US Army Corps of Engineers

AQUATIC PLANT CONTROL RESEARCH PROGRAM

TECHNICAL REPORT A-88-10

# HOST SPECIFICITY AND BIOLOGY OF THE WEEVIL *NEOHYDRONOMUS PULCHELLUS* HUSTACHE, BIOLOGICAL CONTROL AGENT OF WATERLETTUCE (*PISTIA STRATIOTES* L.) IN FLORIDA

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November 1988
Final Report



Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

and

US Army Engineer District, Jacksonville
Jacksonville, Florida 32232-0019

Monitored by Environmental Laboratory
US Army Engineer Waterways Experiment Station
PO Box 631, Vicksburg, Mississippi 39181-0631

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

# REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified

1b. RESTRICTIVE MARKINGS:

2a. SECURITY CLASSIFICATION AUTHORITY:

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE:

3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for public release; distribution unlimited.

4. PERFORMING ORGANIZATION REPORT NUMBER(S):

5. MONITORING ORGANIZATION REPORT NUMBER(S): Technical Report A-88-10

6a. NAME OF PERFORMING ORGANIZATION: See reverse.

6b. OFFICE SYMBOL (If applicable):

7a. NAME OF MONITORING ORGANIZATION: USAEWES Environmental Laboratory

6c. ADDRESS (City, State, and ZIP Code): Gainesville, FL 32601

7b. ADDRESS (City, State, and ZIP Code): PO Box 631 Vicksburg, MS 39181-0631

8a. NAME OF FUNDING / SPONSORING ORGANIZATION: See reverse.

8b. OFFICE SYMBOL (If applicable):

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER:

8c. ADDRESS (City, State, and ZIP Code): Washington, DC 20314-1000; Jacksonville, FL 32232-0019

10. SOURCE OF FUNDING NUMBERS

| PROGRAM ELEMENT NO. | PROJECT NO. | TASK NO. | WORK UNIT ACCESSION NO. |
|---|---|---|---|
| | | | |

11. TITLE (Include Security Classification): Host Specificity and Biology of the Weevil *Neohydronomus pulchellus* Hustache, Biological Control Agent of Waterlettuce (*Pistia stratiotes* L.)

12. PERSONAL AUTHOR(S): Thompson, Catherine R.; Habeck, Dale H.

| 13a. TYPE OF REPORT | 13b. TIME COVERED | 14. DATE OF REPORT (Year, Month, Day) | 15. PAGE COUNT |
|---|---|---|---|
| Final report | FROM ______ TO ______ | November 1988 | 33 |

16. SUPPLEMENTARY NOTATION: Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

17. COSATI CODES

| FIELD | GROUP | SUB-GROUP |
|---|---|---|
| | | |
| | | |

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Biological control; Host specificity; *Neohydronomus pulchellus*; Waterlettuce; Weevil

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

Waterlettuce continues to be a problem plant in Florida waterways where it interferes with recreation and irrigation, impedes water flow, and causes water loss through transpiration. The roots of waterlettuce host several species of mosquito larvae, some species of which are vectors of disease. There is concern that current declines in waterhyacinth populations will be followed by a resurgence in waterlettuce populations.

Although some native natural enemies of waterlettuce have been found in Florida, these are not effective in controlling the weed. Waterlettuce has been successfully controlled in Australia with a weevil, *Neohydronomus pulchellus* Hustache, imported from South America. In the study reported herein, the weevil was imported from Australia and tested in quarantine against 34 plant species in 27 families in nonreplicated no-choice tests. Plants on which the weevils fed or oviposited during this test were retested in a replicated choice test.

(Continued)

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION: Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL:

22b. TELEPHONE (Include Area Code):

22c. OFFICE SYMBOL:

DD Form 1473, JUN 86 — Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

Unclassified

6a. NAME OF PERFORMING ORGANIZATION (Continued).

University of Florida, Institute of Food and Agricultural Sciences

8a. NAME OF FUNDING/SPONSORING ORGANIZATION (Continued).

US Army Corps of Engineers;
USAED, Jacksonville

19. ABSTRACT (Continued).

In no-choice feeding tests, the weevils fed only on other aquatic plants: two duckweed species, frogbit, golden club, mosquitofern, and waterfern. Feeding on the latter two species was slight. In no-choice oviposition tests, the weevils again laid eggs only on aquatic plant species, with the exception of one egg on the terrestrial panda plant. The aquatic plants were frogbit, mosquitofern, waterfern, and two species of duckweed. All of the eggs appeared to be the result of random or accidental oviposition. None were laid in plant leaf punctures as in waterlettuce. When the plants that were oviposited and fed upon were retested in choice tests, the weevils fed and oviposited exclusively on waterlettuce.

While maintaining the weevil colony, a number of aspects of their biology were observed, including larval feeding and mining behavior and patterns of plant damage and ensuing death. Methods of protecting waterlettuce cultures from other native enemies were developed.

The few plants fed or oviposited upon by the weevils during this project were noneconomic plant species generally considered undesirable by aquatic plant managers. The tests indicated that the weevils are sufficiently host-specific to waterlettuce and are unable to complete development on any other plant.

# PREFACE

This research was sponsored by the US Army Engineer District (USAED), Jacksonville, Jacksonville, FL, and the US Army Corps of Engineers (USACE), through the Aquatic Plant Control Research Program (APCRP) of the US Army Engineer Waterways Experiment Station (WES). The USACE Technical Monitor was Mr. E. Carl Brown.

The research described in this report was conducted through Specific Cooperative Agreement No. 58-7B30-3-586 between the US Department of Agriculture (USDA), Agricultural Research Service (ARS), South Atlantic Region (SAR), and the University of Florida (UF) Institute of Food and Agricultural Sciences (IFAS). This report was prepared by Drs. Catherine R. Thompson and Dale H. Habeck, UF, IFAS, Department of Entomology and Nematology. Principal investigators for the UF, IFAS, were Dr. Habeck and Dr. Joseph K. Balciunas, Fort Lauderdale Research and Education Center (FLREC). Principal investigator for the USDA was Dr. Ted Center, ARS, SAR, Aquatic Plant Management Laboratory. Mr. J. Michael Dupes, USAED, Jacksonville, served as point of contact for the work.

