Host Specificity Of *Neochetina bruchi* Hustache (Coleoptera Curculionidae), a Biological Control Agent for Waterhyacinth[1] [2]

B. DAVID PERKINS[3]

Research Entomologist
Agricultural Research Service, U. S. Department of Agriculture
Aquatic Plant Management Laboratory, Fort Lauderdale, Florida 33314

DONALD M. MADDOX[4]

Research Entomologist
Agricultural Research Service, U. S. Department of Agriculture
Biological Control of Weeds Laboratory, Albany, California 94706

ABSTRACT

Waterhyacinth, *Eichhornia crassipes* (Mart.) Solms, is a serious and persistent weed that is naturalized in many areas of the world. Biological control with insects that feed on waterhyacinth in South America, the area of origin of the plant, is a logical way to reduce the abundance of the weed. In Argentina and Albany, California, the amount of feeding on waterhyacinth by adult *Neochetina bruchi* Hustache was compared with the amount on 28 plant species (17 families). The results indicated a high preference of the weevil for waterhyacinth. The fact that little or no feeding, oviposition, or oocyte development occurred on plants other than waterhyacinth suggested that this weevil was safe for introduction into the United States. In 1974, approval was granted by the Federal Working Group for Biological Control of Weeds and by authorities in the states involved to import this weevil for field release in the United States.

INTRODUCTION

Waterhyacinth is the most serious aquatic weed in the world. Up to 15 million dollars are expended annually in chemical and mechanical control of this weed in Florida alone, and loss due to blockage of waterways, pollution, and evapotranspiration is even greater.[5] Due to this high cost and the need to repeat control treatments regularly to combat rapid reinfestation by waterhyacinth, a continuous self-perpetuating control agent is desirable. Thus, the importation of biological control agents that are safe and effective is a logical approach.

The mottled waterhyacinth weevil, *Neochetina eichhorniae* Warner, was introduced in the southeastern United States by the end of 1972 (4). Four more insect species and one mite species that occur in South America were also

[1]Cooperative research of the Agricultural Research Service, U. S. Department of Agriculture, Florida Department of Natural Resources, Fla. Agricultural Experiment Stations, and U. S. Army Corps of Engineers.

[2]Florida Agricultural Experiment Stations Journal Series No. 5941.

[3]Present address: 3205 SW 70th Ave., Fort Lauderdale, Florida 33314.

[4]Present address: 1050 San Pablo Avenue, Albany, California 94706.

[5]Burkhalter, A. P., Aquatic Weed Control Coordinator. Florida Department of Natural Resources, 20 June 1972. Personal Communication.

under consideration for introduction (3). The chevroned waterhyacinth weevil, *N. bruchi,* was first described in 1926 (2). Studies by Silveira Guido in Uruguay indicated that this weevil was among the most likely agents to introduce against this weed.[6] In 1968, studies were begun at the USDA laboratory in Hurlingham, Argentina to determine whether this weevil was host specific to waterhyacinth.

In Argentina *N. bruchi* is present almost everywhere that waterhyacinth occurs, and adult and larval feeding continues in the field whenever the temperature is favorable. The larvae tunnel inside the petiole and crown; the adults feed externally on the leaves and petioles. In the laboratory under conditions of high humidity, feeding by five adult *N. bruchi* and the accompanying tissue deterioration killed a small waterhyacinth plant within 2 weeks.

The weevil apparently has few arthropod enemies; however, in South America, populations of this weevil were infested with the pathogens *Beauveria* sp., *Aspergillus* sp., and *Nosema* sp. and with a nematode.[7] Without these enemies, the potential to control the weed would be strengthened.

Evidence in the literature (2) and from field observations supports the safety of introduction of *N. bruchi*. This insect has never been recorded as a pest of any cultivated crop in South America. The genus *Neochetina* is closely tied to plants of the family Pontederiaceae, the three known species in this genus being recorded only from *Eichhornia* and *Pontederia*. Distribution of this genus in South America does not exceed that of *Eichhornia* spp., and it is limited to an aquatic environment as are other members of the tribe Bagoini to which it belongs. The life cycle can be completed only on waterhyacinth due to the unique underwater pupation site in the roots of this plant. Field examinations of other plant species in Argentina and Uruguay revealed no damage on other than the Pontederiaceae, and damage to members of this family other than *E. crassipes* was rare. This paper presents results of laboratory testing to determine host specificity of *N. bruchi* to waterhyacinth.

