Biological control of ***water hyacinth***

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The weevils *Neochetina bruchi* and *N. eichhorniae*: biologies, host ranges, and rearing, releasing and monitoring techniques for biological control of *Eichhornia crassipes*

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Chapter 1
Introduction

Water hyacinth is widely recognised as the world's worst aquatic weed. Originally exported from its native Amazonia because of its attractive flowers, the species rapidly established and spread throughout tropical, subtropical and warm temperate regions of the world. Water hyacinth forms dense impenetrable mats across water surfaces, limiting access by man, animals and machinery. Navigation and fishing are obstructed, and irrigation and drainage systems become blocked. The consequences are devastating for those communities reliant on water bodies for water, food, sanitation and transport. Programs to control its growth have been initiated in most countries where it occurs.

Photo: M. Julien

Water hyacinth: the world's worst aquatic weed

Photo: M. Julien

Water hyacinth at Port Bell, Uganda

Chemical and mechanical control measures have been used since the early 1900s to combat water hyacinth, but are expensive and ineffective on all but small infestations. Eradication of the weed has been rare because of its rapid growth rate and its ability to reinfest from seeds or isolated plants. Increasing concern about the financial and environmental costs associated with herbicidal control measures and their limited effectiveness has led to growing interest in the use of biological control. Host-specific biological control agents have been identified and researched since the 1960s.

Biological control of water hyacinth offers sustainable, environmentally-friendly, long-term control, and is the only feasible method to provide some level of control to those infestations which cover huge areas, are

Photo: M. Julien

Lush growth in a nutrient-rich pond—Papua New Guinea

difficult to access and/or do not warrant the high cost of physical or chemical control. Several biological control agents have now been introduced into countries having problems with water hyacinth. The species most widely used are the *Neochetina* weevils, *N. bruchi* and *N. eichhorniae*. These have been introduced in more than 30 countries and are contributing to weed control in many areas.

These thoroughly researched, proven biological control agents are readily available for introduction into countries where water hyacinth is a problem. As so much research has already been done, these and other control agents can be introduced into new regions comparatively cheaply. The prospects are excellent for successful and sustainable long-term control of water hyacinth in many situations.

Some definitions

Biological control: The use of natural enemies of a weed or pest to suppress populations of the weed or pest.

Natural enemies: Organisms that attack another organism in its native range and thus contribute to the maintenance of population levels.

Classical biological control of weeds: The use of target-specific natural enemies of a weed to suppress populations of the weed in its exotic range.

Biological control agents: Natural enemies (usually insects but also mites, fungi, nematodes, fish) that have been released to control a weed. They have normally undergone studies to determine the range of plant species that they are capable of damaging and are only released if they do not pose a threat to other organisms.

[Definitions adapted from DeBach (1964), Hokkanen (1985), Huffacker and Messenger (1976), and Waage and Greathead (1988)].

Chapter 2
Water Hyacinth

2.1 Description

Water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach, is a perennial, herbaceous, aquatic plant of the family Pontederiaceae. The genus *Eichhornia* contains a number of other species all of which are aquatic, but only *E. crassipes* has become a serious weed. The leaves of water hyacinth are comprised of a smooth, glossy, circular to kidney-shaped **lamina** and a swollen, spongy **petiole** (Figure 1). The petioles contain air, causing plants to float on the water surface. This characteristic distinguishes water hyacinth from other members of the family Pontederiaceae which remain firmly rooted in the substrate. **Stolons** grow horizontally to produce **daughter plants** from terminal buds. The bisexual **flowers** are bluish purple with a yellow centre and are produced on single **spikes** to 60 cm in length. The flowers can self-fertilise. The **roots** are long, fibrous and feather-like, and are often dark in colour (Harley 1990; Parsons and Cuthbertson 1992; Wright and Purcell 1995).

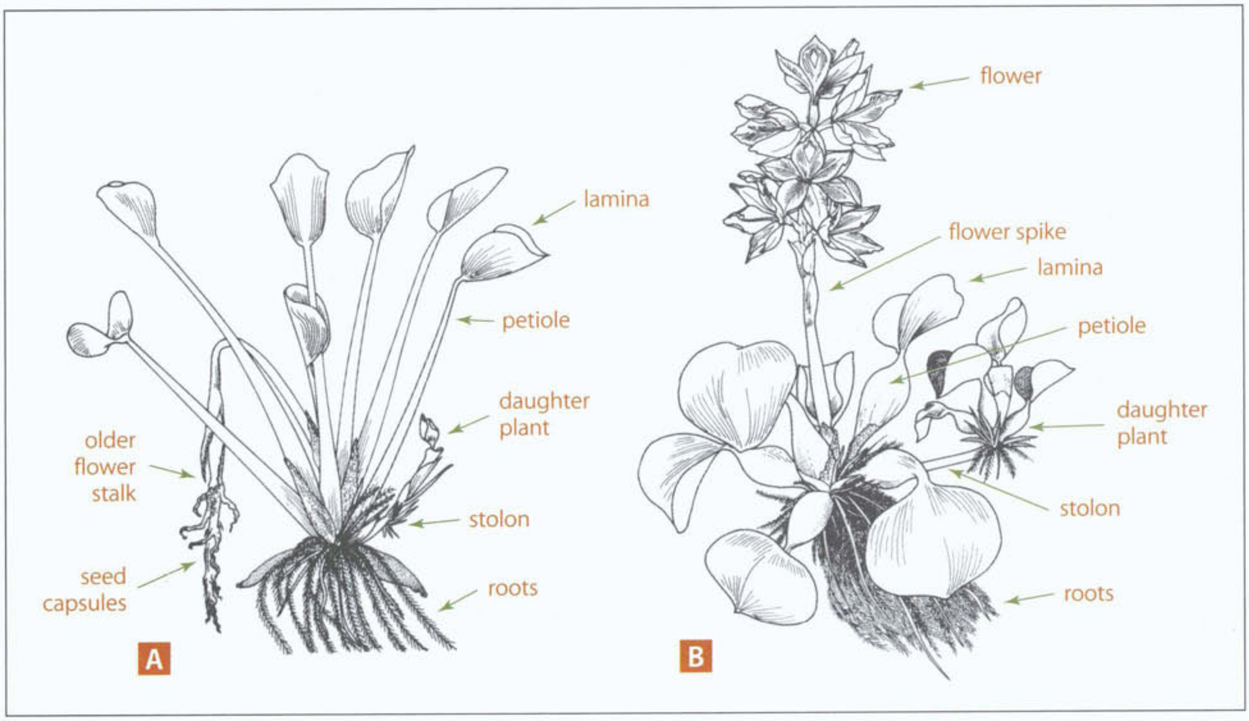


Figure 1. Water hyacinth plants with (A) slender petioles and (B) bulbous petioles (from Wright and Purcell 1995)

Water hyacinth shows considerable variation in both leaf and flower form. The petioles vary from long and slender to swollen or bulbous. The shape of the petiole influences the amount of air contained and consequently the capacity for the plant to float. More slender petioles are typical of plants which occur within dense, crowded infestations, while more bulbous petioles characterise plants in open water or on the open-water margins of infestations. Flowers are of three distinct types, differing in the lengths of the styles and stamen (Barrett 1977). In the introduced range of the species the form with a style of intermediate length predominates, the long-styled form occurs less frequently and the short-styled form has not been recorded (Barrett and Forno 1982). Seeds are produced in large quantities, up to 300 per plant (Wright and Purcell 1995), and are long-lived, remaining viable for 5 to 20 years (Manson and Manson 1958; Matthews 1967; Das 1969;

Photo: M. Julien

Water hyacinth can devastate local communities reliant on waterways for food and transportation—Papua New Guinea

Photo: M. Julien

Water hyacinth limits access to open water—Lake Victoria, Kenya

Photo: M. Julien

Offshoot (daughter) plants connected by a stolon to the main (parent) plant

Photo: A. Wright

Extensive mats of water hyacinth hinder shipping—Kisumu Bay, Lake Victoria, Kenya

Matthews et al. 1977). Seeds sink following release from the seed capsule and may subsequently germinate as water levels fluctuate (see Wright and Purcell 1995).

Vegetative reproduction is common and is largely responsible for the rapid increase and spread of water hyacinth into new areas. The daughter plants produced from the horizontal stolons develop roots and eventually separate from the mother plant following decay or breakage of the connecting stolon. These plants are readily distributed by currents, winds, fishing nets and water craft. Under favourable conditions a single plant can develop into a substantial infestation in a very short time.

2.2 Distribution

The centre of origin of water hyacinth is believed to be Amazonia, Brazil, with natural spread throughout Brazil and to other Central and South American countries (Penfound and Earle 1948; Sculthorpe 1967; Little 1968; Barrett and Forno 1982). The spread of water hyacinth into new areas commenced in the 1880s with its deliberate introduction into the USA as an attractive pond ornamental. Live plants were supposedly handed out to visitors at the 1884 New Orleans Cotton Expo (Center 1994). Thereafter plants continued to be spread around the USA and eventually around the world. Many of these plants were disposed of or spread into ponds and waterways where they rapidly established and continued to expand their range.

The spread of water hyacinth has been spectacular and disastrous. The weed was recorded in Egypt, Australia and southern Asia by the 1890s (Gopal and Sharma 1981), China and the Pacific by the early 1900s (Waterhouse and Norris 1987), East Africa by the 1930s (Chikwenhere 1994), West Africa by the 1970s (van Thielen et al. 1994), and is now established throughout tropical and warm-temperate regions of the world from 40°N (Portugal) to 45°S (New Zealand) (Holm et al. 1977; Julien et al. 1996) (Figure 2). Particularly extensive infestations developed in the southern USA, Mexico, Panama, much of Africa, the Indian sub-continent, Southeast Asia, Indonesia, Australia and the Pacific.

Photo: M. Julien

Dense water hyacinth blocks access to Nveye Lagoon, Ghana

Photo: M. Julien

Water hyacinth seriously restricts transportation—Sepik River, Papua New Guinea

Figure 2. Countries where water hyacinth is a weed and where *Neochetina* spp. have been released

2.3 Habitat

Optimum growth of water hyacinth occurs in eutrophic, still or slow-moving fresh water with a pH of 7, a temperature range between 28° and 30°C, and abundant nitrogen, phosphorus and potassium (Chadwick and Obeid 1966; Knipling et al. 1970; Reddy et al. 1989, 1990, 1991). Plants will, however, tolerate a wide range of growth conditions and climatic extremes. Good growth can continue at temperatures ranging from 22° to 35°C and plants will survive frosting (Wright and Purcell 1995). Although prolonged cold weather may kill plants, the seeds remain viable (Ueki and Oki 1979). Plants can infest pristine, relatively low nutrient waterways (Hitchcock et al. 1949) and will survive for several months in low-moisture substrates. They will tolerate acidic waters but cannot survive in salt or brackish water (Penfound and Earle 1948).

2.4 Impact

In its native range water hyacinth is largely restricted to coastal lowlands and along the margins of lagoons and slow-moving waters. It occurs at relatively low densities, only becoming a problem where the hydrological regime of a water body has been altered by human activities, or where the level of nutrients in the water has been increased.

Within its introduced range, however, the species has enormous social, economic and environmental impacts, earning this plant the title 'world's worst aquatic weed' (Holm et al. 1977). Water hyacinth forms dense, impenetrable mats which cover the water surface. Water bodies which are worst affected are still or slow-moving, and include natural water courses, natural and artificial lakes, irrigation and flood mitigation channels, and dams. The presence of water hyacinth limits access to and use of water by man, animals and birds. The weed chokes intake points for water treatment and supply, and for hydroelectric and other industrial requirements. Navigation is obstructed and irrigation systems become blocked (Harley 1990; Harley et al. 1996). Fishing is often limited or prevented, and the germination and establishment of paddy rice seedlings can be affected. The presence of the weed provides suitable breeding sites for vectors of human and animal diseases, increasing the incidence of diseases such as malaria, encephalitis, schistosomiasis, filariasis, river blindness and possibly cholera (Burton 1960; Seabrook 1962; Spira et al. 1981; Gopal 1987; Viswam et al. 1989). The weed mats also create a habitat attractive to venomous snakes.

The presence of water hyacinth has a direct impact on the hydrological balance of a system. Water hyacinth loses water rapidly through its leaves. This can increase dramatically the rate of water loss from a water body, imposing higher operational costs on water supply schemes (Benton et al. 1978) and threatening their viability in arid regions. During floods, water hyacinth can build up against bridges, culverts, fences etc., thereby obstructing water flow and increasing flood levels, and contributing to loss of life and livestock, damage to property and equipment, and serious soil erosion (Harley 1990).

Extensive mats of water hyacinth also change the physical and chemical composition of the water beneath (Ultsch 1973; Reddy et al. 1983; Aneja and Singh 1992). Light penetration is reduced and oxygen levels decline, resulting in anaerobic conditions. This leads to biological changes in the water body that are unfavourable to communities of aquatic vertebrates, invertebrates and plants (Timmer and Weldon 1967; Ultsch 1973; Willoughby et al. 1993).

2.5 Utilisation

The sheer biomass of plant material in water hyacinth infestations has prompted investigation of various schemes for its utilisation (Monsad 1979; Wolverton and McDonald 1979; see papers in Thyagarajan 1984). Schemes suggested include using the weed:

- as an animal fodder, fertiliser, compost or source of fuel;
- in the manufacture of paper, board, handicraft and furniture;
- in the treatment of waste water; and
- in the management of water quality.

Water hyacinth has limited potential for use in any such programs, and its utilisation is never likely to provide a viable method for controlling or managing the weed. With the exception of small-scale specialist or cottage industries, harvesting for commercial use is unlikely to be viable, because of the complications and high costs associated with accessing and harvesting from infested areas, transporting the plants, and drying, processing and marketing the material. Water hyacinth is 95% water (Harley 1990), making collection costs extremely high for only a 5% dry matter return. The possible advantages of utilising water hyacinth are far outweighed by the enormous problems this weed causes throughout its introduced range. Attempts to control the weed should not therefore be compromised by any consideration of its potential use (Julien et al. 1996).

Chapter 3
Management of Water Hyacinth

The complete removal of water hyacinth is impossible for most areas. Where 'eradication' of an infestation has occurred the effects are usually short-term. The difficulties in achieving effective eradication stem from the ease with which reinfestation can occur in all but small and isolated water bodies, and the subsequent rapid growth and spread of the weed. Plants and/or seeds are readily transported by currents, boats, fishing nets and possibly animals and birds, and only one or a few plants can result in a new infestation. The seeds are long-lived and germination can continue for up to 20 years (see Section 2.1). The aim of any control program is therefore to manage, rather than eradicate, this weed species. In many situations, management extends only to maintaining open water around critical sites, for example village watering points, navigation channels and intake points for water supply, water treatment or hydroelectricity.

Photo: A. Wright

Removal of water hyacinth by hand near Klong Krea Irrigation Project, Thailand

Photo: M. Julien

Mechanical harvester at Port Bell, Uganda

Control methods fall into three main categories: physical, chemical and biological. The application of these methods is not mutually exclusive and 'best practice' is to formulate a management strategy incorporating some or all of these methods, but with reliance on biological control as the most significant component or the long term objective (Harley et al. 1996; Julien et al. 1996). Integration of control measures is discussed further in Section 8.2.

3.1 Physical

Physical removal is historically the most widely used form of control. For the poorer rural communities to whom water hyacinth is so often a threat, removal by hand pulling is often the only available option; an extremely laborious process. In many areas, mechanical

Photo: C. Cilliers

Aerial spraying with herbicide—South Africa

harvesters have been developed which speed the physical removal of water hyacinth. A few of these have been effective in particular situations, but most have been abandoned as ineffective and/or too expensive to operate. Floating booms and barriers are used to maintain areas free of weed and to reduce the downstream spread of an infestation. Plants accumulate rapidly against the booms and must be removed frequently, either physically or by herbicide spraying. Draining a water body will lead to the death of water hyacinth plants, but seeds usually germinate when water is reintroduced.

Photo: A. Wright

Mechanical harvester operating near Bangkok, Thailand

Photo: C. Cilliers

Removal of water hyacinth by hand near Vaal River, South Africa

The rate of growth and invasion by water hyacinth usually exceeds the rate at which it can be cleared. Reinfestation from plant fragments and/or seeds generally occurs rapidly and the process of removal must be repeated continuously. The material removed from the water should be transported away from the site and disposed of appropriately. Physical removal is useful only on small infestations, in delaying the resurgence of the weed following chemical control, and in situations such as ports, hydroelectricity plants, fish landings etc. where the high labour and/or monetary costs can be justified.

3.2 Chemical

Treatment with herbicides has been effective in controlling small infestations of water hyacinth, and infestations in areas climatically unfavourable to the growth of the weed. The herbicides most commonly used are diquat, glyphosate, amitrole, and the amine and acid formulations of 2,4-D, applied as foliar sprays. The application of these compounds requires skilled operators, strict spray regimes, long-term vigilance and frequent reapplication to provide effective, long-term control over the weed and any regrowth. In most situations, chemical control is unacceptably costly in terms of chemicals, equipment, labour and environmental impact. The problem of chemical sprays is compounded by the use of many water hyacinth-infested sites for obtaining drinking water, for washing and for fishing. The costs associated with herbicide application generally limit the applicability of chemical control to an emergency control measure at critical sites rather than for maintenance control over large infestations.

3.3 Biological

In its native range water hyacinth is attacked by a complex of arthropods. Study of the life history and ecology of some of these began in Argentina in 1961 (Center 1994). Research has shown that some are unable to survive on any plant other than water hyacinth, while others may also survive on some very closely related plant species. The first natural enemies were released as control agents in the USA in the early 1970s (Perkins 1973) and to date seven agents have been released in 33 countries (Julien and Griffiths 1998) (Table 1, Figures 5 and 6).

One or more natural enemies have established in most of the countries in which they have been released, and their impact on water hyacinth has been significant in some areas.

The two *Neochetina* species are the most widely distributed of the water hyacinth biological control agents, and to date are the most successful. This dossier discusses the biology, impact, host range and use of these agents.

