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August 1997

Aquatic Plant Control Research Program

Waterlettuce Caterpillar, *Namangana pectinicornis* Hampson, for Biological Control of Waterlettuce, *Pistia stratiotes* L.

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Prepared for Headquarters, U.S. Army Corps of Engineers

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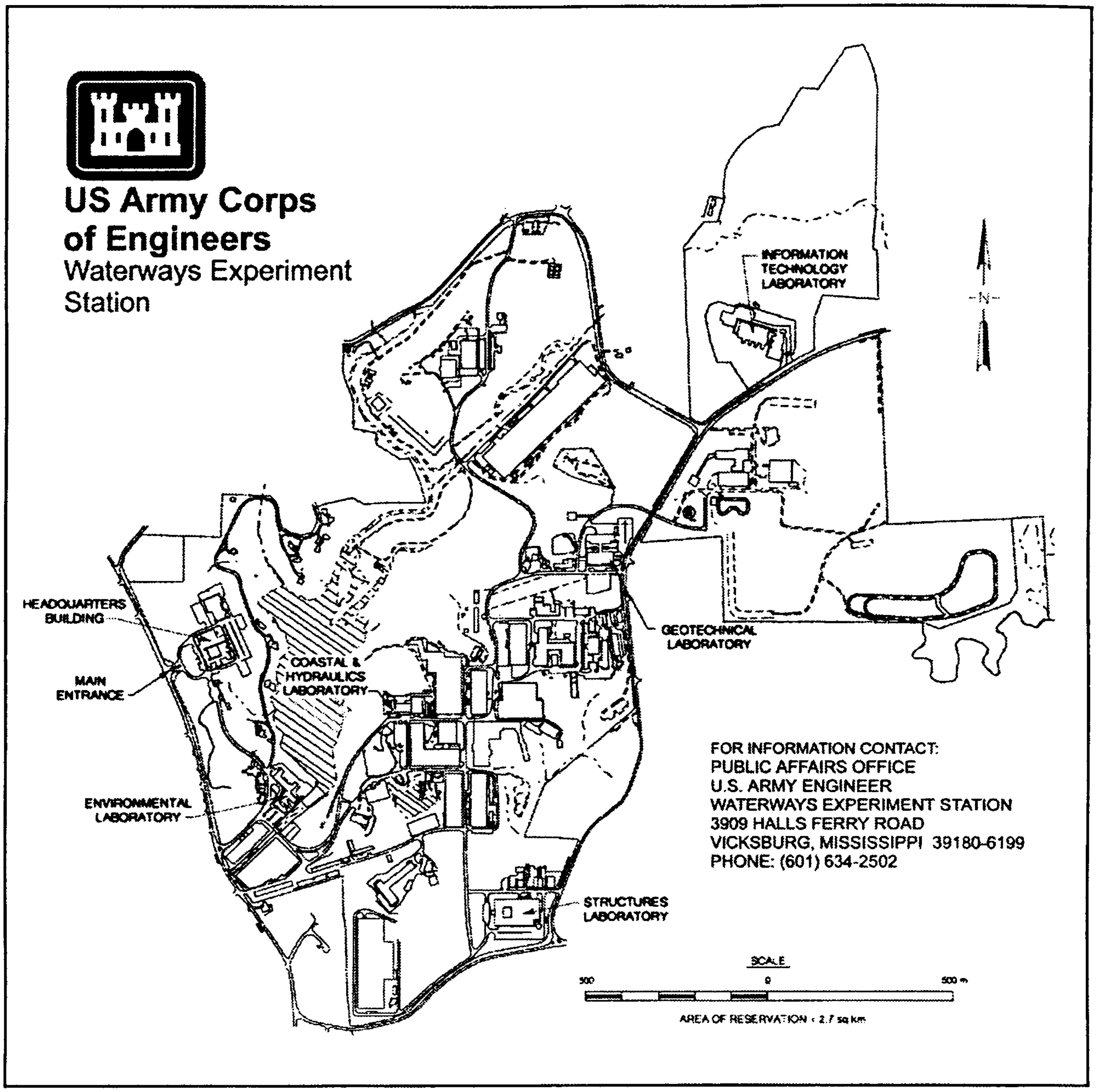

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Contents

Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 33028. 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 Center for Aquatic Plant Research and Technology (CAPRT), Dr. John W. Barko, Director. Mr. Robert C. Gunkel was Assistant Director for the CAPRT. Program Monitor during this study was Ms. Denise White, HQUSACE.

This report was prepared by Dr. Dale H. Habeck, University of Florida (UF), Institute of Food and Agriculture Services (IFAS), Department of Entomology and Nematology, and Ms. Catherine R. Thompson, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL. Principal Investigator was Dr. Habeck.

The research and data analyses were performed by the authors. Assistance with host-specificity testing and colony maintenance was provided by Ms. Lyvia Nong, Mr. Kip Malcolm, Ms. Debbie Matthews, and Mr. John Watts, UF, IFAS, Department of Entomology and Nematology. Ms. Judy Gillmore, UF, IFAS, Department of Entomology and Nematology, assisted in various ways, and Mr. Chris Faircloth, UF, IFAS, Department of Entomology and Nematology, assisted in the preparation of this report. Dr. Gary Buckingham, Agricultural Research Service, U.S. Department of Agriculture, provided some of the test plants. Ms. Chris Bennett, UF, IFAS, Department of Entomology and Nematology, assisted in various quarantine procedures. Moths were provided by Dr. Banpot Napompeth, National Biological Control Research Center, Kasetsart University in Bangkok, Thailand. Dr. Allen Dray, UF, IFAS, Fort Lauderdale Research and Education Center, was helpful in getting shipments through quarantine and customs in Miami.

The study was conducted under the direct supervision of Dr. Alfred F. Cofrancesco, Jr., Chief, Aquatic Ecology Branch, Ecological Research Division (ERD), EL, and under the general supervision of Dr. Conrad J. Kirby, Chief, ERD, and Dr. John Harrison, Director, EL.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin.

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1 Introduction

Pest Status of Waterlettuce

Waterlettuce, *Pistia stratiotes* L., a widely distributed floating aquatic plant, is a serious nuisance plant in Asia and Africa (Cook et al. 1974; Holm et al. 1977). In the southern United States, waterlettuce has generally been considered a minor problem; but in some areas of Florida, it is a serious problem. As a result of the introduction of three biological control agents, as well as maintenance herbicide treatments, there has been a general decline of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) in Florida. This decline in waterhyacinth has provided open water into which waterlettuce has moved. Schardt (1987) indicated that 2,331 ha (5,758 acres) of Florida water were infested with waterlettuce and that an estimated 5,668 ha (14,000 acres) were treated with herbicides. Chemical treatment of waterlettuce is effective but costly and must be repeated regularly for proper management. About four million dollars is spent annually in Florida to control waterlettuce.[1]

Severe waterlettuce infestations create a number of problems: (a) interference with fishing, boating, and other recreational uses; (b) reduction in net flow of water through impacted waterways (causing irrigation problems, among others); (c) water loss through transpiration (such water loss is six times greater from a waterlettuce mat than from open water according to Minden (1899)); (d) limitation of light available to submersed plants and phytoplankton; and (e) reduction in oxygen levels and pH (Yount 1963; Attionu 1976; Sculthorpe 1967).