METHODS AND MATERIALS

Tests were conducted to determine the maximum possible range of plant species to be attacked and the plant species likely to be attacked under natural conditions. Thus, we could ascertain safety before requesting approval to introduce the weevil into the United States. Laboratory testing was conducted by the senior author in Argentina, and quarantine studies were conducted by the second author in California, U.S.A. All tests were conducted at about 30 C ambient temperature.

TESTING IN ARGENTINA: Plant species tested were those that occurred near waterhyacinth in the field in the United States or were taxonomically close to waterhyacinth. These species are listed according to plant family with the Latin name and authority in Table 1. Three types of tests were conducted: (1) starvation, (2) paired plants, and (3) plant group.

Starvation Test: Weevils were caged with only the test plant available as food. If the insects starved to death rather than feed on the plant species, safety to this plant under less restrictive conditions for the insect was assured. Analysis of the relative feeding on plants in this test was used to determine the maximum possible feeding range of the insect.

A test plant was inserted through a hole in the cap of a liter jar so that the roots were in water in the jar. The plant was caged by placing a glass cylinder atop the jar. Adult weevils were confined until death on the caged plants. Feeding damage and number of dead weevils were recorded three times a week for the first month and once a week thereafter. Plants were periodically changed before damage became too severe to count the feeding spots accurately. Water was made available in each cage by providing wet cotton. Five adult insects were placed in each cage, and the number of replications depended on propensity of the insect to feed as well as availability of insects. Test weevils were collected in the field, and only healthy, active adults were used.

Paired Plant Test: Only those plants fed upon in the starvation test were included in this test, except that carrot (*Daucus carota* L.) was included so we could determine relative feeding on waterhyacinth when paired with a plant known not to be harmed by this insect.

TABLE 1. PLANT SPECIES TESTED FOR HOST SPECIFICITY OF *N. bruchi* HUSTACHE AND TYPE OF TEST CONDUCTED[a]

Family	Species	Test
Bromeliaceae	*Ananas comosus* (L.) Merr.	
Commelinaceae	*Commelina coelestis* Willd.	P,G,L
	C. virginica L.	P,G,L
	Tripogandra elongata (G.F.W. Mey.) Woodson	P,G,L
	Tradescantia crassifolia Cav.	P,L
	Zebrina pendula Schnizl.	P,G,L
Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms	P,G,L
	E. azurea (Sw.) Kunth	P,G,L
	Pontederia cordata L.	P,G,L
Liliaceae	*Agapanthus africanus* (L.) Hoffmgg.	
	Allium cepa L.	
	Asparagus officinalis L.	
Polygonaceae	*Polygonum acuminatum* H.B.K.	
Amaranthaceae	*Iresine herbstii* Hook. f.	
Nymphaeaceae	*Nymphaea odorata* Ait.	
	Brasenia schreberi Gmel.	
Cruciferae	*Brassica oleracea* L.	P,L
	Nasturtium officinale R. Br.	
Onagraceae	*Ludwigia arcuati* Walt.	
Umbelliferae	*Daucus carota* L.	P,G
Gentianaceae	*Nymphoides aquatica* (Walt.) Ktze.	
Compositae	*Lactuca sativa* L.	P,G,L
Typhaceae	*Typha latifolia* L.	
Sparganiaceae	*Sparganium americanum* Nutt.	
Alismaceae	*Sagittaria montevidensis* Cham. & Schlect.	
Gramineae	*Saccharum officinarum* L.	
	Oryza sativa L.	
Cyperaceae	*Eleocharis macrostachya* Britt.	
	Scirpus californicus (C. A. Mey.) Steud.	