The research and data analyses were performed by the authors. Assistance with host-specificity testing and colony maintenance was provided by Mr. John Watts and Ms. Debbie Matthews, IFAS, Department of Entomology and Nematology. Mses. Judy Gillmore and Glinda Burnett, also of the Department of Entomology and Nematology, provided assistance in the study and report preparation. Dr. Gary Buckingham, ARS, provided plants for testing, and Dr. David Hall, IFAS, Department of Botany, identified or verified plant names. Ms. Chris Bennett, IFAS, Department of Entomology and Nematology, assisted in various quarantine procedures. Ms. Elizabeth Hall, also of the Department of Entomology and Nematology, prepared the weevil illustration. The report was edited by Ms. Jessica S. Ruff of the WES Information Technology Laboratory.

Weevils were provided by Commonwealth Scientific Industrial Research Organization scientists, T. Sands and R. Kassulke, in Brisbane, Australia. Mr. Alan Dray, IFAS, FLREC, was helpful in getting the shipments through quarantine and customs in Miami, FL.

This research was monitored at WES by Dr. Alfred F. Cofrancesco, Jr., of the Environmental Laboratory (EL), Environmental Resources Division (ERD), Aquatic Habitat Group (AHG). The study was conducted under the general

supervision of Dr. John Harrison, Chief, EL, and Dr. Conrad J. Kirby, Chief, ERD, and under the direct supervision of Mr. Edwin A. Theriot, Chief, AHG. Mr. J. Lewis Decell was Program Manager of the APCRP.

Commander and Director of WES was COL Dwayne G. Lee, EN. Technical Director was Dr. Robert W. Whalin.

This report should be cited as follows:

Thompson, Catherine R., and Habeck, Dale H. 1988. "Host Specificity and Biology of the Weevil *Neohydronomus pulchellus* Hustache, Biological Control Agent of Waterlettuce (*Pistia stratiotes* L.)," Technical Report A-88-10, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

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## CONVERSION FACTORS, NON-SI TO SI (METRIC) UNITS OF MEASUREMENT

Non-SI units of measurement used in this report can be converted to SI (metric) units as follows:

| Multiply | By | To Obtain |
|---|---|---|
| acres | 4,046.873 | square metres |
| gallons (US liquid) | 3.785412 | cubic decimetres |
| square feet | 0.09290304 | square metres |
| tablespoons | 0.00001478 | cubic metres |

# HOST SPECIFICITY AND BIOLOGY OF THE WEEVIL *NEOHYDRONOMUS PULCHELLUS* HUSTACHE, BIOLOGICAL CONTROL AGENT OF WATERLETTUCE (*PISTIA STRATIOTES* L.) IN FLORIDA

## PART I: INTRODUCTION

### Pest Status

1. Waterlettuce, *Pistia stratiotes* L., is a floating aquatic plant widely distributed throughout tropical and subtropical areas of the world. It is a serious problem in Southeast Asia, Africa, and India (Cook et al. 1974, Holm et al. 1977). In Southeast Asia it is estimated to be the third most noxious weed, following waterhyacinth and *Salvinia* (Bennett 1975). Waterlettuce is prominent in the Nile River, where it forms sudds, thick vegetative mats, which have claimed many lives (Holm et al. 1977). In India, the commonest method for waterlettuce control has been mechanical, in which the plants were dumped on the banks to dry. However, improper disposal methods resulted in faster dispersal of the weed (Mangoendihardjo 1983).

2. Despite its status as a minor aquatic weed in the southern United States, waterlettuce creates various problems in many waterways (Table 1). There is concern that the decline in waterhyacinth populations, resulting from waterhyacinth maintenance, herbicide treatments, and the effects of three introduced biological control agents, may be accompanied by waterlettuce invasion. Recent figures on waterlettuce indicate that 5,758 acres* of Florida lakes, ponds, rivers, and other waterways (Table 2) are infested, while an estimated 14,000 acres were treated with herbicides during 1986 (Schardt 1987).

3. Waterlettuce can be controlled with herbicides, but this is expensive and must be repeated. In mixed mats of waterhyacinth and waterlettuce treated with 2,4-D, waterlettuce can quickly take over, since it is poorly controlled by 2,4-D (Aurand 1982). In 1969, 1,610 acres of Florida waterways were infested with waterlettuce, and by 1976 there were 2,010 infested acres.

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* A table of factors for converting non-SI to SI (metric) units of measurement is presented on page 4.

The following year (1977), the US Army Corps of Engineers began chemical control of waterlettuce with diquat, which continues to be the most effective compound for waterlettuce control (Aurand 1982). By 1979 there were 3,500 acres of waterlettuce in 24 (of 67) Florida counties (Aurand 1982). In 1985 the plant reached a high of 7,349 acres (Schardt 1987).

4. Severe waterlettuce infestations can interfere with recreation and irrigation use, impede water flow, and cause water loss. The water lost through transpiration from a mat of waterlettuce has been estimated at six times greater than the water loss from a comparable area of open water (von Minden 1899). Although more recent estimates are not available, water loss from waterlettuce is thought to be considerable (Holm et al. 1977). This aquatic weed also can curtail the light available to phytoplankton and can kill submersed vascular plants underlying the mat. Several authors have reported low oxygen levels and reduced pH in Florida water bodies covered by waterlettuce mats (Yount 1963, Sculthorpe 1967, Attionu 1976).

5. Waterlettuce is detrimental in another way. Larvae and pupae of the mosquito genus *Mansonia* obtain their oxygen by attaching to waterlettuce roots. These mosquitoes are potential transmitters of malaria, encephalomyelitis, and rural filariasis (Holm et al. 1977). Lounibos and Escher (1985) found *Mansonia dyaria* Belkin, Heinemann and Page and *Mansonia titillans* (Walker) commonly breeding in association with waterlettuce. These two species comprised 95.9 percent of the 14 species of mosquitoes identified from nearly 46,000 specimens collected in emergence traps over waterlettuce. In a waterlettuce-covered phosphate pit in Polk County, Florida, the population of *Mansonia* larvae and pupae was estimated at 30 million per acre* of which 85 percent were *M. dyari* and 15 percent were *M. titillans*. *Mansonia titillans* is a ferocious biter of man, while, in Panama, *M. dyari* is the major link in the transmission of St. Louis encephalitis, the most common arbovirus affecting humans in Florida (Lounibos and Escher 1985).