Table 1. Biological control agents released against water hyacinth worldwide

Agent	Type of damage	Countries where released
Insects		
Coleoptera		
Curculionidae		
Neochetina bruchi Hustache	Adults feed on foliage and petioles, larvae tunnel in petioles and crown	see Figure 5
Neochetina eichhorniae Warner	Adults feed on foliage and petioles, larvae tunnel in petioles and crown	see Figure 6
Lepidoptera		
Pyralidae		
Niphograpta albiguttalis Warren (=*Sameodes albiguttalis* (Warren)	Larvae tunnel in petioles and buds	Australia Benin Ghana Malawi Malaysia Panama Papua New Guinea R. South Africa Sudan Thailand USA Zambia Zimbabwe
Pyralidae		
Xubida infusella (Walker) (=*Acigona infusella* Walker)	Larvae tunnel in petioles and buds	Australia Thailand Papua New Guinea
Hemiptera		
Miridae		
Eccritotarsus catarinensis (Carvalho)	Adults and nymphs suck cellular or intercellular fluid from leaves	Malawi R. South Africa Zambia
Mites		
Acarina		
Galumnidae		
Orthogalumna terebrantis Wallwork	Immatures tunnel in laminae	India Malawi R. South Africa Zambia
Fungi		
Hyphomycetes		
Cercospora rodmanii Conway	Punctate spotting and chlorosis of laminae and petioles; necrosis of laminae	R. South Africa

References: Julien and Griffiths (1998), M. Hill, pers. comm.

Chapter 4
The Water Hyacinth Weevils

The genus *Neochetina* is considered to have six species, all of South American origin and all restricted in their feeding to the family Pontederiaceae. The two *Neochetina* species in use as biological control agents are *N. bruchi*, the chevroned water hyacinth weevil, and *N. eichhorniae*, the water hyacinth weevil. A generalised life-cycle of the weevils is shown in Figure 3. The development durations of the different life stages and average fecundities are detailed in Table 2. The following descriptions of the biology and life histories of the weevils are summarised from DeLoach and Cordo (1976), Center (1982, 1994), Jayanth (1987), Harley (1990), and Ogwang and Molo (1997).

4.1 *Neochetina bruchi*

Egg: Eggs are whitish, ovoid and measure 0.8 mm × 0.6 mm. They are laid singly or in groups of up to 25, usually deposited in the 2nd or 3rd layer of aerenchymatous cells in the middle third of the petiole, particularly on older leaves. Eggs are often laid in holes chewed by the female or in small necrotic spots. Eggs fail to hatch below 15°C.

Larva: Larvae are uniformly white with a light-brown head capsule. There are three larval instars. Newly hatched larvae tunnel towards the bases of petioles and into the crowns where they excavate small pockets. From this location larvae often feed on developing axillary buds. Larvae usually occur singly, but occasionally their tunnels become joined and 2 or 3 larvae occur together. As the plant grows, leaves containing feeding larvae are displaced towards the outer edge of the rosette. Consequently, older instar larvae often occur in the older leaves from where they sometimes migrate back into the younger leaves. The impact of feeding on these youngest leaves is particularly severe.

Pupa: Fully grown larvae exit the crown and move down to the roots to pupate under water. They construct a dark, circular cocoon about 2 mm diameter using excised root hairs and attach this to one of the larger roots. A period of dormancy has been reported from South Africa where fully-formed adults remain within the pupal cocoon for several months (M. Hill, pers. comm.).

Photo: A. White

Leaf feeding scars caused by *Neochetina* adults

Figure 3. Generalised life cycle of the *Neochetina* weevils and durations of developmental stages

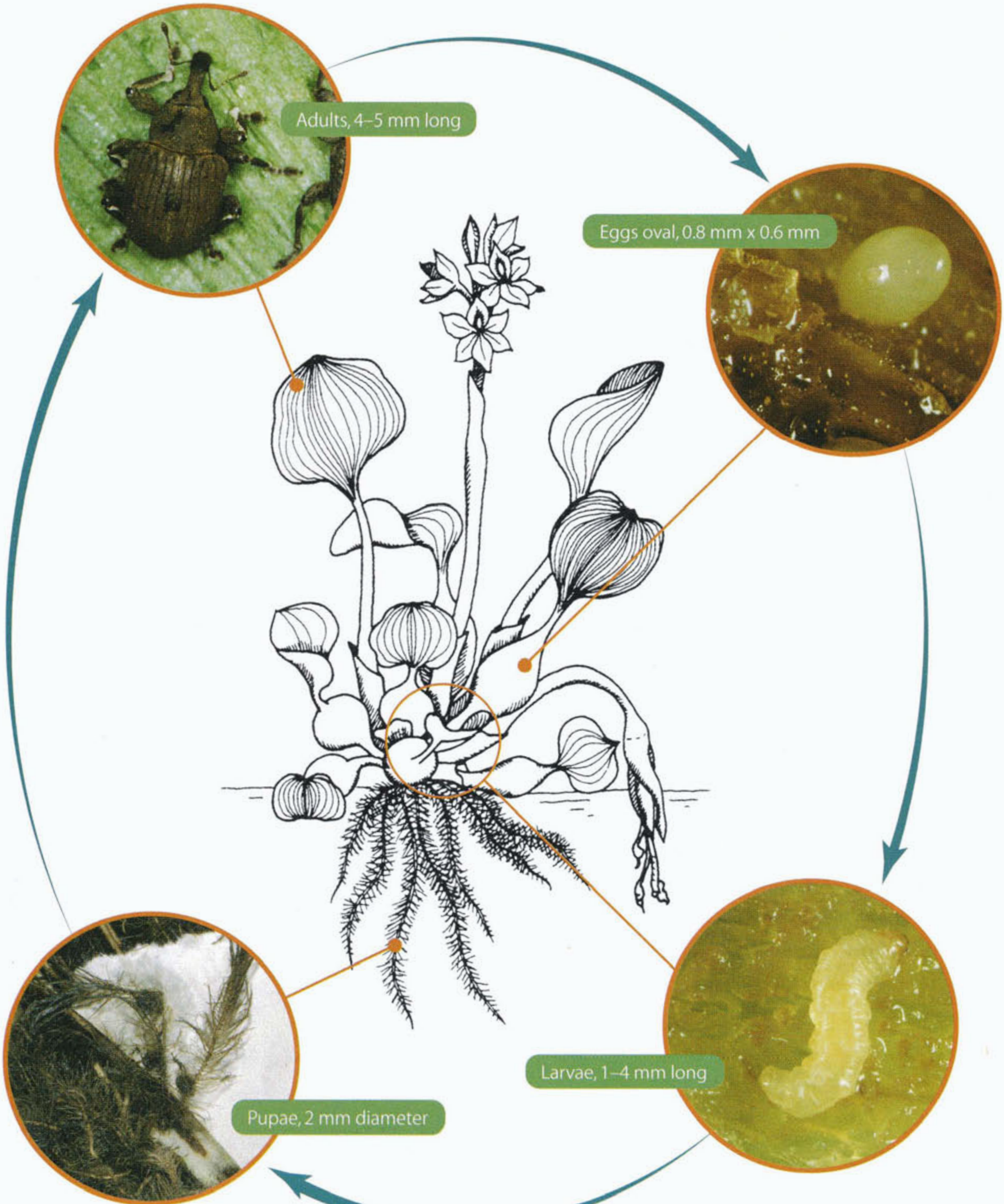


Table 2. Development durations for each life-cycle stage and fecundities for both *Neochetina* species

	Approximate duration (days)					
	N. bruchi			*N. eichhorniae*		
	Argentina[1]	Uganda[2]	India[3]	Argentina[1,4]	Uganda[2]	India[3]
Development stage						
Egg	7.6	7		7–14	10	
Larva						
I instar	10					
II instar	14					
III instar	6					
Total	32	35		75–90	58	
Prepupa	7	10				
Pupa	23	23				
Prepupa + pupa	30			14–20	28	
Generation time	96	72		120	96	
Adult longevity						
Average	89		134			142
Max.				309 (field)		
Fecundity						
Total	293		682			891
Average			4.5			
Daily max.	8.5		26	5–7		

References: [1]DeLoach and Cordo (1976), [2]Ogwang and Molo (1997), [3]Jayanth (1987), [4]Harley (1990)

Adult: Adult beetles are 4–5 mm long and brownish to grey. They are nocturnal and during the day lie concealed near the plant crown. Adults commence feeding within 24 hours of emergence. They feed externally by scraping the epidermal layer and some of the underlying cells to form small, characteristic subcircular scars, which usually do not penetrate through to the other side of the leaf. Adults feed preferentially on the narrow upper third of the petiole and on the upper surface of the lamina, particularly of the first or second youngest leaf. Some feeding may occur during daylight when adults are largely stationary and concealed. Females commence oviposition between 3 and 7 days after emergence. Oviposition peaks during the second week and close to 90% of eggs are laid by the fifth week. The optimal temperature for feeding and oviposition is approximately 30°C. Adults are susceptible to heat and low relative humidity, and exposure to high temperatures may result in a decrease in egg production and death of adults.

Photo: A. Wright

Early stages of larval feeding damage caused by *Neochetina* weevils

Photo: A. Wright

Advanced stages of larval feeding damage caused by *Neochetina* weevils

Photo: M. Julien

Healthy, undamaged water hyacinth plant

4.2 *Neochetina eichhorniae*

Egg: Eggs are more slender and are softer than those of *N. bruchi* and are usually deposited singly just beneath the epidermal layer. The eggs are visible under the epidermis, and cause a slight swelling on the leaf surface. In Argentina, eggs were laid into young central leaves, tender petiole bases or in the ligules, but in Florida eggs were concentrated in mature leaves. At 25°C females lay between 5 and 7 eggs/day, to a total of approximately 300 eggs per female. Eggs require higher temperatures than those of *N. bruchi* to develop normally, and will not hatch at temperatures below 20°C

Larva: Larval behaviour is as for *N. bruchi*. Larvae develop through three instars.

Pupa: The pupal cocoons of the two species are indistinguishable by casual observation, and the pupation behaviour is similar.

Photo: M. Julien

Stunted, heavily damaged water hyacinth plant following feeding by *Neochetina* spp.

Adult: Like *N. bruchi* the adult beetles are nocturnal and feed externally. Adults commence feeding within 24 hours of emergence, and the first eggs are laid approximately 6 days later. Feeding scars of the two species cannot be reliably distinguished, although there is a tendency for *N. bruchi* to produce larger feeding scars than *N. eichhorniae*. Under laboratory conditions feeding scars ranged from 0.5 mm^2 to 25 mm^2, and those of females were slightly larger than those of males. During the day adults are frequently found in the crown of the plant. The sex ratio during rearing is close to 1:1 although in field collections an excess of one or other sex has been recorded. Observations in South Africa suggest that when plants are healthy there is an excess of females while on unhealthy plants males predominate (M. Hill, pers. comm.).

4.3 Key differences

Although *N. eichhorniae* and *N. bruchi* resemble each other in appearance, life history and behaviour, they differ in a number of characteristics:

Adult size

Adult *N. bruchi* are on average larger than *N. eichhorniae*, weighing a mean of 4.53 mg (n = 143) compared with 3.49 mg (n = 34) for (DeLoach and Cordo 1976).

Morphology

Morphologically, the two species are most clearly distinguishable in the adult stage. The main morphological differences are highlighted in Figure 4.

Larval development times

In general the larvae of *N. eichhorniae* develop more slowly than those of *N. bruchi* (Table 2). Development rates vary with temperature and the quality of the plant material.

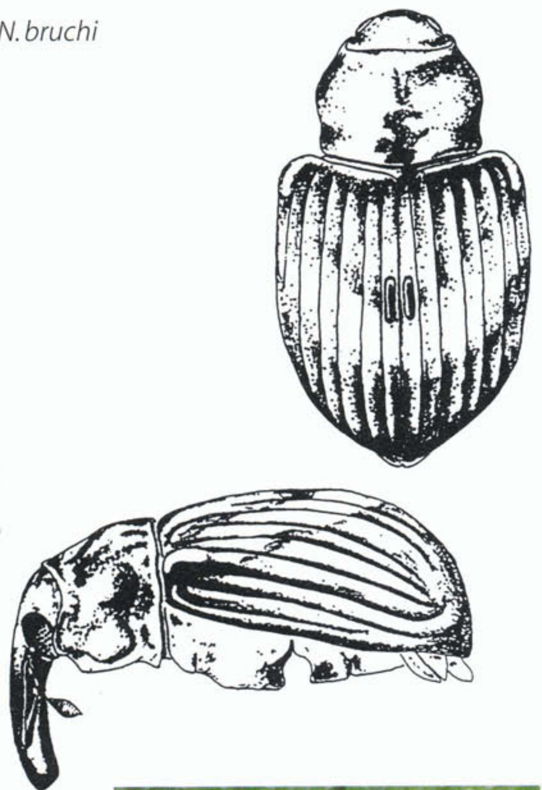
Nutrient requirements

N. bruchi are more dependent on better quality plant material for their successful development than are *N. eichhorniae* (Center 1994; Heard and Winterton, unpub. data). Consequently, the relative abundance of the two species may vary according to the quality of the host plant. Sites of poor plant quality (reflected by lower average tissue nitrogen concentration) tend to have more *N. eichhorniae*, while those of higher plant quality contain a higher proportion of *N. bruchi*.

Figure 4. Key morphological differences between adult *Neochetina bruchi* and *N. eichhorniae*

Character	*N. bruchi*	*N. eichhorniae*
Elytra markings	short and located midway along elytra	long and extending forward on elytra
	generally of equal length	generally of unequal length
Elytra furrows	broader furrows with shallow curvature	narrow furrows with strong curvature
Elytra patterning	scale coloration forms a chevron or V shape across entire elytra - this is most obvious on newly emerged adults but fades as adults age	chevron or V pattern absent from elytra

N. bruchi



Diagrams: T.D. Center

Photo: M. Day

N. eichhorniae

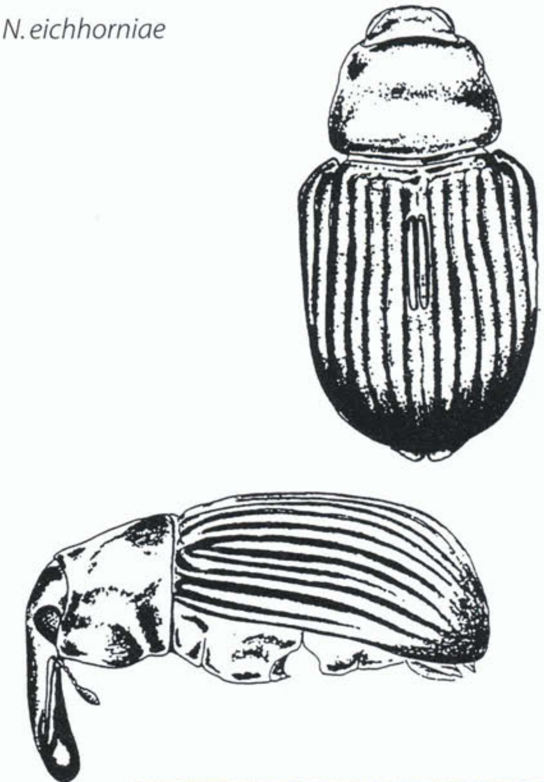


Photo: M. Day

Photo: M. Julien

Neochetina damage kills leaves, causes waterlogging and eventually whole plants die and mats sink—Gerehu Lake, Papua New Guinea

These differences influence the relative suitability of the two species as control agents under different situations. In many situations, one or other species will come to dominate under particular conditions. In general, the two species complement each other, and better control of water hyacinth often occurs when both species are present than when either species is present alone.

4.4 Impact on weed

Damage to water hyacinth comes from both the adults and the larvae. Adult feeding produces distinctive feeding scars on the leaf surface which are clearly visible and easily recognised. Larvae are rarely seen, as they tunnel and remain within the plant tissue from shortly after hatching. Their presence can be determined from the presence of streaks of necrotic tissue just beneath the epidermis of petioles.

Heavy feeding by adult weevils on the lamina causes leaves to desiccate and curl. Under pressure from this damage and from larval feeding the petioles become thin, spindly and brittle, plants become waterlogged and gradually sink. The dense mat of water hyacinth starts to fragment, with patches of water becoming visible between the plants. Areas of newest growth and smallest plants are affected first, so that colonisation by the weevils initially results in a stand of more uniform plant size and structure. The production of flowers, leaves and offshoots is reduced, and plant growth is stunted. Eventually the size of the mat decreases and the area of open water increases. As mats become smaller they are more easily flushed from the system. The speed and efficiency with which control is achieved depends, amongst other factors, on the number of insects released and their distribution through the infestation.

Chapter 5
Host-range Testing

It is crucial that any agent introduced for biological control of a weed does not itself become a pest. Agents must be able to reproduce and sustain a viable population only on the target weed and, possibly, on a number of closely related plants which are also weeds or are plants of no economic or ecological significance to the country of release. To ensure this, *Neochetina bruchi* and *N. eichhorniae* have undergone extensive host testing in numerous countries before their release (see Table 3). The plant species tested and the results of these trials are shown in Appendixes 1, 2 and 3.

The list of plants against which the insects have been tested is long and diverse (Appendix 1), covering 274 plant species in 77 families, representing a wide range of terrestrial, aquatic, economic, exotic and native plant species. The list includes plants taxonomically related to water hyacinth and plants that are taxonomically unrelated but of economic or agricultural importance. Most trials assessed the level of feeding by adults in starvation or choice tests. Some trials noted whether eggs were laid on test plants and monitored the survival of any developing larvae. On a few occasions eggs, newly hatched larvae or older larvae were placed onto plants, usually those species damaged by adult feeding, and their development was monitored.

