Biological control of
water hyacinth 2

The moths *Niphograpta albiguttalis* and *Xubida infusellus*: 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 a dense impenetrable mat across the water surface, 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.

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 via 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.

Photo: M. Julien

Water hyacinth: the world's worst aquatic weed

Photo: M. Julien

Water hyacinth at Lake Gerehu, Papua New Guinea

Biological control offers sustainable, environmentally-friendly, long-term control. In the case of water hyacinth, it offers the only feasible method to provide some level of control to those infestations which cover huge areas, are difficult to access and/or do not warrant the high cost of physical or chemical control. A number of 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

Photo: M. Julien

Lush growth in a nutrient-rich pond, Musoma, Tanzania

N. eichhorniae. The biologies, host ranges, rearing, releasing and monitoring techniques of these two weevils were outlined in Julien et al. (1999). Other insect species used for the biological control of water hyacinth include two moths, *Niphograpta albiguttalis* and *Xubida infusellus*, which have been released in 13 and 3 countries, respectively.

Studies have demonstrated that these biological control agents are host specific. As so much research has already been done, these and other control agents are available and can be introduced into new regions comparatively cheaply. The forecast is 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 the 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), Huffacker and Messenger (1976), Hokkanen (1985), 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 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 thick, spongy, aerenchyma-filled petiole (Figure 1). The large air spaces within the petioles allow the plants to float on the water surface. Water hyacinth floats while all other members of the family Pontederiaceae are rooted in the substrate. Stolons grow horizontally outwards from terminal buds at the base of mature plants. A daughter plant, or ramet, develops at the end of each stolon. The bisexual flowers are bluish-purple with a yellow centre and are produced on single spikes to 60 cm in length emanating from several centimetres below the petiole/lamina junction. 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).

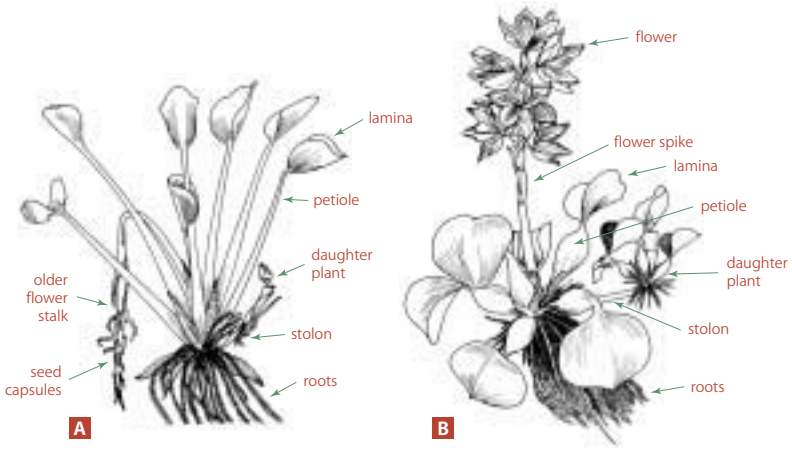


Figure 1. Water hyacinth plants with (A) slender petioles and (B) bulbous petioles (from Wright and Purcell 1995)

Water hyacinth shows considerable variation in both leaf and flower form. The petioles vary from long and relatively 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. Slender petioles are typical of plants that occur within dense, crowded infestations, while bulbous petioles characterise younger plants in open water or on the open-water margins of infestations. Flowers are of three distinct types, differing in the relative length of styles within single flowers (Barrett 1977). In the introduced range of the species, the form with styles of intermediate length predominates, the long-styled form occurs less frequently and the hypothesised short-styled form has not been recorded (Barrett and Forno 1982). Seeds are produced in vast quantities, up to 300 seeds per capsule, and are long-lived, remaining viable for 5–20 years (Manson and Manson 1958; Matthews 1967; Das 1969; Matthews et al. 1977). Seeds sink following release from the seed capsule and may subsequently germinate as water levels change (Wright and Purcell 1995).

Vegetative reproduction is a common form of propagation 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.

Photo: M. Julien

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

Photo: M. Julien

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

Photo: M. Julien

Mats of water hyacinth hinder shipping; Port Bell, Lake Victoria, Uganda

Dispersal of seed is likely to further contribute to the spread of this weed. Seeds are released directly into the water column, from where they can be carried long distances downstream. The numerous and tiny seeds can also be easily transported by vehicles, boats or pedestrians passing through infested areas.

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 to new areas commenced in the 1880s with its deliberate introduction into the United States of America (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.

Photo: M. Julien

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

Photo: M. Julien

Dense water hyacinth blocks access to Nveye Lagoon, Ghana

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, Australia and the Pacific.

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°C 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, allowing the

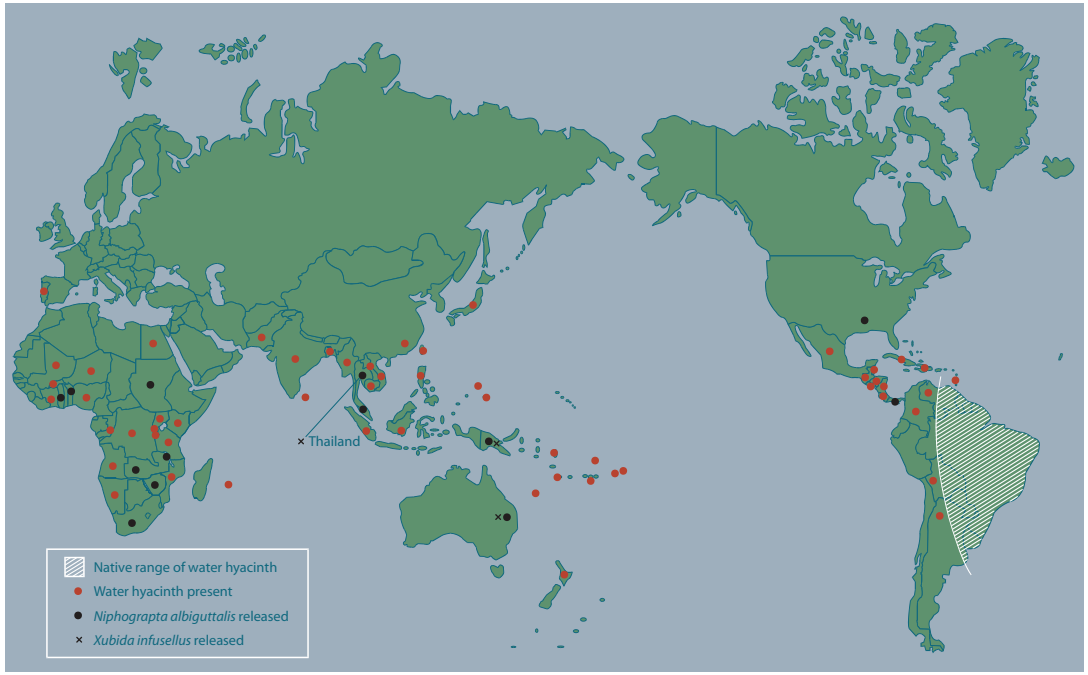


Figure 2. Countries where *Niphograpta albiguttalis* and *Xubida infusellus* have been released and other countries where water hyacinth is present

Photo: M. Julien

Tall, lush growth occurs in tropical conditions

weed to infest countries across a wide range of latitudes and climates (see Figure 2). Good growth can continue at temperatures ranging from 22°C 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 can survive for several months in low-moisture substrates. They can tolerate acidic waters but cannot survive in salt or brackish water (Penfound and Earle 1948).

Photo: M. Julien

Although water hyacinth is damaged by frost, it is not killed and recovers well in summer in warm-temperate climates

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 usually occurs at relatively low densities, but becomes a problem where the hydrological regime of a water body has been altered by human activities, where the level of nutrients in the water has been increased, or where flushing of the weed and natural enemies occurs and the weed population recovers faster than that of the natural enemies. Eventually the balance is restored as the populations of the natural enemies increase to reduce the weed.

Within its introduced ranges, however, the species develops large populations that have enormous social, economic and environmental impacts — earning this plant the reputation of being the 'world's worst aquatic weed' (Holm et al. 1977). Water hyacinth forms dense, impenetrable mats that cover the water surface. The 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 and utilisation of water by man and other animals. The weed chokes intake points for water 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 weed may provide 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 dramatically increase 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. Severe flooding leads 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 into various schemes for its utilisation (Wolverton and McDonald 1979; see papers in Thyagarajan 1984; Lindsay and Hirt 1999). 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.

Photo: A. Wright

Baskets and bags made from water hyacinth

Photo: M. Julien

Water hyacinth handicraft shop, Homa Bay, Kenya

Photo: M. Julien

Biogas generation using cow dung and water hyacinth, Homa Bay, Kenya

Despite these ideas, use of water hyacinth will probably remain restricted to small-scale cottage industries which are highly unlikely to provide a viable method for controlling or managing the weed. 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 on a low-value crop.

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 delayed or otherwise compromised by any consideration of its potential use (Julien et al. 1996).

Chapter 3
Management of Water Hyacinth

The complete removal of water hyacinth has proved impossible for most areas. Where eradication of an infestation has occurred, the effects are usually short-term. The difficulties in achieving effective control 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 seeds are readily transported by currents, boats, fishing nets and possibly by 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, e.g. village watering points, prime recreational areas, 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' aims to incorporate some or all of these methods, but with reliance on biological control as the most significant component and/or the long-term objective (Harley et al. 1996; Julien et al. 1996). Integration of control measures is discussed further in Chapter 8.