6. In several areas of the world it has been demonstrated that, when waterlettuce is removed, populations of *Mansonia* mosquitoes are significantly reduced in that area (Holm et al. 1977). Bidlingmayer (1968) studied central-Florida mosquito populations on aquatic plants, including *P. stratiotes*. He

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* Personal Communication, 1987, C. Morris, Polk County Environmental Services, Bartow, FL.

found that *Mansonia* spp. adults were most abundant during the periods April-May and September-October. Mean numbers of larvae per trap (1 $ft^2$) from October through June were 112, 67, 95, 107, 44, 1, 5, 6, and 16. Dunn (1918) reported collecting 51 *Mansonia* larvae from the roots of one waterlettuce plant.

## Distribution

7. Waterlettuce has been in Florida for at least 200 years. The Bartrams found large numbers of the plants during their 1765 travels through Florida (Stuckey and Les 1984), and there is some debate whether the plant is a native species. However, Pliny referred to its use in Egypt in A.D. 77 for skin problems such as erysipelas and abrasions. In India, waterlettuce leaves boiled in coconut oil were also used on chronic skin problems, while a mixture of the leaves, sugar, and rosewater was taken for asthma or coughs and the leaves were used as a poultice for hemorrhoids (Sculthope 1967). Waterlettuce roots were used as a laxative or diuretic, and ringworms were treated by rubbing plant ashes into the scalp (Sculthorpe 1967). Such a large number of uses for the plant argues for its lengthy existence in several areas of the world. However, Holm et al. (1977) concluded that waterlettuce originated in Africa, since African plants readily produced seeds. North American plants were thought not to set seed (Godfrey and Wooten 1979), but waterlettuce seedlings and plants with seeds attached have recently been found in south Florida.*

8. Adding further fuel to the debate on the origin of *P. stratiotes* is evidence from host-specificity discoveries: many insects are associated with *Pistia* in South America, and a number are host specific, leading some researchers to consider South America to be the origin of waterlettuce (Cordo, DeLoach, and Ferrer 1981). In Southeast Asia, however, the noctuid moth *Namangana pectinicornis* Hampson is reported to be host specific on waterlettuce (George 1963, Suasa-ard and Napompeth 1976). It is generally accepted that host-specific insect-plant relationships evolve only over very long evolutionary time periods. If an insect is host specific on waterlettuce, this

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* Personal Communication, 1987, Ted Center, US Department of Agriculture, Aquatic Plant Management Laboratory, Fort Lauderdale, FL.

would indicate a long association between the insect and the plant and argues a long-term existence of waterlettuce in areas where such relationships exist.

9. Additional evidence has indicated that waterlettuce originated in the Old World. A fossil species, *Pistia sibirica* Dorofeev, was described from the Oligocene and Miocene periods of Western Siberia (Dorofeev 1955, 1958, 1963) and the Miocene period of the German Democratic Republic (East Germany) (Mai and Walther 1983). Still more recently, seeds of *P. sibirica* from the middle Miocene period were found in Denmark (Friis 1985). Thus, based on current information, it appears that *P. stratiotes* is a descendent of *P. sibirica* and originated in Eurasia some 65 million years ago.

## Native Natural Enemies

10. In Florida, some insects attack waterlettuce. The most damaging phytophagous insect that has been observed is the caterpillar *Samea multiplicalis* Guenee. Its larvae tunnel through the leaves and cause severe damage, particularly during autumn months. The *Samea* caterpillar also feeds on *Salvinia*, *Azolla*, and occasionally on waterhyacinth (Knopf and Habeck 1983; Habeck, Haag, and Buckingham 1985). The ability of *Samea* to control waterlettuce is heavily restricted by natural enemies in Florida. This moth was released in Australia as a biocontrol agent, but within 4 years its effectiveness was restricted by a fungus and three hymenopterous parasites (Thomas and Room 1986). Another moth that has been found in close association with waterlettuce is *Petrophila drumalis* (Dyar). This species appears to be limited to the southern half of Florida. The aquatic caterpillars feed on the root hairs, and their presence can be discerned by the absence of lateral hairs along the root. A third species sporadically common on waterlettuce is the water lily leafcutter, *Synclita obliteralis* (Walker) (the most common aquatic caterpillar in Florida). The caterpillars live within a portable case filled with air. They do not crawl very far beneath the water surface and may climb out of the water to feed and to cut leaf portions for their cases. This species is highly polyphagous and has been recorded from more than 40 plant species (Habeck, Haag, and Buckingham 1985).

11. A less common but more obvious caterpillar on waterlettuce is the yellow wooly bear, *Spilosoma virginica* (Fabricius) (D. H. Habeck, personal observation). This caterpillar may reach 50 mm in length and, despite its

common name, may be any color from dirty white to light to dark brown or brownish-yellow. If feeds on a wide variety of both aquatic and terrestrial plants (D. H. Habeck, personal observation).

12. Other natural enemies of waterlettuce include aphids and leafhoppers. The aphid *Rhopalosiphum nymphaeae* L. may occur in high numbers on waterlettuce. Both adults and nymphs suck plant sap and are found on many species of aquatic plants worldwide (Haag, Habeck, and Buckingham 1986). *Draeculacephala inscripta* Van Duzee, a green leafhopper with a pointed yellow head, is common on waterlettuce and other aquatic plants (Haag, Habeck, and Buckingham 1986). This leafhopper, as well as the aphids, may be of importance in transmitting viruses from plant to plant.

13. Several weevil species have also been collected from waterlettuce, including the waterhyacinth weevil and the duckweed weevil. These weevil collections were incidental collections, since none of these species feeds on waterlettuce. No *Neohydronomus pulchellus* weevils were found.