Another harmful aspect of dense waterlettuce growth is the harborage of *Mansonia* mosquito larvae by the plant. Larvae and pupae attach their siphons to waterlettuce roots to obtain oxygen. *Mansonia* mosquitoes are major pests and potential vectors of several human disease pathogens. Two species of *Mansonia* occur in Florida: *M. dyari* Belkin, Heinemann and Page and *M. titillans* Walker. These two species composed 95.9 percent of the 14 species of mosquitoes identified from the 45,932 mosquitoes collected in emergence traps placed over waterlettuce in

[1] Personal Communication, 1996, Don Schmitz, Florida Department of Natural Resources, Tallahassee, FL.

St. Lucie County, Florida (Lounibos and Escher 1985). Removal of waterlettuce may significantly reduce the number of *Mansonia* mosquitoes (Holm et al. 1977).

Origin

Waterlettuce has been in Florida for at least 224 years, since the Bartrams found the plant to be plentiful during their 1765 travels through Florida (Stuckey and Les 1984), leading some to consider waterlettuce as native to Florida. However, Pliny in A.D. 77 reported medicinal uses for waterlettuce in Egypt (Sculthorpe 1967), and Holm et al. (1977) considered Africa to be the home of waterlettuce, since African plants produced seeds while American plants rarely did, indicating an absence of pollinators. Recently, however, waterlettuce seeds and seedlings have been found to be quite common in south Florida (Dray and Center 1989).

The abundant insect association with waterlettuce, including a number that are waterlettuce specific, in South America has caused some researchers to consider that continent the original home of waterlettuce (Cordo, DeLoach, and Ferrer 1981). In Asia, *Namangana pectinicornis* is reported to be host specific on waterlettuce (George 1963; Suasa-ard and Napompeth 1978). Host-specific insect-plant relationships evolve over long periods of time, indicating that those insects specific to waterlettuce have been associated with the plant for a long time.

A fossil waterlettuce species, *Pistia siberica* Dorofeev, has been 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). More recently, Friis (1985) found seeds of *P. siberica* from the middle Miocene in Denmark. It appears that *Pistia stratiotes* is a descendant of *P. siberica*; it originated in Eurasia some 65 million years ago; and it has been widely dispersed for a long time.

Biological Control Investigations

The search for natural herbivores of waterlettuce has been concentrated mainly in South America and Southeast Asia. Bennett (1975) found 15 insect herbivores of waterlettuce, including six weevil species; he suggested using the grasshopper *Paulinia acuminata* (DeGeer) for biological control of waterlettuce in the United States. Surveillance studies in Argentina identified the weevils of two *Onychylis* species, *Ochetina bruchi* Hustache, *Neohydronomus affinis* (reported as *pulchellus* Hustache), four species of *Argentinorhynchus* (DeLoach, DeLoach, and Cordo 1976; Cordo et al. 1978; and Cordo and DeLoach 1982), and the samea caterpillar *Samea multiplicalis* Guenee (DeLoach, DeLoach, and Cordo 1979). The most promising candidate as a potential biocontrol agent appeared to be *Neohydronomus affinis* Hustache (DeLoach, DeLoach, and Cordo 1976). The samea moth, *Samea multiplicalis*, was also investigated in Florida, where it is common on waterlettuce, *Salvinia minima* Baker, *Azolla caroliniana* Willd., and occasionally on water-hyacinth (Knopf and Habeck 1976).

George (1963) reported that *Namangana pectinicornis* Hampson was destructive to waterlettuce in India and did not feed on *Eichhornia speciosa*, *Salvinia auriculata*, or *Oryzae sativa* in the laboratory. Sankaran and Ramaseshiah (1974) reported that *N. pectinicornis* was widespread in India, destrucive to waterlettuce, and not known to feed on any other plants. In Indonesia, Mangoendihardjo and Nasroh (1976) found that newly hatched larvae of *Proxenus hennia* Swinton (= *Namangana pectinicornis* Hampson) starved rather than feed on any of the 26 alternate plant species tested. Later, Mangoendihardjo et al. (1977) reported that 44 species in 21 plant families were tested with similar results. Mangoendihardjo (1983) concluded that *N. pectinicornis* was a promising candidate for biological control of waterlettuce. Alam, Alam, and Ahmed (1980) reported that *Athetis* (= *Namangana*) *pectinicornis* was a natural biological control agent of waterlettuce and waterhyacinth in Bangladesh. Suasa-ard (1976) tested 74 plant species in 34 plant families and concluded that larvae could survive only on waterlettuce. The first report of control of an exotic weed with a native insect was reported by Napompeth (1982), who noted that the use of *N. pectinicornis* (as *Episammia pectinicornis*) had replaced herbicides to control waterlettuce, and that about 300 larvae (mixed instars) per square meter gave control in 2 to 6 weeks.

The weevil *Neohydronomus affinis* (as *pulchellus*) was introduced from Brazil into Australia where it was subsequently released and successfully controlled waterlettuce (Harley et al. 1984). In South Africa, *N. affinis* was released and provided control in the Pafuri area (Cilliers 1987). The weevil was introduced into quarantine in Florida in 1985 and released in south Florida in April 1987 (Thompson and Habeck 1989). *Neohydronomus affinis* has subsequently become established at all release sites, is increasing, and is beginning to have a visible impact on waterlettuce populations.

In 1986, the noctuid moth *Namangana pectinicornis* was introduced into quarantine in Florida. Initial and subsequent shipments have been provided through the kindness and generosity of Dr. Banpot Napompeth of the National Biological Control Research Center, Bangkok, Thailand. The moth has been called *Proxenus hennia* Swinhoe in Indonesian literature; it has also been placed in the genera *Episammia*, *Athetis*, and *Namangana*. The taxonomic confusion of this species is due to inadequate understanding of the distribution of Southeast Asian moths and perhaps, in part, to variations in the wing patterns of the adults.

Adult *N. pectinicornis* are quite variable although most are brown with few or no conspicuous markings. The hind wings are creamy-white. The moths have a wing span of about 16-20 mm, and the females, which are usually slightly larger than males, have filiform antennae (male antennae are pectinate). Adults lived 2-7 days in cages containing waterlettuce plants, spending most of the time on the underside of the leaves with mating and oviposition occurring at night. Eggs were laid in clusters on the upper and lower surface of the leaves. Each cluster was covered with fine hairs from the female's abdomen. The number of eggs per cluster averaged 94.3 ± 50.86 (Suasa-ard 1976). Eggs were very small averaging 0.0315 ± 0.01 mm in diameter (Suasa-ard 1976).