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 harvesters have been developed in an attempt to accelerate the physical removal of water hyacinth. Although a few of these have been effective in particular situations, most have been abandoned as ineffective and/or excessively 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. Drainage of a water body will lead to the death of water hyacinth plants but seeds usually germinate when water is reintroduced to the system.

The rate of growth and invasion by water hyacinth usually exceeds the rate at which it can be cleared. Reinfestation from ramets or seeds generally occurs rapidly and the process of removal must be repeated. The material removed from the water should be transported 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.

Photo: C. Cilliers

Aerial spraying with herbicides, South Africa

Photo: A. Wright

Mechanical harvester operating near Bangkok, Thailand

3.2 Chemical

The treatment of water hyacinth with herbicides has been effective in controlling small infestations of water hyacinth or those 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, ongoing vigilance and frequent reapplication to provide effective, long-term control of the weed

Photo: C. Cilliers

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

and any regrowth. In most situations, chemical control is unacceptably costly in terms of chemicals, equipment, labour and environmental impact. The problems of chemical applications are compounded by the use of many water hyacinth-infested sites for obtaining drinking water, for washing and for fishing. As with physical controls, the costs associated with herbicide applications generally limit their use to emergency control 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 as part of a biological control program (Center 1994).

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 which are plants of no economic importance or ecological

Photo: M. Julien

Water hyacinth covering more than 90% of Warasol Lagoon, Sepik River, Papua New Guinea

Photo: M. Julien

Warasol Lagoon, 1996, 5 years after release of the weevil *Neochetina eichhorniae*

significance to the country of release. Research has shown that a number of agents cannot survive on any plant except 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 1973a) and, to date, 7 agents have been released in 31 countries (Julien and Griffiths 1998; Table 1). 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.

Of these agents, the two *Neochetina* species are the most widely distributed, and to date are the most successful (Julien et al. 1999). In some areas, however, establishment and spread have been slow and/or the weevils have been unable to reduce the weed to acceptable levels. This dossier discusses the biology, impact, host range and utilisation of a further two agents, the pyralid moths *Niphograpta albiguttalis* Warren (previously *Sameodes albiguttalis*) and *Xubida infusellus* (Walker) (previously *Acigona infusella*). Both species are in the family Pyralidae and have larvae that tunnel in the petioles and buds of water hyacinth plants.

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	Australia Burkina Faso Benin Cote D'Ivoire Cuba Ghana Honduras India Indonesia Kenya Malawi Malaysia Mexico Mozambique Nigeria Panama Papua New Guinea People's Rep. China Philippines Rep. South Africa Sudan Taiwan Tanzania Thailand Uganda USA Vietnam Zimbabwe
Neochetina eichhorniae Warner	Adults feed on foliage and petioles, larvae tunnel in petioles and crown	Australia Burkina Faso Benin Cote D'Ivoire Fiji Ghana Honduras India Indonesia Kenya Malawi Malaysia Mexico Mozambique Myanmar Nigeria Papua New Guinea People's Rep. China Philippines

Table 1. Biological control agents released against water hyacinth worldwide (continued)

Agent	Type of damage	Countries where released
N. eichhorniae (continued)		Rep. South Africa Solomon Islands Sri Lanka Sudan Taiwan Tanzania Thailand Uganda USA Vietnam Zambia Zimbabwe
Lepidoptera		
Pyralidae		
Niphograpta albiguttalis Warren (= *Sameodes albiguttalis* (Warren))	Larvae tunnel in petioles and buds	Australia Benin Ghana Malawi Malaysia Panama Papua New Guinea Rep. South Africa Sudan Thailand USA Zambia Zimbabwe
Xubida infusellus (Walker) (= *Acigona infusella* Walker)	Larvae tunnel in petioles and buds	Australia Papua New Guinea Thailand
Hemiptera		
Miridae		
Eccritotarsus catarinensis (Carvalho)	Adults and nymphs suck cellular or intercellular fluid from leaves	Malawi Rep. South Africa Zambia
Mites		
Acarina		
Galumnidae		
Orthogalumna terebrantis Wallwork	Immatures tunnel in laminae	India Malawi Rep. South Africa Zambia
Fungi		
Hyphomycetes		
Cercospora piaropi Tharp, previously *C. rodmanii* Conway	Punctate spotting and chlorosis of laminae and petioles; necrosis of laminae	Rep. South Africa

References: Julien and Griffiths (1998); Julien et al. (1999); M. Hill, pers. comm.

Chapter 4
Niphograpta albiguttalis Warren
(water hyacinth stem borer)

The species has previously been known as *Epipagis albiguttalis* Warren (Bennett and Zwölfer 1968; Bennett 1974) and *Sameodes albiguttalis* (Warren) (Bennett 1984; Harley 1990).

Niphograpta albiguttalis is fairly widespread throughout tropical and temperate regions of South America. The species has been recorded from Trinidad, Guyana, Surinam and Brazil (Bennett and Zwölfer 1968).

4.1 Life history

The following descriptions are summarised largely from Bennett and Zwölfer (1968), DeLoach and Cordo (1978), Center (1981) and Harley (1990). A generalised life cycle of the moth is shown in Figure 3. The duration of development of each life stage and average fecundities are detailed in Table 2.

Egg: Eggs are spherical, creamy white and approximately 0.3 mm in diameter. They are usually deposited singly or in small groups in the leaf tissue. Eggs are concentrated in natural crevices, and at the site of existing injuries, abrasions and feeding punctures. Eggs hatch within 4–5 days at 24°C.

Larva: Larvae undergo five instars, requiring a total larval period of approximately 16–28 days depending on conditions (Table 2). Larvae initially feed on the surface of the lamina or petiole of a young leaf, forming irregularly-shaped scars that penetrate the epidermis and underlying

Table 2. Duration of each developmental stage and fecundities for *Niphograpta albiguttalis*

	DeLoach and Cordo (1978)	Winotai and Napompeth (1996)	Center (1994)
Developmental stage		Approximate duration (days)	
Egg	4	4–5	
Larva	21 (24°C)	17–21	16–18 (23–28°C water temp.) 18 (30°C) 20 (25°C) 28 (20°C)
Pupa	7	7–9	
Generation time	34		27–30
Adult longevity	Av. 5.7 Max. 9	1–5	Av. 4 Max. 6
Number of generations per year			5
Number of instars		5	5
		Fecundity	
Total eggs			Av. 370 Max. 650

parenchyma but not the vascular tissue. After 1–2 days they bore directly into the petioles and buds or occasionally they enter the leaves. Within the petiole, the larvae begin to feed on the parenchyma just below the epidermis, creating transparent areas. Late instar larvae burrow into the central portion of the rosette, destroying the petioles of the youngest leaves and often consuming the entire bud of a plant. Larvae tunnel extensively and frass is extruded from the tunnel entrances. They may emerge and re-enter the same or an adjacent petiole. Two or more larvae are frequently present in the same stem.

Larvae tend to feed on the bulbous petioles that are characteristic of plants growing on the perimeter of water hyacinth mats or in small infestations. They are rarely found in older plants, perhaps due to hardening of the epidermis making penetration by the feeding larvae difficult (Wright and Bourne 1986). However, they may be found on the slender plant form when growth rates are high and the plants are lush and tender.

The larvae are able to survive cold winter temperatures and can tolerate exposure to temperatures as low as 4°C for a number of hours (Center 1984; Stewart and Holwell 1985). They do not survive at constant temperatures of 35°C (Center 1981).

Pupa: Before pupation, the fully developed larva generally exits the petiole where it has been feeding and burrows into a relatively undamaged petiole. Here it excavates an elliptically-shaped pupation chamber within the aerenchyma, with an emergence tunnel leading from one end of the chamber to the epidermis. Only a thin, circular window of epidermis, 2–3 mm in diameter, remains across the exit hole. The larva creates a silken lining within the chamber and along the length of the tunnel, then pupates in a white cocoon within the chamber. After 5–7 days, the adult emerges from the cocoon and crawls up the tunnel to exit the petiole by rupturing the remaining film of the epidermis.

