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Importation, Rearing, Release and Establishment of *Neochetina bruchi* (Coleoptera Curculionidae) for the Biological Control of Waterhyacinth in México

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INTRODUCTION

In México, more than 40,000 ha of dams, lakes, canals and drains are infested with waterhyacinth (*Eichhornia crassipes* (Martius) Solms). This weed was probably introduced in the early 1900s (Novelo 1996). Chemical and mechanical control methods have been used to manage waterhyacinth in México since 1950 with varying degrees of success (Gutiérrez et al. 1994). These methods are providing only temporary conrol and are costly. Due to the severe waterhyacinth infestations, an Integrated Aquatic Weed Control Program was created in 1993. As part of this program, a biological control subproject was initiated that included the use of insects and plant pathogens as part of an overall management strategy.

The host-specific weevils *Neochetina eichhorniae* (Warner) and *Neochetina bruchi* (Hustache) are the leading and most successful biological agents used for the control of *E. crassipes* (Harley 1990). *Neochetina eichhorniae* was introduced into Mexico from the U.S. in the late 1970s (Bennett 1984). However, other reports indicated its presence in some places in Mexico as early as 1967 (O'Brien 1976). Recently, three other waterhyacinth-specific insects, have been identified in Mexico. These include *Sameodes albiguttalis* (Warren), *Cornops aquaticum* (Bruner) and *Orthogalumna terebrantis* (Wallwork), all of which occur naturally in México (Gutiérrez et al. 1996). However, additional agents are needed to supplement the current levels of these natural populations. The present work describes the first results from the release and establishment of *N. bruchi* and the results of a complementary release of *N. eichhorniae*.

METHODS AND MATERIALS

Importation of *Neochetina bruchi*

In 1994, 1,200 adult *N. bruchi* were collected from waterhyacinth growing in three Florida lakes (Lake Alice, Lake Okeechobee and Palm Beach). Insects were transported by plane (6 h of transport) and placed immediately into aquariums with waterhyacinth. All insects arrived alive. To detect possible pathogen infection, one sample of 100 insects was checked according to procedures described by Poinar and Thomas (1984).

To obtain a pathogen free colony, weevils were placed on waterhyacinth (for feeding and oviposition) in 50 aquaria (100 L) and filled with 50% Hoagland's solution. After five days, eggs were removed from the plants with forceps under a dissecting microscope. The egg surfaces were sterilized with 0.2% sodium hypochlorite and subsequently inserted into the petioles of fresh plants through punctures made with forceps. The plants were placed in 2 m^2 tanks covered with greenhouse shade cloth (40% light). The tanks were fertilized with a commercial water soluble fertilizer (N:P:K 20:20:20) and a chelated iron powder at rates calculated to provide 5 ppm nitrogen and 2 ppm iron. Tanks were flushed and fertilized twice a month. The adults that emerged from the sterilized eggs formed the basis for all further production. To detect possible insect infections due to fungi, bacteria, microsporidia or nematodes, a fresh sample of 100 young adults from the first, second and third generation were analyzed according to the procedures described by Poinar and Thomas (1984). In order to release both species at sites where none were present, individuals of *N. eichhorniae* were collected at Lake Chapala then propagated as described above.

Mass Rearing of *Neochetina*

Third generation of *Neochetina* spp. was used for mass production. One pair of adults was placed on each plant (80 plants/m^2) in 2 m^2-tanks which were covered with greenhouse shade cloth (40% light) to prevent escape of the insects. After five days, the adults were removed. After 64 to 75 days, adult insects were harvested twice a week. A sample of 100 adults from each harvest was analyzed to detect pathogens (Poinard and Thomas 1984) and to ascertain the population reproductive capacity using a physiological age-grading system (Grodowitz et al. 1997). Another sample of 100 adults, was sent for examination to a specialist on insect diseases (Dr. Jorge Ibarra, Bioinsecticides Laboratory of Mexican Research Institution, CINVESTAV).

Releasing and monitoring *Neochetina*

The number of insects and release sites are given in Table 1. For sites 1 and 2, only biological control was applied. For

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TABLE 1. WATERHYACINTH INFESTATION AND RELEASE OF *NEOCHETINA* SPP. IN MÉXICO FROM 1994 TO 1998.