Adult *N. bruchi* fed to some extent on 50 species of test plant (Appendix 2). All feeding was significantly less on non-target plants than on water hyacinth. The most consistently damaged plant species were those in the same family as water hyacinth (Pontederiaceae); those in the family Commelinaceae; *Pistia stratiotes* (Araceae); *Lactuca sativa* (Asteraceae); and *Brassica* spp. (Brassicaceae). *N. bruchi* laid one or several eggs on 22 plant species of which 9 (40%) were in the families Pontederiaceae or Commelinaceae. The total numbers of eggs laid on non-target plants was very low and no larvae completed development. Similarly, eggs or larvae placed on plants other than water hyacinth failed to develop and invariably survived for only a few days.

N. eichhorniae made one or several exploratory feeding scars on the foliage of 25 test plants (Appendix 3). Feeding on these plants was much less than on water hyacinth. Most feeding was barely detectable and caused no serious damage to the plants. Eggs of *N. eichhorniae* were laid on 7 species of test plant of which more than half were in the families Pontederiaceae or Commelinaceae. Most of these eggs were infertile and, if larvae hatched, they died soon after. Similarly, larvae inserted into stems of test plants did not feed and died within a short period. The only plant, other than water hyacinth, on which any larval development was observed was *Pontederia cordata* L.

(Pontederiaceae), and no larvae completed development on this plant during testing.

The host-specificity of these insects has been demonstrated during extensive host testing and confirmed by observations after their release. Despite being released widely there are no reports of these weevils seeking out and damaging plants other than water hyacinth. In Australia, feeding and larval damage were observed on *P. cordata* in the field, but only when plants of this species were placed amongst water hyacinth under heavy attack from *Neochetina* spp. (Stanley and Julien unpub. data). Damage has never been observed in other situations. Further support for their specificity comes from knowledge of the life-history of the *Neochetina* spp. The pupation behaviour of these insects, whereby they make a pupal cocoon in the roots of floating water hyacinth (Figure 3), makes it highly unlikely that any substrate-rooted plant could provide a suitable host.

Table 3. Countries and organisations which have undertaken host-specificity trials with *Neochetina bruchi* and *N. eichhorniae*. + indicates that the test list for this country is included in Appendix 1, – indicates that the test list was not available.

Country	*N. bruchi*	*N. eichhorniae*	Organisation	References
Argentina/USA	+	+[a]	United States Department of Agriculture (USDA)	1
Australia	+	+	Commonwealth Scientific and Research Organisation (CSIRO)	2
Egypt[b]	+[a]	+[a]	Department of Biological Control, Plant Protection Research Institute, Agricultural Research Centre	3
India	+	+	Indian Institute of Horticultural Research (IIHR)	4
Indonesia	+[a]	–	SEAMEO-BIOTROP	5
Kenya	+	+	Kenya Agricultural Research Institute (KARI)	6
Malaysia	+	–	Department of Agriculture Malaysia (DOAM), Malaysian Agricultural Research and Development Institute (MARDI), ASEAN Plant Quarantine Centre and Training Institute (PLANTI)	7
Thailand	+	–	National Biological Control Research Centre (NBCRC)	8
Uganda	+	+	National Agriculture Research Organisation (NARO)	9
Vietnam	+	–	Vietnam National Biological Control Research Centre , National Institute of Plant Protection (NIPP)	10
P.R. China	+	+	Biological Control Institute, Chinese Academy of Agricultural Sciences (CAAS)	11
Zimbabwe	+	+	Plant Protection Research Institute (PPRIZ)	12

[a]test results not available for inclusion in Appendixes 2 and 3;
[b]weevils not released in Egypt.
References: 1 DeLoach (1976); 2 Harley (1975), Forno and Wright (1990); 3 Y.H. Fayad, pers. comm.; 4 Jayanth and Nagarkatti (1987), Nagarkatti and Jayanth (1984); 5 S.S. Tjitrosoedirdjo, pers. comm.; 6 G. Ochiel, pers. comm.; 7 Sastroutomo et al. (1991); 8 B. Napompeth, pers. comm.; 9 Ogwang and Molo (1997); 10 Cam (1997); 11 Ding et al.(1998); 12 G. Chickwenhere, pers. comm.

In light of all this evidence, releases of these *Neochetina* spp. into new countries can now be carried out without undertaking exhaustive host testing. Testing, including both multiple choice and no-choice oviposition and larval development trials, should still be carried out on native members of the family Pontederiaceae, if they have not already been tested. By utilising the host-specificity test results already available, the time and cost associated with a release program can be greatly reduced.

Chapter 6
History of Introductions

Based on the results from the various host range trials, these *Neochetina* species have been widely released throughout the distribution of water hyacinth (Julien and Griffiths 1998). *N. bruchi* has been released in 27 countries and *N. eichhorniae* in 30 (Figures 5 and 6). The original source of material for all these releases was Argentina. In most countries, both species have been released and at least one has established and become widespread. There is confirmation that the insects are having a significant impact on the weed in the following countries: Argentina (DeLoach and Cordo 1983); Australia (Wright 1979, 1984); Benin (van Thielen et al. 1994); India (Jayanth 1987, 1988); Papua New Guinea (Julien and Orapa, unpub. data); Republic of South Africa (C. Cilliers, pers. comm.); Sudan (Beshir and Bennett 1985); Tanzania (R. Mohamed and G. Mallya, pers. comm.); Thailand (Napompeth and Wright, unpub. data); Uganda (Ogwang and Molo 1997; J. Ogwang; pers. comm.), United States of America (Goyer and Stark 1984; Center 1994; Cofrancesco 1984) and Zimbabwe (G. Chickwenhere, pers. comm.). In many other countries where releases have been made it is too soon to expect any observable effect.

As well as the recorded introductions, the *Neochetina* weevils have reached some countries by natural dispersal or by unrecorded means (see List D in Julien and Griffiths (1998)).

Figure 5. The origin of *Neochetina bruchi* for each country in which it has been released. Countries in bold type are those where the agent is known to have established. The year of first introduction is given in brackets.

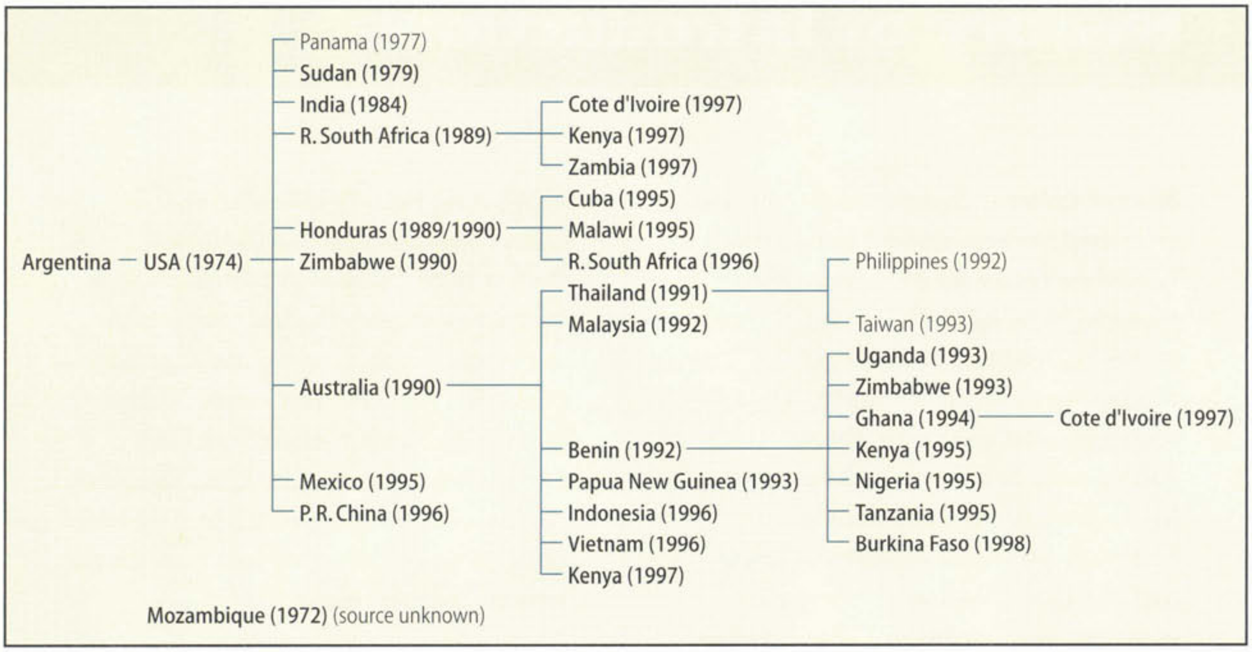
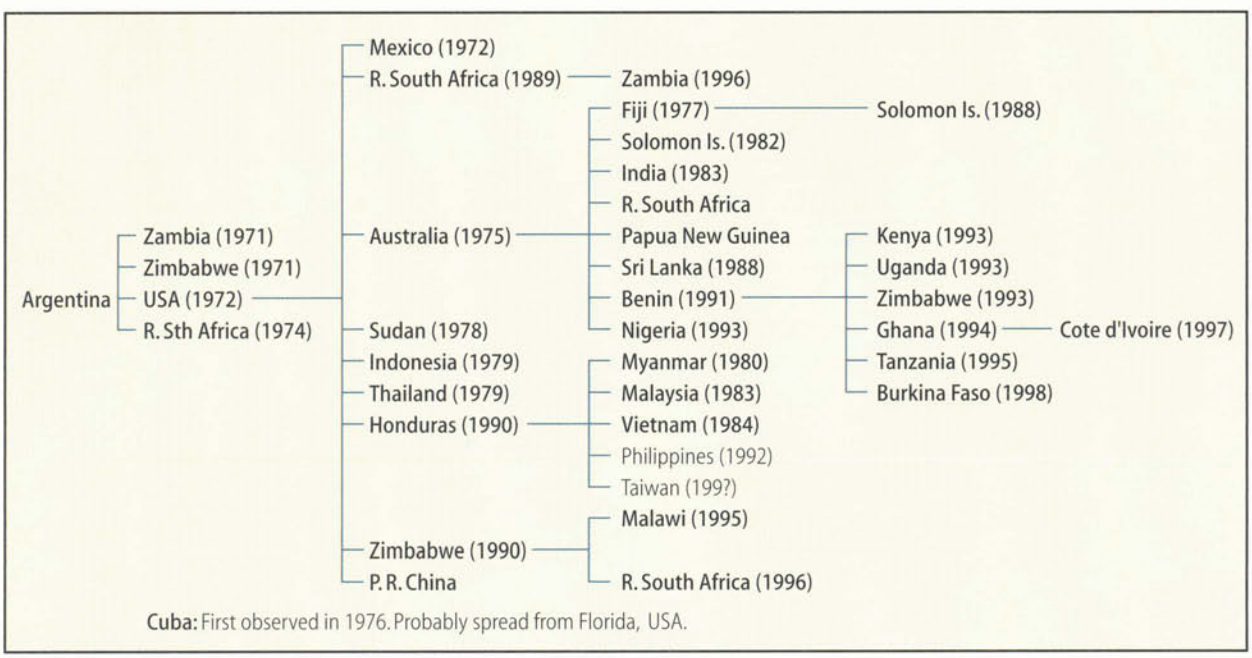


Figure 6. The origin of *Neochetina eichhorniae* for each country in which it has been released. Countries in bold type are those where the agent is known to have established. The year of first introduction is given in brackets.



Chapter 7
Rearing and Distribution

The practices suggested here have been used successfully to rear and release *Neochetina* spp. Alternatives undoubtedly exist that may sometimes better suit particular localities or conditions. In some situations, what is possible or practical may override what is desirable. The rearing times and production figures are indicative only and will vary under different circumstances, according to climate and the condition of the host plants.

7.1 Mass rearing

The aim of any mass rearing program is to produce the maximum number of good quality insects for minimum labour and resource costs. Release of high numbers of insects will increase the likelihood of establishment and reduce the time between release and control of the weed. Despite the advantages of releasing large numbers of insects, agent quality should always be more important than agent quantity.

Rearing techniques are similar for the two *Neochetina* spp., but it is desirable to keep the two species separate during rearing, at least at the early stages in a biological control program. This will ensure that the establishment and success of each species in the field can be more easily monitored and assessed. While *N. bruchi* prefer healthy, young plants, *N. eichhorniae* perform better on older plants. Therefore, there is a risk that, in a mixed culture, *N. eichhorniae* will come

Photo: M. Julien

Photo: M. Julien

The pool should be covered with water hyacinth plants so that the water surface is not visible. The pool on the bottom is well stocked while that on the top is too thinly stocked

to dominate as plants age, and *N. bruchi* may eventually decline in numbers. Colonies can be kept isolated by distance (a distance of several hundred metres between colonies should minimise the risk of invasion between sites), and by placing gauze covers over the rearing containers at night to prevent dispersal by adults. Adult dispersal will also be minimised by ensuring plants are high quality and by regularly harvesting adults, thereby reducing the two factors most likely to result in dispersal.

Two key aspects are fundamental to any successful rearing program.

Proximity to an adequate and constant water source—either a permanent water body or a reliable pumping system. Town supply water, tank water or water from ponds, rivers or wells may be used. If the supply is likely to be disrupted, storage facilities may be required.

Quality of host plants. Field studies have shown that water hyacinth plants which are healthy and have relatively high tissue nitrogen concentration yield more eggs and produce a higher proportion of adults with healthy ovaries than poorer quality (low nutrient) plants (Center 1994). Laboratory studies indicate that high quality plants can extend the duration of the reproductive period and increase the total reproductive output (Center 1994). If the quality of plants used during rearing deteriorates, insect production declines dramatically and insects are likely to disperse from the rearing area. Ultimately, the rearing process may collapse. Nutrient levels influence the growth rate and biomass of water hyacinth, which in turn influence the rate of population increase of the agent.

Where possible, starter colonies for mass-rearing programs should be obtained from neighbouring countries. This reduces both the cost of transporting insects, and the quarantine risks associated with moving insects, and possibly plant material, between regions or continents.

Mass rearing of *Neochetina* weevils has been carried out in large pools, in a technique developed by Commonwealth Scientific and Industrial Research Organisation (CSIRO) Entomology, Australia, and in smaller tubs, as developed by the International Institute of Tropical Agriculture (IITA), Benin.

7.2 Pool rearing

Large pools have been used successfully in a number of countries to rear *Neochetina* spp. for field release. Above-ground metal pools with plastic liners (2–3 m diameter, 60 cm deep, 3000–4000 L capacity) are easily handled and erected by two people, allow easy access to all areas of the water surface, and are large enough to moderate evaporation and overheating. Smaller corrugated iron (approx. 1000 L capacity) have been used successfully in Uganda (Ogwang and Molo 1997), but their smaller size makes them susceptible to overheating. Concrete pools are less easily transported and may need to be lined to prevent the leaching of lime which raises water pH.

Photo: M. Julien

Galvanised iron tanks have been used for rearing *Neochetina* spp. in Kenya

The stages in preparing and inoculating a rearing pool and harvesting *Neochetina* adults are described in Boxes 1a and 1b and in Table 4. Briefly, pools are filled with water and stocked with plants. Adult insects are added, allowed to oviposit, and the larvae develop through undisturbed. After 8–10 weeks the first generation of adults will start to emerge and feed on the leaf surface, and harvesting can commence.

Hand picking of adults from plants is laborious and if done regularly will cause damage to the plants. Insects can be harvested more easily by submerging plants beneath the water surface and collecting the adults as they swim to the surface. A sheet of wire mesh (covering about one third of the pool surface, see Box 1b) is ideal for this purpose and can be managed by one person. Larger mesh sheets covering the entire pool surface have also been used successfully (M. Hill, pers. comm.). Harvesting can continue weekly for approximately 10 months, at which time the pools are likely to require rejuvenation. If managed well the technique produces large numbers of adults with minimal handling. Some of the problems which may be encountered during pool rearing and some suggested solutions are described in Table 5.

Key points

The key to successful pool production is the regular (weekly) collection of adults.

Adult weevils should be harvested from the pool every week, even when not required for release. This will ensure the sustainability of the rearing process. If adults are not removed regularly, larval numbers will increase rapidly

Photo: M. Julien

Insects can be harvested from pools by submerging plants beneath the water surface and collecting adults as they swim to the surface—Angoram, Papua New Guinea

and plants will deteriorate beyond the point of recovery. Weevils which are collected should not be returned to the pools.

Regular and continued maintenance of pools is critical to ensure the continuity of the rearing process.

It is far better to check pools routinely than to try to restore a pool that has deteriorated. Maintenance should be carried out at least fortnightly, and can be done at the same time as insects are harvested. At this time, water levels are checked, plant contaminants removed and the density and condition of plants noted. Fertiliser should be added at intervals of approximately one month.

Fertilise monthly:

Plants require nutrients for healthy growth. To achieve this in rearing pools, between 100 and 200 g of a soluble complete fertiliser containing nitrogen, phosphorus, potassium and trace elements is added monthly to the pool water.

