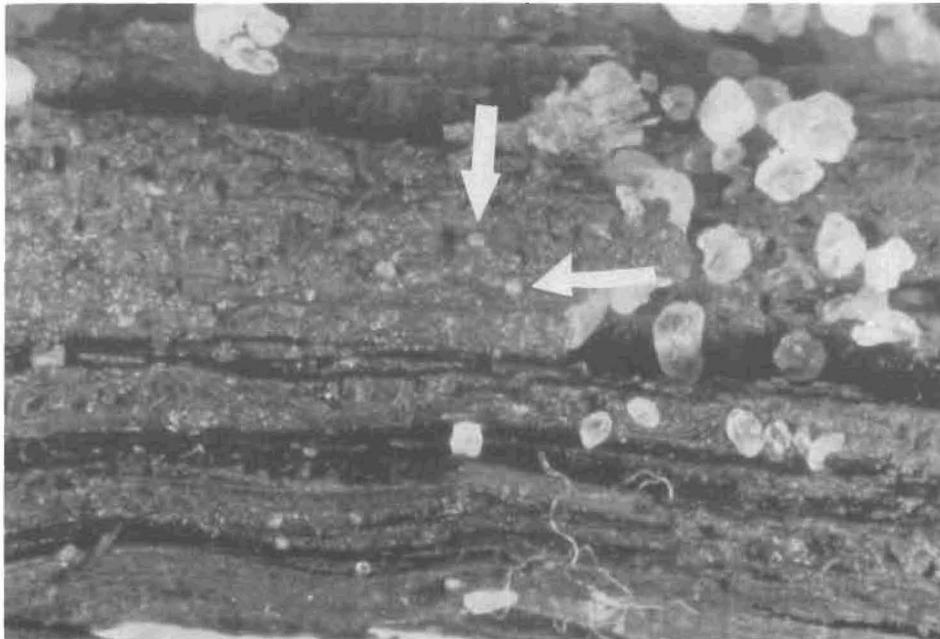
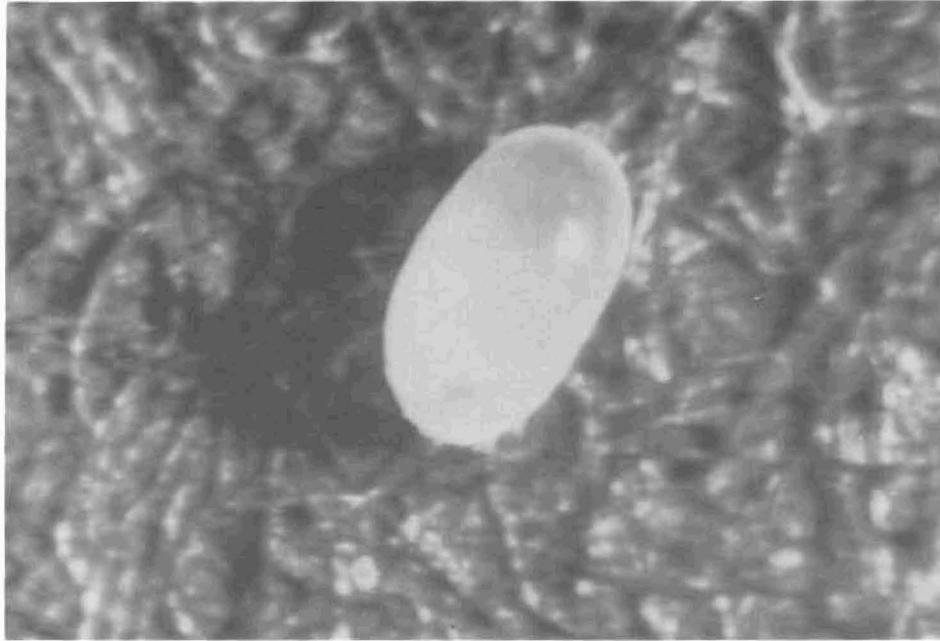


Figure 5. *Bagous affinis* egg (top, photograph by M. C. Thomas, University of Florida at Gainesville) and eggs embedded on moist, waterlogged wood (bottom)