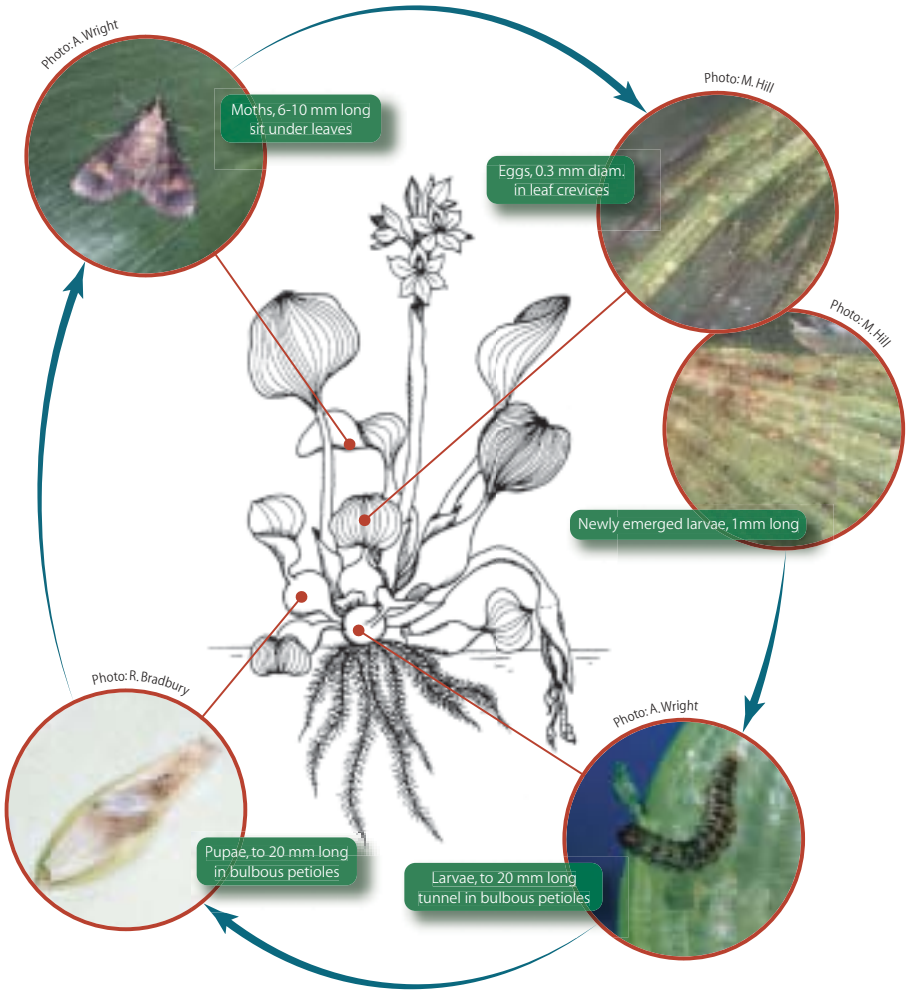
Photo: A. Wright

Photo: R. Bradbury

Female *Niphograpta albiguttalis* moths (top) are generally larger and darker than males (bottom)

Adult: Adult moths are nocturnal and usually rest on the undersurface of a leaf during the day. The adult is small, 6–10 mm in length with a wingspan of 17–25 mm. Colour is extremely variable, ranging from golden-yellow to charcoal grey, with brown, black and white markings. The adults tend to be darker during the winter months. Eggs within the ovaries are fully developed very soon after emergence of the female. Mating occurs shortly after emergence, and oviposition commences after this, with 70% of eggs laid during the second and third nights

Figure 3. Generalised life cycle of the moth *Niphograpta albiguttalis*



after emergence. Female moths lay an average of 370 eggs each, but up to 650 have been recorded. Adult moths live for 4–9 days (Harley 1990).

Female moths are generally darker and larger than the males. The posterior of the abdomen is more pointed in males and tends to be held upwards compared to the females. The female abdomen is wider and has a more rounded posterior.

4.2 Impact on weed

Initial damage from *N. albiguttalis* is characterised by small, dark spots on the leaf petioles which become visible 1–2 days after hatching. Older larvae feed on the developing bud in the centre of the crown, often severing the internal leaves — causing them to wilt and eventually become brown. Generally only the damaged leaf turns brown in the short term and the undamaged leaves remain green for several weeks. Feeding damage can destroy the apical bud, preventing regrowth and ultimately causing the death of that ramet. Daughter plants will continue to grow from axillary meristems on the rhizome and, when populations of the moth are high, these too will be attacked. Larger larvae sometimes move amongst petioles or plants and may damage several plants before pupating.

Photo: A. Wright

Initial *Niphograpta albiguttalis* larval damage is characterised by opaque areas on the petioles

Photo: A. Wright

Further feeding by larvae results in necrosis and waterlogging

Photo: T. Center

Older larvae feed in the crown

Populations of *N. albiguttalis* can increase rapidly due to the relatively high fecundity and short generation time of the insect (Harley 1990). The adults are highly mobile and disperse readily (Center and Durden 1981; Wright 1981, 1996), resulting in rapid distribution within and between infestations of water hyacinth. Dispersal rates averaging 1 km/day and reaching as high as 4 km/day have been recorded (Center 1984).

Although damage from the larvae can be severe and lead to the death of many plants (Wright and Purcell 1995; see Table 3), their distribution and impact are often temporary,

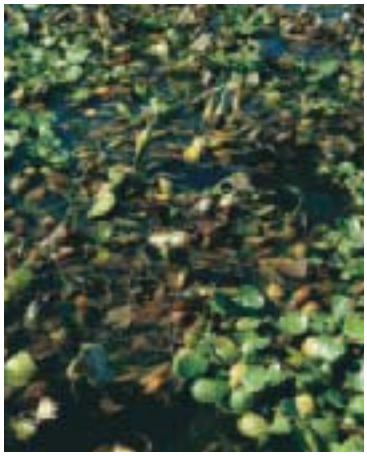


Photo: A. Wright

Damage can be severe and lead to the death of many plants

patchy and seasonal. The centres of larval activity change position irregularly within infestations (Wright 1981). This patchy distribution is apparently due to the preference of the moth to oviposit on, and larvae to develop best on, younger, tender and more bulbous plants (Wright 1981, 1984; Center 1984; Harley 1990). The insect responds to flushes of plant growth, causing extensive damage but then not persisting (Table 3).

N. albiguttalis is likely to be effective in controlling water hyacinth only under certain circumstances. *N. albiguttalis* favours young and tender plants. These are found where rapid new growth occurs, e.g. where plant density is low and along the open water margins of infestations and floating mats. However, defining plants preferred by *N. albiguttalis* is difficult (Center 1984). Often, but not always, these are shorter plants with bulbous petioles. By exploiting new growth, *N. albiguttalis* may play an important role in limiting or preventing further expansion of the weed over open water (Wright 1981; Bennett 1984; Center 1987). Under heavy weed infestation, plant morphology shifts to the tall, slender petiole form that generally characterises older and tougher plants and which are less suitable to the insect (Center 1987).

4.3 Host-range testing

During field surveys in its region of origin, *N. albiguttalis* was not located on any plants other than *E. crassipes* (Bennett and Zwölfer 1968; Silveira-Guido 1971). *N. albiguttalis* has

Table 3. Impact on water hyacinth attributed to *Niphograpta albiguttalis*

Location	Period of assessment	Impact	References
Florida, USA	18 months post-release	Spread throughout peninsular Florida with limited impact in spring only	1, 2
Maitland, New South Wales, Australia	March to June 1992	Reduced water hyacinth mass by 20%, water hyacinth returned in spring to have an overall cover of 80%	3
Ingham, Queensland, Australia	November 1978 to June 1980	Reduction of weed cover from 85% to ~5%; by November 1981 this had returned to 65%	4
Bangkok, Thailand	September 1995 to September 1997	At one site nearly 100% of plants showed larval damage; 87 pupae and 56 larvae in a 2 m x 2 m quadrat	3

References: 1. Center (1984); 2. Center (1987); 3. A. Wright and A. Winotai, pers. comm.; 4. Wright (1984).

undergone extensive host testing in numerous countries (Table 4). The list of plants against which the species has been tested is long and diverse (Appendix 1), covering 136 plant species in 60 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.

The tests verified that *N. albiguttalis* is specific to the family Pontederiaceae with a strong preference for water hyacinth (Appendix 2). Post-release evaluation has confirmed that the insect does not damage plants other than water hyacinth.

4.4 History of introductions

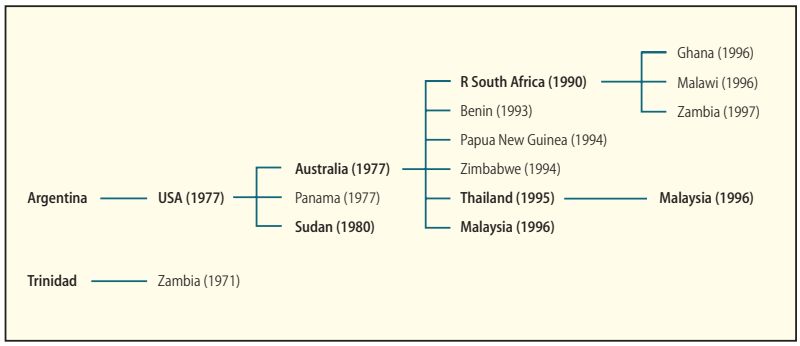
Based on the results from the various host-specificity trials, *N. albiguttalis* has been released in 13 countries throughout the distribution of water hyacinth (Figure 4; Julien and Griffiths 1998). The original source of material for most of these releases was Argentina. The source of the first release made in Zambia was Trinidad.

Table 4. Countries where *Niphograpta albiguttalis* has been released and organisations that have undertaken host-specificity trials

Country	Organisation	References	Appendix 1
Australia	Commonwealth Industrial and Scientific Research Organisation, Entomology (CSIRO Entomology)	1	Included
Benin	International Institute of tropical Agriculture (IITA)	2	Not tested
Ghana	Environment Protection Agency	3	Not tested
India*	CABI Bioscience (formerly CIBC)	4	Included; not released
Malawi	Malawian Fisheries Department	5	Not tested
Malaysia	Malaysian Agricultural Research and Development Institute (MARDI); Department of Agriculture Malaysia (DOA)	6	Included
Panama	Panama Canal Commission	7	Not tested
Papua New Guinea	Department of Agriculture and Livestock	8	Not tested
Republic of South Africa	Plant Protection Research Institute	3	Limited additional testing undertaken; included
Sudan	University of Khartoum	9	Tested; results not available
Thailand	National Biological Control Research Center (NBCRC)	10	Included
USA**	United States Department of Agriculture (USDA)	4, 11	Included
Vietnam***	Vietnam Biological Control Research Centre (VNBCRC)	12	Included; not released
Zambia	CABI Biosciences	5	Not tested
Zimbabwe	Plant Protection Research Institute	13	Not tested

* Tests were conducted in India by CABI Biosciences but the insect was not released.
** Tests were conducted for USA in USA (ref. 11) and in Argentina (ref. 12).
*** Tests were conducted in Vietnam by VNBCRC but the insect was not released.
References: 1. CSIRO Entomology, unpublished report; 2. O. Ajuonu, pers. comm.; 3. C. Cilliers, pers. comm.; 4. Cordo and DeLoach (1978); 5. M. Hill, pers. comm.; 6. Anwar et al. (1997); 7. Parris (1980); 8. W. Orapa, pers. comm.; 9. Beshir and Bennett (1985); 10. Winotai and Napompeth (1996); 11. N. Spencer, pers. comm. (USDA unpublished report); 12. A. Wright, pers. comm. (ACIAR Unpublished Report PN9320, 1995–1997); 13. G. Chickwenere, pers. comm.