Table 4. Stages in setting up and maintaining a rearing pool

Setting up	
Requirements	▸ pool and liner ▸ level area slightly larger than the pool diameter for each pool ▸ adequate, regular water supply ▸ fine sand or soil for bedding under the pool liner
Prepare site	▸ smooth out bedding sand/soil to 5-10 cm depth ▸ apply broad-spectrum herbicide or layer of salt if weeds are present (e.g., *Cyperus rotundas*, nutgrass) ▸ mark a circle the diameter of the pool. Cut a piece of string to be half the diameter of the pool. Anchor one end of the string where the centre of the pool is to go and extend the string to mark a circle around the central point.
Construct pool	▸ unpack the pool parts, set out the pool wall around the marked circle and join the ends (follow manufacturers instructions) ▸ place the liner into the pool, unwrap around pool sides and press into joins between walls and base to remove creases ▸ fit clips to top of wall to hold liner in place
Prepare for plants	▸ fill pool with water to 20 cm below the top ▸ if the water is chlorinated allow pool to sit for several days to allow sunlight to reduce chlorine level. ▸ check for any leakage
Fertilise	▸ dilute 100–200 g soluble complete fertiliser in a bucket of water ▸ prepare iron supplement (if available) ▸ add fertiliser/s and stir through water column
Add plants	▸ collect field plants that are in good condition (healthy, undamaged) ▸ cover the pool surface with plants so that no water is visible between the plants ▸ remove other plants or insects ▸ allow plants to settle in for at least 3–4 days before adding insects
Inoculate with insects	▸ add approximately 200 young adults per pool ▸ distribute throughout pool

Table 4. (continued)

Harvesting–weekly (commencing 2–3 months after set up or when fresh feeding scars are visible)	
Submerge plants	▶ place mesh over plants and push below surface ▶ hold mesh below surface with weights (stones or bricks) ▶ skim off any weeds floating to surface with a sieve
Collect adults	▶ collect adults rising to the surface with a small sieve ▶ place adults in collecting container ▶ keep collecting container in shade ▶ when no more adults float to surface (after 20–40 minutes) raise mesh and repeat process over a new section of the pool ▶ record number of insects collected in each pool on each sampling date (Box 2) ▶ determine and record number of each weevil species present in collection
Maintenance–fortnightly from set up	
Pool maintenance	▶ check that no water is visible between plants, add new plants if necessary ▶ check water level is within 20 cm from top; top-up if necessary ▶ remove any contamination from other weeds (for floating weeds it is best done when plants are submerged for harvesting) and insects
Monitor plant quality	▶ check that plants are not sinking and look healthy without excessive feeding damage, chlorosis or necrosis
Review adult numbers	▶ if numbers are low for two consecutive weeks consider troubleshooting options
Fertilise–monthly from set up or as required	
Fertilise	▶ dilute 100–200 g soluble complete fertiliser in water ▶ prepare iron supplement (if available) ▶ add fertiliser/s at several points around pool perimeter
Rejuvenating pools - 9-10 monthly	
Clean and restock	▶ collect adults but retain for reinoculating pool ▶ remove water hyacinth plants ▶ drain water ▶ remove sludge build-up from base of pool ▶ check condition of liner, repair or replace as required ▶ set-up pool (see above)

Box 1a. The stages in preparing pools for rearing *Neochetina* weevils

Photo: M. Julien

1. Setting up Above-ground metal pools with plastic liners can be used for rearing. These should be erected on a level surface close to a water supply.

Photo: M. Julien

3. Mixing fertiliser A soluble complete fertiliser should be added when the pool is set up and monthly thereafter. The fertiliser should be diluted in water.

Photo: M. Julien

5. Adding plants and insects Water hyacinth plants should be chosen which are healthy with minimal insect damage, and should be crowded into the pool so that water is not visible from above. To commence breeding, a starter colony of 200 adults (100 females) per pool is recommended.

Photo: A. Wright

2. Filling the pools Pools can be filled with town supply water, tank water or water from ponds, rivers or wells. Pools should be filled to within approximately 20 cm of the top and this level should be maintained. If the water supply is chlorinated the filled pools should be allowed to sit for several days.

Photo: M. Julien

4. Adding fertiliser Fertiliser should be added to the pool water and stirred well, not applied to the plant foliage.

Photo: M. Hill

6. A rearing facility A series of rearing pools set up near Lusaka, Zambia.

Box 1b. The stages in harvesting *Neochetina* weevils from rearing pools

Photo: M. Julien

1. Preparing to harvest Numerous fresh feeding scars seen on young leaves 8–10 weeks after initial stocking indicate that a new generation of adults is emerging and that harvesting should commence.

Photo: M. Julien

2. Mesh used to submerge the water hyacinth A sheet of wire mesh can be used to force plants below the water surface. The edges of the mesh should be covered to prevent damage to the plastic pool liner. Hosepipe around the edges, held in place with tape or string, provides good protection.

Photo: M. Griffiths

3. Placing the mesh The mesh should be placed over the plants and pushed down to submerge the water hyacinth.

Photo: M. Griffiths

4. Submerging the plants The mesh can be held beneath the water surface by weights (heavy bricks or rocks). Unwanted weeds will float to the surface and can be skimmed off.

Photo: A. White

5. Collecting weevils Within a few minutes weevils will float to the water surface where they can be collected using a small strainer. Collection should commence within, at most, 10 minutes of submersion and can continue for 20 to 40 minutes. Insects should be harvested weekly even if not required for release.

Photo: M. Julien

6. Containing collected weevils during harvest Collected adults can be held in plastic containers with several water hyacinth leaves. Care must be taken not to leave the container in direct sunlight during or after collecting as overheating will kill the insects.

Table 5. Troubleshooting during pool rearing

Problem/Symptom	Possible cause	Possible solution
Pool factors		
▶ pool losing water	▷ hole in pool	■ patch hole or replace plastic liner
	▷ excessive evaporation	■ provide shade
▶ water surface visible	▷ too few plants	■ add new plants
▶ excessive algal growth	▷ too much fertiliser	■ reduce amount of fertiliser added
	▷ too few plants allowing sunlight on water surface	■ add new plants
Plant factors		
▶ yellow or light green plants	▷ nutrient deficiency	■ fertilise
▶ blue colour to roots	▷ nutrient deficiency	■ fertilise
▶ spots or streaks of chlorosis or necrosis on leaves	▷ nutrient deficiency	■ fertilise
▶ thinning plant density—water visible between plants	▷ insect density too high	■ add new plants/harvest insects
▶ plants dying and/or sinking	▷ insect density too high	■ add new plants/harvest insects
▶ excessive stunting of plants	▷ insect density too high	■ add new plants/harvest insects
▶ excessive spider mite	▷ plants stressed	■ fertilise, add new plants/harvest insects
Insect factors		
▶ insect production poor	▷ plant quality deteriorated	■ see plant factors above
	▷ too hot	■ provide shade
	▷ too cold	■ insulate pool exterior
▶ high proportion of *N. eichhorniae* in *N. bruchi* pools	▷ natural invasion by *N. eichhorniae*	■ rejuvenate pools and restock with *N. bruchi*, assess whether plant quality has deteriorated or pools require more regular harvesting to limit dispersal, consider moving pools further apart or covering pools
▶ collected adults die before release	▷ overheating	■ keep collecting container in shade during collection and until release

The addition of an iron supplement such as iron sulphate or iron chelate, if available, will further enhance the growth of water hyacinth, which requires higher levels of iron than that provided by most complete fertilisers. The amount of fertiliser required will vary according to the condition of the plants, with more required during periods of active growth. Over-fertilising, especially when plant density is low, promotes algal growth.

Optimise water temperature:

Production from pools is optimal when water temperature beneath the weed mat is approximately 25°C. At cooler temperatures weed growth, insect development and, ultimately, production figures will decline. Electric immersion heaters can be used to heat pools but if not available, insulating material such as grass or straw packed around the sides of the pool may reduce heat loss. Overheating will lead to death of plants and insects. If air temperatures above 35°C are common, partial shading of pools is recommended.

Remove plant contaminants:

Plant species other than water hyacinth should be removed from pools. Submersion of the plants to harvest adults provides an ideal opportunity to remove populations of *Salvinia molesta* (salvinia or water fern), *Azolla* spp., *Lemna* and *Spirodela* spp. (duck weeds) and algae which will float to the surface and can be skimmed off with a sieve. Larger weedy contaminants including *Utricularia* spp. (bladderwort), *Pistia stratiotes* (water lettuce) and larger *Salvinia molesta* plants require hand-weeding.

Remove animal contaminants:

Generalist feeders such as larvae of *Spodoptera* spp. can be excluded with the use of tight-fitting gauze covers placed over the pool at night and held tightly around the pool lip. Spider mite can be controlled using non-insecticidal miticide. Predatory mites are not an effective control option as they do not tolerate submersion during harvesting of *Neochetina* spp. Mosquitoes will breed in pools, causing potential health problems. Insectivorous fish have been tried in pools in Australia but were unsuccessful because the fish, at high densities, also fed upon *Neochetina* pupae.

Maintain high plant density:

At all times plants should cover the pool area so that the water surface is not visible. If the plants are too thin new plants should be added to achieve the correct density. Regular declines in plant density may indicate that adults need to be harvested more frequently or that additional fertiliser is required.

The numbers of adults collected and the relative proportions of the two *Neochetina* species in a pool should be monitored continuously.

In addition to knowing the number of insects being released into the field it is important to monitor the productivity of the pools as an indicator of pool quality. Although insect numbers are likely to fluctuate from week to week, a continual decline over a number of weeks may indicate problems in the rearing process and that the maintenance and harvesting programs should be reviewed.

In addition to total insect numbers it is important to monitor the relative abundance of the two *Neochetina* spp. Experience has shown that if *N. eichhorniae* adults invade a *N. bruchi* pool they often come to dominate. Invasion can occur from nearby natural infestations or from neighbouring pools and is more likely to occur if plant quality in the source pool is poor, encouraging adults to disperse in search of better quality plants. It is important to monitor the numbers of each species collected and, when the levels of *N. eichhorniae* increase to as much as 50% of the population, to review rearing. At high levels it becomes necessary to rejuvenate the pools and restock with *N. bruchi*. At this time it may also be beneficial to move the rearing pools further apart to limit dispersal between pools.

A suggested recording sheet to monitor insect numbers and relative abundance is shown in Box 2.

If managed well, rearing pools should be sustainable for up to ten months.

If pools are kept filled with water and are fertilised, and adults are harvested regularly, the system should be sustainable for 9–10 months. After this time plant quality may start to deteriorate and production figures will decline. Pools should be rejuvenated by draining, cleaning, refilling with water and restocking with new plants and insects (see Table 4). Plants can be collected from field infestations or from neighbouring pools which have been established for shorter periods. The plastic liners can be patched if leaks develop but will probably require replacing after 3–4 years. The longevity of the liner can be extended by maintaining the water level to within 20 cm of the top, reducing the risk of exposure and deterioration of the liner.

Multiple pools

A set-up including 4–6 pools can be maintained and harvested by two or three people working 2–3 days/week and, if well managed, will produce large quantities of insects (425–1100 adults/pool/month) for field release. The average for 5 pools in PNG was about 700 adults/month/pool (see Table 6). When operating a number of pools, cleaning and rejuvenating should be staggered so that not all pools are restarted at the same time. This prevents a decline in the numbers of insects harvested and spreads the workload over a longer period.

7.3 Tub rearing

The tub-rearing technique was initially developed by IITA in Benin and has subsequently been modified for use in other countries (O. Ajuonu, pers. comm.; G. Ochiel, pers. comm.; Ogwang and Molo 1997; van Thielen et al. 1994). The following account is based on these sources.

Photo: M. Julien

An operation including four to six pools can be maintained and harvested by two people

Box 2. Rearing productivity sheet — biological control of water hyacinth

Date	No. and percent of each weevil spp.									Total adults	Fertiliser added	Comments
	Pool 1				Pool 2							
	Ne	%	Nb	%	Ne	%	Nb	%				

Weevils are reared in tubs measuring about 50 cm diameter and 33 cm deep with a 50 L capacity. These containers are readily available in most areas, being widely used as washing bowls. Rearing can be carried out in a greenhouse or outdoors in semi-shade (e.g. under shade trees). Tubs are filled with water and a general fertiliser added (approximately 3 g). Buckets are stocked with 5 to 15 water hyacinth plants, depending on size, and adult weevils are placed on the plants. The number of adults used ranges from one pair per tub to one pair per plant. Adults are allowed to feed, mate and lay eggs on the plants for up to one week, and then removed.

Weevil eggs are extracted from the plant under a dissecting microscope using forceps and placed onto thin layers of water hyacinth tissue. These tissues are then inserted into holes made in fresh plants and the plants are placed in the field within 15 to 20 days. This technique allows accurate recording of the number of eggs released but is tedious and requires skilled labour. There have been no estimates of the number of eggs that survive this process and complete development to adult. Adults for rearing are obtained from a separate culture, maintained in similar tubs, but with eggs allowed to hatch and larvae left to develop through to adults. Adults are harvested by hand three times per week throughout the year, and are stored in plastic jars with water hyacinth leaves until required. A single tub containing nine plants can yield 180 adults per month.

Table 6. Production figures for the mass-rearing techniques used most widely for *Neochetina* spp.

	Pools	Tubs (modified technique–adults collected)
Location	Sepik River, Papua New Guinea	Kisumu, Kenya
Duration of assessment	21 months	21 months
No. containers assessed	5 pools	100 tubs
Insects collected/month	3542	1000
Labour requirements (approx.)	3 workers, 2 days/week (harvesting)	4 workers, 5 x 0.5 days/week (harvesting)
		4 workers, 0.5 days/week (plant collection)
	= 6 worker days/week	= 12 worker days/week
	= 24 worker days/month	= 48 worker days/month
Insects /worker/month	148	21
Information source	A. White, pers. comm.	G. Ochiel, pers. comm.

Photo: M. Julien

Tub rearing of *Neochetina* spp.—Kibaha, Tanzania

The tub-rearing technique has been modified in a number of ways.

- Following the removal of adults, infested plants are retained in the tubs until adults start to emerge. These are then collected and released into the field. This method is less labour intensive and permits an accurate record of the number of adults released.

- Following the removal of adults, infested plants are placed directly in the field. This technique requires very little labour input but gives no indication of the number of insects released.

7.4 Comparison of techniques

Both pool rearing and tub rearing have been successful in producing *Neochetina* weevils. The pool-rearing technique has been used in Australia, Papua New Guinea, South Africa, Malawi, Uganda and Malaysia, and the small container technique in Benin, Ghana, Kenya, Tanzania and Thailand. We recommend pool rearing for the production of *Neochetina* spp. for the following reasons:

- Large numbers of insects can be produced at relatively low cost. Workers using the pool-rearing technique have produced averages of 425 adults/pool/month in Makhanga, Malawi; 708 adults/pool/month in the Sepik River, Papua New Guinea; and 1100 adults/pool/month in Brisbane, Australia averaged over a 1–2 year period.

- The numbers of insects released into the field are known with some accuracy.

- Pools are readily available, relatively inexpensive, easily transported and erected, and long-lasting.

- Less labour than for other techniques is needed during rearing and harvesting. As a guide, production figures are shown in Table 6 for pool rearing in Papua New Guinea and for tub rearing using a modified method whereby adults are collected for field release in Kenya. The figures from Kenya were the only ones available to the authors for the tub-rearing technique.

Photo: M. Julien

Tub rearing: adult weevils being collected by hand—Kibaha, Tanzania

Photo: M. Julien

Neochetina weevils being harvested by hand from an established field site for redistribution to new areas

- The process does not involve the transfer of large amounts of plant material, which is costly and risks the spread of other weed species.

- Plants do not need to be regularly collected from the field for use during rearing.

7.5 Field harvesting

Mass rearing of insects is often labour intensive and requires special facilities. To overcome these problems, *Neochetina* spp. can be harvested from natural infestations for wider distribution. The process is convenient, cost effective, and ensures insects are well adapted to field situations. However, at least two years may be required to enable populations to build up at a site before field harvesting can commence. Adult weevils can be hand picked from plants in the field, or the plants themselves can be collected for redistribution. Although the latter method is rapid and ensures that all stages of the insect are included in the shipment, large numbers of plants are required to ensure sufficient insects are present. This can amount to a considerable mass of plant material. Infested plant material requires protection from extreme heat during transportation to the release sites. Care should be taken to ensure that other weed species are not spread to new locations with the collected water hyacinth.

7.6 Storage and transportation

Adult insects can be stored and transported in small containers that have air holes or gauze covers for ventilation. It is very important to prevent overheating of the containers, so direct sunlight must be avoided at all times.

The method of transport depends on the distance insects will travel and the time for which they will be in storage.

- For transfers of short duration, containers should be stocked with clean, fresh water hyacinth leaves. Containers of approximately 11 cm diameter, 11 cm depth, and a capacity of 800 mL will hold 200–250 adults. If held for more than two days adults should be transferred to clean containers with fresh leaves. Failure to regularly replace the leaves and transfer to new containers will result in reduced fecundity, poor health, disease and death of the weevils.

- To transfer between countries, plant material may be replaced by clean, damp (not wet) rag. This should be placed loosely in the container to allow free movement of the adults. Lids should be taped to prevent accidental opening during transit. Adults can

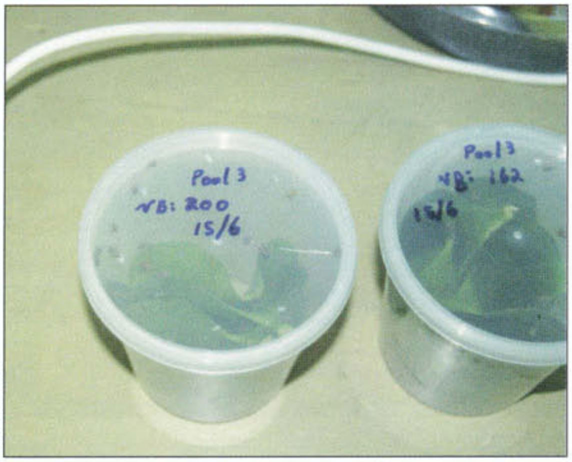


Photo: A. White

Insects can be stored and transported in small containers with leaf material

be stored for no longer than 3 days under these conditions. Containers should be loosely packed in a strong, sealed box that will protect the insects from heat. The box should be clearly marked 'LIVING INSECTS' and 'PROTECT FROM HEAT'. Import permits should accompany the package and the recipient should be advised of the package transport number, the flight number and arrival details.

- If necessary, storage can be prolonged by holding the adults at 12–15°C. At these cooler temperatures, activity, including egg laying, is reduced, and up to 100 adults can be stored in small containers for 1–2 months. Insects should be checked every week and transferred to clean containers with fresh leaves when necessary.

Adults should be released into the field as soon as possible after collecting, maximising the opportunity for oviposition on plants in the field rather than in the container, where the eggs are wasted.

7.7 Field releasing

When releasing weevils onto field infestations a number of factors should be considered.

- Releases should be made away from critical locations where herbicidal or physical controls may occur. Such control measures may prevent establishment or affect populations of control agents.