[a] All plants listed were tested in starvation or no choice tests.
Other tests are represented by symbols:
P = Paired plant
G = Plant group
L = Larval feeding

[6]Silveira Guido, A. 1965. Natural enemies of weed plants. Final report (unpublished report, Department Sanidad Vegetal, Univ. de La Republic, Montevideo, Uruguay), 128 pp.

[7]Allen, G., Professor of Entomology; Univ. of Florida, Gainesville, August 21 and September 17, 1974 Personal Communication.

Aquaria measuring 29.5 by 37 by 35 cm were used as cages. A sheet of heavy, clear plastic was cut to fit exactly inside the aquarium and serve as a false bottom. This plastic sheet rested atop 2 jars of water placed inside the aquarium. A hole was cut in the sheet above each jar. The test plant in question was placed in one of the holes, and a waterhyacinth plant was placed in the other. Roots of these plants were in water in the jars below the plastic sheet. Weevils (five to ten) were placed in the center of the plastic sheet between the two plants, and a sheet of heavy brown paper in which holes had been punched was taped over the aquarium top to prevent escape of the insects. Generally, after 13 to 15 days, the plants were examined, and the number of feeding spots on the two species was counted. A few tests were continued beyond 15 days.

Plant Group Test: Under natural conditions, weevils would potentially encounter a number of plant species adjacent to an area of heavily infested waterhyacinth. Therefore, a test was conducted to determine the degree of feeding that could possibly occur on these plants. This test was conducted in the open in the laboratory garden during summer.

Test plant species fed upon in the starvation test were placed, two each, opposite each other, so as to surround a pool containing 10 waterhyacinth plants. A cage measuring 1 m^2 was placed over the waterhyacinth plants after 60 adult *N. bruchi* had been placed on them. These plants and the first cage were covered with a second cage (1.3 m^2) to exclude other insects which could attack the plants. After 10 days, which allowed the 60 weevils to become accustomed to their new location, the inner cage was removed, and the outer cage was replaced. Thus, the insects could remain on the waterhyacinth plants or move to the test plants.

Larval Tests: Punctures were made in each of five test plants of each species attacked in the adult starvation test, and five first-instar larvae were introduced head first at the point of injury. The acceptance or rejection of the test plant and survival on the plant were recorded. Plants tested were: *B. oleracea, C. coelestis, C. virginica, E. azurea, E. crassipes, L. sativa, P. cordata, T. crassifolia, T. elongata,* and *Z. pendula* (Table 1).

TESTING IN QUARANTINE: Four plant species, *Brasenia schreberi* Gmel. *Ludwigia arcuati* Walt., *Nymphoides aquatica* (Walt.) Ktze., and *Sparganium americanum* Nutt., were tested. The Federal Working Group for Biological Control of Weeds suggested the first three species, and the fourth was included because of its importance in Florida. Test plants were collected by J. K. Balciunas, University of Florida graduate student, and N. R. Spencer, USDA Research Entomologist, Gainesville, from natural habitats in Florida and sent by air to the quarantine laboratory in Albany, California. All plants were kept in hydroponic solutions (Hoagland's #1) in the greenhouse. Adult *N. bruchi* were collected in the field in Argentina and shipped by air to Albany. The weevils were kept about 1 month on waterhyacinth plants in the quarantine laboratory before testing.

No-choice Test: This type of test corresponded to the starvation test, but was not continued until death of the insect by starvation. A male and a female *N. bruchi* adult and a cut piece of test plant or waterhyacinth (control) were placed into No. 802 Sealright® cartons.[8] Each carton was lined with aluminum foil and capped with nylon mesh covered by the carton cover in which four pin holes had been punched. A piece of coarse filter paper, 9 cm in diameter, was placed on the foil in the bottom of the carton. Water was poured into the container to a depth of 6 mm to provide water for the insects and high humidity to prevent drying of the plant material. The test was continued for 15 days and was replicated 10 times for each test species and 20 times for the control. All female insects were preserved in 70% alcohol at the end of the test period and later dissected to determine oocyte development. Plant tissue was changed as necessary after deterioration from feeding damage or aging. Data were recorded three times a week. The number of feeding spots (ca. 3 mm in diameter), the number of eggs deposited, and the number of dead weevils were recorded.