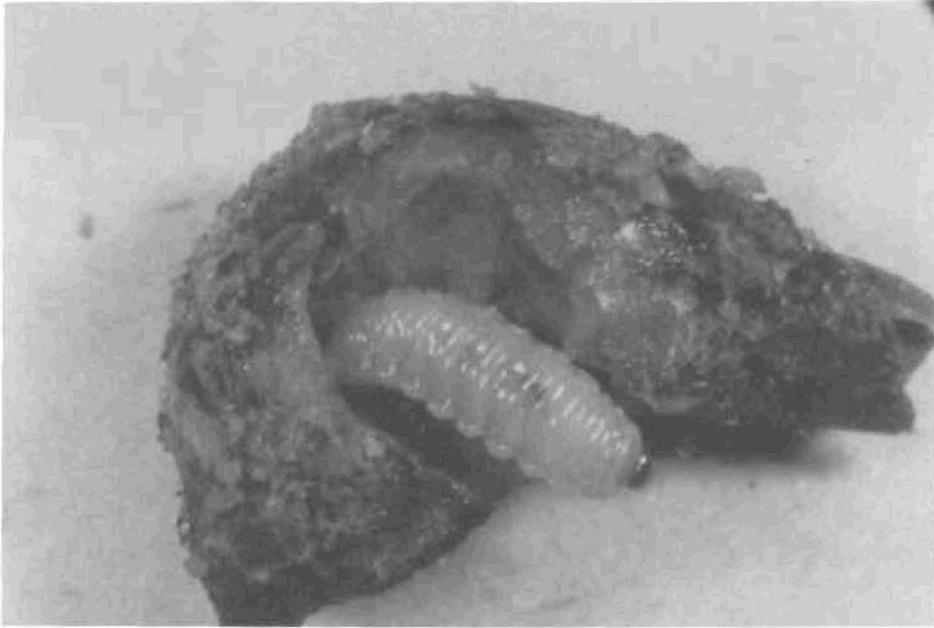


Figure 6. *Bagous affinis* larva (third instar) in a hydrilla tuber



Figure 7. Hydrilla tubers attacked in the field by *Bagous affinis* larvae



Figure 8. Hydrilla tubers destroyed by *Bagous affinis* larvae (three on right) and by adult feeding (two on bottom left). Undamaged tuber (upper left)

the majority less than 15 cm deep.¹ In the horizontal board test, the percentage of attack decreased as distance from the central release site increased; for example, 90 percent of the tubers were attacked at the center ($n = 10$); $n = 20$ at all other distances, 70 percent at 5 cm, 40 percent at 10 cm, none at 15 cm, 30 percent at 20 cm, 10 percent at 25 cm, none at 30 and 35 cm, and 10 percent at 40 cm.

Stem turions were also attacked by larvae. Sixteen adults emerged from 16 buried stem turions.

Pupae

The newly formed pupa was whitish yellow with scattered minute reddish-brown bristles on the head and dorsum. It turned darker yellow as it matured. One day before eclosion, eyes were dark, mandible tips red, and leg joints had begun to darken. In the laboratory, the naked pupae were found both inside tubers and in the soil. Teneral adults remained in the tubers or in the soil, often for 1 to 2 days, while they hardened and darkened. Measurements and duration of the pupal stage are reported in Table 1.

¹ Personal observation, 1989, Gary R. Buckingham, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL, and Christine A. Bennett, IFAS, University of Florida, Gainesville, FL.

Baloch, Sana-Ullah, and Ghani (1980) reported that larvae in the field pupated inside tubers. In our colony rearing pots, most pupae were in the soil; but when tubers were in individual cups with vermiculite, pupae were inside the tubers. In the rearing pot with almost a 2:1 tuber to larva ratio, more pupae ($n = 4$) and teneral adults ($n = 6$) were found inside tubers than in the soil ($n = 2$). Although numbers were low, this result suggests that pupation in soil in the rearing pots was because of the high larval densities, which destroyed the tubers.

Host Range Studies

Larval tests

Thirty-eight plant species in 20 families were tested for larval attack and subsequent larval development (Tables 4 and 5). Larval feeding was found only on the crowns of water celery and a dwarf arrowhead, the subterranean stems of turions of American pondweed, the tubers of sago pondweed, and the stems of egeria. A few larvae developed on four of the preceding five species plus on the underground stem of Richardson's pondweed in a preliminary test. These results were neither surprising nor alarming. *Bagous affinis* coexists in India and Pakistan with the very similar *Bagous laevigatus* (O'Brien and Pajni 1989). *Bagous laevigatus* were also tested in the laboratory, and its principal host plants were hydrilla and sago pondweed, which was preferred (unpublished data). These two weevil species may have divided the resources with *B. affinis* specializing on the more common and abundant hydrilla tubers and *B. laevigatus* on the less abundant but larger sago pondweed tubers. Since host finding by larvae of *B. laevigatus* would be more "risky" than that by *B. affinis* larvae, *B. laevigatus* is not as specialized. *Bagous affinis* is so strongly specialized that few adults were produced on attacked plants other than hydrilla. However, because the native host plant of *B. laevigatus* has not yet been reported, this scenario is still mostly conjecture. Several mature *B. affinis* larvae were produced on sago pondweed tubers in the preliminary tests conducted during the first months of observations, but no adults were produced. In the series of tests reported in Table 4, adults were not produced on sago pondweed, and only two adults were produced on American pondweed from a total of 105 eggs and 305 eggs, respectively.