- Establishment is more likely if the plants are in good condition and the weed mat is stable and unlikely to be flushed downstream. Releases are likely to be more effective in slow-moving water bodies or in protected sections of a river system, such as coves, inlets or small lakes, than in fast-flowing rivers, where severe and/or repeated flooding flushes out the weed. In catchments, weevils should be released at source infestations as high up the system as possible.

- In an extensive infestation, release of large numbers of insects into a number of well distributed sites is recommended.

Photo: M. Julien

Releasing *Neochetina* adults at Madang, Papua New Guinea

Neochetina spp. do not disperse rapidly, so multiple releases ensure that large areas are brought under control more rapidly.

- The number of insects to be released depends on the size of the infestation and the number of adults available. Although successful establishment will result from releases of low numbers of insects, the greater the numbers released, the more rapid will be any impact on the weed. Normally, releases comprise 250 to 1000 adults

- Adults can be tipped from containers directly into the infestation, either from a boat or from the waters edge. In one instance in Thailand, adults were reportedly dropped from a low-flying plane over an isolated infestation and established.

Photo: M. Julien

Releasing *Neochetina* adults from a boat—Sepik River, Papua New Guinea

- Records should be kept of the estimated number and stages of insects released.

An example of a consignment sheet to accompany each shipment of insects being released is shown in Box 3.

Box 3. Consignment sheet — biological control of water hyacinth

CONSIGNMENT SHEET

Insect sp:. .... Date consigned: ....

Life stage: .... Date released: ....

Number of insects dispatched: ....

Recipient: ....

Carrier: ....

Released by: ....

Condition of insects/material on arrival:

Any evidence of

- insect mortality? ....
- deterioration of plant material? ....
- over-heating during/after shipment ....

Other comments: ....

....

....

Number of insects released: ....

Details of release site: ....

Site name: ....

Type (dam, lake, stream etc): ....

Location ....

Estimated size: ....

Water hyacinth cover (%): ....

Other insect agents present: ....

Other comments: ....

....

....

(On reverse, please sketch site to show point of release.)

Please return this form to:

.... (Contact person and address)

....

Chapter 8
Post-release Management

8.1 Evaluation

Monitoring the establishment, spread and impact of a control agent is a very important stage in a biological control program. It provides information on the effectiveness of an agent in establishing and reducing the weed problem, and allows assessments to be made of the potential effectiveness of the agent if introduced to other regions. In addition, observations on the interactions between agent and host, and between different agents, provides important biological information and allows an evaluation of alternative or complementary management techniques if the level of control achieved is less than that desired.

As part of any biological control release on water hyacinth the following information should be recorded:

- whether the agent established following release in a particular area;
- the rate of natural spread of the agent;
- the time taken for the agent to reach a damaging population; and
- the progressive impact on the weed infestation and the eventual level of control achieved.

The success of the *Neochetina* spp. weevils in establishing and the level of control achieved will vary according to the following factors:

- Nutrient levels and temperature of the water body;
- occurrence and degree of flooding;
- number of insects released; and
- amount of disruption to the system by chemical and/or physical control measures.

Neochetina spp. should be monitored after release, at a number of different scales.

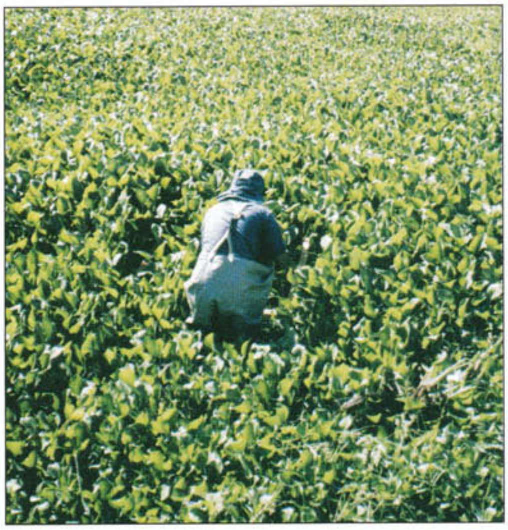


Photo: A. Wright

Placement of the quadrat during monitoring in Kenya

Quadrat monitoring

Quadrat monitoring from a boat or by wading into the infestation allows assessment of establishment, changes in weevil abundance, and impact on the weed population. Monitoring at different distances from the original release sites gives an indication of dispersal of the agents. Monitoring is recommended at one to three-monthly intervals following initial release. In a monitoring system developed by CSIRO scientists in Australia, a series of six randomly located quadrats is sampled and 10 plants are removed from each for close assessment (additional plants should be taken from next to the quadrat if there are less than 10 within the quadrat). The remaining plants and ramets within the quadrat are then counted. In locating the quadrats it is important to place them within the mat rather than on the edge to avoid irregular results due to an edge effect. An example of a monitoring sheet is shown in Box 4.

Site planimetry

Planimetry techniques enable measurement of the changes in the size of a weed infestation over time. They involve measuring the area of an irregular plane surface, e.g. the area of a water hyacinth mat or a water body. This can be done using a special instrument, a planimeter, or can be estimated using graph paper. When using graph paper, an outline of the water body and/or infestation is drawn on a sheet of graph paper and the number of squares occurring within that outline are counted. Initially the outline and size of a water body are determined from aerial photographs or satellite images. This outline is replicated for each sampling occasion. The portion of the water body covered with water hyacinth is mapped on each occasion, preferably by two or more independent assessors, and the percentage weed cover is determined using a planimeter or by counting the squares on graph paper. The data can be presented as a percentage cover by water hyacinth or can be converted to actual cover in square metres or kilometres.

Assessments can be made from an elevated position on the ground, or preferably from aerial surveys. Ground surveys will be effective only on water bodies which are relatively small and easily accessible, while aerial surveys allow coverage of water bodies which are larger or more widely distributed within a catchment. Surveys conducted six monthly or annually provide an indication of the effectiveness of the control program. The results should be combined with monitoring of the abundance and impact of released agents to link any reduction in weed biomass to the activity of control agents. Examples of mapping diagrams and the information which can be derived from them are shown in Box 5.

Satellite imagery has been tested in Uganda using SPOT images and spectral analysis, in conjunction with ground assessments, to delineate water hyacinth (Neuville et al. 1995). Large infestations on lagoons of the Sepik River flood plain could be observed on LANDSAT images in natural colour, but no measurements or comparisons over time were made. Satellite images have not yet been used as part of a practical monitoring technique.

Box 4. Monitoring sheet — biological control of water hyacinth

MONITORING SHEET

Site: .... Date: ....

Name of collector: ....

Site characteristics: Water hyacinth cover (%): ....

Open water (%): ....

Water temp. (°C): ....

General comments (including photo details): ....

Quadrat samples (0.5 x 0.5 m, randomly located, avoiding edges)

Sample no.: .... of 6 No. of plants: .... per quadrat No. of ramets: .... per quadrat

Take 10 plants from this quadrat or immediately nearby if there are not 10 in the quadrat and record the following. Repeat this for Sample 2, Sample 3, etc.

Plant No.	No. of N.b.	No. of N.e.	Second youngest leaf				Comments
			Leaf length (mm)	Total feeding scars	Leaf area (cm^2)	Wet weight (gm)	
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Definitions

Plant — Single plant including all attached stolons and daughter plants ie a number of clones joined by stolons.

Ramet — An individual clone ie, the mother plant and each daughter plant counted separately.

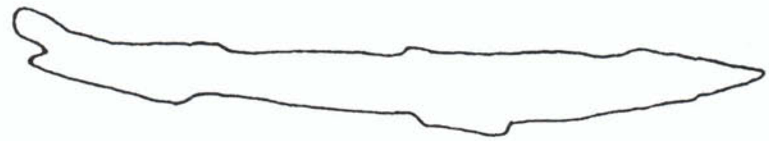
Leaf length — Measured from the tip of the lamina to the base of the petiole.

Box 5. Process of planimetry monitoring for assessing the impact of a biological control agent. An outline of the lake surface, obtained from an aerial photograph (A), is used to chart the change in weed cover during three-monthly surveys following the release of the agent (B). This information can be used to generate tables (C) and figures (D) summarising the impact of the control agent on the weed infestation. The example given is for information collected for *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) released on *Salvinia molesta* D.S. Mitchell in northern Australia (Julien and Storrs, unpublished data).

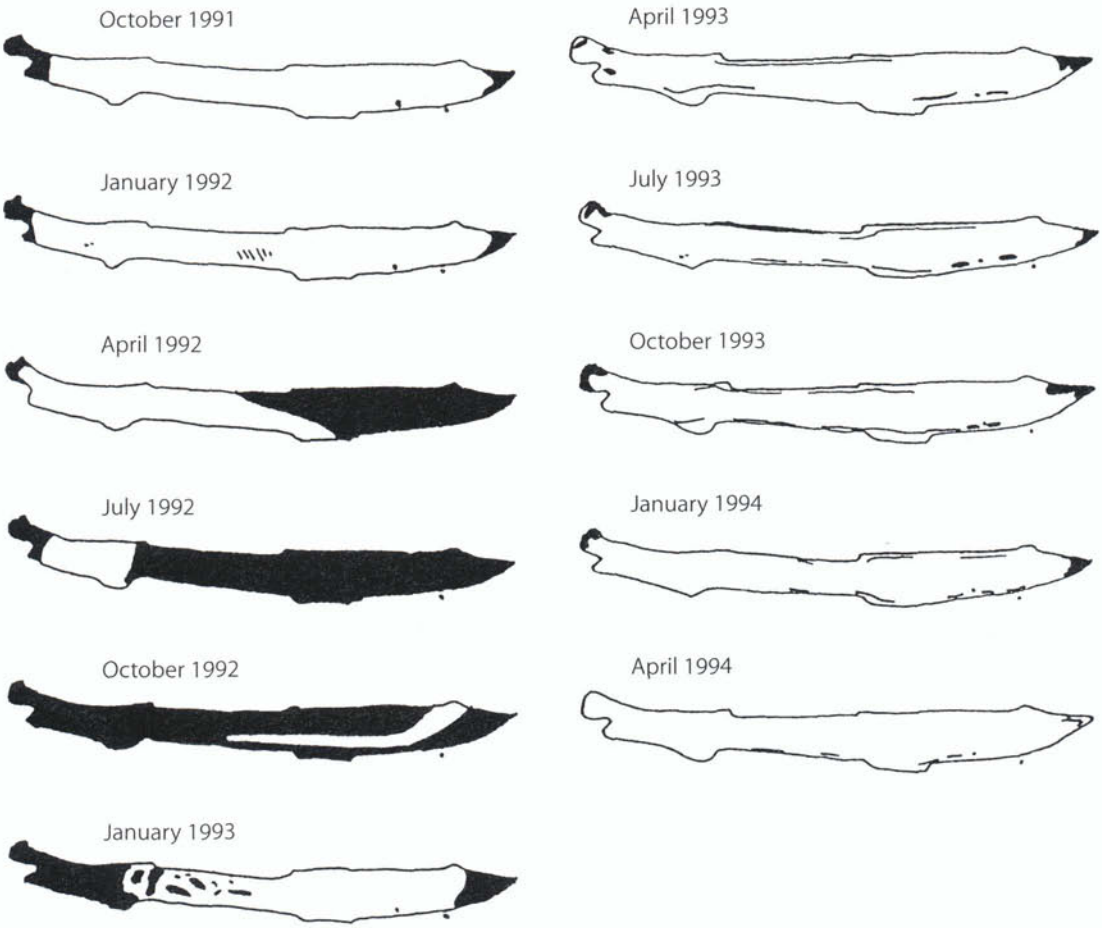
A.

Location: Jabiluka Billabong

Area: 0.116 km^2



B.



Box 5. (continued)

C. Results of planimetry assessment of a water body (0.116 km^2) during a 30-month period following the release of a biological control agent.

Month	Weed cover (%)	Weed cover (m^2)	Comments
Oct 1991	8.2	9,512	Relatively small amounts of weed
Jan 1992	11.9	13,804	Water levels very low
Apr 1992	52.5	60,900	Extensive weed cover
Jul. 1992	81.2	94,192	Weed showing signs of weevil damage
Oct 1992	86.8	100,688	Damage levels increasing
Jan 1993	27.8	32,248	Weed cover markedly reduced
Apr 1993	1.8	2,088	Generally very little weed
Jul 1993	2.2	2,552	Some new growth of weed
Oct 1993	1.8	2,088	Weed heavily damaged
Jan 1994	0.5	580	Very small amounts of heavily damaged weed
Apr 1994	0.1	116	Very sparse weed cover

D. Change in weed cover (%) on a natural water body following the release of a biological control agent.

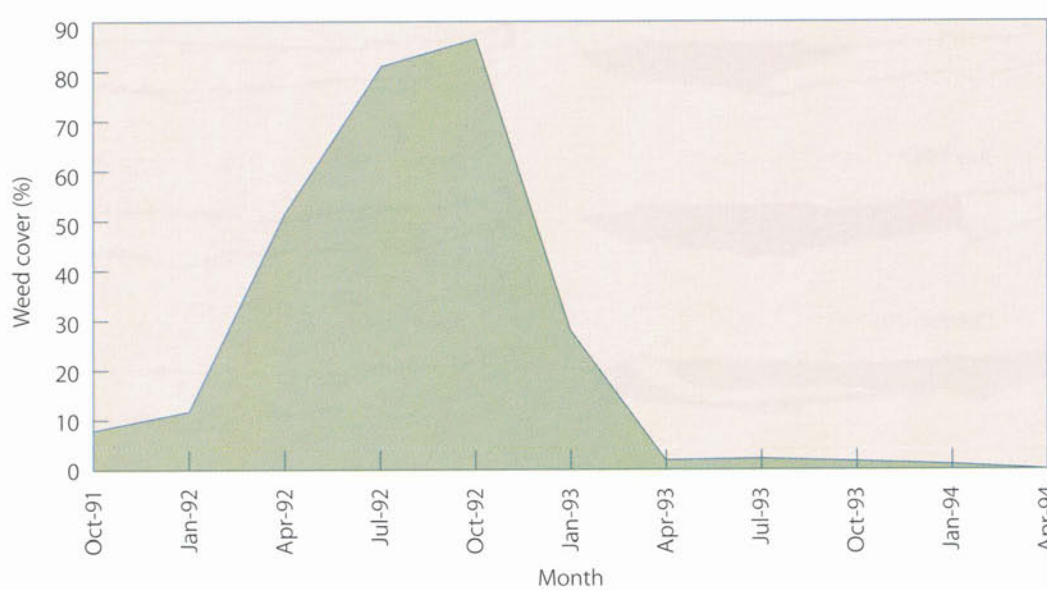


Photo: M. Julien

Measuring leaves and counting weevil feeding scars during monitoring in the Sepik River, Papua New Guinea

Time series photography

If resources for detailed, long-term planimetry studies are not available, then a series of photos of an infestation taken before and at regular intervals after the release of a control agent provides a good visual record of the impact of the agent. As with planimetry techniques, such photos are best combined with ground monitoring techniques.

In taking before and after photos it is important to:

- select a photography site which allows a good view of the water body or infestation (e.g. an elevated site);
- select a site which is easily located even after several years (this may require marking the site);
- include in all photos the same natural landmarks, which are unlikely to change;
- carry a copy of the initial photo as a reference on each occasion; and
- standardise the lens type and settings for each photo.

Several photographs demonstrating the decline in water hyacinth cover on three water bodies are shown in Box 6.

8.2. Integrated management

Biological control can provide the key component in any water hyacinth control program. However, the management of the whole system needs to be considered and additional control and management strategies may be required. Integrated management programs are site-specific and will depend greatly on the hydrological and nutrient status of the system, the extent of the infestation, the climate of the area and the usage, if any, of the water body.

Photo: M. Julien

Aerial surveys, in conjunction with ground monitoring provide important information on the impact and spread of *Neochetina* weevils

Box 6. Time series photographs showing sites before and after control by *Neochetina* weevils.

Location: Taway Lagoon, Sepik River, Papua New Guinea (insects released upstream in Sepik River, March 1989, control achieved by mid 1995)

Before Control

Photo: M. Julien

February 1995

After Control

Photo: M. Julien

August 1997

Location: Waigani Lake, Papua New Guinea (insects released March 1993, control achieved by August 1995)

Before Control

Photo: M. Julien

November 1993

After Control

Photo: M. Julien

August 1995

Location: Mt Spencer, Australia (insects released 23 November 1977, control achieved by November 1981)

Before Control

Photo: A. Wright

June 1977

After Control

Photo: A. Wright

November 1981

Table 7. Level of water hyacinth control and time taken to achieve this following the introduction of *Neochetina* spp.

Country/site	Agent	Time (yrs)	Level of control achieved	References
Argentina/Dique Los	*N. bruchi*	4	weed cover reduced by 67%	1
		6	weed cover reduced 90-95%	1
Australia/Crescent Lagoon	*N. eichhorniae*	2	-	2
India/Hebbal Tank	*N. eichhorniae*	2.7	weed cover reduced by 95%	3
India/Agram Tank	*N. bruchi*	3.25	weed cover reduced by 90%	3
USA/Louisiana	*N. eichhorniae*	1.2	-	4
USA/Texas	*N. bruchi*	3	weed cover reduced by 90%	5
Papua New Guinea/eutrophic lake	Both spp.	2.5	weed cover reduced from 70% to 20%	6
Papua New Guinea/floodplain lagoon	*N. eichhorniae*	6	weed cover reduced from 30–80% in numerous lagoons to less than 10%	6
Zimbabwe/Manyame River	Both spp.	5	weed cover reduced by 55%*	7
Zimbabwe/Lake Chivero	Both spp.	5	weed cover reduced by 85%*	7
Uganda/Lake Kyoga	Both spp.	4	lake nearly free of weed	8

*achieved in conjunction with herbicides
References: 1. DeLoach and Cordo (1983); 2. Wright (1979); 3. Jayanth (1987); 4. Goyer and Stark (1984); 5. Cofrancesco (1984); 6. Julien and Orapa, unpub. data; 7. G. Chikwenhere, pers. comm. 8. Ogwang and Molo (1997).