RESULTS

TESTING IN ARGENTINA: Of the 25 plants included in the starvation test, only 11 were fed on (Table 2). The greatest degree of feeding occurred on *E. azurea, E. crassipes,* and *P. cordata*. The greatest survival, i.e., average longevity, occurred on *B. oleracea, E. crassipes, L. sativa,* and *P. cordata*.

In the paired plant tests, besides waterhyacinth, only *E. azurea* and *P. cordata* were fed upon notably (Table 3), but *C. virginica* and *T. elongata* were fed on slightly. The ratio of damage to these plants was: *E. crassipes* to *E. azurea* = 8:1; to *P. cordata* = 30:1; to *C. virginica* = 385:1; to *T. elongata* = 397:1.

In the plant group tests, only the Pontederiaceae were fed on and then only lightly compared with waterhyacinth (Table 4). *Eichhornia azurea* had only 18 feeding spots, and *P. cordata* had none; the waterhyacinth plants had a total of 2,463. Of the 60 insects liberated on waterhyacinth, 32 were still alive and healthy and resting on the waterhyacinth plants at the end of 22 days.

The first-instar larvae entered *E. azurea, E. crassipes, L. sativa, P. cordata,* and *T. elongata*. Plants were dissected 2 weeks later, and larvae were found dead either on or off the plant in all except *E. crassipes*. Of the five larvae on *E. crassipes,* three were tunneling in the petiole and two were off the plant.

TESTING IN QUARANTINE: Table 5 indicates feeding, oviposition, oocyte development, and mortality of *N. bruchi*. Feeding occurred on *E. crassipes, L. arcuati,* and *S. americanum,* but no feeding occurred on *B. schreberi* or *N. aquatica*. Much greater oviposition and oocyte development occurred on *E. crassipes* than on the other plant species. Feeding on *E. crassipes* totaled 1,619 feeding spots (89.25% of the total), on *S. americanum,* 185 feeding spots

[8]Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

TABLE 2. FEEDING DAMAGE AND LONGEVITY OF ADULT *N. bruchi* HUSTACHE CONFINED ON INDIVIDUAL TEST PLANTS IN ARGENTINA UNTIL DEATH.

Family and species	Feeding Spots/ insect/day ($\overline{X}$)	Insect-days lived	Days insect lived ($\overline{X}$) (Total)
Typhaceae			
T. latifolia	0.01	647	25.9
Alismaceae			
S. montevidensis	0	512	20.5
Gramineae			
S. officinarum	0	124	9.5
O. sativa	0	121	24.2
Cyperaceae			
E. macrostachya	0	817	32.5
Scirpus sp.	0	50	10.0
Bromeliaceae			
A. comosus	0	324[a]	17.1
Commelinaceae			
C. coelestis	0.13	126	25.2
C. virginica	0.20	136	27.2
T. elongata	0.41	170	34.0
T. crassifolia	0.03	77	7.7
Z. pendula	0.69	49	9.8
Pontederiaceae			
E. crassipes	4.59	542	60.2
E. azurea	1.59	104	20.8
P. cordata	1.17	277	55.4
Liliaceae			
A. africanus	0	50[a]	10.0
A. cepa	0	99[a]	19.8
A. sativum O.		23	4.6
A. officinalis	0	157	26.2
Polygonaceae			
P. acuminatum	0	158	31.6
Amaranthaceae			
I. herbstii	0	79	15.8
Nymphaeaceae			
Nymphaea sp.	0	192	19.2
Cruciferae			
B. oleracea	0.41	201	40.2
N. officinale	0	267	20.5
Umbelliferae			
D. carota	0	188	24.5
Compositae			
L. sativa	0.50	566	70.9

[a] Plants were provided soil, and insects were found under soil particles upon examination.