## Exotic Biological Control Agents

14. Waterlettuce problems have triggered searches for natural enemies in several regions of the world. In Java and Sulawesi, the most promising natural enemy is the noctuid moth *Proxenus hennia* Swinhoe (Mangoendihardjo et al. 1977). The moth was not able to survive on any plant except waterlettuce in tests of 44 plants belonging to 21 families (Mangoendihardjo et al. 1977). Unfortunately, the larva was parasitized by larvae of two different orders, a dipteran larva and a coleopteran larva, and laboratory larval mortality was 79 percent (Mangoendihardjo and Nasroh 1976). A second promising moth is *N. pectinicornis*. Biocontrol workers in Thailand count among their successes the exploitation of *N. pectinicornis* for control of *Pistia* (Napompeth 1982). They report that *N. pectinicornis* has replaced pesticides for waterlettuce control. They achieved control in 6 to 10 weeks with ca. 300 larvae (mixed sizes of instars) per square metre.

15. In South America, Bennett (1975) found 13 possible insect enemies on waterlettuce. He suggested the acridid grasshopper *Paulinia acuminata* (De Geer) as a possible biocontrol agent for *P. stratiotes* in the United States. Among the most promising of the South American insects were weevils (family Curculionidae). These included *Onychylis cretata* Oliv., *O.* nr.

*nigrirostris* (Boheman), *Ochetina bruchi* Hustache, *N. pulchellus* Hustache, and four species of *Argentinorhynchus*, particularly *A. bruchi* (Hustache) (Cordo and De Loach 1982).

16. In-depth studies of many of these insects, however, have revealed serious disadvantages. For example, studies of the weevil *A. bruchi* (Cordo et al. 1978) showed that it was large, caused heavy damage to waterlettuce, and had high fecundity (1,575 eggs/female). However, laboratory efforts to rear it failed. The fourth instar larvae fell into the water beneath the plants and drowned. Other problems encountered by Cordo et al. (1978) were cannibalism, eggs laid in highly exposed areas, rarity in the field, and high mortality from handling. DeLoach, DeLoach, and Cordo (1976) concluded, however, that the weevil *N. pulchellus* (Figure 1) looked promising. They found that there were three generations of *N. pulchellus* per year in Argentina and that populations reached 250 to 600 adults per square metre of waterlettuce (they calculated that 250 weevils/$m^2$ = 8 weevils/plant).

17. The first usage of *N. pulchellus* as a biocontrol agent was in Australia. Aston (1973) states that the first Australian waterlettuce appeared in the Northern Territory in 1946. Subsequently, other areas up to 153 km from the port of Darwin became infested, and in 1967 waterlettuce was reported as a pest in Queensland (Aston 1973). A large reservoir near Brisbane was covered with the weed, with the entire population originating from three plants (Aston 1973). Harley et al. (1984) reported that, in 1981, the Commonwealth Scientific and Industrial Research Organization (CSIRO) imported *N. pulchellus* into Australia. Following specificity testing, the weevils were released at five sites from March to November 1982. The initial release was 3,000 adults and larvae, followed by a June release of 1,000 adults and larvae. Six months later the weevils had spread through the entire *Pistia* infestation. In 20 months (March 1982-October 1983), infestations of waterlettuce had been reduced 100, 93, and 82 percent in the three reservoirs in which the weevils had been released (Harley et al. 1984).

## Host-Specificity Tests

18. DeLoach, DeLoach, and Cordo (1976) conducted preliminary host-specificity testing with *N. pulchellus* adults in Argentina. They tested 24 species of plants in 16 families in starvation tests (Table 3). The

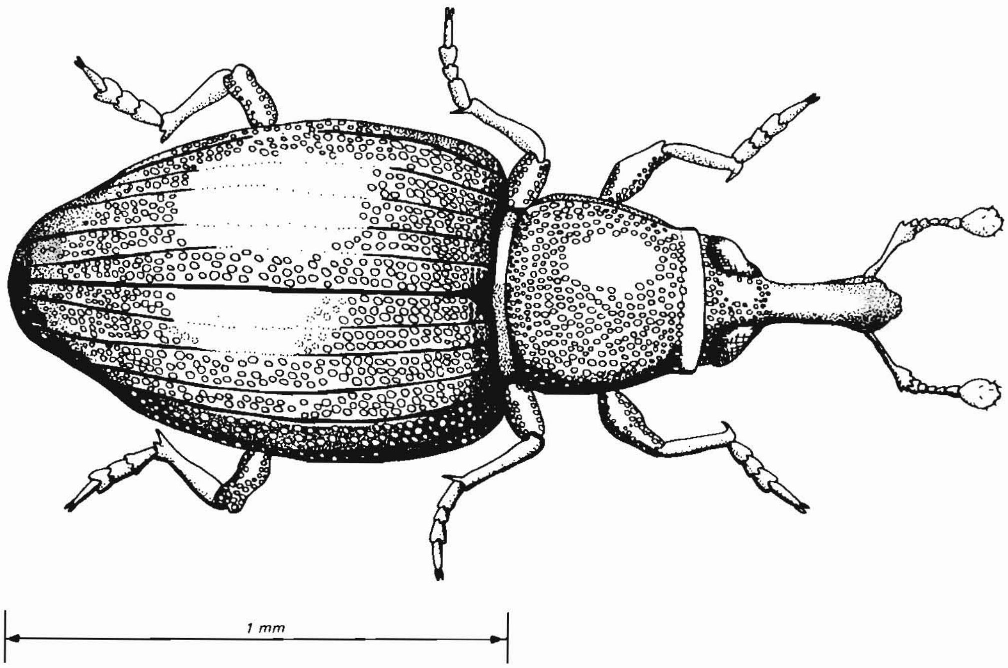


Figure 1. *Neohydronomus pulchellus* Hustache, biological control agent of waterlettuce

weevils fed heavily on waterlettuce, slightly on frogbit (*Limnobium stoloniferum*) (G. W. Meyer) Griseb., giant duckweed (*Spirodela polyrhiza*) (L.) Schleid, and duckweed (*Lemna* sp.). There was also very slight feeding on two species of dayflower (*Commelina coelestis* Willd and *C. virginica* L.), lettuce (*Lactuca sativae* L.), wandering jew (*Zebrina pendula* Schizl.), waterhyacinth (*Eichhornia crassipes* (Mart.) Solms), pickerel-weed (*Pontederia lanceolata* Nutt.) and *Reussia rotundifolia* (L.f.) Castellanos. The feeding on frogbit, giant duckweed, and duckweed occurred in the presence of waterlettuce.