The aim of any biological control program is not to eradicate the weed, but to reduce its abundance to a level where it no longer causes a problem. Small infestations of water hyacinth will continue to harbour populations of the control agents so that if regrowth of the weed occurs the control agents can build up rapidly to restore control. Once established, the process should be largely self-perpetuating and self-regulating. Additional releases or redistribution from other areas may be required as new catchments are invaded by water hyacinth, or if severe flooding flushes out water hyacinth and the associated control agents, and weed reinfestation occurs.

Biological control will take some years to reduce water hyacinth levels (Table 7). Although it is not possible to generalise about the time required to achieve biological control, it is evident that, under favourable conditions, very high levels of control can be achieved in 3–5 years. Until adequate control occurs other management strategies, including physical removal and the prudent use of herbicides, may be required to maintain critical areas of water bodies weed-free. Once biological control has reduced the population of the weed, additional controls should not be required in most areas. However, at some critical sites continued monitoring and the judicious use of physical and chemical control options will be required to prevent short-term reinfestation of the weed.

Good watershed management will help reduce the water hyacinth problem. High nutrient levels, brought about through processes such as deforestation, agricultural and urban run-off, and discharge of industrial and urban waste, promote the growth of water hyacinth (Harley et al. 1996). Reducing nutrient inputs from these sources will slow the rate of growth and spread of the weed, and further improve the effectiveness of control agents.

Chapter 9
Conclusion

Biological control offers sustainable, environmentally friendly, long-term control of water hyacinth. Many of the costs associated with control using natural enemies occur early in a program and relate to collecting and identifying potential agents, conducting detailed host-specificity tests to determine their safety, and establishing a mass-rearing program to obtain large numbers for release. For water hyacinth, however, there is extensive experience with biological control in several countries, and implementation now amounts to a transfer of technology at a relatively low cost.

The two *Neochetina* weevils should be the first agents used in a biological control program. They have been widely studied and have a proven record of providing significant reductions in water hyacinth infestations.

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Glossary

aerenchyma
Plant tissue containing large intercellular air spaces.

anaerobic
Lacking oxygen.

aquatic plant
Plant which lives in or is closely associated with water.

arthropod
Member of the phylum Arthropoda, including insects, mites, spiders and crustaceans.

axillary buds
Buds arising in leaf axils.

chlorosis
Yellowing of normally green plant parts.

cocoon
Silky covering or envelope spun by larvae of many insects, in which the pupal stage develops.

crown
The point at which the root of a plant joins the stem.

daughter plant
Plant that results from vegetative growth from another plant.

epidermis
Outermost layer of cells in plant or animal.

eradicate
Remove or destroy completely.

eutrophic
Rich in nutrients.

head capsule
Sclerotised cuticle surrounding head of insect.

herbaceous
Non-woody seed-bearing plant.

herbivorous
Feeding on plants.

host-specific
Restricted to a particular host.

hydroelectric
Generating electricity by utilisation of water power.

hydrological regime
Fluctuations in the level and/or flow of water.

instar
Stage between moults during larval or nymphal development.

invertebrate
An animal not having a backbone.

lamina
Flat sheet-like structure, e.g. the blade of a leaf.

ligule
Narrow projection from the base of a leaf.

morphology
The form or appearance of an organism.

necrosis
Death of a piece of tissue.

nocturnal
Active by night.

ovoid
Oval with one end more pointed than the other.

parenchyma
Tissue consisting of living thin-walled cells with inter-cellular spaces containing air.

perennial
Persisting for a number of years.

petiole
Slender stalk joining leaf to stem.

pristine
In original condition, unspoiled.

propagation
Breed by natural processes from the parent stock.

pupa
An insect in the stage of development between a larva and an adult.

pupate
Become a pupa.

ramet
An individual member of a clone i.e. the mother plant and each daughter plant separately.

self-fertilise
The fusion of male and female gametes from the same individual as opposed to cross fertilisation in which the gametes come from different individuals.

stamen
Male fertilising organ of a flowering plant.

stolon
Horizontal stem or branch that develops roots at points along its length, forming new plants.

style
Narrow extension of the ovary supporting the stigma, within the female flower.

sustainable
Able to be maintained continuously.

vascular tissue
Tissue containing vessels for conducting sap.

vector
A carrier of disease.

vegetative reproduction
Reproduction by non-sexual means, involving unspecialised plant parts which may become reproductive structures, in the case of water hyacinth this is the stem.

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Appendix 1

Plants tested in studies of the host specificity of the *Neochetina* weevils, the countries in which they were tested, and the types of test undertaken. The test designs used were: 1. Oviposition - multiple choice with host; 2. Oviposition - multiple choice, presence of host unknown; 3. Oviposition - no choice; 4. Oviposition - unknown design; 5. Adult feeding/survival - multiple choice with host; 6. Adult feeding/survival - multiple choice, presence of host unknown; 7. Adult feeding/survival - paired choice; 8. Adult feeding/survival - no choice; 9. Adult feeding/survival - unknown design; 10. Development following placement of eggs or larvae; 11. Unknown design.

N. bruchi	*N. eichhorniae*
Agavaceae	
Agave americana L. [century plant]	
China (2,6)	China (2,6)
Agave sisalana Perrine [sisal]	
Indonesia (11)	
Cordyline fruticosa (L.) A.Chev.	
Indonesia (11)	
Polyanthes tuberosa L. [tube rose]	
India (3,8)	India (3,8)
Alismataceae	
Limnocharis flava Buch. [limnocharis]	
Indonesia (11)	
Thailand (9)	
Sagittaria graminea Michx. [sagittaria]	
Australia (1,5)	Australia (1,5)
Sagittaria montevidensis Cham. & Schltdl. [arrowhead]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
	Australia (1,5)
Sagittaria trifolia L. [arrowhead]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Amaranthaceae	
Alternanthera philoxeroides (Martius) Griseb. [alligator weed]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Thailand (9)	
Amaranthus hybridus L. [pig weed, slim amaranth]	
Zimbabwe (9)	Zimbabwe (9)
Amaranthus sp.	
Indonesia (11)	
Anacardiaceae	
Mangifera indica L. [mango]	
India (3,8)	India (3,8)
Malaysia (5)	Zimbabwe (9)
Thailand (9)	
Vietnam (9)	
Zimbabwe (9)	

N. bruchi	*N. eichhorniae*
Annonaceae	
Annona squamosa L. [custard apple, sugar apple, sweet sop]	
India (3,8)	India (3,8)
Thailand (9)	
Apiaceae	
Coriandrum sativum L. [coriander]	
India (3,8)	India (3,8)
Hydrocotyle ranunculoides L.f. [water pennywort]	
Argentina (3,8)	
Apocynaceae	
Nerium oleander L. [oleander]	
Thailand (9)	
Araceae	
Amorphophallus sp. [yam]	
Amorphophalus campanulatus	
Indonesia (11)	
Arum colocasia L. [colocasia]	
Egypt (11)	Egypt (11)
Anthurium spp. [anthurium]	
Thailand (9)	
Colocasia esculenta (L.) Schott. [taro]	
India (1,3,5,8)	India (3,8)
Malaysia (5)	
Vietnam (9)	
Colocasia sp.	
Kenya (5,8)	
Malaysia (5)	
Dieffenbachia sp.	
Indonesia (11)	

N. bruchi	*N. eichhorniae*
Pistia stratiotes L. [water lettuce]	
Argentina (3,8)	India (1,3,5,8)
India (1,3,5,8)	Zimbabwe (9)
Indonesia (11)	
Thailand (9)	
Vietnam (9)	
Zimbabwe (9)	
Syngonium sp.	
India (3,8)	India (3,8)
Zantedeschia aethiopica (L.) Spreng. [calla]	
	China (2,3,6,8,10)
Arecaceae	
Cocos nucifera L. [coconut]	
India (3,8)	India (3,8)
Malaysia (5)	
Thailand (9)	
Asteraceae	
Ambrosia artemissifolia L. [annual ragweed]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Chrysanthemum indicum L. [garden chrysanthemum]	
Thailand (9)	
Chrysanthemum morifolium	
China (2,6,10)	China (2,6,10)
Conyza canadensis (L.) Cronq. [Canadian fleabane]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Gerbera jamesonii L. [gerbera]	
Thailand (9)	
Gynura crepidioides Benth	
Indonesia (11)	
Helianthus annuus L. [sunflower]	
Australia (1,5)	Australia (1,5)
China (2,3,6,8,10)	China (2,3,6,8,10)

N. bruchi	N. eichhorniae
India (3,8)	India (3,8)
Zimbabwe (9)	Zimbabwe (9)
Lactuca sativa L. [lettuce]	
Argentina (1,3,5,7,8)	Argentina/USA (4,5,7,8)
Egypt (11)	Australia (1,5)
India (1,3,5,8)	Egypt (11)
Thailand (9)	India (3,8)
Pluchea indica Less.	
Indonesia (11)	
Tithonia diversifolia (Hemsley) A.Gray [Mexican sunflower]	
Indonesia (11)	

Azollaceae

N. bruchi	N. eichhorniae
Azolla pinnata R. Br. [ferny azolla, green azolla]	
Australia (1,5)	India (3,8)
India (3,8)	
Thailand (9)	

Begoniaceae

N. bruchi	N. eichhorniae
Begonia sp.	
India (1,3,5,8)	India (1,3,5,8)

Bombacaceae

N. bruchi	N. eichhorniae
Durio zibethinus Murray [durian]	
Malaysia (5)	

Brassicaceae

N. bruchi	N. eichhorniae
Brassica campestris L. [Chinese cabbage]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Brassica caulorapa (DC.) Pasq. [kohlrabi]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Brassica chinensis var. *parachinensis* [Chinese cabbage]	
Thailand (9)	
Brassica chinensis L. [Chinese white cabbage]	
Indonesia (11)	
Thailand (9)	
Brassica juncea (L.) Czernj. & Cosson [Chinese mustard]	
Australia (1,5)	
India (3,8)	
Thailand (9)	
Brassica nigra (L.) S. Kohl ex Koch [mustard]	
India (3,8)	India (3,8)
Brassica oleracea var. *botrytis* (L.) Alef. [cauliflower]	
Thailand (9)	
Brassica oleracea var. *capitata* (L.) Alef. [cabbage]	
Argentina (1,3,5,7,8)	Argentina/USA (4,5,8)
Egypt (11)	Australia (1,5)
India (3,8)	Egypt (11)
Indonesia (11)	Zimbabwe (9)
Kenya (5,8)	
Thailand (9)	
Vietnam (9)	
Zimbabwe (9)	
Brassica oleracea L. [Chinese kale]	
Thailand (9)	
Brassica pekinensis Lour. Rupr. [Chinese cabbage]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Brassica rapa var. *rapa* L. [turnip]	
China (2,3,6,8)	China (2,3,6,8)
Thailand (9)	
Brassica sp. [sukuma wiki]	
Kenya (5,8)	
Nasturtium sp.	
Indonesia (11)	

N. bruchi	N. eichhorniae
Raphanus sativus L. [radish]	
China (2,3,6,8,10)	China (2,3,6,8,10)
India (1,3,5,8)	India (1,3,5,8)
Rorippa nasturtium-aquaticum (L.) Hayek = *Nasturtium officinale* L. [watercress]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
Australia (1,5)	Australia (1,5)
Bromeliaceae	
Ananas comosus (L.) Merr. [pineapple]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
India (3,8)	India (3,8)
Indonesia (11)	Zimbabwe (9)
Zimbabwe (9)	
Buxaceae	
Buxus sinica	
China (2,3,6,8,10)	China (2,3,6,8,10)
Cannaceae	
Canna edulis Ker Gawler [arrowroot]	
Indonesia (11)	
Canna indica L. [canna, Indian shoot]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Egypt (11)	Egypt (11)
India (1,3,5,8)	India (1,3,5,8)
Thailand (9)	
Caricaceae	
Carica papaya L. [papaya, paw paw]	
India (3,8)	India (3,8)
Malaysia (5)	
Thailand (9)	

N. bruchi	N. eichhorniae
Ceratophyllaceae	
Ceratophyllum oryzetorum	
China (2,3,6,8,10)	China (2,3,6,8,10)
Chenopodiaceae	
Atriplex nummularia Lindley [old man saltbush]	
	Australia (1,5)
Beta vulgaris var. *folloisa* [sugar beet]	
Egypt (11)	Egypt (11)
Beta vulgaris var. *rapae* [vegetable beet]	
Egypt (11)	Egypt (11)
Beta vulgaris L. [beetroot]	
India (3,8)	Australia (1,5)
Indonesia (11)	India (3,8)
Zimbabwe (9)	Zimbabwe (9)
Spinacia oleracea L. [spinach]	
Egypt (11)	Egypt (11)
Commelinaceae	
Commelina coelestis Willdenow = *C. tuberosa*	
Argentina (1,3,5,7,8)	Argentina/USA (4,5,8)
Commelina nudiflora	
Indonesia (11)	
Commelina virginica L. [day flower]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
Tradescantia crassifolia Cavanilles [spiderwort]	
Argentina (1,3,5,7,8)	Argentina/USA (4,5,8)
Tradescantia fluminensis Velloso	
India (1,3,5,8)	India (1,3,5,8,10)
Tripogandra elongata (G.F.W. Mey) Woodson	
Argentina (1,3,5,7,8)	Argentina/USA (4,5,8)
	India (1,3,5,8)

N. bruchi	*N. eichhorniae*
Zebrina pendula Schnizlein [wandering Jew]	
Argentina (1,3,5,7,8)	Argentina/USA (4,5,7,8)
India (1,3,5,8)	India (1,3,5,8)
Indonesia (11)	
Convolvulaceae	
Convolvulus arvensis L. [field bindweed]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Ipomea aquatica Forsk. [potato vine, morning glory]	
Australia (1,5)	
Indonesia (11)	
Thailand (9)	
Vietnam (9)	
Ipomea batatas (L.) Lam. [sweet potato]	
Australia (1,5)	Australia (1,5)
India (3,8)	India (3,8)
Indonesia (11)	
Malaysia (7)	
Vietnam (9)	
Cucurbitaceae	
Citrullus lanatus (Thunb.) Matsum. & Nakai. [melon]	
	Zimbabwe (9)
Zimbabwe (9)	
Citrullus vulgaris Schrad. [watermelon]	
India (3,8)	India (3,8)
Thailand (9)	
Cucumis melo L. [musk melon]	
Thailand (9)	
Cucumis sativus L. [cucumber]	
China (2,3,6,8,10)	China (2,3,6,8,10)
India (3,8)	India (3,8)
Indonesia (11)	Zimbabwe (9)
Zimbabwe (9)	

N. bruchi	*N. eichhorniae*
Cucurbita maxima Duchesne ex Lam. [pumpkin]	
India (3,8)	Australia (1,5)
Vietnam (9)	India (3,8)
Cucurbita moschata (Duchesne ex Lam.) Duchesne ex Poiret [pumpkin]	
Indonesia (11)	
Cucurbita pepo L. s.lat. [marrow]	
Thailand (9)	
Lagenaria leucantha Rusby [bottle gourd]	
Thailand (9)	
Momordica charantia L. [bitter gourd, balsam pear]	
Thailand (9)	
Sechium edule (Jacq.) Sw.	
Indonesia (11)	
Cupressaceae	
Biota orientalis L. = *Thuja orientalis* [thuja]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Cyperaceae	
Cyperus esculentus L. [cypress, yellow nutgrass]	
Zimbabwe (9)	Zimbabwe (9)
Cyperus papyrus L. [papyrus]	
Egypt (11)	Egypt (11)
Cyperus rotundus L. [nutgrass]	
Indonesia (11)	
Eleocharis haumaniana Barros	
Argentina (8)	
Eleocharis macrostachya Britton	
	Argentina/USA (4,5,8)
Scirpus californicus (C.A. Mey.) Steud.	
	Argentina/USA (4,5,8)
Argentina (1,5,8)	
Scirpus grossus L. [giant bulrush]	
Indonesia (11)	

N. bruchi	*N. eichhorniae*
Euphorbiaceae	
Acalypha australis L.	
China (2,3,6,8,10)	China (2,3,6,8,10)
Codiaeum variegatum (L.) Adr. Juss. [croton]	
India (3,8)	India (3,8)
Hevea brasiliensis Muell. Arg.[rubber]	
Malaysia (5)	
Manihot esculenta Crantz = *M. utilissima* [tapioca]	
India (3,8)	India (3,8)
Vietnam (9)	
Phyllanthus acidus Skeels [star gooseberry]	
Thailand (9)	
Ricinus communis L. [castor oil plant]	
Egypt (11)	Egypt (11)
India (3,8)	India (3,8)
Sauropus androgynus L.	
Vietnam (9)	
Fabaceae	
Arachis hypogaea L. [groundnut, peanut]	
India (3,8)	India (3,8)
Indonesia (11)	Zimbabwe (9)
Vietnam (9)	
Zimbabwe (9)	
Dolichos lablab L. = *Lablab purpureus* [lablab]	
India (1,3,5,8)	India (3,8)
Glycine max (L.) Merr. [soybean]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Indonesia (11)	Zimbabwe (9)
Thailand (9)	
Vietnam (9)	
Zimbabwe (9)	

N. bruchi	*N. eichhorniae*
Medicago sativa L. [lucerne]	
Australia (1,5)	Australia (1,5)
Phaseolus aureus Roxb. = *Vigna radiata* [mungbean]	
Thailand (9)	
Phaseolus vulgaris L. [bean]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Indonesia (11)	Zimbabwe (9)
Kenya (5,8)	
Thailand (9)	
Zimbabwe (9)	
Pisum sativum L. s.lat. [pea]	
China (2,3,6,8,10)	China (2,3,6,8,10)
India (3,8)	India (3,8)
Thailand (9)	
Psophocarpus tetragonolobus (L.) DC [winged bean]	
Thailand (9)	
Sesbania formosa (F. Muell.) N.Burb. = *S. grandiflora* (L.) Poiret [sesbania]	
Thailand (9)	
Trifolium subterraneum L. [subterranean clover]	
Australia (1,5)	Australia (1,5)
Vicia faba var. *equina* Pers. [horse bean]	
Egypt (11)	Egypt (11)
Vicia faba L. [faba bean, field bean]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Vigna sesquipedalis (L.) Fruwirth[yard long bean]	
Thailand (9)	
Vigna sinensis (L.) Hassk. [cowpea]	
India (1,3,5,8)	India (3,8)
Haemodoraceae	
Anigozanthos manglesii D. Don [kangaroo paw]	
Australia (1,5)	