Only six adults or other mature stages were produced on sago pondweed in clay vases and only four adults on American pondweed versus 1,027 and 411 on the hydrilla controls, respectively. Two adults were produced on Richardson's pondweed compared with 412 adults produced on hydrilla. Both of those adults were in no-choice vases (Table 5). The single adult that was produced on the egeria stems and the two adults on water celery crowns (Table 4) were produced under highly artificial conditions that prevented plant desiccation. Two mature stages were produced on water celery in clay vases that more closely approximated natural conditions, but the companion hydrilla control produced 870 mature stages (Table 5). When *B. affinis* larvae had a choice between hydrilla and water celery, the attack on water celery was

Table 4
Summary of No-Choice Feeding Tests with *Bagous affinis* Larvae

Family and Species	Common Name	Test ¹ Symbol	Total Insects Tested		% Larvae ² Attacking		% Adults ³	
			Test Plant	Hydrilla	Test Plant	Hydrilla	Test Plant	Hydrilla
Allismataceae								
<i>Echinodorus cordifolius</i> (L.) Griseb.	Upright burhead	B	18	18	0	28	0	11NS
	Upright burhead	C	5	30	0	40NS	0	27NS
<i>Sagittaria Latifolia</i> Willd.	Common arrowhead	A	30	30	0	57	0	13NS
<i>Sagittaria rigida</i> Pursh.	Deep water duck potato	A	25	5	0	80	0	100
	Deep water duck potato	A	30	30	0	40	0	23
<i>Sagittaria</i> spp.	A broadleaf arrowhead	A	30	30	0	30	0	33
	A dwarf arrowhead	A	30	30	0	37	0	33
Apiaceae								
<i>Daucus carota sativus</i> (Hoffm.) Arcang.	Carrot	A	29	30	0	57	0	13NS
	Carrot	A	30	30	0	43	0	33
Sheet 1 of 7								
¹ Test symbol defined in Table 1. Florida hydrilla tubers used as controls in all tests. ² NS = difference between test plant and hydrilla not significant ($p > .05$); unmarked numbers are significantly different. ³ 1A = one male, 0.4%.								

Table 4 (Continued)								
Family and Species	Common Name	Test Symbol	Total Insects Tested		% Larvae Attacking		% Adults	
			Test Plant	Hydrilla	Test Plant	Hydrilla	Test Plant	Hydrilla
	Carrot	J	8	20	0	30NS	0	10NS
	Carrot	J	30	15	0	60	0	33
<i>Hydrocotyle umbellata</i> L.	Water pennywort	G	16	--	0	--	0	--
Aponogetonaceae								
<i>Aponogeton undulatus</i> Roxb.	Aponogeton	A	30	30	0	53	0	37
Araceae								
<i>Colocasia esculenta</i> (L.) Schrad.	Taro	A	31	29	0	45	0	38
Brassicaceae								
<i>Brassica napus</i> L.	Rutabaga	A	30	30	0	57	0	13NS
<i>Brassica rapa</i> L., Rapifera Group	Tumip	A	30	30	0	47	0	30
<i>Raphanus sativus</i> L.	Radish	A	30	15	0	67	0	67
	Radish	A	30	30	0	43	0	33
Sheet 2 of 7								

Table 4 (Continued)								
Family and Species	Common Name	Test Symbol	Total Insects Tested		% Larvae Attacking		% Adults	
			Test Plant	Hydrilla	Test Plant	Hydrilla	Test Plant	Hydrilla
Cannaceae								
<i>Canna edulis</i> Ker	Canna	A	30	30	0	40	0	27
Chenopodiaceae								
<i>Beta vulgaris</i> L.	Beets	A	30	30	0	43	0	33
Convolvulaceae								
<i>Ipomoea batatas</i> (L.) Lam.	Sweet potato	A	30	30	0	47	0	30
Cyperaceae								
<i>Cyperus distinctus</i>	Sedge	E	50	50	0	24	0	2NS
<i>Cyperus esculentus</i> L.	Chufa, Yellow nutsedge	A	50	50	0	24	0	20
	Chufa, Yellow nutsedge	I	50	25	0	16	0	12
<i>Scirpus fluviatilis</i> (Torr.) Gray	River bulrush	A	30	30	0	53	0	37
Equisetaceae								
<i>Equisetum</i> sp.	Horsetail	A	30	30	0	40	0	27

Table 4 (Continued)								
Family and Species	Common Name	Test Symbol	Total Insects Tested		% Larvae Attacking		% Adults	
			Test Plant	Hydrilla	Test Plant	Hydrilla	Test Plant	Hydrilla
Hydrocharitaceae								
<i>Egeria densa</i> Planch.	Egeria	L	235	—	—	—	1A	—
<i>Hydrilla verticillata</i> (L.f.) Royle	Hydrilla (in India)	A	88	—	—	—	60	—
	(Potomac R.)	A	33	35	45	49NS	21	29NS
	(Rhizome + Tub.)	F	32	30	53	30NS	31	27NS
	(Crown)	A	10	10	30	60NS	30	60NS
<i>Limnobium spongia</i> (Bosc) Steud.	Frogbit	A	30	30	0	73	0	47
<i>Vallisneria americana</i> Michx.	Water celery	A	30	30	0	37	0	33
	Water celery	A	18	18	6	67	9	22
	Water celery	A	30	29	0	45	0	38
Iridaceae								
<i>Iris</i> sp.	Iris, aquatic species	B	54	18	0	28	0	11