Hatching occurred 4-6 days following oviposition. The color of the eggs changed from yellow-green to light green and brown, and 24 hr before eclosion, red eyespots appeared. First instar larva were clear to light yellow in color and extremely mobile. First instar larvae fed on leaf hairs, then burrowed into the leaf surface, or they entered the leaf beneath the egg mass itself. The first larval instar time period was about 2 days.

The third and succeeding larval instars spent their time feeding externally or burrowing into the thicker parts of the leaf. Larger larvae in later instars moved toward the thick basal areas of leaves. The mature larvae (Figures 1 and 2), up to 25 mm long, pupated within the leaves parallel to the large leaf ribs, in the crowns, or in a hollowed-out leaf base. Larval development was completed in 17-20 days. Suasa-ard (1976) reported seven instars. Both larvae and pupae are characterized by conspicuous enlarged spiracles that extend outward from the body surface. The dorsal surface of new pupae remained bright green for approximately 24 hr; subsequently, the entire pupa gradually darkened to dark brown (Figure 3). The pupal stage lasted 4–7 days.

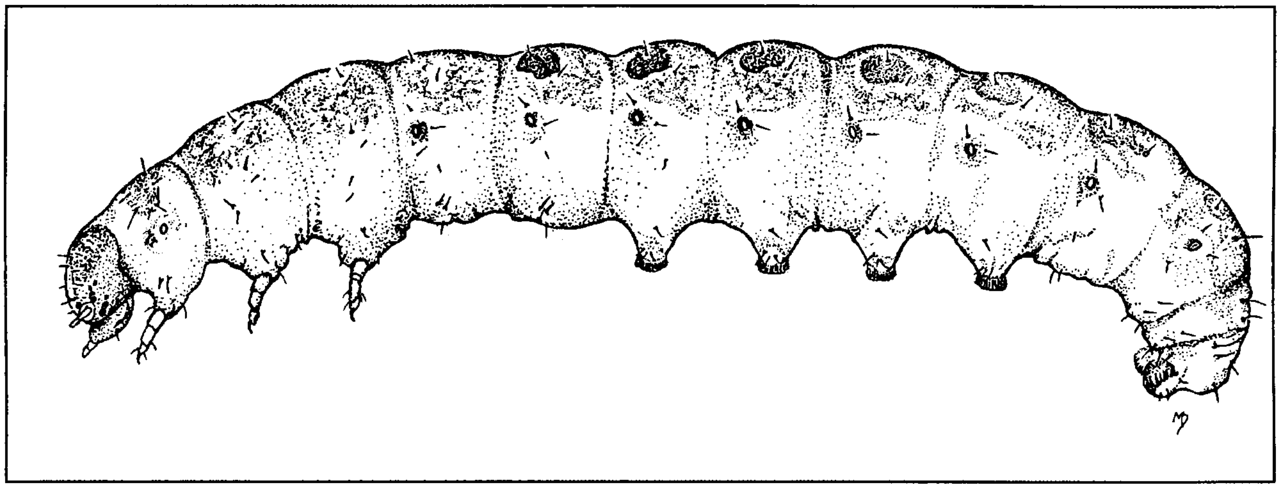

Figure 1. Mature larva of *Namangana pectinicornis*

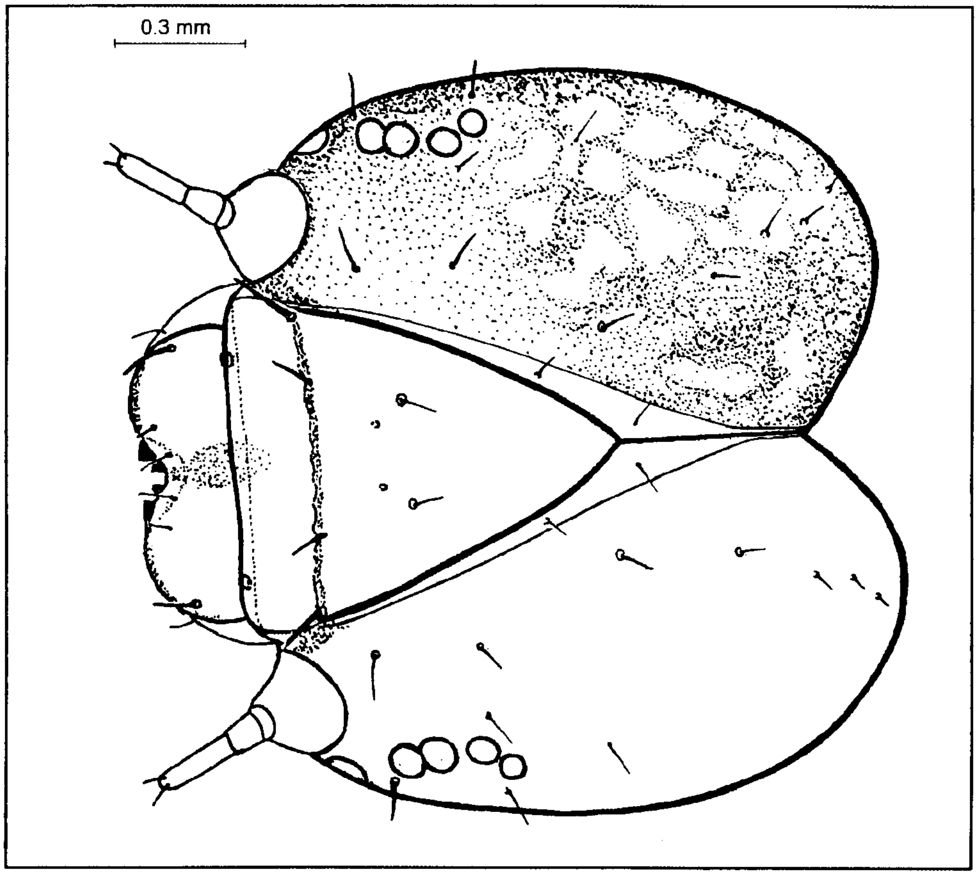

Figure 2. Head capsule of mature larva

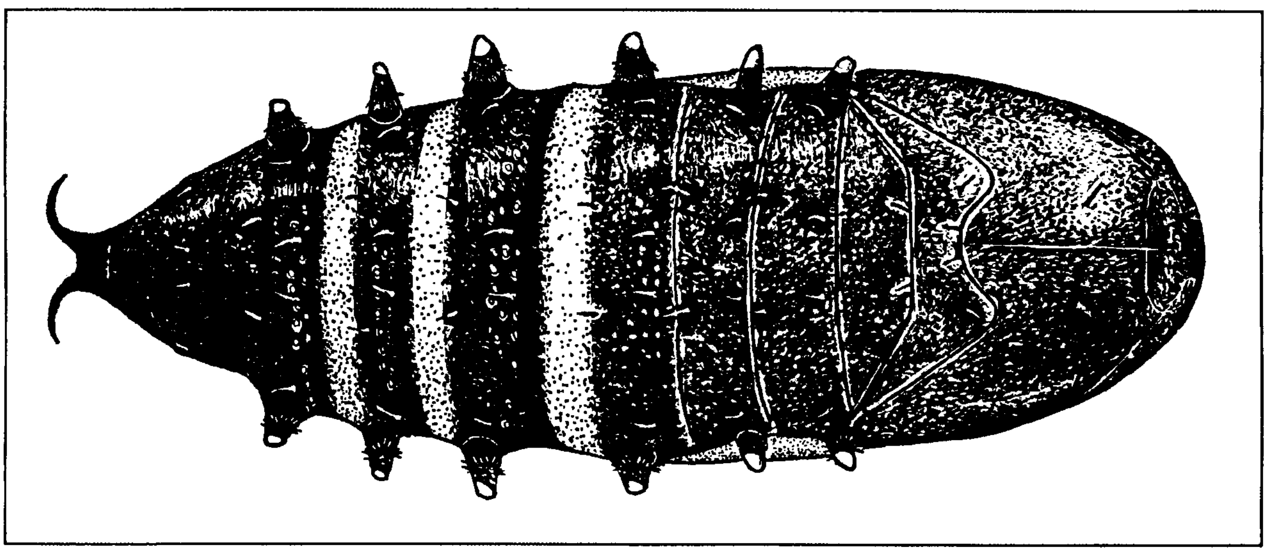

Figure 3. *Namangana pectinicornis* pupul stage

2 Methods and Materials

Moth Rearing

The first shipment of *Namangana pectinicornis* pupae arrived from Thailand in September 1986. Additional shipments were received in December 1987 and March and May 1988. The 511 survivors of the first shipment were placed in two glass-topped wooden cages containing approximately 10 small (about 12-cm-diam) waterlettuce plants floating in shallow trays. All water surfaces were covered with plants. Plants showing damage from hatched larvae were transferred to other cages, and new plants were provided until all the moths were dead. When plants deteriorated from larval feeding damage, the damaged plants were placed over new plants to allow larvae to transfer. Following pupation, the pupae were removed and placed in cups on the floor of a cage with 2-3 young waterlettuce plants placed individually in small containers of water. Following emergence, time was allotted for mating and oviposition, and plants containing eggs were removed and again placed in cages with shallow trays of young plants. The moth has now been reared through approximately 25 generations, and rearing methods have evolved considerably since the beginning of the project.

Host Specificity Tests

Newly hatched first instar larvae were tested for host specificity in no-choice tests (Table 1). Each replicate consisted of 10 larvae placed in a 0.00002975-m^3 (1-oz) clear plastic cup with test plant leaves and/or stems. A piece of black filter paper was moistened to allow runoff excess moisture. One to six replicates were performed for each plant species; all but three species had at least three replicates. The larvae were transferred with a camel hair brush directly on to the previously examined plant material. Rearing cups were checked daily for evidence of feeding and to determine whether larvae were still alive. Plants that deteriorated were replaced as needed. Each group of tests included at least one replicate with waterlettuce as a control. Sixty-one plant species in 32 plant families were tested against first instar larvae (Table 2).