N. bruchi	N. eichhorniae
Haloragaceae	
Myriophyllum aquaticum (Vell. Conc) Verdc. [parrots feathers, Brazilian water milfoil]	
Zimbabwe (9)	Zimbabwe (9)
Hydrocharitaceae	
Hydrilla sp.	
India (1,3,5,8)	India (1,3,5,8)
Ottelia ovalifolia (R. Br.)L.C.Rich [swamp lily]	
Australia (1,5)	
Vallisneria sp.	
India (1,3,5,8)	India (1,3,5,8)
Lamiaceae	
Coleus amboinicus	
Indonesia (11)	
Mentha arvensis L. [mint]	
	India (3,8)
India (1,3,5,8)	
Mentha cordifolia Opiz [kitchen mint]	
Thailand (9)	
Ocimum sanctum L. [holy basil, tulsi]	
Thailand (9)	
Lauraceae	
Persea americana Miller [avocado]	
Zimbabwe (9)	Zimbabwe (9)
Lemnaceae	
Lemna sp. (either *L. gibba* or *L. parodiana* Giaredelli) [duckweed]	
Argentina (3,8)	
Lemna trisulca L. [narrow-leaved duck weed]	
Thailand (9)	
Spirodela intermedia Koch [giant duckweed]	
Argentina (3,8,10)	

N. bruchi	N. eichhorniae
Liliaceae	
Agapanthus africanus Lam. [African lily]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
Allium ampeloprasum L. [Leek]	
Thailand (9)	
Allium ascalonicum L. [shallot]	
Thailand (9)	
Allium cepa L. [onion]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
Egypt (11)	Australia (1,5)
India (3,8)	Egypt (11)
Indonesia (11)	India (3,8)
Thailand (9)	Uganda (4,9)
Uganda (4,9)	Zimbabwe (9)
Zimbabwe (9)	
Allium fistulosum L. [leek, Welsh onion]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Thailand (9)	
Allium sativum L. [garlic]	
Indonesia (11)	
Allium tuberosum	
China (2,6,10)	China (2,6,10)
Amaryllis sp.	
India (1,3,5,8)	India (1,3,5,8)
Asparagus officinalis L. [asparagus]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
Australia (1,5)	Australia (1,5)
Bulbine bulbosa (R.Br.) Haw. [native leek, bulbine lily]	
Australia (1,5)	
Burchardia umbellata R.Br. [milkmaids]	
Australia (1,5)	

N. bruchi	*N. eichhorniae*
Chlorophytum comosum (Thunb.) Jacques [spider plant]	
China (2,6)	China (2,6)
Crinum asiaticum L. [giant lily]	
Thailand (9)	
Crinum pedunculatum R. Br. [swamp lily]	
Australia (1,5)	
Dianella caerulea Sims. [blue flax lily]	
Australia (1,5)	
Hymenocallis sp. [spiderlily]	
India (3,8)	India (3,8)
Lilium sp.	
Indonesia (11)	
Protasparagus plumosus (Baker) Oberm. = *Asparagus setaceus* (Kunth) Jessop [climbing asparagus fern]	
China (2,6)	China (2,6)
Malvaceae	
Abelmoschus esculentus (L.) Moench = *Hibiscus esculentus* [okra, bhendi]	
India (3,8)	India (3,8)
Thailand (9)	
Gossypium arboreum L. [cotton]	
India (3,8)	Australia (1,5)
	India (3,8)
Gossypium barbadense L. [sea island cotton]	
Egypt (11)	Egypt (11)
Gossypium herbaceum L. [desi cotton]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Gossypium hirsutum L. [cotton]	
Zimbabwe (9)	Zimbabwe (9)
Gossypium sp. [cotton]	
Indonesia (11)	
Thailand (9)	

N. bruchi	*N. eichhorniae*
Hibiscus sabdariffa L. [rosella]	
Indonesia (11)	
Thailand (9)	
Hibiscus syriacus L. [Syrian hibiscus]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Marantaceae	
Maranta sp. [arrowroot]	
Kenya (5,8)	
Marsileaceae	
Marsilea crenata C. Presl. [water clover]	
Thailand (9)	
Menyanthaceae	
Nymphoides indica (L.) Kuntze [water snowflake]	
Australia (1,5)	
Vietnam (9)	
Mimosaceae	
Acacia podalyriifolia Cunn. ex G. Don [Queensland silver wattle]	
Australia (1,5)	
Albizia lebbeck (L.) Benth. [Indian siris]	
India (3,8)	India (3,8)
Mimosa pigra L. [giant sensitive plant]	
Thailand (9)	
Neptunia natans [neptunia]	
Thailand (9)	
Neptunia oleraceae Lour [watercress]	
Vietnam (9)	

N. bruchi	N. eichhorniae

Moraceae

Artocarpus heterophyllus Lam. =
Manilkara apota [jack-fruit]

India (3,8)	India (3,8)

Ficus carica L. [fig]

India (3,8)	India (3,8)

Morus alba L. [white mulberry]

Zimbabwe (9)	Zimbabwe (9)

Musaceae

Musa paradisiaca (L.) [banana]

Australia (1,5)	Egypt (11)
Egypt (11)	India (1,3,5,8)
India (1,3,5,8)	Uganda (4,9)
Indonesia (11)	
Malaysia (5)	
Uganda (4,9)	

Musa sapientum L. [banana]

Thailand (9)	

Musa sp. [banana]

Vietnam (9)	Zimbabwe (9)
Zimbabwe (9)	

Myrtaceae

Eucalyptus saligna Sm. [Sydney blue gum]

Australia (1,5)	Australia (1,5)

Eucalyptus tereticornis Sm. [forest red gum]

Australia (1,5)	Australia (1,5)

Eugenia sp. [roseapple]

Thailand (9)	

Psidium guajava L. [guava]

India (3,8)	India (3,8)
Thailand (9)	

N. bruchi	N. eichhorniae

Nelumbonaceae

Nelumbo lutea (Willd. Pers.

	Argentina/USA (4,5,8)

Nelumbo nucifera Gaertner [Indian lotus, sacred lotus]

China (2,3,6,8,10)	China (2,3,6,8,10)
Thailand (9)	
Vietnam (9)	

Nelumbium nelumbo [lotus]

Indonesia (11)	

Nymphaeaceae

Nuphar advena (Aiton) Aiton f.
[waterlilly, spatterdock]

	Argentina/USA (4,5,8)

Nymphaea lotus L. [lotus]

Thailand (9)	

Nymphaea sp. [waterlily]

India (3,8)	Argentina/USA (4,5,8)
Indonesia (11)	India (3,8)

Oleaceae

Jasminum nudiflorum Lindl. [jasmine]

India (3,8)	India (3,8)

Jasminum sambac Ait. [Arabian jasmine]

Thailand (9)	

Onagraceae

Jussiaea repens L. = *Ludwigia adscendens* (L.) Hara
[creeping water primrose]

Thailand (9)	

Ludwigia peploides (Kunth) Raven [water primrose]

Argentina (3,8)	Australia (1,5)
Australia (1,5)	
Vietnam (9)	

N. bruchi	*N. eichhorniae*
Orchidaceae	
Dendrobium sp. [orchid]	
Australia (1,5)	
Malaysia (5)	
Thailand (9)	
Vanilla fragrans Andrews = *V. planifolia* Andr [vanilla orchid]	
India (1,3,5,8)	India (1,3,5,8)
Palmae	
Areca catechu L. [betel nut]	
India (3,8)	India (3,8)
Elaeis guineensis Jacq. [oil palm]	
Malaysia (5)	
Philydraceae	
Philydrum lanuginosum Banks & Sol. [frogsmouth]	
Australia (1,5,7)	
Pinaceae	
Cedrus libani A. Rich. [taurus cedar]	
Egypt (11)	Egypt (11)
Piperaceae	
Peperomia sp	
India (3,8)	India (3,8)
Poaceae	
Bambusa blumeana [bamboo]	
Thailand (9)	
Bambusa spp.	
Vietnam (9)	
Bambusa tulda Roxb. [bamboo]	
India (3,8)	India (3,8)

N. bruchi	*N. eichhorniae*
Brachiaria mutica (Forsskal) Stapf [paragrass]	
Thailand (9)	
Eleusine coracana (L.) Gaertn. [ragi, Indian millet]	
India (3,8)	India (3,8)
Eleusine indica (L.) Gaertner [crowsfoot grass]	
Zimbabwe (9)	Zimbabwe (9)
Oryza sativa L. [rice]	
Argentina (8)	Argentina/USA (4,5,8)
Australia (1,5)	Australia (1,5)
China (2,3,6,8,10)	China (2,3,6,8,10)
India (3,8)	India (3,8)
Indonesia (11)	Uganda (4,9)
Malaysia (5)	
Thailand (9)	
Uganda (4,9)	
Vietnam (9)	
Saccharum *officinarum* L. [sugarcane]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
India (3,8)	India (3,8)
Thailand (9)	Zimbabwe (9)
Zimbabwe (9)	
Sorghum bicolor (L.) Moench = *S. vulgare* (Pers.) [sorghum, jowar]	
India (3,8)	India (3,8)
Thailand (9)	Zimbabwe (9)
Zimbabwe (9)	
Triticum aestivum L. = *T. vulgare* [wheat]	
Australia (1,5)	Australia (1,5)
China (2,3,6,8,10)	China (2,3,6,8,10)
Egypt (11)	Egypt (11)
India (3,8)	India (3,8)
Zimbabwe (9)	Zimbabwe (9)

N. bruchi	N. eichhorniae
Zea mays L. [maize]	
Australia (1,5)	Australia (1,5)
China (2,3,6,8,10)	China (2,3,6,8,10)
Egypt (11)	Egypt (11)
India (3,8)	India (3,8)
Indonesia (11)	Zimbabwe (9)
Kenya (5,8)	
Malaysia (5)	
Thailand (9)	
Vietnam (9)	
Zimbabwe (9)	

Polygonaceae

N. bruchi	N. eichhorniae
Polygonum acuminatum H.B.K. [smartweed]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
Polygonum lapathifolium L. = *Persicaria lapathifolium* minus Hudson [knotweed, smartweed]	
Australia (1,5)	Australia (1,5)
Polygonum sp.	
Indonesia (11)	
Vietnam (9)	
Rumex brownii Campdera [swamp dock]	
	Australia (1,5)

Pontederiaceae

N. bruchi	N. eichhorniae
Eichhornia azurea (Sw.) Kunth [anchored waterhyacinth]	
Argentina (1,3,5,7,8,10)	Argentina/USA (4,5,7,8)
Monochoria cyanea (F. Mueller) F. Mueller [water hyacinth]	
Australia (1,3,5,7,8)	Australia (3,5)
Monochoria hastata (L.) Solms-Laub. [phak top thai]	
Thailand (9)	
Vietnam (9)	
Monochoria korsakowii	
China (2,3,6,8,10)	China (2,3,6,8,10)
Monochoria vaginalis (Burman f.) C.Presl ex Kunth	
Australia (1,3,5,7,8)	Australia (3,5)
China (2,3,6,8,10)	China (2,3,6,8,10)
Indonesia (11)	
Thailand (9)	
Pontederia cordata L. [pickerel weed]	
	Argentina/USA (4,5,7,8)
	Australia (1,5)
Pontederia lanceolata Nutt [pickerel weed]	
Argentina (1,3,5,7,8,10)	
Reussia rotundifolia (L.f.) Castellanos	
Argentina (3,8,10)	

Potamogetonaceae

N. bruchi	N. eichhorniae
Potamogeton crispus L. [curly pondweed]	
Australia (1,5)	

Punicaceae

N. bruchi	N. eichhorniae
Punica granatum L. [pomegranate]	
India (3,8)	India (3,8)
Thailand (9)	

Rhamnaceae

N. bruchi	N. eichhorniae
Ziziphus mauritiana Lamk. [Indian jujube]	
Thailand (9)	

Rosaceae

N. bruchi	N. eichhorniae
Fragaria × *ananassa* Duchesne [strawberry]	
Australia (1,5)	Australia (1,5)
Malus domestica Borkh. = *Pyrus malus* [apple]	
Australia (1,5)	Australia (1,5)

N. bruchi	N. eichhorniae
Malus pumila Mill. [apple]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Prunus armeniaca L. [apricot]	
Zimbabwe (9)	Zimbabwe (9)
Prunus persica (L.) Batsch [peach]	
China (2,3,6,8,10)	China (2,3,6,8,10)
Zimbabwe (9)	Zimbabwe (9)
Prunus sp.	
Vietnam (9)	
Rosa alba (L.) [rose]	
India (3,8)	India (3,8)
Rosa chinensis Jacq.	
China (2,3,6,8,10)	China (2,3,6,8,10)

Rubiaceae

N. bruchi	N. eichhorniae
Coffea arabica L. [coffee]	
Zimbabwe (9)	Zimbabwe (9)
Coffea liberica [coffee]	
Malaysia (5)	
Coffea robusta Linden = *C. canephora* [coffee]	
India (3,8)	India (3,8)
Coffea sp.	
Indonesia (11)	
Ixora sp.	
Indonesia (11)	
Morinda citrifolia L. [Indian mulberry, rotten cheesefruit]	
Thailand (9)	

Rutaceae

N. bruchi	N. eichhorniae
Citrus aurantifolia (Christm.) Swingle [lime]	
Thailand (9)	
Citrus grandis Hassk. [pomelo]	
Malaysia (5)	
Vietnam (9)	
Citrus limon (L.) Burm. f. [lemon]	
Australia (1,5)	Australia (1,5)
Zimbabwe (9)	Zimbabwe (9)
Citrus medica L. [citron]	
India (3,8)	India (3,8)
Citrus microcarpa Bunge [calamondin, China orange]	
Malaysia (5)	
Citrus paradisi Macfad. [grapefruit]	
Zimbabwe (9)	Zimbabwe (9)
Citrus reticulata Blanco [mandarin]	
	Australia (1,5)
Citrus sinensis (L.) Osbeck [sweet orange]	
	Australia (1,5)
Citrus spp.	
Vietnam (9)	
Murraya exotica (L.). = *M. paniculata* L. Jack [curry leaf]	
India (3,8)	India (3,8)

Salviniaceae

N. bruchi	N. eichhorniae
Salvinia molesta D.S. Mitchell [salvinia]	
	Australia (1,5)
Salvinia sp.	
Vietnam (9)	

Sapindaceae

N. bruchi	N. eichhorniae
Dimocarpus longan Lour. [longan]	
Thailand (9)	
Vietnam (9)	
Nephelium lappaceum L. [rambutan]	
Malaysia (5)	

Sapotaceae

Achras zapota (L.) van Royen = *Manilkara apota* [sapota]

N. bruchi	N. eichhorniae
India (3,8)	India (3,8)

Mimusops kauki [sapota]

N. bruchi	N. eichhorniae
Thailand (9)	

Solanaceae

Capsicum annuum var. *grossum* Sendtn. [sweet pepper]

N. bruchi	N. eichhorniae
Thailand (9)	

Capsicum annuum L. [chilli]

N. bruchi	N. eichhorniae
India (3,8)	India (3,8)
Vietnam (9)	

Carpocapsae minimum [bird chili pepper]

N. bruchi	N. eichhorniae
Thailand (9)	

Lycopersicon esculentum Miller [tomato]

N. bruchi	N. eichhorniae
Australia (1,5)	Australia (1,5)
China (2,3,6,8,10)	China (2,3,6,8,10)
India (3,8)	India (3,8)
Indonesia (11)	Zimbabwe (9)
Thailand (9)	
Zimbabwe (9)	

Nicotiana tabacum L. [tobacco]

N. bruchi	N. eichhorniae
China (2,3,6,8,10)	China (2,3,6,8,10)
Indonesia (11)	Zimbabwe (9)
Thailand (9)	
Zimbabwe (9)	

Solanum melongena L. [brinjal, eggplant]

N. bruchi	N. eichhorniae
China (2,3,6,8,10)	China (2,3,6,8,10)
India (3,8)	India (3,8)
Indonesia (11)	
Thailand (9)	

Solanum torvum Sw. [cluster eggplant, devil's fig]

N. bruchi	N. eichhorniae
Thailand (9)	

Solanum tuberosum L. [potato]

N. bruchi	N. eichhorniae
China (2,3,6,8,10)	China (2,3,6,8,10)
India (3,8)	India (3,8)
Uganda (4,9)	Uganda (4,9)
Zimbabwe (9)	Zimbabwe (9)

Solanum xanthocarpum [eggplant]

N. bruchi	N. eichhorniae
Thailand (9)	Uganda (4,9)
Uganda (4,9)	

Sparganiaceae

Sparganium americanum Nutt.

N. bruchi	N. eichhorniae
	Argentina/USA (4,5,7,8)

Sterculiaceae

Theobroma cacao L. [cocoa]

N. bruchi	N. eichhorniae
Malaysia (5)	

Taxodiaceae

Metasequoia glytostroboides

N. bruchi	N. eichhorniae
China (2,3,6,8,10)	China (2,3,6,8,10)

Theaceae

Thea sinensis (L.) O.Kuntze = *Camelia sinensis* [tea]

N. bruchi	N. eichhorniae
India (3,8)	India (3,8)

Tiliaceae

Corchorus capsularis L. [jute]

N. bruchi	N. eichhorniae
Thailand (9)	

Corchorus olitorius L.