Sheet 4 of 7

Table 4 (Continued)								
Family and Species	Common Name	Test Symbol	Total Insects Tested		% Larvae Attacking		% Adults	
			Test Plant	Hydrilla	Test Plant	Hydrilla	Test Plant	Hydrilla
Nymphaeaceae								
<i>Nelumbo lutea</i> (Willd.) Pers.	American lotus	A	30	30	0	30	0	27
<i>Nuphar luteum</i> (L.) Sibth. & Sm.	Spatterdock	A	30	30	0	30	0	27
<i>Nymphaea</i> sp.	Waterlily	A	30	30	0	30	0	27
<i>Nymphaea tuberosa</i> Paine	White waterlily	A	25	5	0	80	0	100
Poaceae								
<i>Oryza sativa</i> L.	Rice	A	30	30	0	67	0	53
	Rice	E	50	50	0	24	0	2NS
Polygonaceae								
<i>Polygonum amphibium</i> L.	Water smartweed	A	30	30	0	57	0	13NS
Pontederiaceae								
<i>Pontederia cordata</i> L.	Pickereelweed	A	30	30	0	40	0	27
<i>Sheet 5 of 7</i>								

Table 4 (Continued)										
Family and Species	Common Name	Test Symbol	Total Insects Tested		% Larvae Attacking		% Adults			
			Test Plant	Hydrilla	Test Plant	Hydrilla	Test Plant	Hydrilla		
Potamogetonaceae										
<i>Potamogeton nodosus</i> Poir	American pondweed	J	10	20	0	30NS	0	10NS		
	American pondweed	A	90	90	6	52	0	13		
	American pondweed	A	30	30	0	40	3	23		
	American pondweed	A	60	60	7	43	3	43		
	American pondweed	A	60	60	0	48	0	10		
	American pondweed	A	30	29	0	45	0	38		
<i>Potamogeton pectinatus</i> L.	Sago pondweed	A	30	30	3	40	0	23		
	Sago pondweed	J	10	20	60	30NS	0	10NS		
	Sago pondweed	A	30	30	10	43	0	33		
	Sago pondweed	A	30	29	10	45	0	38		
Solanaceae										
<i>Solanum tuberosum</i> L.	White potato	A	30	30	0	47	0	30		

Sheet 6 of 7

Table 4 (Concluded)								
Family and Species	Common Name	Test Symbol	Total Insects Tested		% Larvae Attacking		% Adults	
			Test Plant	Hydrilla	Test Plant	Hydrilla	Test Plant	Hydrilla
	Red potato	A	30	30	0	47	0	30
Typaceae								
<i>Typha latifolia</i> L.	Common cattail	A	30	30	0	53	0	37
Zingiberaceae								
<i>Zingiber officinale</i> Roscoe	Ginger	A	30	30	0	40	0	27
Sheet 7 of 7								

Table 5
Summary of Feeding Tests with *Bagous affinis* Larvae in Clay Vases

Test Plant ¹	Test Symbol ²	Total Replicates	No. Plant Parts ³ Tested (Initial)		% Plant Parts Attacked		% Total Plant ⁴ Parts Attacked		No. Mature ⁵ Stages	
			Test	Hydrilla	Test	Hydrilla	Test	Hydrilla	Test	Hydrilla
No-Choice Vases⁶										
Water celery	N	1	2(15)	79(100)	50	52	--	--	0	96
	M	1	5	45(50)	40	93	--	--	1	102
	M	1	2(5)	60	100	15	--	--	0	3
	O	1	3(6)	44(50)	66	14	--	--	0	5
	M(a)	2	8(17)	72	38	85	--	--	2	127
	M	3	22(27)	151	27	95	--	--	1	572

Sheet 1 of 3

¹ Scientific names listed in Table 4 except Richardson's Pondweed, *Potamogeton richardsonii* (A. Bann.) Rydb.; gladiolus, an ornamental hybrid *Gladiolus* sp.; caladium, *Caladium bicolor* (Ait.) Vent.

² Test symbol defined in Table 1. () indicates that "no-choice" and "choice" vases with same letter were companions in the same test.

³ Number of plants parts in good condition when checked for attack (number of plant parts at initiation of test). Difference is due to plants rotting or drying; volume of test plant parts and hydrilla tubers at initiation approximately equal.

⁴ $\frac{\text{Number Plant Parts Attacked (test or hydrilla)}}{\text{Number Test Plant Parts Attacked} + \text{Number Hydrilla Turions Attacked}} \times 100$

⁵ Number of mature larvae, pupae, and adults present when the test was terminated; (a) indicates adults only; these tests were held longer than others to recover maximum number of adults.

⁶ One vase with test plant parts and one with hydrilla tubers per replicate. Some tests also had an additional vase per replicate with mixed test plant parts and hydrilla tubers.