19. Plants were also tested for oviposition. In normal oviposition, DeLoach, DeLoach, and Cordo (1976) report that eggs are inserted beneath the epidermis of the leaf, and the opening is then sealed with a dark substance. They observed this type of oviposition only on waterlettuce. A few eggs (one or two) were laid on the surface of *Sagittaria montevidensis* Cham. and Schlect., *Pontederia lanceolata, C. coelestis*, and *Oryza sativa* L.

20. Feeding tests in Australia included 36 plant species in 22 families exposed in choice tests with waterlettuce present (D. Sands and R. Kassulke, CSIRO, personal observation). In addition, four varieties of rice seedlings were exposed to the weevils in no-choice tests. No feeding or oviposition was observed on any plant other than waterlettuce.

21. Biological control of waterlettuce, while feasible, was not considered earlier partly because of the high cost of foreign exploration and other studies necessary to introduce a biological control agent. Australian scientists at CSIRO, however, introduced the weevil *N. pulchellus* from Brazil into Australia for control of waterlettuce. The weevils were tested in quarantine and released, and very successfully controlled waterlettuce (Harley et al. 1984). Reports from Australia* and South Africa** indicate that *N. pulchellus* has not been found or observed on any plants except waterlettuce. This success, coupled with the increasing abundance of waterlettuce populations in Florida, and the support and encouragement of the Aquatic Plant Control Research Program of the US Army Engineer Waterways Experiment Station and the US Army Engineer District, Jacksonville, provided the motivation to attempt biological control of waterlettuce in Florida.

* Personal Communication, 1986, D. Sands, CSIRO, Brisbane, Australia.
** Personal Communication, 1986, C. J. Cilliers, Plant Protection Research Institute, Pretoria, South Africa.

# PART II: METHODS AND MATERIALS

## Host Specificity

22. The first shipment of *N. pulchellus* from Australia was admitted into Gainesville quarantine in late September 1985. The Florida host-specificity studies were conducted in a quarantine facility with nonreplicated no-choice tests (Table 3). Thirty-four plant species representing 27 families were tested. Emphasis was placed on noneconomic plant species, since considerable testing of crop plants had already been done in Australia and Argentina. Individual plants were placed in plastic petri dishes (150 mm in diameter, 22 mm deep). The bottom was covered with a water-saturated, size 14 Hercules clarifying filter disc. Whole or partial plants were placed in the dish, usually with stem ends inserted into a hole in the filter disc. Ten unsexed adult weevils were placed in each dish, and the dishes were kept in the quarantine greenhouse where the weevil colony was being maintained. Each test lasted for 10 days. Every 3 to 5 days, the dishes were checked for signs of feeding and oviposition. The few dead weevils that were found were replaced with live ones. Most plants remained in good condition for several days. Plants that deteriorated were replaced as needed.

23. Duckweed species and *Salvinia* were tested similarly, but in small petri dishes (35 mm wide by 10 mm deep) containing 5 to 7 mm of water. Plants were checked before use to eliminate extraneous insects and damaged plants.

24. Plants on which feeding or oviposition had occurred in the no-choice tests were retested in choice tests. Whole plants (except for frog-bit where two leaves were used) were placed in petri dishes and then arranged at random in a plastic shoe box. Three boxes (replicates) were established and 25 weevils were placed in each box. The boxes were held in an incubator at 27° C and standard photoperiod (16 hr light, 8 hr dark) for 10 days. Boxes were checked every few days, and plant material was replaced as needed. After the experiment was terminated, all plants were examined under the microscope for signs of feeding and oviposition. Plant material removed before the end of the experiment was examined similarly.

## Weevil Rearing

25. All weevil adults were maintained in a screened cage on waterlettuce plants. The plants were removed weekly from the cage and examined for weevils. Weevils were counted, removed with soft-tip forceps, and placed into jars containing waterlettuce leaves. The plain tap water was changed, new plants of various sizes were placed in the cage, and the weevils were replaced by scattering them randomly over the foliage.

26. Plants containing weevil eggs were held in labeled trays containing plain tap water. New adults were placed in the parent colony. The quarantine greenhouse was maintained at 16° to 31° C. To counteract the extreme drying effect of the cooler system in the greenhouse, a waterhose was kept running 24 hr/day to wet the cement floor.

## PART III: RESULTS AND DISCUSSION

### Host-Specificity

27. Feeding and oviposition were always observed on waterlettuce (Table 4). The weevils fed on the duckweeds *Lemna minor* L., *Spirodela punctata* (Meyer) Thomps., and *S. polyrhiza*; frogbit; golden club (*Orontium aquaticum* L.); carolina waterfern (*Azolla carolininana* Willd.); and salvinia (*Salvinia minima* Baker). Feeding on the latter two species was very slight. Feeding on golden club was confined to the cut end of the petiole where a weevil made a small hole about 2 mm deep. The leaf was replaced with a new leaf, and no further feeding occurred. Feeding on duckweed was characterized by the presence of small holes in the dorsal surface of the leaves. The feeding on frogbit was restricted to the spongy tissue on the undersides of the leaves. Under natural conditions, the undersides of these leaves rest on the water surface. This would render the spongy tissues inaccessible to the weevils since it has been observed that the weevils cannot penetrate the water surface. When the weevils fall into water, they are initially buoyant and often float until their tarsi contact a surface on which to climb.

28. No attempt was made to quantify feeding damage in the tests. In most cases, weevils on plants other than those discussed above seemed disinterested in the plants and were found around the edges of the dishes. Sometimes they hid under the leaves even when they did not feed. The no-choice feeding tests in which the weevils were exposed to a plant other than waterlettuce for 10 days resulted in heavy delayed mortality (ca. 50 percent) of the weevils. Such high mortality shortly after completion of the feeding tests indicates nearly universal unsuitability of plants other than waterlettuce as food resources for *N. pulchellus*.