Figure 4. The origin of *Niphograpta albiguttalis* 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 included in parentheses



The agent is confirmed to have established in six countries: Australia, Republic of South Africa, Sudan, USA, Thailand and Malaysia (Figure 4). It established readily and is widespread in the first four countries (Wright 1981; Beshir 1984; Center 1984, 1994; Beshir and Bennett 1985; C. Cilliers, pers. comm.). In Malaysia and Thailand, releases were made relatively recently, and low numbers are present at a few sites (Julien and Griffiths 1998).

This insect failed to establish in the following countries: Benin, released in 1993 (C.J. Lomer, pers. comm.); Zambia, where 50 larvae only were released in 1971 (Hill 1997); and Papua New Guinea, despite the release of over 8000 larvae or pupae from 1994 to 1996 (W. Orapa and M. Julien, unpublished information). Establishment has not been evaluated in Panama where it was released in 1977, nor has it been confirmed for the more recent releases in Zimbabwe (1994) and Ghana and Malawi (1996).

Chapter 5
Xubida infusellus (Walker)
(water hyacinth stalk borer)

The native range of *Xubida infusellus* is fairly extensive through Central and South America from Guatemala, Trinidad, Colombia, Guyana, Surinam, Amazonian Brazil, Peru, Paraguay, Uruguay and Argentina (Bennett and Zwölfer 1968; Silveira-Guido 1971; DeLoach et al. 1980).

The taxonomy of the species has been in a state of flux and the list of synonyms is extensive. The synonyms as listed by Bleszynski (1967) in DeLoach et al. (1980) include *Acigona infusella* (Walker) = *comparella* Felder 1875, = *surinamella* Moschler 1882, = *purpurealis* Hampson 1896, = *ignitalis* Hampson 1917, = *cayugella* Schaus 1922. Since this listing, the species has also been referred to as *Acigona infuscatella* (Walker) (Bennett 1974).

5.1 Life history

The following descriptions are summarised from Bennett and Zwölfer (1968), Silveira-Guido (1971), Perkins (1973b), DeLoach (1975), DeLoach et al. (1980) and Sands and Kassulke (1983). These studies have reported large differences in the performance and fecundity of the species during rearing (Table 5). The variation may be due to some studies being conducted on colonies infected with *Nosema* being compared to studies on colonies free of the disease.

A generalised life cycle of the moth is shown in Figure 5. The duration of development of each life stage and average fecundities are detailed in Table 5. This moth utilises the taller, slender form of water hyacinth. This form is typical of crowded mats and is normally older and tougher than the smaller, bulbous form that grows at the uncrowded edge of mats.

Egg: Individual eggs are creamy to white in colour and approximately 0.8 mm long and 0.5 mm wide. They are laid in a continuous extrusion up to several centimetres long and covered by a white secretion. The eggs and secretion are deposited simultaneously but originate from different openings. Under laboratory conditions, estimates of the average number of eggs in each mass range from 14 (DeLoach et al. 1980) to

Photo: M. Julien

The taller, slender form of water hyacinth utilised by *Xubida infusellus*

Table 5. Duration of each developmental stage and fecundities for *Xubida infusellus*

Developmental stage	Silveira-Guido (1971)	M. Julien and J. Stanley, unpublished data	Sands and Kassulke (1983)	DeLoach et al. (1980)
		Approximate duration (days)		
Egg	8–11 (23–24°C, 60% humidity)		6–7	
Larva	40 (summer)	57 (30°C) 64 (25°C) 149 (20°C) Estimated developmental zero = 18°C	48 (26°C) (range 36–90)	64.1 (range 48–86)
Pupa	9		9 (range 8–10)	
Generation time	58			
Adult longevity	1–8 (females)		5 (range 4–7 days)	
Number of generations per year	3 (Rocha, Uruguay)			3
Number of instars	7–10	6	7–10	
		Fecundity		
Eggs/mass		Max. 300	171 (range 82–225)	14 (range 1–42)
Total eggs	150		640	

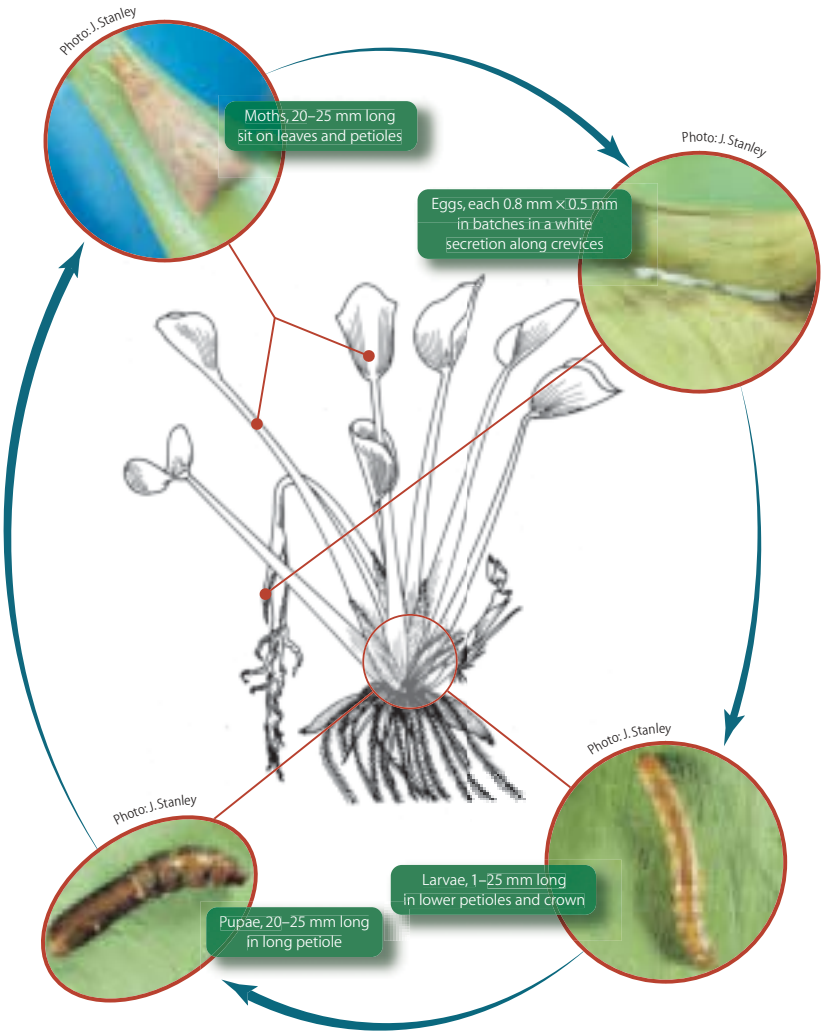
171 (Sands and Kassulke 1983). The much lower egg production recorded by DeLoach et al. (1980) suggests that disease may have influenced their results. The egg masses are deposited on leaves or leaf petioles, concentrated in crevices and grooves, between overlapping or curling leaves, and other irregularities in the plant tissue. In the absence of appropriate crevices, much lower numbers of eggs are scattered individually or in small groups on the plant surface. Occasionally three or four groups of eggs may be laid on a single water hyacinth leaf.

Larva: Newly-hatched larvae frequently disperse after hatching either by walking or lowering themselves on silken threads. Eventually the larvae settle and mine in the leaf lamina. From here they enter the petiole either by tunnelling directly from the lamina or emerging from the lamina, crawling and entering the petiole at a lower level. Frequently, small larvae tunnel around the periphery of petioles just below the epidermis at about mid-height, girdling the petiole and causing a characteristic yellow ring. Above this

Photo: M. Julien

Girdling by larvae leads to death of the petioles above the girdle

Figure 5. Generalised life cycle of the moth *Xubida infusellus*



point the lamina and petiole die. Larvae continue to tunnel downward into the base or crown of the plant from where they may tunnel upwards into another petiole. Generally there is only one larva per petiole and each larva attacks only one plant, but occasionally a larva transfers from one plant to another by tunnelling through the interconnecting stem. Larvae are usually more abundant in larger plants with tall, slender petioles than in smaller, bulbous plants. Larvae develop more rapidly in plants growing in high nutrient conditions than those in lower nutrient conditions.

Pupa: Larvae pupate within the petiole but do not spin a cocoon. Before pupation, the larva cuts a circular window in the petiole, leaving only the epidermis across the opening. The tissue within the circle dies and turns brown, leaving a weak but protective screen to the pupal tunnel. If the screen is breeched during excavation, the larva repairs it with webbing. Adults emerge usually in the late afternoon and exit the plant through the prepared window. The pupal case is left projecting from the emergence hole 5–10 cm above the water surface (Bennett and Zwölfer 1968; Wright and Purcell 1995). The sex of pupae can easily be determined by different structures on the posterior, underside of the abdomen (Sands and Kassulke 1983) (see Figure 6).