Table 5 (Continued)										
			No. Plant Parts Tested (Initial)		% Plant Parts Attacked		% Total Plant Parts Attacked		No. Mature Stages	
Test Plant	Test Symbol	Total Replicates	Test	Hydrilla	Test	Hydrilla	Test	Hydrilla	Test	Hydrilla
American pondweed	N	1	15	79(100)	0	52	--	--	0	96
	M(b)	3	27(33)	149	0	64	--	--	0	137a
Sago pondweed	M(c)	3	29(34)	117	52	96	--	--	3	586
Richardson pondweed	M(d)	3	7(42)	148	0	93	--	--	2a	241a
Choice Vases¹										
Water celery	M(a)	2	13(16)	73	8	67	2	98	0	87
American pondweed	M	3	27	238	7	20	4	96	0	26
	M(b)	3	28(33)	149	0	66	0	100	4a	152a
Sago pondweed	M(c)	3	34	125	26	83	8	92	3	441
Richardson pondweed	M(d)	3	12(42)	146	0	75	0	100	0	171a
A dwarf arrowhead	M	3	6	174	17	70	1	99	0	167
Red potato	P	2	4	70	0	71	0	100	0	34
Taro	P	2	5	70	0	71	0	100	0	34
<i>Sheet 2 of 3</i>										
¹ Test plant parts and hydrilla tubers exposed together—one vase per replicate or in some tests combined with two single plant vases per replicate.										

Table 5 (Concluded)										
			No. Plant Parts Tested (Initial)		% Plant Parts Attacked		% Total Plant Parts Attacked		No. Mature Stages	
Test Plant	Test Symbol	Total Replicates	Test	Hydrilla	Test	Hydrilla	Test	Hydrilla	Test	Hydrilla
Gladiolus	P	2	5	70	0	71	0	100	0	34
Caladium	P	2	5	70	0	71	0	100	0	34

Sheet 3 of 3

greatly reduced. The successful production of a few individuals on water celery and egeria was not surprising since they are in the same family as hydrilla. All attempts to produce a second generation with the small numbers of adults on these test plants were unsuccessful.

Several tests with hydrilla material other than Florida tubers are also reported in Table 4. One test was conducted by these authors at the Commonwealth Institute of Biological Control Station, Bangalore, India, using the quarantine techniques of these authors with eggs from field-collected females and with field-collected tubers. More adults were produced than in most of the Florida tests, but the percentage was still within the range obtained in Florida. Florida tubers with attached rhizomes, crowns without attached tubers, and tubers from the Potomac River were compared at various times with Florida tubers. There were no significant differences ($P > .05$).

Adult feeding tests

Adult feeding tests are reported in Tables 6-8. Thirty-nine species, including both aquatic and crop plants, in 23 families were tested. All aquatic plants were tested outside of water, since the weevils fed little in water. Even when placed with submersed hydrilla, most weevils crawled out of the water and remained exposed. Only nibbling to moderate feeding was observed on plants outside the family Hydrocharitaceae. The heaviest of that feeding--50 to 60 percent of the control--was on sago pondweed tubers. The plants related to hydrilla that were heavily eaten were water celery, frogbit, and egeria.

Oogenesis tests

Newly emerged females produced small numbers of viable eggs on water celery, egeria, and elodea, with oviposition periods shorter than on hydrilla (Table 9). Although feeding was not measured in the oogenesis tests, little feeding on common cattail and leaf lettuce was observed. Mean egg production on hydrilla in these tests was small, apparently because some females generated wing muscles at the expense of eggs. Fully developed wing muscles were observed during dissections. These tests demonstrated the ability of females to produce eggs on species of Hydrocharitaceae other than hydrilla, but they may have underestimated the potential fecundity of *B. affinis* on those species.

Table 6
Summary of No-Choice Feeding Tests with *Bagous affinis* Adults

Family and Species	Common Name	Plant ¹ Structure	Test ² Symbol	Total Replicates	Total Adults	Feeding ³
Allismataceae						
<i>Sagittaria</i> sp.,	Dwarf arrowhead	Cr	M	5	25	0
Apiaceae						
<i>Hydrocotyle umbellata</i> L.	Water pennywort	Lv,St,Sto	M	5	25	++
Araceae						
<i>Pistia stratiotes</i> L.	Waterlettuce	P1	M	5	25	+ *
Asteraceae						
<i>Lactuca sativa</i> L.	Leaf lettuce	Lv	L	5	25	+ *
Brassicaceae						
<i>Brassica oleracea</i> L.	Broccoli	P1	M	5	25	0
Sheet 1 of 4						
¹ Cr = Crown, Lv = Leaves, St = Stem, Sto = Stolon, P1 = Entire plant, Fr = Fruit, Rt = Root, Tu = Tuber. ² Test symbols are defined in Table 2. ³ 0 = no feeding, + = nibbling, <2 percent of control, ++ = 10 to 40 percent of control, +++ = 41 to 65, ++++ = 66 to 99, +++++ = equal to or greater than control; * differs significantly ($p \leq .05$) from feeding on companion hydrilla control.						