Site		Area (ha)	Date/number/species Nb = *N. bruchi*/Ne = *N. echhorniae*			Establishment and dispersion	*Height (cm); wet weight (kg/m²); no. plants/m² Initial			After 4 years		
1. Yecapixtla, Morelos								n	**N		n	**N
Altitude:	1,700 m	Flooded area: 10	1994	6,500 Nb;	1,400 Ne	After 2 years WDS = 2	12.5 ± 0.14	12	0.01	12.0 ± 0.17	12	0.02
Climate	Subtropical	(reservoir)	1995	1,850 Nb;	300 Ne	After 4 years WDS = 4	15.6 ± 0.39	12	0.06	15.0 ± 0.38	12	0.06
Temperature	20C	Infested area: 10	1996	4,000 Nb;	400 Ne	40 feeding scars/leaf, 7 Nb and 3 Ne/m²	80 ± 3	12	0.17	79 ± 3	12	0.16
2. San Miguel Regla, Hidalgo												
Altitude:	2,320 m	Flooded area: 50	1995	400 Ne		After 2 years WDS = 2	32.3 ± 1.48	12	0.21	31.9 ± 1.34	12	0.18
Climate	Temperate	(dam)	1996	2,000 Nb;	250 Ne	After 4 years WDS = 4	43.3 ± 1.05	12	0.06	43.0 ± 1.03	12	0.06
Temperature	14.8C	Infested area: 8	1997	5,218 Nb;	2,114 Ne	60 feeding scars/leaf,	192 ± 3	12	0.02	194 ± 3	12	0.02
			1998	1,452 Nb;		6 Nb and 4 Ne/m²						
3. Cuitzeo, Michoacán												
Altitude:	1,820 m	Flooded area: 42,000	1995	206 Nb	206 Nb		29.4 ± 1.31	12	0.20			
Climate	Temperate	(lake)	1996	500 Nb	500 Nb	No establishment	35.7 ± 0.46	12	0.02			
Temperature	17C	Infested area: 650	1997	2,600 Nb	2,600 Nb		122 ± 3	12	0.07			
4. Niágara, Aguascalientes												
Altitude:	1,850 m	Flooded area: 290				After 1 year WDS = 2	14.2 ± 1.75	12	1.53			
Climate	Temperate	(dam)	1998	13,000 Nb	348 Ne	3 feeding scars/leaf,	35.5 ± 0.95	12	0.08			
Temperature	18C	Infested area: 290				1 Nb and 0 Ne/m²	100 ± 5	12	0.23			
5. Rojo Gómez Hidalgo												
Altitude:	2,109 m	Flooded area: 350	1995	200 Nb		No establishment	57.6 ± 1.13	12	0.04			
Climate	Temperate	(dam)	1996	3,700 Nb	250 Ne	Frost decreased water-	32.4 ± 1.13	12	0.12			
Temperature	16C	Infested area: 250	1997	2,652 Nb	2,112 Ne	hyacinth infestation	192 ± 3	12	0.02			
6. Santa Cruz and San Nicolás, Jalisco												
Altitude:	1,524 m	Flooded area: 20.5;29.5	1997	14,871 Nb		After 1 year WDS = 3	19.4 ± 1.16	12	0.36	25.8 ± 1.78	12	0.47
Climate	Subtropical	(dams)	1998	12,078 Nb		60 feeding scars/leaf,	38.5 ± 1.14	12	0.09	43.2 ± 1.10	12	0.06
Temperature	19C	Infested area: 20.5;29.5				5 Nb and 6 Ne/m²	217 ± 3	12	0.02	133 ± 3	12	0.06
7. Chapala, Jalisco												
Altitude:	1,524 m	Flooded area: 108,000	1995	1,010 Nb		After 2 years WDS = 2	23.5 ± 7.34	12	9.77	33.1 ± 7.81	12	5.57
Climate	Subtropical	(lake)	1996	5,000 Nb		After 3 years WDS = 4	35.9 ± 6.64	12	3.41	34.1 ± 9.20	12	7.27
Temperature	19C	Infested area: 18,000				70 feeding scars/leaf, 3 Nb and 48 Ne/m²	148 ± 11	12	0.58	116 ± 19	12	2.76

Weevil dispersal score (WDS): 0 = no weevil feeding scars; 1 = feeding scars only at the release site, 2 = feeding scars 100m from the release site, 3 = feeding scars 500 m from the release site; 4 = feeding scars beyond 500 m.
*Data are the average taken in the peak growth waterhyacinth season (April-May).
**N (minimum sample size) = According to Madsen, 1993.

sites 3 to 7, *Neochetina* releases were made two months after chemical applications using glyphosate or following mechanical control at sites where no chemical treatment had been used. The sites and insect releases were approved by the Mexican plant protection service. One day before the release, adults were placed in plastic containers with water and fresh waterhyacinth leaves. Weevil establishment at all release sites, was evaluated by monitoring the number of each species of *Neochetina* adults and the number of feeding scars observed per m^2 of waterhyacinth. By using a weevil dispersal score (Van Thielen et al. 1994), insect dispersion was evaluated from the release site to places where insects or feeding scars were found. Monitoring was carried out every four months using an airboat. The effect of *Neochetina*, was evaluated by measuring plant height, number of plants and wet weight per m^2 of waterhyacinth. Table 1 contains the data for waterhyacinth at the peak of growth.