N. bruchi	N. eichhorniae
Vietnam (9)	

N. bruchi	N. eichhorniae
Trapaceae	
Trapa bicornis Osb. [water caltrops]	
Thailand (9)	
Trapa bispinosa Roxb. = *T. natans* (Linn.) [water chestnut]	
India (1,3,5,8)	India (1,3,5,8,10)
Typhaceae	
Typha domingensis Pers. = *T. angustifolia* [lesser reedmace]	
Thailand (9)	
Typha latifolia L. [cattail]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
Typha orientalis C. Presl. [bullrush, broad leaved cumbungi]	
Australia (1,5)	Australia (1,5)
Umbelliferae	
Daucus carota L. [carrot]	
Argentina (1,5,8)	Argentina/USA (4,5,8)
India (3,8)	Australia (1,5)
	India (3,8)
Unknown	
Morkara sp.	
Malaysia (5)	

N. bruchi	N. eichhorniae
Verbenaceae	
Tectona grandis L.f. [teak]	
India (3,8)	India (3,8)
Vitidaceae	
Vitis vinifera L. [grape]	
India (3,8)	India (3,8)
Thailand (9)	
Xanthorrhoeaceae	
Lomandra longifolia Labill [longleaf matrush]	
Australia (1,5)	
Zingiberaceae	
Alpinia siamensis [greater galangal]	
Thailand (9)	
Curcuma longa (L.) possibly *C. domestica* Val. [turmeric]	
India (3,8)	India (3,8)
Malaysia (5)	
Vietnam (9)	
Elettaria cardamomum (L.) Maton [cardamom]	
India (3,8)	India (3,8)
Zingiber officinale Rosc. [ginger]	
Australia (1,5)	China (2,3,6,8,10)
China (2,3,6,8,10)	India (3,8)
India (3,8)	
Malaysia (5)	

Appendix 2

Summary results of host-specificity tests carried out on *N. bruchi* for which some damage was recorded. Results detail the country in which the test was made, the basic test design, and the outcome of the trial. The test designs used were: 1. Oviposition - multiple choice with host; 2. Oviposition - multiple choice, presence of host unknown; 3. Oviposition - no choice; 4. Oviposition - unknown design; 5. Adult feeding/survival - multiple choice with host; 6. Adult feeding/survival - multiple choice, presence of host unknown; 7. Adult feeding/survival - paired choice; 8. Adult feeding/survival - no choice; 9. Adult feeding/survival - unknown design; 10. Development following placement of eggs or larvae.

Alismataceae

Sagittaria trifolia

China

8 fed and survived for 29 days, no feeding in multiple choice tests (unclear to which weevil species this refers)

Apiaceae

Hydrocotyle ranunculoides

Argentina

3 less than 0.5 eggs/female/day vs 3.5 on water hyacinth

8 less than 3 feeding spots/weevil/day vs 17/day for water hyacinth

Araceae

Amorphophallus sp.

India

8 slight feeding (scraping of leaf epidermis) and survived for 31 days, no feeding in multiple choice tests

Colocasia esculenta

India

8 slight feeding (scraping of leaf epidermis) and survived for 43 days, no feeding in multiple choice tests

Pistia stratiotes

Argentina

3 less than 0.5 eggs/female/day vs 3.5 on water hyacinth

8 less than 3 feeding scars/weevil/day vs 17/day for water hyacinth

India

8 fed and survived for 76 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

Vietnam

9 65 feeding scars vs 220-338 on water hyacinth

Zimbabwe

9 0.03 feeding scars/weevil/day vs 9.83 on water hyacinth (unclear to which weevil species this refers)

Asteraceae

Lactuca sativa

Argentina

1 1 egg laid vs 749 on water hyacinth

3 less than 0.25 eggs/female/day vs 3.0/day for water hyacinth

5 0.031 feeding scars/weevil/day vs 4.01 on water hyacinth

7 0.02 feeding scars/weevil/day vs. 13.49/day on water hyacinth (on average)

8 0.85 feeding scars/weevil/day vs 7.58 on water hyacinth

India

4 adult fed and survived for 34 days, 2 fertile eggs laid, larvae died within 2 days of hatching, no feeding in multiple choice tests

Begoniaceae

Begonia sp.

India

8 slight feeding (scraping of leaf epidermis) and survived for 24 days, no feeding in multiple choice tests

Brassicaceae

Brassica caulorapa

China

8 fed and survived for 36 days, no feeding in multiple choice tests (unclear to which weevil species this refers)

Brassica oleracea

Kenya

8 1 feeding scar vs a maximum of 376 on water hyacinth, no feeding in multiple choice tests vs 313 feeding scars on water hyacinth

8 137 feeding scars vs a maximum of 376 on water hyacinth

Brassica oleracea var. *capitata*

Argentina

1 3 eggs laid vs 749 on water hyacinth

3 less than 0.25 eggs/female/day vs 3.0/day for water hyacinth

5 0.003 feeding scars/weevil/day vs 4.01 on water hyacinth

7 0.01 feeding scars/weevil/day vs. 13.49/day on water hyacinth (on average)

8 0.83 feeding scars/weevil/day vs 7.58 on water hyacinth

Zimbabwe

9 0.01 feeding scars/weevil/day vs 9.83 on water hyacinth (unclear to which weevil species this refers)

Brassica pekinensis

China

8 fed and survived for 24 days, no feeding in multiple choice tests (unclear to which weevil species this refers)

10 some feeding but unable to complete development

Raphanus sativus

India

8 slight feeding (scraping of leaf epidermis) and survived for 50 days, no feeding in multiple choice tests

Rorippa nasturtium-aquaticum

Argentina

5 0.001 feeding scars/weevil/day vs 4.01 on water hyacinth

Bromeliaceae

Ananas comosus

Argentina

1 1 egg laid vs 749 on water hyacinth

Cannaceae

Canna indica

India

8 fed and survived for 34 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

Ceratophyllaceae

Ceratophyllum oryzetorum

China

10 some feeding but unable to complete development (unclear to which weevil species this refers)

Commelinaceae

Commelina coelestis

Argentina

3 less than 0.25 eggs/female/day vs 3.0/day for water hyacinth

8 0.56 feeding scars/weevil/day vs 7.58 on water hyacinth

Commelina virginica

Argentina

5 0.001 feeding scars/weevil/day vs 4.01 on water hyacinth

8 0.01 feeding scars/weevil/day vs 7.58 on water hyacinth

Tradescantia crassifolia

Argentina

1 2 eggs laid vs 749 on water hyacinth

3 less than 0.25 eggs/female/day vs 3.0/day for water hyacinth

5 0.001 feeding scars/weevil/day vs 4.01 on water hyacinth

7 0.04 feeding scars/weevil/day vs. 13.49/day on water hyacinth (on average)

8 1.39 feeding scars/weevil/day vs 7.58 on water hyacinth

Tradescantia fluminensis

India

8 fed and survived for 51 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

Tripogandra elongata

Argentina

1 1 egg laid vs 749 on water hyacinth

3 less than 0.25 eggs/female/day vs 3.0/day for water hyacinth

5 0.017 feeding scars/weevil/day vs 4.01 on water hyacinth

8 0.21 feeding scars/weevil/day vs 7.58 on water hyacinth

Zebrina pendula

Argentina

1 2 eggs laid vs 749 on water hyacinth

3 less than 0.25 eggs/female/day vs 3.0/day for water hyacinth

5 0.011 feeding scars/weevil/day vs 4.01 on water hyacinth

7 0.04 feeding scars/weevil/day vs. 13.49/day on water hyacinth (on average)

8 0.92 feeding scars/weevil/day vs 7.58 on water hyacinth

India

8 fed and survived for 36 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

Cucurbitaceae

Cucumis sativus

China

3 eggs laid but unable to complete life-cycle (unclear to which weevil species this refers)

8 fed and survived for 38 days, no feeding in multiple choice tests (unclear to which weevil species this refers)

10 some feeding but unable to complete development (unclear to which weevil species this refers)

Cyperaceae

Eleocharis haumaniana

Argentina

8 0.09 feeding scars/weevil/day vs 7.58 on water hyacinth

Fabaceae

Dolichos lablab

India

8 slight feeding (scraping of leaf epidermis) and survived for 18 days, no feeding in multiple choice tests

Vigna sinensis

India

8 slight feeding (scraping of leaf epidermis) and survived for 37 days, no feeding in multiple choice tests

Hydrocharitaceae

Hydrilla sp.

India

8 slight feeding (scraping of leaf epidermis) and survived for 47 days, no feeding in multiple choice tests

Vallisneria sp.

India

4 adult fed and survived for 38 days, 3 fertile eggs laid, larvae died within 2 days of hatching, no feeding in multiple choice tests

Lamiaceae

Mentha arvensis

India

8 slight feeding (scraping of leaf epidermis) and survived for 39 days, no feeding in multiple choice tests

Lemnaceae

Lemna sp. (either *L. gibba* or *L. parodiana* Giaredelli)

Argentina

3 less than 0.5 eggs/female/day vs 3.5 on water hyacinth

8 less than 3 feeding spots/weevil/day vs 17/day for water hyacinth

Spirodela intermedia

Argentina

3 1 egg/female/day vs 3.5 on water hyacinth

8 less than 3 feeding spots/weevil/day vs 17/day for water hyacinth

Liliaceae

Allium cepa

Argentina

8 0.03 feeding scars/weevil/day vs 7.58 on water hyacinth

Amaryllis sp.

India

4 adult fed and survived for 40 days, single infertile egg laid in decaying plant tissue

Asparagus officinalis

Argentina

5 0.001 feeding scars/weevil/day vs 4.01 on water hyacinth

8 0.12 feeding scars/weevil/day vs 7.58 on water hyacinth

Musaceae

Musa paradisiaca

India

8 fed and survived for 56 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

Uganda

9 63 feeding scars vs 1838 on water hyacinth in petri dishes but no damage in cage tests

Musa sp.

Vietnam

9 53 feeding scars vs 220-338 on water hyacinth

Onagraceae

Ludwigia peploides

Argentina

3 less than 0.5 eggs/female/day vs 3.5 on water hyacinth

8 less than 3 feeding scars/weevil/day vs 17/day for water hyacinth

Vietnam

9 70 feeding scars vs 220-338 on water hyacinth

Orchidaceae

Vanilla fragrans

India

8 slight feeding (scraping of leaf epidermis) and survived for 36 days, no feeding in multiple choice tests

Philydraceae

Philydrum lanuginosum

Australia

7 average of 1.3 feeding scars vs 830.7 feeding scars

Poaceae

Saccharum officinarum

Argentina

1 2 eggs laid vs 749 on water hyacinth

Pontederiaceae

Eichhornia azurea

Argentina

1 14 eggs laid vs 749 on water hyacinth

3 less than 0.25 eggs/female/day vs 3.0/day for water hyacinth

5 0.125 feeding scars/weevil/day vs 4.01 on water hyacinth

7 7.36 feeding scars/weevil/day vs. 13.49/day on water hyacinth (on average)

8 4.86 feeding scars/weevil/day vs 7.58 on water hyacinth

10 placement of eggs, resulting in 2 larvae vs 9 pupae in water hyacinth

10 placement of first instars, 3 possible points of larval tunneling vs 12/15 larvae or pupae after 15 days in water hyacinth

Monochoria cyanea

Australia

3 eggs laid, 2 larvae recovered resulting in 0 adults; vs. 69 larvae recovered from water hyacinth, 30 larvae transferred to new plants resulted in 23 adults

7 average of 1.7 feeding scars vs 910.0 on water hyacinth

8 average of 45 feeding scars/plant after 5 days vs 234.3 on water hyacinth

Monochoria hastata

Thailand

9 feeding occurred but unable to complete life-cycle

Vietnam

9 84 feeding scars vs 220-338 on water hyacinth

Monochoria vaginalis

Australia

3 eggs laid, 12 larvae recovered resulting in 1 adult; vs. 51 larvae recovered from water hyacinth, 30 larvae transferred to new plants resulted in 20 adults

7 mean 20.0 feeding scars vs 768.0 on water hyacinth; 1 egg laid

8 average of 54.7 feeding scars/plant after 5 days vs 147.0 on water hyacinth

Thailand

9 feeding occurred but unable to complete life-cycle

Pontederia lanceolata

Argentina

3 less than 0.25 eggs/female/day vs 3.0/day for water hyacinth

5 0.01 feeding scars/weevil/day vs 4.01 on water hyacinth

7 2.55 feeding scars/weevil/day vs. 13.49/day on water hyacinth (on average)

8 3.48 feeding scars/weevil/day vs 7.58 on water hyacinth

10 placement of eggs, resulting in 1 larval tunnel vs 9 pupae in water hyacinth

Reussia rotundifolia

Argentina

3 ~0.2 eggs/female/day vs ~2.75 on water hyacinth

8 5.91 feeding scars/weevil day vs. 9.80 on water hyacinth,

10 placement of first instars, 2 pupae, 1 lge larva from 32 eggs vs 16 pupae from 16 eggs on water hyacinth

Salviniaceae

Salvinia sp.

Vietnam

9 10 feeding scars vs 220-338 on water hyacinth

Trapaceae

Trapa bispinosa

India

4 adult fed and survived for 42 days, 5 fertile eggs laid, larvae died within 3 days of hatching, no feeding in multiple choice tests

10 placement of 25 older larvae, no survival beyond 4 days

Typhaceae

Typha latifolia

Argentina

5 0.001 feeding scars/weevil/day vs 4.01 on water hyacinth

Umbelliferae

Daucus carota

Argentina

8 0.1 feeding scars/weevil/day vs 7.58 on water hyacinth

Appendix 3

Summary results of host-specificity tests carried out on *N. eichhorniae* for which some damage was recorded. Results detail the country in which the test was made, the basic test design, and the outcome of the trial. The test designs used were: 1. Oviposition - multiple choice with host; 2. Oviposition - multiple choice, presence of host unknown; 3. Oviposition - no choice; 4. Oviposition - unknown design; 5. Adult feeding/survival - multiple choice with host; 6. Adult feeding/survival - multiple choice, presence of host unknown; 7. Adult feeding/survival - paired choice; 8. Adult feeding/survival - no choice; 9. Adult feeding/survival - unknown design; 10. Development following placement of eggs or larvae.

Alismataceae

Sagittaria trifolia

China

8 fed and survived for 29 days, no feeding in multiple choice tests (unclear to which weevil species this refers)

Araceae

Amorphophallus sp.

India

8 slight feeding (scraping of leaf epidermis) and survived for 34 days, no feeding in multiple choice tests

Pistia stratiotes

India

8 fed and survived for 75 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

Zimbabwe

9 0.03 feeding scars/weevil/day vs 9.83 on water hyacinth (unclear to which weevil species this refers)

Begoniaceae

Begonia sp.

India

8 slight feeding (scraping of leaf epidermis) and survived for 33 days, no feeding in multiple choice tests

Brassicaceae

Brassica caulorapa

China

8 fed and survived for 36 days, no feeding in multiple choice tests (unclear to which weevil species this refers)

Brassica oleracea

India

8 slight feeding (scraping of leaf epidermis) and survived for 43 days, no feeding in multiple choice tests

Brassica oleracea var. *capitata*

Zimbabwe

9 0.01 feeding scars/weevil/day vs 9.83 on water hyacinth (unclear to which weevil species this refers)

Brassica pekinensis

China

8 some feeding but unable to complete development (unclear to which weevil species this refers)

10 fed and survived for 24 days, no feeding in multiple choice tests (unclear to which weevil species this refers)

Raphanus sativus

India

8 slight feeding (scraping of leaf epidermis) and survived for 23 days, no feeding in multiple choice tests

Cannaceae

Canna indica

India

8 fed and survived for 27 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

Ceratophyllaceae

Ceratophyllum oryzetorum

China

10 some feeding but unable to complete development

Commelinaceae

Tradescantia fluminensis

India

8 fed and survived for 25 days, eggs laid, no feeding in multiple choice tests

Zebrina pendula

India

8 fed and survived for 33 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

USA

7 0.2 feeding scars/weevil/day vs 13.2 on water hyacinth

Cucurbitaceae

Cucumis sativus

China

3 eggs laid but unable to complete life-cycle (unclear to which weevil species this refers)

8 fed and survived for 38 days, no feeding in multiple choice tests (unclear to which weevil species this refers)

10 some feeding but unable to complete development (unclear to which weevil species this refers)

Hydrocharitaceae

Hydrilla sp.

India

8 slight feeding (scraping of leaf epidermis) and survived for 16 days, no feeding in multiple choice tests

Vallisneria sp.

India

8 fed and survived for 16 days, eggs laid, no feeding in multiple choice tests

Liliaceae

Amaryllis sp.

India

8 fed and survived for 32 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

Musaceae

Musa paradisiaca

India

8 fed and survived for 28 days, no feeding in multiple choice tests

10 placement of 3 first instars, no survival beyond 3 days

Orchidaceae

Vanilla fragrans

India

8 slight feeding (scraping of leaf epidermis) and survived for 34 days, no feeding in multiple choice tests

Pontederiaceae

Eichhornia azurea

USA

7 0.7 feeding scars/weevil/day vs 13.2 on water hyacinth

Monochoria cyanea

Australia

3 eggs laid, 1 larva recovered resulting in 0 adults; vs. 37 larvae recovered from water hyacinth, 24 larvae transferred to new plants resulted in 15 adults

5 average of 8.0 feeding scars vs 1583.0 on water hyacinth

8 average of 33.7 feeding scars/plant after 5 days vs 318 on water hyacinth

Monochoria vaginalis

Australia

3 eggs laid, 1 larva recovered resulting in 0 adults; vs. 37 larvae recovered from water hyacinth, 24 larvae transferred to new plants resulted in 15 adults

5 average of 1.3 feeding scars vs 1583.0 on water hyacinth

8 average of 111.7 feeding scars/plant after 5 days vs 318.0 on water hyacinth

Pontederia cordata

Australia

5 adult feeding and oviposition occurred, larvae unable to complete development

USA

7 10.1 feeding scars/weevil/day vs 13.2 on water hyacinth

10 larval development observed

Sparganiaceae

Sparganium americanum

USA

7 3.1 feeding scars/weevil/day vs 13.2 on water hyacinth

Trapaceae

Trapa bispinosa

India

8 fed and survived for 35 days, eggs laid, no feeding in multiple choice tests