29. Eggs were observed on six plant species tested in addition to waterlettuce. An egg was found on the leaf of a panda plant, *Kalanchoe tomentosa* Baker, even though no feeding was observed. One egg was found on frogbit, one on mosquitofern, four on waterfern, and several on duckweeds. These eggs were the result of random oviposition; none was placed in a puncture or otherwise deposited in a normal way. The female weevil apparently merely dropped the eggs. The eggs on duckweed were placed in punctures on the dorsal surface of the leaf. However, because the leaves were so small, no egg

was ever inserted more than about one third of its width, leaving most of the egg exposed. Larvae would be unable to complete their development in leaves as small as duckweed. For that reason, *Wolfia* and *Wolfiella*, which have even smaller leaves, were not tested, although the study working group had recommended they be tested.

30. In retrospect, with the assistance of taxonomic botany, it is not surprising that the weevils fed on the duckweeds *L. minor* and *S. punctata*. Both these plants are in the family Lemnaceae. Botanists have long argued whether the Lemnaceae are derived from the aroids or Araceae, to which waterlettuce belongs (Sculthorpe 1967). The fact that the weevils feed on plants in both families is strong evidence for the close affinity of these two plant groups.

31. The plants, except *Kalanchoe tomentosa* and *O. aquaticum*, on which feeding or oviposition had occurred in the no-choice tests (Table 4), were retested in choice tests. The *Kalanchoe* and *Orontium* species had been retested in no-choice tests and were negative, indicating a truly nonspecific reaction of the weevils. No feeding or oviposition was observed on any plants except waterlettuce.

## Weevil Rearing

32. A number of observations concerning the biology of *N. pulchellus* have resulted from the rearing of ca. 20 generations of the weevil. *Neohydronomus pulchellus* is a small weevil. Males average slightly less and females slightly more than 2 mm long (DeLoach, DeLoach, and Cordo 1976). They vary considerably in color, ranging from brown to bluish-gray, often with color patterns like a checkerboard or concentric circles of light and dark on the abdomen. Adult feeding produces characteristic round holes in the leaves. These holes completely penetrate the thinner areas of the leaves near the apex but penetrate only one surface in the basal leaf areas where the leaves are thicker. Eggs are generally laid in punctures on the outer third of the leaves. Larvae hatch in 2 to 3 days between the leaf epidermises, eventually moving to the basal portion of the leaf. They complete development through three instars in approximately 20 days, then pupate within the leaves. Development from oviposition of the egg to adult emergence requires about a month.

33. The larvae of this weevil migrate, as they mature, along the inside of waterlettuce leaf ribs. Late instars often emerge from the leaves and wander on leaf surfaces, apparently searching for a pupation location. The last instar is easily identified both by its size and by the pronounced brown anal shield on an otherwise yellow body. Final instar larvae excavate a small pocket in the largest (basal) leaf rib areas or in the inflated leaf bases near the centers of the plants. Here they pupate with the last larval exuviae beside them. This area of the plant leaf is the last to sink, thereby affording pupae the longest possible air supply and protection.

34. In the laboratory it was necessary to keep heavily infested plants crowded together since, as the plants underwent increasingly more severe attack, the large leaves began to sink and die. Dying leaves were kept partially above water by suckers in the crown which grew larger. By crowding the plants, large dying leaves were kept afloat, resulting in higher pupal survival. Under natural conditions, waterlettuce plants are usually crowded together in thick mats that would keep a dying plant afloat unless the entire mat had been weakened. The general pattern of death of the mother plants and survival of suckers, followed by attack on those suckers before they are full-sized by a new generation of weevils, is the same pattern observed by Harley et al. (1984). Newly emerged weevils almost inevitably sought out the youngest foliage available. These were generally the suckers produced by dying plants.

35. In the quarantine greenhouse, some problems were experienced in keeping infested waterlettuce alive long enough to allow emergence of a weevil generation. The only way to save larvae when this occurred was to invert the infested old plants onto healthy new plants. Dessication time of the old plants was decreased by clipping off the roots before inverting them. A further observation that was made concerning *Pistia* was that two varieties, or at least two morphological forms of the plant, were noticed in the greenhouses. One form has flat, large rosettes, with relatively small floats and limited pubescence. The other form is generally darker green, smaller, more pubescent, and has larger floats. There may be at least four (Hooker 1851) or as many as nine varieties of waterlettuce (Neal 1965). The weevils attacked both plant forms; however, rearing of the hairier variety was not attempted in this study because it is more difficult to recover weevils from these plants.

36. Two people searching for approximately 3 hr can examine about 4 $m^2$ of waterlettuce for 500 weevils. The adult insects take advantage of overlapping leaves, particularly leaf edges, to conceal themselves. More than 90 percent of the weevils recovered were found on the undersurface of the leaves, and many hide in the crevices formed between the bases of the leaf ribs. Weevils were seldom found on outer leaves, apparently because they prefer the younger leaves near the center of the plant. They have not shown any discernible preference for plants of a certain age or size. Occasionally they can be found in the flowers, and often in the curly edges of emerging new leaves. They often employ a defense mechanism of dropping off a leaf or "playing dead" for up to a minute if disturbed.

37. In some experiments, numbers of weevils of known sex were needed. Sexing weevils morphologically is a time-consuming task involving some risk of damaging the insects. However, known quantities of each sex are quite easily obtained by collecting pairs in copula. Despite heavy handling of the adults, which involved movement to new plants weekly, mortality was relatively low. It averaged 10 percent per week in the laboratory colony. Adults lived an average of 3 months under the rearing conditions of the study.

38. Insects were much less likely to crawl out of a container if a piece of waterlettuce leaf was put in the bottom of the container with them. These insects gravitate to a host plant or host plant leaf extremely quickly and spend most of their time on ventral leaf surfaces.

39. The critical number of weevils needed to maintain a culture is seldom known with certainty, but fewer than 20 weevils in the 0.12-$m^3$ cages resulted in colony loss. During a Gainesville winter, the weevils required 6 weeks per generation in a temperature-controlled quarantine greenhouse (16° to 31° C), but ca. 4 weeks during the summer in a nonquarantine greenhouse where temperatures frequently exceeded 35° C. Weevil development was slowed by cool temperatures. When the greenhouse mean temperature was raised from 21° to 27° C, weevil production increased over 65 percent (adult numbers) in the following generation and remained at that level.