Table 6 (Continued)						
Family and Species	Common Name	Plant Structure	Test Symbol	Total Replicates	Total Adults	Feeding
<i>Nasturtium officinale</i> R. Br.	Watercress	Lv,St	M	5	25	0
<i>Raphanus sativus</i> L.	Radish	P1	I	1	8	0
Chenopodiaceae						
<i>Beta vulgaris</i> L.	Beet	P1	I	1	8	0
Cucurbitaceae						
<i>Cucumis melo</i> L.	Musk melon	Fr	J	5	25	0
<i>Cucumis melo</i> L.	Honeydew	Fr	J	5	25	0
<i>Cucurbita pepo</i> L.	Zucchini	Fr	K	5	25	0
<i>Cucurbita pepo</i> L.	Yellow squash	Fr	K	5	25	0
Cyperaceae						
<i>Eleocharis coloradoensis</i> (Britt.) Gilly	Dwarf spikerush	P1	M	5	25	0
Hydrocharitaceae						
<i>Egeria densa</i> Plauch	Egeria	Lv,ST	M	5	25	+++++
<i>Elodea canadensis</i> Michx.	Elodea	Lv,St	M	5	25	++ *

Table 6 (Continued)						
Family and Species	Common Name	Plant Structure	Test Symbol	Total Replicates	Total Adults	Feeding
<i>Limnobium spongia</i> (Bosc) Steud.	Frogbit	Cr	R	3	78	+ *
<i>Vallisneria americana</i> Michx.	Water celery	Cr	L	5	25	++
Juncaceae						
<i>Juncus elliotii</i> Chapm.	Rush	Cr	S	3	30	0
Nymphaeaceae						
<i>Nelumbo nucifera</i> Gaertn.	Sacred lotus	Rt,St	M	5	25	0
<i>Nuphar luteum</i> (L.) Sibth. & Sm.	Spatterdock	Cr	M	5	25	0
Polygonaceae						
<i>Polygonum punctatum</i> Ell.	Dotted smartweed	Lt,St	M	5	25	0
Pontederiaceae						
<i>Eichhornia crassipes</i> (Mart.) Solms	Waterhyacinth	P1	M	5	25	0
Potamogetonaceae						
<i>Potamogeton nodosus</i> Poir.	Longleaf pondweed	Lv,St	M	5	25	++
<i>Potamogeton pectinatus</i> L.	Sago pondweed	St,Tu	M	6	30	+++
Sheet 3 of 4						

Table 6 (Concluded)

Family and Species	Common Name	Plant Structure	Test Symbol	Total Replicates	Total Adults	Feeding
Scrophulariaceae						
<i>Bacopa</i> sp.	Waterhyssop	Lv,St	M	5	25	0
Solanaceae						
<i>Capsicum annum</i> L.	Green pepper	P1	M	5	25	0
<i>Lycopersicon esculentum</i> Mill.	Small fry tomato	P1	M	4	20	0
<i>Solanum melogena</i> L.	Eggplant	P1	M	5	25	+ *
Sheet 4 of 4						

Table 7
Summary of No-Host Multichoice Feeding Tests with *Bagous affinis* Adults

Test Plant ¹	Plant Structure ²	Test Symbol ³	Total Replicates	Total Insects	Feeding ⁴
Test 1		C	3	33	
Potato (red)	Rt				0
Potato (white)	Rt				0
Carrot	Rt				0
Sweet potato	Rt				0
Turnip	Rt				0
Radish	Rt				+
Beet	Rt				0
Test 2		C	3	33	
River bulrush	Rt				0
A dwarf arrowhead	Cr				+
Sago pondweed	Tu				+
Common cattail	Cr				0
Water celery	Cr				+++++
Spatterdock	Rt				0
American pondweed	Rt				0
Test 3		G	2	103	
Banana	Lv				+
Canna	Lv				0
Ginger	Lv				0
Lily	Lv				0
Iris	Lv				0
Pickeralweed	Lv				++
Test 4		H	2	60	
Portulaca	Lv,St				0
Wandering Jew	Lv,St				0
Crassulacæae	Lv,St				0
Rice	Lv,St				0

(Continued)

¹ Test plants in each test exposed together. Scientific names listed in Tables 4 and 6 except for the following: Upright burhead, *Echinodorus cordifolius* L. (Griseb.) (Alismataceae); celery, *Apium graveolens* L. var. *dulce* (Mill.) Pers. (Apiaceae); cabbage, *Brassica oleracea* L. Capitata Group (Brassicaceae); Wandering Jew, *Zebraea pendula* Schnizl. (Commelinaceae); Crassulacæae, unidentified ornamental species; green beans, *Phaseolus vulgaris* L. (Fabaceae); iris, ornamental *Iris* sp. (Iridaceae); lily, ornamental *Lilium* sp. (Liliaceae); banana, *Musa X paradisiaca* L. (Musaceae); curly dock, *Rumex crispus* L. (Polygonaceae); smartweed, *Polygonum densiflorum* Meissner (Polygonaceae); portulaca, ornamental *Portulaca* sp. (Portulacaceae).

² Plant structures are defined in Table 6.

³ Test symbols are defined in Table 2.

⁴ Feeding code is defined in Table 6. Only Tests 1 and 2 had companion hydrilla controls. There was no significant difference between feeding on water celery and hydrilla, but the other three species in these two tests were eaten significantly less than hydrilla. A standard hydrilla feeding rate of 0.29 mm³/insect/day, which was obtained in another test conducted during the same time period, was used for comparison in Tests 3-12.