RESULTS AND DISCUSSION

Success or failure of a biological control agent to establish itself depends partially on the health of the individuals released (Riba and Silvy 1989). The results of ours tests for detecting pathogens on the ovipositing parents, showed that these insects were healthy. However, in order to detect if the ovipositing parents carried a possible pathogen, three generations of *Neochetina* spp. were checked before insect releases were made. No pathogens were found at our laboratory or at CINVESTAV. These procedures ensured that colonies that were produced were free of pathogens.

The physiological age-grading system provided a means of assessing whether *Neochetina* females reared at our facilities, were able to reproduce in the field. In a three generation sample of 300 females, 162 showed a stage **P3**; 78 were stage **P2**, 42 were stage **P1** and 18 were stage **N3**. Stage **P1**, **P2** and **P3**, are parous stages with fully developed eggs. The mean number of eggs oviposited was 2 for stage **P1**, 12 for stage **P2** and 38 for stage **P3**. Stage N3 is a nulliparous stage characterized by females with fully developed follicles and ready for ovulation. These results showed that 94% of females contained functioning ovaries and 6% were young individuals.

The physiological age-grading system and tests to detect pathogens used in studies of three generations of *Neochetina* spp. ensured the colonies produced were able to reproduce and were free of pathogens. With this system, it was possible to make repeated releases of *Neochetina* spp. with healthy and fecund individuals.

Four years after release, *N. bruchi* was well established in 5 of the 7 release sites (Table 1), where the climate varied from temperate (Hidalgo: 10C in winter) to subtropical (Morelos: 35C in summer). The weevils were released in areas where the waterhyacinth had not been disturbed and allowed to remain untouched for at least three months.

At site 3 (Cuitzeo), fishermen removed the waterhyacinth plants on which *Neochetina* was released. Five sites (except San Miguel Regla and Yecapixtla), had been included in an integrated weed control program with the use of glyphosate, mechanical and manual controls to reduce the infestation (Gutiérrez et al. 1994, 1996).

From April 1994 to August 1998, 84,911 *Neochetina* spp. adults were released (Table 1). At sites 1 and 2, only biocontrol was applied. Two years after release, the plants showed numerous feedings scars and weevils had advanced 100 m from the release site. Four years after the original release, at least 7 *N. bruchi* and 4 *N. eichhorniae* per m^2 of waterhyacinth were observed; 90% of all leaves showed numerous feeding scars and weevils were found more than 500 m from the release site.

At sites 3 and 5 establishment was not obtained due to manual extraction of infested plants and frost. In Jalisco (sites 6 and 7), only *N. bruchi* was released because *N. eichhorniae* had been identified there in 1975 (Bennett 1984). At Chapala Lake, climatic conditions (prevailing winds, heavy swell) and a decrease in water volume ($5{,}050\ m^3 \times 10^6$ in 1994 and $3{,}090\ m^3 \times 10^6$ in 1998) due to drought, caused a significant reduction of waterhyacinth populations (Gutiérrez et al. 1998).

In summary, although *Neochetina* was introduced and established in México, a substantial increase in insect populations was not observed (Table 1). Despite numerous feeding scars found on most plants, no substantial reduction in plant size, wet weight and number of plants per m^2 were observed after four years of *Neochetina* spp. release (Table 1). Center (1980) observed that *Neochetina* accelerates the senescence of waterhyacinth leaves but that this has little affect on insect survivorship. According to Perkins (1978), *Neochetina* population increases slowly and therefore weevil density is often too low for plant control. Some authors (Center and Wright 1991, Center 1992), suggested that plant quality may influence the abundance of *Neochetina*. Center and Dray (1992) found that weevil population growth was satisfactory on higher-quality plants (healthy and green plants) and such plants are associated with eutrophic conditions. In this study, the waterbodies where *Neochetina* spp. were released were eutrophic. These conditions are propitious for rapid plant growth. Therefore plant reproduction may have occurred a faster rate than the weevils could inflict damage. Moreover, these insects have relatively long life cycles (66 to 75 days for *N. bruchi* and 96 to 120 for *N. eicchorniae*); therefore, population build-up is slow. It is important to continue monitoring waterhyacinth populations in order to determine the impact of these insects.

Even though *Neochetina* is well established in México, the effectiveness of biological control requires the use of additional agents to complement existing ones (Charudattan 1986). Studies have begun in México to determine indigenous species of pathogens and evaluate how the most promising of these may be applied as biological herbicides in areas where *Neochetina* is present to enhance the control effect (Martínez and Charudattan 1998, Martínez and Gutiérrez 2001).

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