40. The plants in the rearing program were periodically beseiged by unwanted phytophagous organisms, including the moth *S. multiplicalis*, various snails, and aphids. *Samea* can be extremely destructive to plants containing immature weevils. It apparently causes the greatest problems not by competing with the weevil directly for plant material, but by consuming beetle larvae or

pupae while feeding on the leaves. Mechanical control was effective when moth densities were low, since the larvae leave obvious webbing where they are feeding on the plants. When densities increased, however, the microbial insecticide *Bacillus thuringiensis* was applied at the rate of 3 tbsp/gal. To ensure that this insecticide did not affect the weevils, treated plants were not placed in contact with weevils for 1 week following treatment. It has not been possible to find a satisfactory control for snails that would not adversely affect either the weevils or the *Pistia*; therefore, mechanical means are used to control these animals. However, successful control of the aphid *R. nymphae*, which can reach extremely high numbers on waterlettuce, has been achieved with the native wasp parasite *Lysiphlebus testaceipes* (Cresson). In a 21-$m^2$ experimental waterlettuce area, the aphid and parasite have maintained themselves at low numbers without additional attention.

41. Although the laboratory colony has continued to increase in numbers, production rates appear to be far below those of which the weevils are capable. If one assumes that the sex ratio is 50:50 and that a female lays one egg per day (DeLoach, DeLoach, and Cordo 1976), then a colony of 400 weevils would produce 200 eggs/day. That would be 1,400 eggs in the 7 days that weevils are retained on a batch of plants. Maximum production during this study was well below this theoretical amount, although a 24-hr production rate of 120 percent was obtained in one experiment. The factors that are responsible for the low weevil production are unknown. Thomas and Room (1986) discovered that nitrogen levels in the plants had to be raised to obtain satisfactory numbers of the weevil *Cyrtobagous salviniae* Calder and Sands for control of the aquatic weed *Salvinia*.

42. Additional shipments of weevils were received from Australia in February 1986 (455 live weevils) and April 1987 (496 live weevils). One shipment was also received from Hugo Cordo in Argentina in May 1986 (372 live weevils) and was held as a separate colony from the Australian stock. The Argentine colony, however, gradually declined and died in February 1987. Ten weevils were removed from each shipment and examined by an insect pathologist for microsporidia and nematodes using whole-body squashes viewed at 500 and 1,000X. No weevils infected with these organisms have been found.

## PART IV: CONCLUSIONS

43. The few plants on which the weevils fed during the studies reported herein are also considered to be undesirable by most aquatic plant managers. When offered a choice between these plants and waterlettuce, the weevils always fed on the waterlettuce. Oviposition tests indicated that these weevils are host specific on waterlettuce and are unable to complete development on any plant other than waterlettuce. In view of the host specificity exhibited in the field and under laboratory conditions in Argentina, Australia, and Florida, the weevil is safe to introduce into Florida.

44. Permission for field release of the weevils was obtained 14 November 1986. Since population numbers were low, the weevils were retained in a nonquarantine greenhouse to increase their number prior to field release. Based on the results of the Australians, waterlettuce reductions could be visible within a year. The weevil, with its 30-day life cycle, should produce six to eight generations per year in northern Florida, and perhaps more in southern Florida. Initial field release was made on 29 April 1987 in Lake Okeechobee. Sufficient numbers of weevils were retained to maintain a Gainesville colony and to allow a north-Florida release at a later date. Three shipments of weevils have been sent to start a weevil colony in south Florida for further releases in south Florida waterways.

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Table 1

The Distribution of Waterlettuce in Florida Waterways

| Water Body | 1982 | 1983 | 1984 | 1986 |
|---|---|---|---|---|
| Lakes | | | | |
| Number | 49 (26)* | 59 (28) | 61 (32) | 68 (33) |
| Acres | 2,104 (19) | 4,056 (18) | 6,342 (13) | 4,122 (22) |
| Rivers | | | | |
| Number | 17 (16) | 22 (19) | 21 (23) | 24 (23) |
| Acres | 1,238 (3) | 842 (9) | 265 (17) | 974 (7) |
| Canals | | | | |
| Number | 38 (6) | 37 (7) | 37 (7) | 35 (8) |
| Acres | 937 (7) | 765 (8) | 742 (8) | 662 (8) |
| Total | | | | |
| Number | 104 (18) | 118 (22) | 119 (24) | 127 (29) |
| Acres | 4,279 (12) | 5,663 (17) | 7,349 (14) | 5,758 (18) |

Source: Schardt 1987.
* Rankings are in parentheses.

Table 2

Acreage, County Distribution, and Problem Ranking of Water Lettuce Infestations in Florida

| Parameter | 1982 | 1983 | 1984 | 1986 |
|---|---|---|---|---|
| Total acreage | 4,298 | 5,663 | 7,349 | 5,758 |
| Overall rank | 12 | 17 | 14 | 18 |
| Total counties | 31 | N/A | N/A | N/A |
| Water bodies with problem populations | | | | |
| Severe | 0 | 2 | 4 | 6 |
| Moderate | 5 | 21 | 6 | 7 |
| Water bodies with waterlettuce | 104 | 118 | 119 | 127 |
| Rank | 18 | 22 | 24 | 29 |

Source: Schardt and Nall 1983, Schardt 1987.