Photo: J. Stanley

Tunnelling larvae of *Xubida infusellus* frequently girdle the petiole, causing a characteristic yellow ring

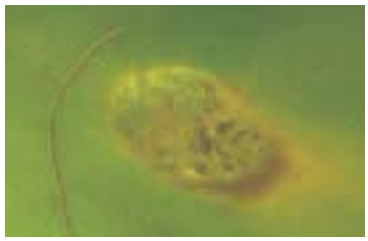
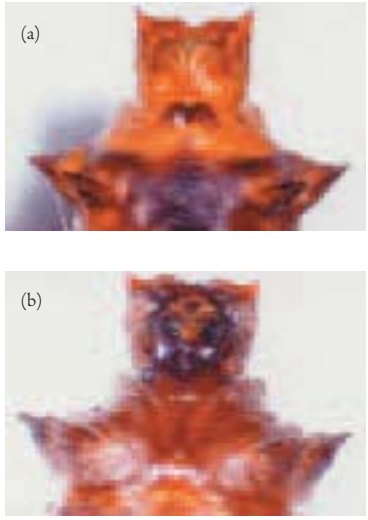


Photo: J. Stanley

The larva cuts an exit window, leaving a thin layer of epidermis through which the adult will emerge

Adult: Adults are tan to red–brown in colour, measuring approximately 20–25 mm in length. The male moths are smaller than the females. Adults are nocturnally active and good fliers. During the day they can often be seen resting on leaf stalks of water hyacinth. The sex ratio



Photos: A. Winotai

Figure 6. Terminalia of male (a) and female (b) pupae of *Xubida infusellus*, ventral view

Photo: A. White

Larval feeding in the lower petiole and crown causes extensive damage

is approximately 1:1, with male moths emerging before females. Adults mate soon after emergence of the female and egg-laying occurs during the next 2–4 nights after which the female dies (Julien and Stanley 1999).

X. infusellus cannot develop below around 18°C but can survive colder winter temperatures. No diapause has been noted for this insect. The life cycle of about 2 months at 25°C and 30°C increased to 5 months at 20°C (J. Stanley and M. Julien, unpublished data). This insect undergoes three generations per year and overwinters as larvae within plants.

5.2 Impact on weed

X. infusellus larvae are capable of inflicting heavy damage to water hyacinth even when at low densities. Plants are severely weakened distal to the point of larval entry, and often die as a result of damage caused by the tunnelling larvae. Larvae developing within the crown and lower petioles cause extensive damage to plants, reducing growth rates and leaf production. In the native range of water hyacinth, damage from *X. infusellus* can result in patches of dead and dying plants several metres in diameter (Bennett and Zwölfer 1968). Although plants often die as a result of the damage, one or more daughter plants may be produced before the plant succumbs (Wright and Purcell 1995).

Cage experiments in Australia revealed that the dominant detrimental influence of *X. infusellus* on water hyacinth was in reducing the leaf production rate of the main plant. This attribute suggests great potential for the species to impact significantly on the weed, as the rapid leaf production of water hyacinth is considered to be one of the principle attributes which allows the plant to recover from the impact of insect damage (Center 1985).

5.3 Host-range testing

Field observations showed that in its native range *X. infusellus* is able to develop on a number of other species from the family Pontederiaceae, including *Eichhornia azurea* (Sw.) Kunth, *E. heterosperma* Alexander, *E. paniculata* (Spreng.) Solms-Laub, *Pontederia cordata* L. and *P. (Reussia) rotundefolia* L. (Bennett and Zwölfer 1968; Silveira-Guido 1971; DeLoach 1975; DeLoach et al. 1980). *E. azurea* appeared particularly suitable as a host, receiving high numbers of eggs (Perkins 1973b) and supporting

Photo: K. Harley

In its native range, feeding by larvae of *Xubida infusellus* kills patches of plants

large numbers of larvae (Silveira-Guido 1971; DeLoach 1975; DeLoach et al. 1980). Eggs of *X. infusellus* were also frequently located on plants other than those in the family Pontederiaceae, especially on plants growing above the water hyacinth, e.g. *Polygonum ferrugineum* Wedd., *P. stelligerium* Cham. and *Sagittaria montevidensis* Cham. & Schltdl. (DeLoach et al. 1980). However, larvae hatching from these eggs caused no damage to the plants on which they were laid, but dropped on silken threads to the water hyacinth beneath.

The host-specificity of *X. infusellus* has been tested in few countries (Table 6) and the outcomes have been variable. The species has been tested under a range of 'choice' (where a range of test plant species are included, with or without the target plant species) and 'no choice' (where a single test plant species only is available) designs against 66 plant species in 30 families in numerous laboratory and field trials (Silveira-Guido 1971; Deloach et al. 1980; Sands and Kassulke 1983; Appendixes 3 and 4). In a number of these trials, insects were offered cut sections of plant or were placed into holes specifically made in the plant stem. Such trials enable larvae to avoid many of the physical and chemical characteristics of an undamaged plant; characteristics which may be crucial in the host selection and identification process of the insect. The results of such trials may therefore not reflect realistic host suitability. In particular, during the early trials in the USA, a number of crop species were implicated as hosts (DeLoach et al. 1980) but were subsequently shown to be unlikely hosts (Sands and Kassulke 1983).

In light of the above findings, the most reliable indicators of host suitability are field records throughout the native range of the insect, and laboratory trials allowing complete development of the insect on whole growing plants. Despite the extensive geographical range over which *X. infusellus* occurs, incorporating many agricultural areas, the species has been reported only from the Pontederiaceae.

Based on the results of the host-specificity trials, *X. infusellus* was released in Australia in 1981 but failed to establish. The species was reconsidered for release in 1995 at which time it underwent testing on closely-related species within the Pontederiacae. These were the native Australian *Monochoria* species, *M. cyanea* (F.

Table 6. Countries in which host-specificity tests with *Xubida infusellus* were undertaken and organisations that carried out the tests. Note that this insect has only been released in Australia, Thailand and Papua New Guinea

Country	Organisation	References	Appendix 3
Uruguay	United States Department of Agriculture (USDA)	1	Included; testing was for USA
Argentina	United States Department of Agriculture (USDA)	2	Included; testing was for USA
Australia	Commonwealth Scientific and Industrial Research Organisation (CSIRO)	3	Included
India	CABI Bioscience (formerly CIBC)	2	Included; testing was for CABI Biosciences
Trinidad		4	Included; testing was for CABI Biosciences
Thailand	National Biological Control Research Center (NBCRC)	5	Included
Papua New Guinea		6	Not tested

References: 1. Silveira-Guido (1971); 2. DeLoach et al. (1980); 3. Sands and Kassulke (1983); 4. Bennett (1970); 5. A. Wright, pers. comm. (ACIAR Report P/N9320 September 1997); 6. M. Julien and W. Orapa, unpublished information

Muell) F. Muell. and *M. vaginalis* (Burman f.) Kunth, which had not been tested before the earlier release, and the American species *P. cordata*. Initial tests showed that each of these species could support the development of *X. infusellus* (Sands and Kassulke 1983; J. Stanley and M. Julien, unpublished data) and so the impact of the insect on these plant species relative to the impact on water hyacinth was assessed as follows.

Monochoria species

Four species of *Monochoria* are present in Australia, of which the two most abundant, *M. vaginalis* and *M. cyanea*, were tested. *Monochoria* often inhabits temporarily flooded ditches and drains, where the seeds germinate readily in response to submergence following rain. Plants then grow rapidly and set large quantities of seed.

Cage trials did not reveal any oviposition preference between water hyacinth and the *Monochoria* species. Over 95% of eggs were laid apparently at random on the cage walls, even in large flight cages 2 m × 2 m × 10 m long. The propensity of the moths to lay on non-host plants in the field and on the cage sides in captivity suggests that the final choice of oviposition site by the females is relatively non-discriminatory as long as it is physically suitable and in the vicinity of the host. The extensive dispersal of neonate larvae (see Section 5.1) suggests that the placement of eggs directly onto the host plant may not be critical to the survival of this species.

X. infusellus completed development on *M. vaginalis* and *M. cyanea*. The impact of larval tunnelling was more pronounced on *Monochoria* due to the smaller size and greater softness of the petioles.

Despite the evidence of oviposition and feeding on *Monochoria* species it was considered that *X. infusellus* would have low or no impact for the following reasons:

- The duration between forming substantial emergent leaves and setting seed is likely to be insufficient to allow *X. infusellus* to establish and develop populations sufficiently large to impact on seed production.
- *Monochoria* probably relies on seed production for the maintenance of future generations. This almost ephemeral habit means it is highly unlikely to support populations of *X. infusellus* throughout the year.
- *Monochoria* is often present in areas that are geographically or temporally isolated from water hyacinth infestations. In such areas, *X. infusellus* populations would be unlikely to persist on *Monochoria*.

Where *Monochoria* grows in the vicinity of water hyacinth, local damage is expected but the impact of water hyacinth itself is potentially more damaging to local populations of *Monochoria* and other organisms. In view of the huge detrimental impact of water hyacinth in Australia and the limited potential impact on *Monochoria* species, the decision was made to release *X. infusellus*. The first releases were made in September 1996.