Table 1
Plant Species Tested as Food for Larvae of *Namangana pectinicornis* in Indonesia (I), Thailand (T), and Florida (F)

			1st Instar		3rd Instar	
Family	**Genus and Species**	**Common Name**	**I**	**F**	**T**	**F**
Alismataceae						
	Sagittaria montevidensis Cham and Schlecht.	Calif. Arrowhead		X		
Amaranthaceae						
	Alternanthera philoxeroides (Mart.) Griseb.	Alligatorweed		X	X	X
	Amaranthus hybridus L.	Smooth pigweed	X			
	Amaranthus retroflexus L.	Redroot pigweed		X		
Amaryllidaceae						
	Crinum asiaticum L.	Asian crinum			X	
Anacardiaceae						
	Mangifera indica L.	Mango		X	X	
Annonaceae						
	Annona squamosa L.	Sugar-apple			X	
Apocynaceae						
	Nerium oleander L.	Oleander			X	
Apiaceae						
	Cicuta mexicana Coult and Rose	Waterhemlock				X
	Daucus carota L. var. *savita* DC	Carrot	X	X		
	Hydrocotyle umbellata L.	Waterpennywort		X		X
Araceae						
	Aglaonema sp.	Aglaonema		X		
	Anthurium sp.	Anthurium		X	X	
	Arisaema dracontium (L.) Schott	Green dragon		X		X
	Arisaema triphyllum (L.) Schott and Endl.	Jack-in-the-pulpit		X		
	Colocasia and esculenta (L.) Schott	Taro	X			
	Dieffenbachia sp.	Dumb cane		X		
	Orontium aquaticum L.	Goldenclub		X		X
	Peltandra virginica (L.) Kunth	Arrowarum		X		X
	Pistia stratiotes L.	Waterlettuce	X	X	X	X
	Spathiphyllum sp.	Spathe flower		X		
Asteraceae						
	Bidens mitis (Michx.) Sherff.	Beggar-tick		X		
	Chrysanthemum hortorum Host.	Chrysanthemum			X	
	Cirsium horridulum Michx.	Yellow thistle		X		
	Gerbera jamesonii Hooker	Gerbera daisy			X	

(Sheet 1 of 6)

Table 1 (Continued)

Family / Genus and Species	Common Name	1st Instar		3rd Instar	
		I	F	T	F
Asteraceae (continued)					
Gnaphalium obtusifolium L.	Fragrant cudweed				X
Gnaphalium purpureum L.	Purple cudweed		X		
Lactuca sativa var. *crispa* L.	Lettuce		X	X	X
Balsaminaceae					
Impatiens sp.	Impatiens		X		X
Brassicaceae					
Brassica campestris var. *napobrassica* (L.) DC	Rutabaga		X		
Brassica chinensis L.	Chinese whitecabbage	X		X	
Brassica chinensis var. *parachinensis* L.	Chinese cabbage			X	
Brassica deraceae var. viridis L.	Kale		X		
Brassica juncea Coss	Chinese mustard			X	
Brassica oleracea L.	Chinese kale	X		X	
Brassica oleracea var. botrytis Miller	Cauliflower			X	
Brassica oleracea L. var. *capitata* Hort.	Cabbage			X	
Brassica rapa L.	Turnip			X	
Nasturtium officinale R. Br.	Watercress				X
Raphanus sativus L.	Radish	X			
Butomaceae					
Limnocharis flava (L.) Buchenau		X			
Cannaceae					
Canna flaccida Salisb.	Golden Canna		X		
Chenopodiaceae					
Beta vulgaris L.	Beet		X		
Commelinaceae					
Commelina diffusa Burm, f	Spreading dayflower				X
Tradescantia sp.	Wanderingjew		X		
Convolvulaceae					
Ipomoea aquatica Forsk	Swamp morningglory	X		X	
Ipomoea batatas (L.) Lam.	Sweetpotato	X	X		
Crassulaceae					
Crassula argentea Thunb.	Jade		X		