(10.20%), and on *L. arcuati*, 10 feeding spots (0.55%). Eggs were deposited on the test plants as follows: *E. crassipes*, 326 eggs (93.95% of total), *S. americanum*, 9 eggs (2.59%), *L. arcuati*, 2 eggs (0.58%), *B. schreberi*, 3 eggs (0.86%), and *N. aquatica*, 7 eggs (2.02%). Eggs on *E. crassipes* were all placed within the plant tissue as would normally occur in nature; eggs on the other plants were placed on the surface of the plants or on the container. Oocyte development, based on development in the proximal zone of the ovary, was classed as maximum, average, minimum, or none. Maximum development occurred in 78% of the weevils on *E. crassipes*, 33% on *S. americanum*, 30% on *L. arcuati*, 20% on *B. schreberi*, and 11% on *N. aquatica*. Mortality was least on *E. crassipes* and *B. schreberi* (each 2.5%), highest on *N. aquatica* (40%), and moderate on *S. americanum* (25%) and *L. arcuati* (15%).

DISCUSSION AND CONCLUSIONS

Testing of insects by confronting them with feeding or starvation serves to indicate the maximum condition they can encounter. The relative feeding and survival on plants in these tests indicated that damage would be negligible outside the family Pontederiaceae. The feeding in the paired plant test confirmed this. Lettuce, interestingly, was fed upon notably in the starvation test (not surprising, considering its blandness and high percentage of water) but was completely unharmed when the insects were offered waterhyacinth and lettuce together. Some damage continued to occur on members of the family Commelinaceae in the paired plant test, but this damage was negligible compared with damage to waterhyacinth. When species of Commelinaceae were exposed with waterhyacinth and other plants as a group that more closely simulated field conditions, no damage occurred to plants in this family. The slight, essentially negligible, damage that occurred to *E. azurea* was not unexpected since it shares the same genus with waterhyacinth; but *E. azurea*

TABLE 3. AMOUNT OF FEEDING BY ADULT *N. bruchi* CONFINED TO TEST PLANTS PAIRED WITH WATERHYACINTH IN ARGENTINA.

Test plant (family and species)	Total number feeding spots per plant species		Duration of test (Days)	Total number insects tested
	Test plant	*E. crassipes*		
Commelinaceae				
C. coelestis	0	435	14,44[a]	15
C. virginica	1	385	13,24[a]	15
T. elongata	2	794	13,13,31[a]	19
T. crassifolia	0	471	13	10
Z. pendula	0	330	13	10
Pontederiaceae				
E. azurea	45	355	13,26[a]	20
P. cordata	17	520	13,35[a]	15
Cruciferae				
B. oleracea	0	525	14,26[a]	20
Compositae				
L. sativa	0	64	24	10
Umbelliferae				
D. carota	0	590	14	20

[a] Two replications with the number of days indicated, respectively, were conducted, except for the test with *T. elongata* in which three replications were conducted.

TABLE 4. PLANTS DAMAGED WHEN CAGED TOGETHER WITH WATERHYACINTH INFESTED WITH *N. bruchi*[a] IN ARGENTINA.

Species damaged	No. of plants	Feeding spots $\overline{X}$ per plant	Feeding spots Total	Weevils on plants $\overline{X}$ per plant	Weevils on plants Total
E. azurea	3	6.0	18	0	0
E. crassipes	18	136.8	2,463	1.8	32

[a] Species included in test, but undamaged and not bearing insects; *P. cordata, C. coelestis, C. virginica, T. elongata, Z. pendula, D. carota,* and *L. sativa.*

TABLE 5. FEEDING, OOCYTE DEVELOPMENT, OVIPOSITION, AND MORTALITY OF *N. bruchi* TESTED ON PLANTS IN QUARANTINE.

Plant species	Feeding spots No.	Feeding spots % of total	Eggs No.	Eggs % of total	Oocyte development % females on plnt specs. Max.	Ave.	Min.	None	Mortality % on plant species
E. crassipes (Control 1)	1,619	89.25	151	94.0	78	5	17	0	2.5
E. crassipes (Control 2)	1,141	—[a]	175	—[a]					
S. americanum	185	10.20	9	2.6	33	0	11	56	25.0
L. arcuati	10	0.55	2	0.6	30	0	0	70	15.0
B. schreberi	0	0	3	0.8	20	0	0	80	2.5
N. aquatica	0	0	7	2.0	11	11	45	33	40.0

[a] This control was conducted separately and is not considered with the total for the purpose of determining percentage.

does not occur in the United States. Larval acceptance also took place only on waterhyacinth, which tends to indicate that larvae moving from nearby waterhyacinth would reject other plants. A larva was observed in the laboratory to move from a tunnel in one waterhyacinth plant to an adjacent waterhyacinth plant where it tunneled. Larval transfer was noted only this once, however.