Pontederia cordata

This plant is a native wetland species of southern USA of ecological significance as food and shelter for fish and wildfowl (Melton and Sutton 1991). It grows rooted in the soil with stems partially submerged, making it a valuable species for flood mitigation, shoreline restoration and roadside revegetation (Melton and Sutton 1991). *P. cordata* grows in close proximity to water hyacinth in the USA and is a known host of *X. infusellus* within its native range in South America. Further information was required on the likelihood and extent of damage to *P. cordata* before release in the USA could be considered. In Australia, *P. cordata* is an exotic ornamental of minimal value.

In cage experiments in Australia, extensive tunnelling occurred within the petioles and rootstock of both *P. cordata* and water hyacinth. Insects completed their life cycle similarly on both plant species. When both species were artificially infested with a range of larval densities, the same density of larvae had a more severe impact on *P. cordata*. This was true for several of the most important aspects of plant growth measured, including total shoot dry weight, number of new shoots, number and size of daughter plants, number of green petioles, and rate of leaf production (J. Stanley and M. Julien, unpublished data).

Photo: J. Stanley

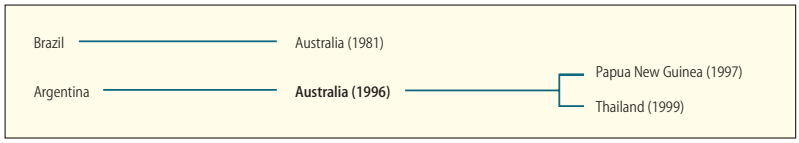
Field trials were carried out in Australia to determine the impact of *Xubida infusellus* on *Pontederia cordata*

Field experiments were carried out in Australia to further assess the relative impact of *X. infusellus* on *P. cordata* and water hyacinth. Potted plants were placed adjacent to water hyacinth infestations where *X. infusellus* had been released. The studies were inconclusive due to the early poor establishment of *X. infusellus*, high levels of *Neochetina* species weevils, and physical disturbance at the sites. It was concluded that the small population levels of *P. cordata* present in Australia made it impossible to accurately assess the degree of impact. However, it was considered likely that *P. cordata* could sustain considerable damage if *X. infusellus* were to become established in the USA (Julien and Stanley 1999). The insect has not been released in the USA.

5.4 History of introductions

X. infusellus has been released in relatively few countries (Figure 7). The absence of absolute host-specificity has limited its consideration and use as a biological control agent for water hyacinth. *X. infusellus* imported from Brazil was released in Australia in 1981. Although insects were recovered from two field sites for up to 9 months following liberation, populations were not sustained and eventually died out (Wright and Purcell 1995; Wright 1996; D. Sands, pers. comm.). The reasons for the failure are not clear and include destruction of one site through development and dry conditions at the other.

Figure 7. The origin of *Xubida infusellus* 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 included in parentheses



In addition, only limited numbers were released before the project terminated.

In 1995, *X. infusellus* was again imported into Australia, this time from Argentina, and released in 1996. It is established at one site in south-eastern Queensland but apparently not at the many other release sites. *X. infusellus* was imported into Papua New Guinea in 1997 and released without further testing. It has been recovered from one site near Port Moresby on several occasions 3–15 months after release. It was imported into Thailand, where testing was conducted before release in 1999, and has since been recovered (B. Napombeth, pers. comm. 1999). The insect also underwent host testing in India during early 1970s but was not released (Bennett 1984).

Chapter 6
Rearing

6.1. General methods

Eliminating disease from insect cultures

When biological control agents are introduced to a new country they should be obtained from an organisation that can verify that they are disease- and parasite-free. Alternatively, they are held in a quarantine facility for at least a generation while the colony is checked for diseases and parasites. Only disease- and parasite-free agents should be released so that they can reach their full potential as control agents and so that new diseases or parasites are not introduced that might affect other organisms.

Eliminating parasites is relatively easy, however eliminating a disease requires careful rearing techniques and strict hygiene. Both *Niphograpta albiguttalis* and *Xubida infusellus* are known to have endemic disease caused by the microsporidian *Nosema*. High incidence of *Nosema* in laboratory colonies has caused problems during studies of the biology, rearing and host testing of the insects both in their native range and in countries of introduction (Andres and Bennett 1975; Wright 1981). Procedures to eliminate disease are detailed below.

Photo: M. Julien

Strict hygiene procedures are required to prevent contamination between lines

Disease clearance involves identifying and eliminating diseased individuals — not curing them.

Collection

Efforts to minimise the likelihood of collecting and importing infected insects in the starter colony could greatly increase the chances of success.

- Starter colonies should be obtained from existing 'clean' cultures in other countries.
- If obtaining insects from the native range:
  - collect early spring generations. Microsporidian infection rates in *X. infusellus* are known to increase throughout the summer;
  - collect from diverse locations and keep the material from each population separate so that 'clean' populations do not become contaminated; and
  - collect only healthy, robust individuals.

Shipment procedures

Insects such as *N. albiguttalis* and *X. infusellus*, with a known endemic pathogen, should be accepted into quarantine with additional precautions. Care at this stage could

Photo: A. White

Maintaining each line separately will help prevent contamination between lines

improve the chances of obtaining a clean colony or reduce the time taken to achieve it. Larvae should be packaged for transport individually or in very small groups, depending on the size of the shipment. Each individual or group of individuals should be treated as a separate unit, and handled with the same strict hygiene procedures used in line rearing (see below). Checking for disease level at this stage may indicate the chances of success, i.e. high levels of disease might suggest ordering a new shipment. However, checking requires destruction of individuals and this may not be possible unless the shipment is large.

Rearing to eliminate disease

Nosema spp. are thought to be transmitted both orally, when the faeces of infected individuals contaminate food and are ingested by non-infected larvae, and transovarially through the egg. Following hatching, *X. infusellus* larvae remain around the egg mass and feed upon the hatched egg cases, increasing the risk of cross-infection. Because of this high likelihood of cross-contamination, the priority should be to obtain clean lines rather than clean individuals. If any insect from a line is thought to be infected then the entire line should be destroyed. Only when insect numbers are very low should each insect be considered separately and destroyed only when it is thought to be infected.

Recommended rearing procedures for disease elimination include the following.

- *Identification of infected adults*. The eggs from a female should be kept separately for future rearing or destruction. Both the male and female of the pair should be checked. Identification is required by an experienced insect pathologist, or following training by a pathologist. Important points in preparing material for examination are outlined below.
- *Destruction of progeny from adults shown or suspected to be infected*. If either or both adults of the pair show signs of disease, all eggs or larvae from that female should be destroyed.
- *Line rearing of progeny from mating pairs that are considered to be disease-free*. To minimise laboratory selection and the possibility of genetic bottlenecks occurring, it is recommended that approximately 20 apparently clean lines, each with 100 to 150 individuals, are maintained.
- *Minimisation of contamination across lines*. Strict hygiene is required to prevent the spread of disease across lines. See below.
- *Maximisation of disease transmission and expression within a line*. To ensure that infected lines are identified so that they can be eliminated from the colony, conditions should allow maximum infection rates. This can be achieved by crowding 10–15 early instar larvae together over the first few weeks of development, as infection of early instars is often more likely and early infection leads to greater expression of the disease. Sterile procedures within a line should be avoided to maximise infection rates.

Photo: M. Julien

Instead of using a brush that requires sterilising, a strip torn from a water hyacinth leaf can be used to transfer a larva and then discarded

- *Routine testing of progeny and destruction of all siblings of any infected larvae*. Approximately 10% of the larvae in each line are tested for the presence of disease. In order to maximise disease expression, testing is best carried out on last instar larvae that have been starved for 6 days before testing. A pathologist can then prepare abdominal smears from these individuals and test for the presence of disease. If any larvae show signs of disease the entire line should be destroyed. Important points in preparing material for examination are outlined below.
- *Removal of slower developing individuals*. Culling of these individuals, which are considered more likely to harbour disease, may help clear the colony. During disease clearance procedures for *X. infusellus* in Australia, each line of approximately 100 1st instars resulted in 60–70 late instar larvae. The first 50% of any line to emerge as adults (30–35 adults) were retained for breeding and the slower developing individuals were destroyed.

The above rearing procedure is laborious and may need to be repeated over several generations to acquire a colony free of a microsporidian. If infection rates are high, it may not be possible to remove the disease from the colony and a fresh colony may be required. The colony can be considered clear of *Nosema* once three to four generations have passed without disease being found in a single individual at the 10% larval sampling frequency and 100% checking of breeding pairs.

Culture hygiene

The elimination of cross-contamination between lines is paramount to obtaining a clean colony, requiring strict hygiene procedures. As the priority during rearing should be to obtain clean lines rather than clean individuals, sterile procedures within the processing of a line are not required.

Hygiene procedures include the following.

- Using disposable containers and fresh food material.
- Using disposable gloves, disposable bench covers and disposable wipes to be renewed when commencing a new line.
- Preparing and storing all rearing containers away from rearing facilities.
- Sterilising all utensils used during rearing, e.g. brushes, by immersing in 5% hypochlorite solution for at least 2 minutes before starting work on a new line.
- Transferring insects using small sections of plant is preferable to any reused implement such as a brush. Water hyacinth leaves tear easily into strips that can be used for moving larvae to a new container or plant.
- Treating lines as separate units, e.g. keeping all containers from one line stacked together on a separate tray, and isolated from all other containers. This will allow trays to be collected for processing of a single line, without needing to move other containers. This ensures that the gloves used for each line do not contact surfaces touched by gloves used to process other lines.