(Sheet 2 of 6)

Table 1 (Continued)

Family / Genus and Species	Common Name	1st Instar		3rd Instar	
		I	F	T	F
Cucurbitaceae					
Citrullus vulgaris Schrad.	Watermelon			X	
Cucumis melo L.	Cantaloupe			X	X
Cucumis sativus L.	Cucumber		X	X	X
Cucurbita pepo L.	Pumpkin			X	
Cucurbita pepo var. *melopepo* (L.) Alef	Summer squash		X		
Lagenaria leucantha Rusby	Bottle gourd			X	
Momordica charantia L.	Balsamapple			X	
Ericaceae					
Rhododendron indicum (L.) Sweet	Azalea		X		
Euphorbiaceae					
Manihot esculenta Crantz	Cassava	X			
Phyllanthus distichus Muell.	Star gooseberry			X	
Fabaceae					
Arachis hypoqaea L.	Peanut	X			
Glycine max (L.) Merr.	Soybean	X		X	
Lathyrus odoratus L.	Snowpea		X		
Phaseolus aureus Roxburgh	Mungbean			X	
Phaseolus vulgaris L.	Bean		X	X	
Pisum sativum L.	Garden pea		X	X	
Psophocarpus tetragonolobus (L.) Decardolle	Winged bean		X		
Sesbania grandiflora (L.) Pers.	Corkweed tree			X	
Vigna sesquipedalus L. Fruwirth	Yard long bean			X	
Vigna sinensis (Torner) Savi	Black-eyed pea	X			
Fagaceae					
Quercus virginiana Mill.	Liveoak		X		
Haloragaceae					
Myriophyllum aquaticum (Vell.) Verdc.	Parrotfeather		X		
Hydrocharitacea					
Limnobium spongia (Bosc.) Steud.	American frogbit		X		
Labiatae					
Mehtha piperita L.	Peppermint			X	
Ocimum sanctum L.	Basil			X	
Lemnaceae					
Lemna minor L.	Common duckweed		X	X	
Spirodela punctata (Meyer) Thomps.	Giant duckweed		X		

(Sheet 3 of 6)

Table 1 (Continued)

Family / Genus and Species	Common Name	1st Instar		3rd Instar	
		I	F	T	F
Liliaceae					
Allium ascalonicum L.	Shallot			X	
Allium cepa L.	Onion	X	X	X	
Allium fistulosum L.	Spanish onion	X			
Allium porrum L.	Leek		X	X	
Asparagus officinalis L.	Asparagus		X		
Malvaceae					
Corchorus capsularis L.	Jute			X	
Gossypium hirsutum L.	Cotton		X		X
Gossypium sp.	Cotton			X	
Hibiscus esculentus L.	Okra				X
Hibisucs schizopetalus Hooker	Coral hibiscus			X	
Hibiscus subdariff L.	Roselle			X	
Hibiscus sp.	Hibiscus		X		
Marsileaceae					
Marsilea crenata Presl.	Waterclover	X		X	
Musaceae					
Musa sapientum L.	Banana			X	
Myrtaceae					
Eugenia sp.	Rose apple			X	
Psidium quajava L.	Guava			X	
Nymphaceae					
Nelumbi nucifera Gaertner	Indian lotus			X	
Oleacea					
Jasminum sambac Ait	Arabian jasmine			X	
Onagraceae					
Ludwigia adscendens (L.) Hara		X			
Ludwigia repens L.	Creeping water primrose		X		
Poaceae					
Brachiaria mutica Stapl.	Paragrass			X	
Oryza sativa L.	Rice	X	X	X	X
Saccharum officinarum L.	Sugarcane		X	X	X
Sorghum vulgare Persoon	Sorghum	X		X	
Triticum aestivum L.	Wheat		X		
Zea mays L.	Corn	X		X	
Zea mays var. saccharata (Sturtev.) Bailey			X		
Polygonaceae					
Polygonum densiflorum Meisn	Smartweed		X		
Rumex sp.	Dock		X		

(Sheet 4 of 6)

Table 1 (Continued)

Family	Genus and Species	Common Name	1st Instar I	1st Instar F	3rd Instar T	3rd Instar F
Pontederiaceae						
	Eichhornia crassipes (Mart.) Solms	Waterhyacinth	X	X	X	X
	Monochoria vaginalis (Burm. f) Kunth	Monochoria	X			
	Pontederia cordata L.	Pickerel weed		X		X
Punicaceae						
	Punica granatum L.	Pomegranate			X	
Rhamaceae						
	Zizyphus mauritiana L.	Indian jujube			X	
Rosaceae						
	Fragaria chiloensis Duchesne var. *ananassa* Bailey	Strawberry		X		
Rubiaceae						
	Gardenia jasminoides Ellis	Gardenia		X		
	Morinda citrofolia L.	Indian mulberry			X	
Rutaceae						
	Citrus aurantifolia Swing	Lime			X	
	Citrus limon (L.) Burm.	Rough lemon		X		X
Salviniaceae						
	Azolla caroliniana Willd.	Car. mosquitofern		X		X
	Salvinia cucullata Roxb.		X			
	Salvinia minima Baker	Waterfern		X		X
	Salvinia molesta Mitchell	Karibaweed	X			
	Salvinia natans (L.) All.		X			
Sapindaceae						
	Euphoria longana Lark.	Longan			X	
Sapotaceae						
	Mimusops kauki Dub.	Sapote			X	
Solanaceae						
	Capsicum annum var. *grassum* Sendt	Sweetpepper			X	
	Capsicum minimum Roxburgh	Bird chili pepper			X	
	Lycopersicon esculentum L.	Tomato		X	X	X
	Nicotiana tabaccum L.	Tobacco			X	
	Physalis sp.	Ground cherry		X		
	Solanum melongena L.	Eggplant		X	X	X
	Solanum tarvum Swartz	Eggplant			X	
	Solanum tuberosum L.	Potato		X		
	Solanum xanthocarpum (Schrad.) Wendl.	Eggplant			X	

(Sheet 5 of 6)

Table 1 (Concluded)

Family / Genus and Species	Common Name	1st Instar I	1st Instar F	3rd Instar T	3rd Instar F
Theaceae					
Camellia japonica L.	Camellia		X		
Typhaceae					
Typha latifolia L.	Common cattail		X		
Vitaceae					
Vitis vinifera L.	Grape			X	
Zingiberaceae					
Alpinia siamensis K. Schum	Greater galangal			X	
Kaempferia galanga L.		X			

(Sheet 6 of 6)

Third instar tests were conducted in the same way as for first instars except that larvae, which were 5-7 days old, were handled with soft-tipped forceps. All treatments were placed in an incubator at 27 °C and standard photoperiod (16 hr light, 8 hr dark). Twenty-five plant species in 14 plant families were tested against third instar larvae (Table 3).