Table 7 (Concluded)					
Test Plant	Plant Structure	Test Symbol	Total Replicates	Total Insects	Feeding
Test 5 Canna Rice Pickerelweed	Lv Lv,St Lv	F	1	29	+ + 0
Test 6 Green beans Cabbage Radish (red) Sweet potato Turnip Celery Carrot Potato (white) Zucchini Leaf lettuce	Fr Lv Lv,Rt Rt Rt Lv,St Rt Rt Fr Lv	G	1	200+	0 0 0 0 0 0 0 0 + +
Test 7 Spatterdock Deep water duck potato Common cattail Curly dock Smartweed	Lv Lv Lv Lv Lv,St	F	1	48	0 0 + 0 0
Test 8 Upright burhead Common cattail Smartweed	Lv Lv Lv,St	F	1	38	0 0 0
Test 9 Smartweed Frogbit Sedge	Lv,St Cr Lv	F	1	58	0 +++++ 0
Test 10 Curly dock Frogbit	Lv Lv	F	1	34	0 0
Test 11 Curly dock Waterlettuce	Lv Lv	F	1	8	0 0
Test 12 Common cattail Smartweed Frogbit Sedge	Lv Lv,St Lv Lv	F	1	24	<+++ 0 0 0

Table 8
Summary of Multichoice With-Host Feeding Tests with *Bagous affinis* Adults

Test Plant ¹	Plant Structure ²	Test Symbol ³	Total Replicates	Total Insects ⁴	Feeding ⁵
Experiment 1					
Carrot	Rt	B	1	N/C	0
Turnip	Rt	B	1	N/C	0
Radish	Rt	B	1	N/C	0
Peanut	Rt	B	1	N/C	0
Taro	Rt	B	1	N/C	0
Lettuce	Lv	B	1	N/C	+
Sacred lotus	Rt	B	1	N/C	0
Experiment 2					
Water celery	Cr	D	3	63	++++
Experiment 3					
Water celery	Cr	E	6	60	++++
Experiment 4					
Frogbit	St,Cr	P	3	30	++++
Egeria	Lv,St	P	3	30	++++ *
Elodea	Lv,St	P	3	30	++
Water celery	Cr	P	3	30	+++
Experiment 5					
Frogbit	St,Cr	Q	10	20	++
Egeria	Lv,St	Q	10	20	++
Elodea	Lv,St	Q	10	20	++
Water celery	Cr	Q	10	20	+++
Experiment 6					
Sago pondweed	St,Tu	M	6	30	+++

¹ Scientific names listed in Table 4 or Table 6.

² Plant structures defined in Table 6.

³ Test symbols defined in Table 2.

⁴ N/C = Not Counted, ca. 100 to 200 in lab colony.

⁵ Feeding code defined in Table 6. Control feeding not estimated in Test 1, + indicates nibbling on lettuce. * indicates significantly different ($p \leq .05$) from control. Feeding on egeria in Experiment 4 was greater than on control.

Table 9
Summary of Oogenesis Tests with *Bagous affinis*

Test Plant ¹	Test Symbol ²	Total Replicates	Total Females ³	Mean Eggs Female ⁴		% Hatching		Days to Last ⁵ Oviposition (X)	
				Test	Hydrilla	Test	Hydrilla	Test	Hydrilla
Frogbit	F	1	16	0	9.5	—	98	—	60
Water celery	F	1	21	0.6	9.5	77	98	37	60
Common cattail	F	1	14	0	9.5	—	98	—	60
Leaf lettuce	R	3	30	0a	7.8 ± 3.6b	—	—	—	42(38)
Egeria	S	3	30	1.9 ± 2.7a	20.9 ± 13.1b	96	100	28(17)	63(55)
Elodea	S	3	30	1.1 ± 0.7a	1.5 ± 1.5a	97	85	7-22 o	29 o

¹ Scientific names are listed in Tables 4 or 6.

² Test symbols are defined in Table 2. Duration of Test F was 66 days.

³ Total females at initiation of test; some females were dissected during the tests; frogbit, water celery, and common cattail had the same hydrilla control with 21 females.

⁴ Means in the same row followed by different letters are significantly different ($P \leq .05$).

⁵ (o) indicates test terminated by dissecting all females on Day 29 when elodea supply was exhausted; females on elodea had stopped ovipositing, but those on hydrilla were still ovipositing.

4 Conclusions

Drawdowns of lakes and canals successfully eliminate the aboveground portions of hydrilla, but the tubers survive extensive dry periods. The tubers germinate when the water returns, and hydrilla density may be even greater after a drawdown than before. *Bagous affinis* should be released to help reduce the density of tubers that survive these drawdowns. Laboratory tests indicated that this species presents little risk to other plants. Although it is possible that adults might feed a little in water celery or frogbit crowns and small numbers of larvae might develop in pondweed or water celery during the early stages of a drawdown, the minor damage to these nonhost plants should be of no consequence. Not only would aboveground portions of the plants desiccate during drawdown, but all these plant species reproduce also from seeds, which would be unaffected by *B. affinis*.