Photo: M. Julien

Slides of the haemolymph and fat bodies of insect samples are inspected by an expert under a light-diffracting microscope to identify the presence of *Nosema* spores

Key points in the preparation of insect material for disease identification

1. Smears can be made from both larvae and adults. Adults should be tested after eggs have been deposited, and both the male and female of the pair should be checked. A 10% sub-sample of larvae should be tested from each line after they have been starved for 6 days.
2. All insects should be alive before preparation of the smears. The number of background organisms present in dead insects increases rapidly, making it difficult to identify disease. Smears can be made directly from live individuals or following freezing of live specimens at –8°C.
3. Wet smears are made from abdominal haemolymph containing fat body tissue. The abdomen of the insect is crushed and a drop containing haemolymph and fat is placed onto a microscope slide.
4. Correct disease identification requires an experienced insect pathologist.

General practices for growing plants and rearing moths in pools

Practices suggested here have been used by CSIRO Entomology, Australia, to rear and release *Niphograpta albiguttalis* and *Xubida infusellus*. Alternatives undoubtedly exist that may better suit particular localities or conditions. In some situations, what is possible or practical may override what is desirable. Both species have been reared using large, plastic-lined pools. These should be located within a mesh cage, shade house or glasshouse to prevent the dispersal of emerging adult moths, and to limit infestation from other insects, particularly the *Neochetina* spp. In cooler climates, growth lamps suspended over the pool will compensate for radiation losses due to shading and will promote plant growth. The techniques below, other than those relating to the moths, generally apply when growing insect-free water hyacinth plants for use in rearing.

The general methods for growing insect-free plants and rearing moths in pools are outlined in Box 1 and Table 7 and some hints on troubleshooting are given in Table 8 and Figure 8. Procedures specific to each moth species are detailed in Sections 6.2 and 6.3.

Three key aspects are fundamental to any successful rearing program.

- Elimination of disease. The rearing of both of these moth species has been problematic due to the presence of pathogens in colonies. Thorough screening and strict hygiene are required to identify infected individuals and produce a clean culture. Procedures to do this are outlined above.
- 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

Box 1. The stages in preparing and managing pools for rearing *Niphograpta albiguttalis* or *Xubida infusellus*

Photo: M. Julien

1. Requirements. Above-ground pools with plastic liners (2–3 m diameter, 60 cm deep, 3000–4000 L capacity) are easily handled and erected by two people. These allow easy access to all areas of the pool and are large enough to moderate evaporation and overheating.

Photo: A. Wright

3. Filling the pools. 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 before adding plants.

Photo: A. White

2. Setting up. Pools should be erected on a level surface close to a water supply. Ideally, rearing pools should be located in a cage to contain adult moths.

Photo: M. Julien

4. 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.

Box 1. (continued)

Photo: M. Julien

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

Photo: M. Julien

6. Removing weed contaminants. The plants can be submerged or compressed to expose contaminants which can then be skimmed off the surface with a strainer or by hand.

wells may be used. If the supply is likely to be disrupted, adequate storage facilities may be required.

- Quality of host plants. Mass-rearing of water hyacinth insects requires a reliable source of good quality water hyacinth plants. Plants are easily grown in pools located outside in warm climates or in glass or shade houses in cooler areas or during winter.
  During the rearing process, plants are frequently destroyed by the insects or removed from rearing pools to harvest insects or for field releases. To maintain plant densities, it may be necessary to restock regularly. If plants are not readily available from the field it is useful to maintain a number of additional pools stocked with water hyacinth plants which have been cleared of *Neochetina* weevils and other insects. This is especially so for rearing *N. albiguttalis* which requires the young, bulbous form of the plant.

Key points in pool establishment and maintenance

- *Regular and continued maintenance of the pools is critical to ensure healthy plant growth*
  It is easier to routinely check pools than to restore a pool that has deteriorated. Maintenance should be carried out weekly when water levels should be checked and plant and insect contaminants removed. Note that maintenance when growing small, bulbous plants for *N. albiguttalis* must be done daily or every second day to ensure that crowding does not occur.
- *Fertilise fortnightly*
  Plants require nutrients for healthy growth. To achieve suitable conditions in rearing pools, between 100 and 200 g of a soluble complete fertiliser containing nitrogen, phosphorus, potassium and trace elements is added to the pool water fortnightly. Water hyacinth requires higher levels of iron than

Table 7. Stages in setting up a pool to grow plants or to rear *Niphograpta albiguttalis* or *Xubida infusellus*

Setting up	
Requirements	▶ Pool and liner ▶ Level area slightly larger than the diameter of each pool ▶ Adequate, regular water supply ▶ Fine sand or soil for bedding under the pool liner ▶ Mesh cage to cover pool
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 manufacturer's 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.
Erect cage	▶ Erect cage, ensuring that there is room to move around the pool inside the cage. ▶ The door of the cage can be zippered, clipped or just overlapped and held closed with weights on the floor.
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 leaks and fix if necessary.
Fertilise	▶ Dilute 200 g soluble complete fertiliser in a bucket of water. ▶ Add iron supplement if available, e.g. 2 litres of chelated iron (EDTA). ▶ Add fertilisers to pool and stir through water column.
Add plants	▶ Collect field plants that are in good condition (healthy, undamaged). ▶ For *N. albiguttalis*: • collect small bulbous plants; • cover half to three-quarters of the water surface with plants. ▶ For *X. infusellus*: • collect tall, slender plants; • loosely cover the surface of the water. ▶ Remove other plants or insects. ▶ Allow the plants to settle in for at least 3–4 days before adding insects.
Inoculate with insects	▶ *N. albiguttalis*: • place larvae onto plants, place plants already containing larvae into the pool or release moths into the cage. ▶ *X. infusellus*: • place eggs or larvae on plants, place plants already containing larvae into the pool, or release moths into the cage.

Table 8. Troubleshooting while growing plants in pools or rearing insects in pools

Problem/symptom	Possible cause	Possible solution
Pool factors		
▶ pool losing water	▷ hole in pool	☐ patch hole or replace plastic liner
	▷ excessive evaporation	☐ provide shade
▶ excessive algal growth	▷ too much fertiliser	☐ reduce amount of fertiliser added
Plant factors (also see Figure 8)		
▶ 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
▶ plants dying and/or sinking	▷ 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
	▷ using the wrong plant form	☐ restock pools with bulbous plant form if rearing *N. albiguttalis*
		☐ prevent crowding by regular thinning
	▷ too hot	☐ provide shade
	▷ too cold	☐ insulate pool exterior
▶ high proportion of *N. eichhorniae* and/or *N. bruchi* in pools	▷ invasion by *Neochetina* weevils	☐ rejuvenate pools and restock with insect free plants
		☐ fix holes in cage

that provided by most complete fertilisers. The addition of an iron supplement, such as iron sulfate or iron chelate, if available, will further enhance plant growth. The amount of fertiliser required will vary according to the condition of the plants, with more required during periods of active growth. Over-fertilising should be avoided as it promotes the growth of algae.

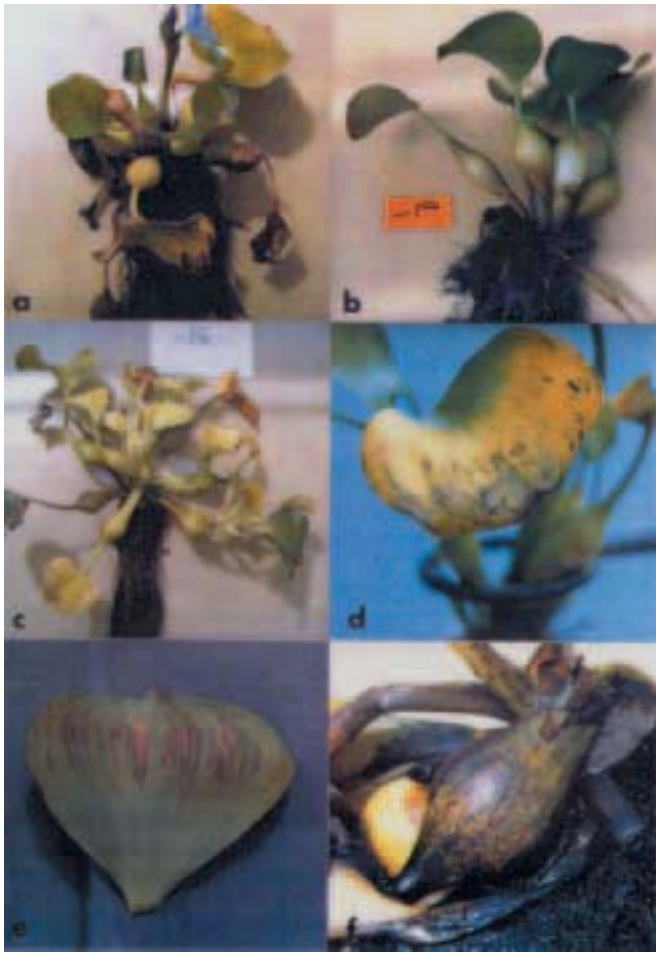
▶ *Optimise water temperature*

Growth will be optimal when the water temperature beneath the weed mat is approximately 26°C. At cooler temperatures, weed growth 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 the plants. 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. Likely contaminants are *Salvinia molesta* (salvinia or water fern), *Azolla* spp., *Lemna* and *Spirodela* spp. (duck weeds) and algae. These can be skimmed off the surface with a sieve. Larger weedy contaminants including *Utricularia* spp. (bladderwort), *Pistia stratiotes* (water lettuce) and larger *Salvinia molesta* plants require hand weeding.