In a separate test, three impatiens plants (4-5 in. high) in individual pots were placed in a cage. Each plant was infested with 10 third instar larvae. Plants were observed daily for evidence of feeding. After 5 weeks, the plants were removed from the cage and examined closely for larvae on the foliage or in the stems. The soil was also checked for pupae.

Oviposition Tests

Thirty-five plant species in 21 plant families were tested for oviposition preference in the quarantine greenhouse. Plant stems and/or leaves were inserted into 4-dram vials placed at random in vial racks. Waterlettuce, Carolina mosquito fern, and waterfern were placed in shallow plastic (5.2-cm-diam) petri dishes. Each of the three replicates occupied a separate cage. Thirty unsexed moths were placed in each cage; after 24 hr, dead moths were replaced. After 48 hr, the plants were carefully examined for egg masses. Each cage and vial rack was completely disassembled and again examined for eggs.

Table 2
Host Specificity Tests of First Instar Larvae of *Namangana pectinicornis* (Hampson)

Plants Tested			
Family — Genus and Species	**No. Replicates**	**Days Lived**	**Feeding**
Alismataceae			
Sagittaria montevidensis	3	2	-
Amaranthaceae			
Alternanthera philoxeroides	2	1	-
Amaranthus retroflexus	3	2	-
Anacardiaceae			
Mangifera indica	3	2	-
Apiaceae			
Hydrocotyle umbellata	3	2	-
Daucus carota var. *sativa*	3	2	-
Araceae			
Aglaonema sp.	3	1	-
Anthurium sp.	3	1	-
Arisaema dracontium	6	4	Slight
Arisaema triphyllum	6	4	Slight
Dieffenbachia sp.	3	1	-
Orontium aquaticum	1	1	-
Peltandra virginica	6	2	-
Pistia stratiotes	30+	*	
Spathiphyllum sp.	3	1	-
Asteraceae			
Bidens mitis	5	2	-
Cirsium horridulum	3	1	-
Gnaphalium purpureum	6	1	-
Lactuca sativa var. *crispa*	3	2	-
Balsaminaceae			
Impatiens sp.	3	6	Some
Brassicaceae			
Brassica campestris var. *napobrassica*	3	1	-
Brassica deraceae var. *viridis*	3	2	-
Cannaceae			
Canna flaccida	3	1	-
Chenopodiaceae			
Beta vulgaris	3	2	-

(Sheet 1 of 3)

Note: * = Larvae survived and completed development.

Table 2 (Continued)

Plants Tested			
Family Genus and Species	**No. Replicates**	**Days Lived**	**Feeding**
Commelinaceae			
Tradescantia sp.	3	3	Slight
Convolvulaceae			
Ipomoea batatas	3	2	-
Crassulaceae			
Crassula argentea	3	1	-
Cucurbitaceae			
Cucumis stativus	3	2	Slight
Cucurbita pepo var. *melopepo*	3	2	Very slight
Ericaceae			
Rhododendron indicum	3	1	-
Fabaceae			
Lathyrus odoratus L.	3	1	-
Phaseolus vulgaris	3	3	-
Pisum sativum L.	3	1	-
Fagaceae			
Quercus virginiana	3	1	-
Haloragaceae			
Myriophyllum aquaticum	4	2	-
Hydrocharitaceae			
Limnobium spongia	3	2	-
Lemnaceae			
Lemna minor	3	1	-
Spirodela punctata	3	2	-
Liliaceae			
Allium cepa	3	1	-
Asparagus officinalis	3	2	-
Malvaceae			
Gossypium hirsutum	3	1	-
Hibiscus sp.	3	1	-
Poaceae			
Oryza sativa	3	1	-
Saccharum officinarum	3	2	-

(Sheet 2 of 3)

Table 2 (Concluded)

Plants Tested			
Family Genus and Species	**No. Replicates**	**Days Lived**	**Feeding**
Poaceae (continued)			
Triticum aestivum	3	1	-
Zea mays var. *saccharata*	3	2	-
Polygonaceae			
Polygonum densiflorum	3	2	-
Rumex sp.	2	2	-
Pontederiaceae			
Eichhornia crassipes	3	2	-
Pontederia cordata	3	1	-
Rosaceae			
Fragaria chiloensis var. *ananassa*	3	1	-
Rubiaceae			
Garenia jasminoides	3	1	-
Rutaceae			
Citrus limon	3	1	-
Salviniaceae			
Azolla caroliniana	3	2	-
Salvinia minima	3	1	-
Solanaceae			
Lycopersicon esculentum	3	2	-
Physalis sp.	3	1	-
Solanum melongena	3	1	-
Solanum tuberosum	3	1	-
Theaceae			
Camellia japonica	3	1	-
Typhaceae			
Typha latifolia	2	2	-

(Sheet 3 of 3)

Table 3 **Summary of Host Specificity Studies of Third Instar *Namangana pectinicornis* Larvae**					
		Days Until			
Family Plant	**No. of Reps**	**50% Dead**	**90% Dead**	**100% Dead**	**Feeding**
Amaranthaceae					
Alternanthera philoxeroides	3			2	None
Apiaceae					
Cicuta mexicana	1	1		2	None
Hydrocotyle umbellata	4		3	4	None
Araceae					
Arisaema dracontium	3	3	4	5	Some
Orontium aquaticum	3	3	4	5	Extensive-1st 24 hr
Peltandra virginica	8	2	4	6	Some
Pistia stratiotes	10				Complete development
Asteraceae					
Gnaphalium obtusifolium	3	2	3	4	Very slight
Lactuca sativa	1	2		3	1 larva fed some
Balsaminaceae					
Impatiens sp.	5	4	16	25	Extensive
Brassicaceae					
Nasturtium efficinale	2		2	4	None
Commelinaceae					
Commelina diffusa	4		3	4	Some
Cucurbitaceae					
Cucumis melo	1			4	Slight 1st 24 hr
Cucumis sativus	1	1		2	Some
Malvaceae					
Gossypium hirsutum	1	2		3	None
Hibiscus esculentus	1			4	Some 1st 24 hr
Poaceae					
Oryza sativa	3	3	5	6	A few feeding, 1 extensively
Saccharum afficinarum	1		3	4	None
Pontederiaceae					
Eichhornia crassipes	1	2	3	4	None
Pontederia cordata	4		2	3	Slight
					(Continued)