Pondweeds are heavily eaten by wildlife (Martin et al. 1951), and thus their populations have evolved to sustain large amounts of herbivory. Sago pondweed, one of the important wildlife species, produces an abundance of seed along with tubers. Interestingly, Harris and Marshall (1963) found that both it and Richardson's pondweed produced excellent seed crops during a drawdown in a Minnesota marsh. Both sago and American pondweed occur along with hydrilla in India, but they are apparently not attacked by *B. affinis*.

It seems unlikely that *B. affinis* would be able to establish field populations in cold northern areas, perhaps not even in northern Florida. The absolute minimum temperature near Lahore, Pakistan, at the northern limit of the known range of *B. affinis* is -2.2°C , and the mean daily minimum of the coldest month is 4.4°C (Walter and Lieth 1960). The lack of a seasonal dry period in most of the United States would also reduce the chances of establishment in most areas. In Pakistan, it was found near Lahore but not near Rawalpindi (Baloch, Sana-Ullah, and Ghani 1980) even though other hydrilla insects were found in both areas. The major climatic difference between the two areas appears to be a long (October-May) arid season at Lahore versus two short (October-November, April-May) arid seasons at Rawalpindi (Walter and Lieth 1960). It thus appears that this species will need to be managed in many areas much like the alligatorweed flea beetle in North and South Carolina. Weevils could be collected from rearing ponds in southern Florida or southern California and released in early summer in more northerly locations. They appear to

be readily attracted to black lights when in a migratory phase, and thus newly emerging adults might be easily trapped at the rearing ponds.

The establishment of a viable field population of *B. affinis* on one of the test plant species would appear to be almost impossible based upon laboratory studies. Although females produced eggs on test plants, small egg numbers combined with low larval success should result in a very small percentage of adults emerging at the same time in the field. There is no evidence from India or Pakistan, however, to suggest any field development on these plants attacked in the laboratory.

Hydrilla and other exotic species present a much greater risk to native aquatic plant populations through competition and displacement than do potential biocontrol agents through occasional nonhost feeding. Hydrilla invades and displaces quickly through vegetative reproduction similar to the manner that some exotic grasses have displaced native grasses. Most of the grasses observed daily throughout the country are exotics, not natives. Hydrilla infested 90 percent of the canals, 45 percent of the rivers, and 41 percent of the lakes surveyed in Florida during 1988 (Schardt and Nall 1989). Chemical controls are and will continue to be used in many economically important waterways, but they will not be practical for the majority of rivers and small lakes. In those waterways, hydrilla will dominate and will reduce plant diversity. Herbivorous fish can be used in some waterways, but their grazing on native plants will be much greater than the occasional nonhost feeding by insect biocontrol agents.

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13. ABSTRACT (Maximum 200 words) <p><i>Hydrilla verticillata</i> (L.f.) Royle (common name hydrilla) is a noxious aquatic plant introduced into the United States from Africa through the aquarium industry. Two reproductive structures that enable hydrilla to withstand extremely harsh weather conditions are turions or winter buds (dense clusters of apical leaves that are produced in the leaf axils, green and ovoid-conical shaped buds) and bubbl-like hibernacular structures, commonly, but incorrectly, called tubers (formed at the ends of stolons buried in the substratum). Tubers can remain dormant in the sediment for several years and remain viable. Hydrilla plants may be found in lakes, rivers, drainage and irrigation canals, ponds, and streams. Severe infestations of hydrilla can restrict boat traffic and interfere with fisheries and waterflow.</p> <p>Hydrilla is a nuisance adventive submersed aquatic plant that reproduces by fragmentation, tubers (which may remain dormant in the substrate for several years and yet remain viable), turions, as well as by seeds (in the monoecious variety). This plant is one of the most prolific problem aquatic plants in the United States, causing problems in many lakes and reservoirs with recreation and navigation. It is extremely difficult to control because of its varied methods of reproduction. Hydrilla is found in many southern states, California, and recently Virginia; it is removed in most cases by mechanical methods or by using herbicides. Mechanical removal tends to increase the</p> <p style="text-align: right;">(Continued)</p>				
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spread of hydrilla because of fragmentation; while herbicides are used in various places, a major concern exists for the environment and water quality.

This report has been prepared to consolidate information on the biology and host range studies of *Bagous affinis* Hustache (a tuber feeder on hydrilla). The results from this study showed that although the mean length for females grown in cultures was significantly larger than field-collected females, significant differences did not exist between males. In adult feeding preference tests on hydrilla stems, leaves, stem turions and tubers, stems were preferred 4:1 by newly emerged adults, while older adults fed only on the stems. In host specificity studies, *B. affinis* did feed on a few other species, however, not to any great extent; therefore, it does not appear likely that this insect will cause any major problems with native species.

Although drawdowns of lakes, canals, and reservoirs will eliminate the aboveground biomass of hydrilla, the tubers can withstand extensive periods of drought.

Based on laboratory studies, it appears unlikely that *Bagous affinis* could be established in the field in the cold northern areas of the United States and probably not even northern Florida. Because of the lack of a seasonal dry period in most areas of the United States, establishment of field populations seems unlikely. However, the insects could be reared in southern Florida and transported to sites during the early summer to aid in tuber reduction.