Tests with *B. schreberi, E. crassipes, L. arcuati, N. aquatica,* and *S. americanum* in quarantine indicated that *N. bruchi* will feed very heavily on *E. crassipes* and negligibly, or not at all, on the other species. Oocyte development, which indicates the possibility of an insect species reproducing on a plant species (1), was greater in weevils feeding on *E. crassipes,* and oviposition was much higher on this species. Thus, the feeding response of *N. bruchi* in the various tests indicated specificity for waterhyacinth. Other members of the family Pontederiaceae would probably be fed upon to some degree should they occur near waterhyacinth infested with *N. bruchi.* However, Pontederiaceae is represented only by waterhyacinth and pickerelweed *(P. cordata)* in the United States, and the larvae of *N. bruchi* reject pickerelweed. Moreover, this plant lacks the underwater roots required by *N. bruchi* for pupation. According to principles set forth by Zwölfer and Harris (5), the question of whether an insect can develop on a plant species, not just feed on it, should be the criterion for judging the safety of an insect before introduction. Populations of *N. bruchi* would not develop on isolated stands of pickerelweed since interrupting the life cycle of the insect at any point would eliminate the next adult generation.

Strongest emphasis in this study was given adult feeding since this stage selects the host plant for feeding and oviposition and since it is almost entirely responsible for distribution of the species. The adult, being able to fly, is largely responsible for infestations in stands of the weed distant from the original host plant. In addition, the adult would be most responsible for moving this species from a drifting waterhyacinth plant to another waterhyacinth plant contacted by these infested plants. If any alternate host existed, the adult stage, as it moved from its original host, would have the opportunity for selecting this new host for feeding and oviposition. Rejection of a plant species by the adult, thus, tends to preclude contact of the plant species with other stages of the weevil species. For this reason, oviposition testing was minimized in testing in Argentina. It was used to support the quarantine study, however, when feeding in the starvation test was abundant on an unusual host.

The studies supported the safety of *N. bruchi* in the United States as a biological control agent for waterhyacinth. From these and other studies, a request for approval to import *N. bruchi* was submitted to the Federal Working Group for Biological Control of Weeds and to states where waterhyacinth was a problem. Approval to import the insect was granted, and the first introductions were made in July, 1974.

ACKNOWLEDGMENT

The authors wish to gratefully acknowledge the assistance of P. M. Berger and H. A. Cordo, Insect Research Helpers, USDA laboratory, Hurlingham, Argentina, in the studies in Argentina.

LITERATURE CITED

1. Andres, L. A. and G. W. Angalet. 1963. Notes on the ecology and host specificity of *Microlarinus lareynii,* and *M. lypriformis*

(Coleoptera: Curculionidae) and the biological control of puncturevine, *Tribulus terrestris*. J. Econ. Entomol. 56:333-40.

2. Hustache, A. 1926. Contribution à l' étude des curculionides de la République Argentina (première note). Mus. Nac. Hist. Rivadavia, Buenos Aires 34:155-261.
3. Perkins, B. D. 1973a. Potential for waterhyacinth management with biological agents. Proc. Tall Timbers Conf. Ecol. Anim. Control Habitat Manage. 4:53-64.
4. Perkins, B. D. 1973b. Release in the United States of *Neochetina eichhorniae* Warner, an enemy of waterhyacinth. Proc. 26th Ann. Meeting S. Weed Sci. Soc. 368 (Abstr.)
5. Zwölfer, H. and P. Harris. 1971. Host specificity determination of insects for biological control of weeds. Annu. Rev. Entomol. 16:159-178.