Table 3 (Concluded)

Family Plant	No. of Reps	Days Until 50% Dead	Days Until 90% Dead	Days Until 100% Dead	Feeding
Rutaceae					
Citrus limon	1	2		3	None
Salviniaceae					
Azolla caroliniana	1	2		3	None
Salvinia minima	1	2	3	4	None
Solanaceae					
Lycopersicon esculentum	1	2		3	None
Solanum melongena	1		2	3	Very slight

3 Results and Discussion

Moth Rearing

A number of changes were made in the initial moth-rearing program in quarantine. Adults had been allowed to oviposit on young plants in trays on the floor of a cage. Such large numbers of eggs were laid that the resulting larvae fed upon the plants too heavily, and many of the young larvae apparently starved. The procedure was altered so that bare pupae might be placed on moist cotton in large, open petri dishes instead of individually in cups. Moth drowning, which had occurred with whole trays of plants, was eliminated by placing two to three young plants in containers large enough to hold only the plant roots and a small amount of water. These small plant units were changed daily while egg production was heavy.

Plants with egg masses were removed from the oviposition cage, and individual egg masses were cut out of the leaves with scissors. Removed egg masses were placed in 0.00002975-m^3 (1-oz) cups with moist cotton in the bottom. Many of the larvae, however, wandered around the tops of the cups and died or remained undetected in the leaf under the egg mass, which eventually became moldy and was discarded. Attempts to move eggs with fine brushes or needles were unsuccessful, as the eggs stuck to each other or to leaf surface; such eggs rarely hatched. As an alternative, egg-leaves were cut off at the stem bases and placed in knotted plastic bags. The massed egg-leaves kept humidity levels high, and the leaves lasted several days.

Once larvae reached the second instar, the leaves were removed from the bags and placed in 104-mm-diam petri dishes with Celu-max filter pads (Seitz Filters, Kingston, NY). Leaves were added as needed, and larvae were moved to new dishes when grass and leaf fragments made the dishes too wet. Fourth instar larvae were placed individually into 0.00002975-m^3 (1-oz) plastic cups and fed until pupation occurred. Leaving older larvae in petri dishes saved labor, but resulted in much greater mortality, apparently from fungal infection (probably *Beauvaria*). Considerable mortality also occurred in prepupates which, although full size, failed to pupate, turned black, and died. Healthy pupae were selected and placed in the oviposition cage to start the new generation.

As with the rearing of the weevil *Neohydronomus affinis* (Thompson and Habeck 1989), *Samea* larvae invaded waterlettuce leaves being fed to the

Namangana larvae. When *Samea* populations became too high, a microbial insecticide, *Bacillus thuringiensis*, was applied at 3T/gal. Treated plants were not exposed to *Namangana* larvae for 2 or more weeks.

Host Specificity Tests

Newly hatched larvae were provided in no-choice tests with 61 species of plants in 32 families (Table 2). The number of exposed neonates was 1,900, and each plant was tested in 1-6 replicates of 10 neonate larvae each (these figures exclude waterlettuce, which was included in each test date as a control). Of 1,900 larvae tested, only 11 lived more than 72 hr, and none lived longer than 6 days, except on waterlettuce. First instars fed very slightly on summer squash (*Cucurbita pepo* var. *Melopepo*), fed slightly on cucumber (*Cucumis sativus*), wanderingjew (*Tradescantia* sp.), and two species of waterlettuce relatives, green dragon (*Arisaema dracontium*) and jack-in-the-pulpit (*Arisaema triphyllum*), and fed moderately on *Impatiens*.

The results of host specificity testing of third instar larvae are shown in Table 3. Third instars fed slightly on egg plant (*Solanum melongena*) and fragrant cudweed (*Gnaphalium obtusifolium*). There was moderate feeding on spreading dayflower (*Commelina diffusa*), cucumber (*Cucumis sativus*), and on the waterlettuce relatives green dragon (*Arisaema dracontium*) and arrowarum (*Peltandra virginica*). Feeding was slight to extensive, but occurred only during the first 24 hr on golden club (*Orontium aquaticum*), cantaloupe (*Cucumis melo*), and okra (*Hibiscus esculentus*). On lettuce (*Lactuca sativa*) and rice (*Oryza sativa*), feeding was moderate to extensive, but only one to a few larvae were eating, the others having died previously. No third instar larvae lived longer than 6 days. In most cases, larvae seemed disinterested in the plants other than waterlettuce and were found around the edges of the dishes. Although the larvae rarely fed, they were occasionally found in plant material.

Impatiens was the exception to the above tests. First instar larvae had fed moderately on the plant, but the third instar larvae fed and plant damage was extensive; one third instar larva lived for 25 days. The larvae were unable, however, to complete development on *Impatiens*. Additional tests were performed using entire *Impatiens* plants in cages in the quarantine greenhouse. Three *Impatiens* plants, each infested with 10 third instar larvae, were observed frequently for about 5 weeks. Initially, a few larvae were observed feeding on the lower leaves and later boring into the stems. After 5 weeks, the plants were examined carefully, the stems dissected to check for boring larvae, and the soil sifted for pupae; no larvae or pupae were found, and the plants appeared to be growing well, having increased from 10-13 cm originally to 20-26 cm high. There was no evidence of fresh boring, and the larvae probably had died at least 7 to 10 days earlier.

Although there is some concern about the amount of feeding on *Impatiens*, the problem is not serious since the larvae were not able to complete development. Furthermore, first instar larvae fed only marginally on *Impatiens* and did not survive more than 6 days. Also, *Impatiens* are rarely grown near wet areas where waterlettuce occurs. Adult moths also appear to be rather specific to the waterlettuce habitat and do not seem to fly far under laboratory conditions.

Oviposition Tests

In the oviposition choice tests, over 70 percent of the 91 egg masses laid were on waterlettuce, with 19 (roughly 21 percent) placed on the cage interior or vial racks rather than on plants other than waterlettuce. Of the remaining eight egg masses, four plant species received one egg mass each: tomato (*Lycopersicon esculentum*), fragrant cudweed (*Gnaphalium obtusifolium*), beet (*Beta vulgaris*), and the waterlettuce relative, goldenclub (*Orontium aquaticum*). The only nonwaterlettuce plant with more than one egg mass was egg plant (*Solanum melongena*), which had four egg masses. Six of the eight egg masses on plants other than waterlettuce were on tomato, eggplant, and fragrant cudweed, all plants with hairy leaves similar to waterlettuce.