Table 3

Plants Included in Host-Specificity Tests for Adult *N. pulchellus* Hustache in Argentina and Florida (Partially Adapted from DeLoach, DeLoach, and Cordo 1976)

| Family | Genus and Species | Common Name | Argentina | Florida |
|---|---|---|---|---|
| Alismataceae | *Sagittaria montevidensis* Cham and Schlecht. | California arrowhead | - | - |
| Amaranthaceae | *Alternanthera philoxeroides* (Mart.) Griseb. | Alligatorweed | - | - |
| Amaryllidaceae | *Agapanthus africanus* (L.) Hoffm. | African lily | | |
| Anacardiaceae | *Mangifera indica* L. | Mango | | - |
| Apiaceae | *Cicuta mexicana* Coult and Rose | Water hemlock | | - |
| | *Hydrocotyle ranunculoides* L. | Floating pennywort | - | - |
| | *Hydrocotyle umbellata* L. | Water pennywort | | - |
| Aracea | *Aglaonema* sp. | Aglaonema | | - |
| | *Arisaema dracontium* (L.) Schott | Green dragon | | - |
| | *Dieffenbachia* sp. | Dumb cane | | - |
| | *Orontium aquaticum* L. | Golden club | | + |
| | *Peltandra viginica* (L.) Schott and Endl. | Arrow arum | | - |
| | *Pistia stratiotes* L. | Waterlettuce | ++++ | ++++ |
| | *Spathiphylum* sp. | Spathe flower | | - |
| Asteraceae | *Bidens mitis* (Michx.) Sherff. | Beggar trick | | - |
| | *Lactuca sativa* L. | Lettuce | + | |
| Balsaminaceae | *Impatiens balsamina* L. | Impatiens | | - |

(Continued)

Notes: - = no feeding, + = very slight feeding, ++ = slight feeding, +++ = moderate feeding, and ++++ = heavy feeding.

(Sheet 1 of 3)

Table 3 (Continued)

| Family | Genus and Species | Common Name | Argentina | Florida |
|---|---|---|---|---|
| Brassicaceae | *Brassica oleracea* var. *capitata* L. | Cabbage | - | |
| | *Nasturtium officinale* R. Br. | Watercress | - | |
| Bromeliaceae | *Ananas comosus* (L.) Merr. | Pineapple | - | |
| Cannaceae | *Canna flaccida* Salisb. | Golden canna | | - |
| Commelinaceae | *Commelina coelestis* Willd. | Dayflower | + | |
| | *Commelina virginica* L. | Dayflower | - | |
| | *Tradescantia crassifolia* Cav. | Spiderwort | - | |
| | *Zebrina pendula* Schizl. | Wandering jew | + | |
| Convolvulaceae | *Ipomoea batatas* (L.) Lam. | Sweet potato | | - |
| Crassulaceae | *Crassula argentea* Thunb. | Jade plant | | - |
| | *Kalanchoe tomentosa* Baker | Panda plant | | - |
| Cyperaceae | *Scirpus californicus* (C. A. Mey) Steud. | Southern bulrush | - | |
| Haloragaceae | *Myriophyllum aquaticum* (Vell.) Verdc. | Parrotfeather | | - |
| Hydrocharitaceae | *Limnobium spongia* (Bosc.) Steud. | American frogbit | | ++ |
| | *Limnobium stoloniferum* (G. W. Meyer) Griseb. | Frogbit | ++ | |
| Lemnaceae | *Lemna minor* L. | Common duckweed | | ++ |
| | *Lemna* sp. | Duckweed | ++ | |
| | *Spirodela intermedia* Koch | Giant duckweed | ++ | |
| | *Spirodela punctata* (Meyer) Thomps. | Duckweed | | ++ |
| | *Spirodela polyrhiza* (L.) Schleid. | | | ++ |

(Continued)

Table 3 (Concluded)

| Family | Genus and Species | Common Name | Argentina | Florida |
|---|---|---|---|---|
| Onagraceae | *Ludwigia repens* Forst. | Floating water primrose | | - |
| | *Ludwigia uraguayensis* (Camb.) Hara | Uruguayan water primrose | | - |
| Poaceae | *Oryza sativa* L. | Rice | - | - |
| | *Saccharum officinarum* L. | Sugarcane | - | |
| Polygonaceae | *Polygonum densiflorum* Meisn. | Smartweed | | - |
| | *Rumex* sp. | Dock | | - |
| Pontederiaceae | *Eichhornia azurea* (Swartz) Kunth. | Anchored waterhyacinth | - | |
| | *Eichhornia crassipes* (Mart.) Solms | Waterhyacinth | + | |
| | *Pontederia cordata* L. | Pickerel-weed | | - |
| | *Pontederia lanceolata* Nutt. | Lanceolate-leaved pickerel-weed | + | |
| | *Reussia rotundifolia* (L.f.) Castellanos | -- | + | |
| Potamogetonaceae | *Potamogeton nodosus* Poir. | American pondweed | | - |
| Rosaceae | *Fragaria chiloensis* Duchesne var. *ananassa* Bailey | Strawberry | | - |
| Rutaceae | *Citrus paradisi* Macfed. | "Duncan" grapefruit | | - |
| Salviniaceae | *Azolla caroliniana* Willd. | Mosquitofern | | + |
| | *Salvinia minima* Baker | Waterfern | | + |
| Saururaceae | *Saururus cernuus* L. | Lizard's tail | | - |
| Solanaceae | *Lycopersicon esculentum* Mill. | Tomato | | - |
| Typhaceae | *Typha domingensis* Pers. | Southern cattail | | - |
| | *Typha latifolia* L. | Cattail | - | |

(Sheet 3 of 3)

Table 4

Plants Tested in Quarantine in Florida on Which Feeding or Oviposition by *N. pulchellus* Hustache Adults Occurred in a No-choice Test Situation and Subsequently in a Choice Test

| Plant Family | Plant Name | No Choice | | Choice | |
|---|---|---|---|---|---|
| | | Feeding* | Oviposition** | Feeding | Oviposition |
| Araceae | *Pistia stratiotes* L. | + | + | + | + |
| | *Orontium aquaticum* L.† | + | - | NT†† | NT |
| Crassulaceae | *Kalanchoe tomentosa* Baker† | - | + | NT | NT |
| Hydrocharitaceae | *Limnobium spongia* (Bosc.) Steud | + | + | - | - |
| Lemnaceae | *Lemna minor* L. | + | + | - | - |
| | *Spirodela punctata* (Meyer) Thompson | + | + | - | - |
| | *Spirodela polyrhiza* (L.) Schleid. | - | - | - | - |
| Salvinaceae | *Azolla caroliniana* Willd. | + | + | - | - |
| | *Salvinia minima* Baker | + | + | - | - |

* Feeding: *O. aquaticum* had hole 2 to 3 mm deep in broken petiole end; *Azolla* and *Salvinia* had slight feeding.

** Oviposition: appeared accidental or atypical on all except *Pistia*.

† No-choice test was repeated, and results for feeding and oviposition were negative.

†† Not tested.