4 Conclusions

Currently, about four million dollars are spent annually to control waterlettuce in Florida.[1] Successful biological control would reduce that figure considerably and have the added effect of reducing the amounts of herbicides introduced into the environment.

The use of *Namangana pectinicornis* for biological control of waterlettuce in Thailand is an indication of the insect's ability to successfully control this nuisance plant. If *N. pectinicornis* fed extensively, or completed its life cycle, on other plants, release from quarantine would not be permitted. Reports from India (George 1963; Sankaran and Ramaseshiah 1974), Indonesia (Mangoendihardjo and Nasroh 1976; Mangoendihardjo et al. 1977), Bangladesh (Alam, Alam, and Ahmed 1980), and Thailand (Suasa-ard 1976; Suasa-ard and Napompeth 1978; Napompeth 1982) and research of these authors all indicate that this insect is specific to waterlettuce.

Table 1 summarizes the tests conducted on the host specificity of *Namangana pectinicornis*. More than 136 plant species representing 50 families were tested, including 84 each on both first and third instar larvae. Few insects imported and released into the United States for biological control of weeds have been tested so extensively in either the number of plant species or families.

Namangana pectinicornis is able to complete development only on waterlettuce, indicating that it is highly host specific. Furthermore, it did not feed extensively on any other member of the Araceae or on other aquatic or semiaquatic species. *Namangana pectinicornis* should be considered safe for release into the environment and should have considerable impact on waterlettuce in Florida and other areas where it may be subsequently released.

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[1] Personal Communication, 1996, Don Schmitz, Florida Department of Natural Resources, Tallahassee, FL.

Table 4
Oviposition Tests for *Namangana pectinicornis* Moths

Family	Genus and Species	Common Name	No. of Egg Masses: Cage 1	Cage 2	Cage 3	Total
Amaranthaceae						
	Alternanthera philoxeroides (Mart.) Griseb.	Alligatorweed	0	0	0	0
Apiaceae						
	Cicuta mexicana Coult and Rose	Waterhemlock	0	0	0	0
	Daucus carota L. var. *sativa* D.C.	Carrot	0	0	0	0
	Hydrocotyle umbellata L.	Waterpennywort	0	0	0	0
Araceae						
	Orontium aquaticum L.	Goldenclub	0	0	1	1
	Peltandra virginica (L.) Kunth	Green arum	0	0	0	0
	Pistia stratiotes L.	Waterlettuce	19	25	20	64
Asteraceae						
	Gnaphalium obtusifolium L.	Fragrant cudweed	0	0	1	1
	Lactuca sativa var. *crispa L.*	Lettuce	0	0	0	0
Balsaminaceae						
	Impatiens sp.	Impatiens	0	0	0	0
Brassicaceae						
	Brassica campestris var. *Napobrassica* (L.) D.C.	Rutabaga	0	0	0	0
	Brassica oleracea var. *capitata* L.	Cabbage	0	0	0	0
Cannaceae						
	Canna flaccida Salisb.	Golden canna	0	0	0	0
Chenopodiaceae						
	Beta vulgaris L.	Beet	0	1	0	1
Convolvulaceae						
	Ipomoea batatas (L.) Lam.	Sweetpotato	0	0	0	0
Cucurbitaceae						
	Cucumis sativus L.	Cucumber	0	0	0	0
Fabaceae						
	Pisum sativum L.	Sugar snap pea	0	0	0	0
Hydrocharitaceae						
	Limnobium spongia (Bosc.) Steud.	American frogbit	0	0	0	0

(Continued)

Table 4 (Concluded)

Family	Genus and Species	Common Name	No. of Egg Masses Cage 1	Cage 2	Cage 3	Total
Liliaceae						
	Allium cepa L.	Onion	0	0	0	0
Malvaceae						
	Gossypium hirsutum L.	Cotton	0	0	0	0
	Hibiscus esculentus L.	Okra	0	0	0	0
Poaceae						
	Oryza sativa L.	Rice	0	0	0	0
	Saccharum officinarum L.	Sugarcane	0	0	0	0
	Zea mays var. *saccharata* (Sturtev.) Bailey	Sweetcorn	0	0	0	0
Polygonaceae						
	Polygonum densiflorum Meisn.	Smartweed	0	0	0	0
Pontederiaceae						
	Eichhornia crassipes (Mart.) (Sturtev.) Bailey	Waterhyacinth	0	0	0	0
	Pontederia cordata L.	Pickerel weed	0	0	0	0
Rutaceae						
	Citrus limon Burm.	Rough lemon	0	0	0	0
Salviniaceae						
	Azolla caroliniana Willd.	Carolina mosquitofern	0	0	0	0
	Salvinia minima Baker	Waterfern	0	0	0	0
Solanaceae						
	Lycopersicon esculentum Mill.	Tomato	0	1	0	1
	Solanum melongena L.	Eggplant	0	1	3	4
	Solanum tuberosum L.	Potato	0	0	0	0
	Solanum sp.	Banana pepper	0	0	0	0
Typhaceae						
	Typha latifolia	Cattail	0	0	0	0
Miscellaneous						
	(plastic vial racks, sponges, cage walls, or floor)	Cage sleeves				

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13. ABSTRACT *(Maximum 200 words)*

As waterhyacinth populations have declined, waterlettuce is increasing, and about $4 million is expended annually in Florida to control this aquatic weed. Waterlettuce increases water loss through transpiration and interferes with recreation and irrigation use. The roots are used as an oxygen source by several species of mosquito larvae.

A number of insects are associated with waterlettuce in Florida and South America, but the only insect used for biological control is a South American weevil, *Neochetina affinis* Hustache. This weevil was used successfully in Australia and South Africa; it was also released in Florida in 1987, where it has established and is gradually increasing and spreading.

A second insect, *Namangana pectinicornis* Hampson, was introduced into quarantine in September 1986. This noctuid moth, native to southeast Asia, was introduced from Thailand, where it was used successfully to control waterlettuce, which is considered exotic there. Earlier studies in India, Indonesia, and Thailand had indicated that this insect was host specific to waterlettuce. In Florida, first and third instar larvae fed on only a few plants and did not live more than 6 days on any test plant except *Impatiens*. Some third instar larvae fed on *Impatiens* and became almost mature, but none were about to complete development. Over 136 plant species in 50 families have been offered to larvae of *Namangana pectinicornis* in tests in India, Indonesia, Thailand, and Florida. Larvae fed

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on only a few of these and were unable to complete development on any of them. In oviposition studies, moths clearly preferred to oviposit on waterlettuce instead of other plants offered.

This extensive testing leads one to conclude that *Namangana pectinicornis* is sufficiently host specific to release in the United States as a biological control agent against waterlettuce.

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