Figure 8. Nutrient deficiency symptoms in water hyacinth (Newman and Haller 1988): (a) nitrogen-deficient plant showing reduced growth and leaf chlorosis; (b) phosphorus-deficient plant exhibiting shortened leaves and petioles and deep green leaf colouration; (c) typical iron deficiency symptoms including overall chlorosis and yellow striations of the leaf; (d) magnesium-deficient leaves typically first show a necrotic spot, followed by chlorosis advancing from the leaf tip toward the petiole; (e) potassium deficiency symptoms are characteristic brown bands on the distal end of the leaf; (f) calcium deficiency symptoms appear rapidly with brown spots occurring on the leaves and petioles



- *Exclude and remove animal contaminants*
  Many of the areas in which moth rearing is to be carried out will be close to areas where *Neochetina* spp. weevils have been released. Consequently, plants harvested from natural weed infestations may harbour populations of the weevils, and outdoor pools may become infested. Field-collected plants will need to be cleared of any existing insects. The use of pyrethrin sprays and the physical removal of any plants showing signs of active insect damage characteristic of other species will eliminate unwanted insects. The use of cages will reduce infestation of the pools, but if these are not available, insects can be excluded with the use of tight-fitting gauze covers placed over the pool at night. Pools require regular inspection and removal of unwanted insects or plants that have feeding marks as they are likely to have eggs. Spider mite can be controlled using non-insecticidal miticide. Predatory mites are an effective control option for spider mites. Mosquitoes will breed in pools, causing potential health problems. Insectivorous fish may be an option for mosquito control.

The aim of any mass-rearing program is to produce the maximum number of good quality insects for minimum cost, in terms of both labour and resources. Release of sufficient numbers of healthy, fecund insects, at as wide a range of locations over as many years as possible will increase the likelihood of establishment. For all biological control agents, quality (healthy, well fed, and for adults, young and fecund) should always be more important than agent quantity.

6.2. *Niphograpta albiguttalis* rearing

Receiving a newly-imported colony

Suitable plant material should be collected or grown before receiving a colony. Newly-imported colonies are likely to arrive as larvae, though some may have pupated, particularly if the colony was delayed.

If the authorities are satisfied that the insect is host specific (refer to Section 4.3 and Appendices 1 and 2) and can be released in your country and you have received a parasite- and disease-free colony, it may not be necessary to rear the insect in quarantine. However, the package should be opened in a quarantine facility or in a secure laboratory. All unhealthy insect material, all plant material and the material in which the insects were packaged should be destroyed in the quarantine or secure laboratory.

Containers should be prepared with either young laminae for young larvae or excised bulbous petioles for mid-size and larger larvae. The container-rearing techniques described below should be followed until sufficient numbers are available to begin mass-rearing using the combined pool and laboratory techniques.

If the colony received is not known to be free of parasites or diseases, larvae from different locations should have been packaged separately for transportation, and they should be transferred and maintained in separate containers inside a quarantine facility. All unhealthy insect material and all plant material imported with the colony and the packaging material must be destroyed in quarantine. Fresh, young laminae or bulbous petioles should be prepared and larvae transferred carefully to them. Sterile techniques should be used when transferring larvae from different locations to minimise the possibility of cross-contamination.

It may be necessary to follow the container-rearing techniques, described below, until sufficient numbers are available to assess presence or absence of disease. Techniques to manage a colony to determine and eliminate disease are outlined in Section 6.1.

Recommended rearing methods

The recommended method for rearing *N. albiguttalis* uses a combination of laboratory rearing and rearing in large pools. This provides maximum numbers for field release at the lowest labour cost. The pool culture provides infested plants for field release as well as adults to allow mass-rearing of larvae in the laboratory for release. Laboratory-reared larvae can be released in the field or grown to the adult stage and released into the rearing pool cage as required to maintain the pool population.

The critical issue in rearing *N. albiguttalis* is to use small plants which are healthy and rapidly growing, with tender, bulbous petioles. Such plants are best for insect performance, and are also most convenient for rearing, being easy to manage. These plants are often not consistently available from the field so it is usually necessary to grow them in pools. Plant-growing pools will provide the laminae and petioles required for laboratory rearing and the whole plants required for the rearing pool.

Maintaining both a laboratory and a pool culture also insures against the loss of the entire colony. This is especially important when first importing material to commence a release program. If one culture fails, the whole colony is not lost. The potential for failure is higher in the laboratory than in a pool, due to mishaps such as failed air conditioning, or lack of attention over weekends or holidays.

In its native range, *N. albiguttalis* is infested with a *Nosema* (microsporidian) disease. When diseased colonies are imported for use as biological control agents, it is important to eliminate such a disease before it is released into the field. The processes for eliminating disease from a culture are outlined in Section 6.1.

Growing plants to support laboratory and pool rearing

The most important aspect of rearing *N. albiguttalis* is growing suitable plants. This moth requires tender plants typical of the small bulbous form. Once plants elongate (petioles become thin and long) they generally toughen and are unsuitable for rearing this insect. However, bulbous plants grown under adverse conditions may be unsuitable, while slender plants growing under ideal conditions may be suitable.

To begin a plant culture, plants can be collected from the field as seedlings or the very young, small offshoots from larger plants, and placed into one or more pools. They will require some days to grow into bulbous plants before they can be used. Plants will remain small and bulbous so long as they are not crowded. Crowding stimulates petioles to elongate and become unsuitable for rearing. To maintain plants of the required shape, it is essential to regularly thin the plants in the pools. As the plants will grow rapidly in the uncrowded pool conditions, they will require frequent checking and regular thinning. Thinning should be carried out in anticipation of crowding, not after crowding has occurred. Each day, or at least every 2 days, plants should be checked and removed to maintain water cover of 75–90%. Colonies of ramets should be broken apart so that the offshoot plants are separated from the older plants. During thinning, the older plants should be discarded and the younger, more tender plants kept. Once petioles have elongated they cannot revert to the bulbous stage and such plants should be discarded.

These plants will provide the whole plants required for rearing the moth in pools and the laminae and petioles required for laboratory rearing. Two plant-growing pools will be required to support every moth-rearing pool and associated laboratory rearing.

It may be necessary to cage the plant-growing pools to prevent contamination by the moth or by the water hyacinth weevils, *Neochetina* species.

Rearing the moth in pools

The general methods for establishing and maintaining pools for rearing moths are outlined in Section 6.1. Details specific to rearing *N. albiguttalis* are shown in Box 2 and Table 9.

Pool rearing is the simplest method of maintaining a colony of the moth. It is essential to provide the moth with small, tender, uncrowded plants as these are most suitable for the larvae to feed and develop and for adults to lay eggs.

The rearing pool should be caged to keep the moths in and to prevent contamination by other organisms such as *Neochetina* species. The surface of the pool should be no more than three-quarters covered with bulbous water hyacinth plants and mated females or pairs of adults released into the cage to lay eggs on the plants. Alternatively, mid-sized larvae can be gently placed onto the youngest petioles of plants and allowed to tunnel in. The maintenance of this pool is the same as for other pools, as described above. It is most important to thin the plants so that they do not elongate. This means discarding some plants with eggs, larvae or pupae in them. These discarded plants can be searched and the larvae or pupae returned to the pool or they can be released in the field.

When the larvae in the plants in the rearing pool have reached mid instar, 15–18 days after oviposition, half of the plant material can be taken from the pool and released in the field. New insect-free plants are then added to the rearing pool from the plant-growing pools, taking care to maintain the rearing pool surface at less than three-quarters full of plants. The larvae that remain will develop to adults, lay eggs on the plants and begin the next cohort of larvae. New adults should emerge after about 28 days in warm conditions and later in cooler conditions. Again, when larvae are above mid-size, repeat the process and release half of the plant material.

Photo: R. Chan

The bulbous form of the water hyacinth plant required by the moth *Niphograpta albiguttalis*

Rearing the moth in the laboratory

Laboratory rearing permits large numbers of larvae to be grown for release. This technique takes advantage of the oviposition preferences of the female moth. Under field conditions, eggs are generally laid on leaf tissue in which the epidermis has been damaged either by previous feeding punctures or mechanically. This situation is replicated under laboratory conditions by making grooves in, or scraping a portion of, the surface of the leaf. The two youngest laminae of healthy plants (from the plant-growing pools) are removed and, using a narrow, blunt instrument, such as forceps, four to five grooves are made along the length of the upper surface of the lamina, running parallel to the veins. The grooves should not penetrate through to the other side of the leaf. Alternatively, up to a third of the upper epidermis can be scraped off using an instrument such as a scalpel.

Box 2. *Niphograpta albiguttalis* rearing

Pool rearing

Photo: A. Wright

1. Plants used for rearing should be small, healthy and rapidly growing, with tender, bulbous petioles.

Photo: A. Wright

2. Plants should not be crowded, and should cover no more than 75% of the water surface.

Photo: A. Wright

3. Two plant-growing pools are required to support each insect-rearing pool.

Container rearing

Photo: A. Wright

4. Plants are prepared for oviposition by making grooves on the upper surfaces of the laminae with a narrow, blunt instrument.

Photo: A. Wright

5. Grooved leaves are provided to female moths in ventilated containers with a paper towelling substrate. Eggs are maintained in the containers in which they were laid.

Photo: A. Wright

6. Young larvae can be encouraged to move to new leaves by sandwiching the damaged leaf containing feeding larvae between two new leaves.

Photo: J. Stanley

7. The sandwiched leaves with larvae should be separated every few days and new leaves provided. During this process the numbers of growing larvae per container (or per leaf) should be reduced.

Photo: A. Winotai

8. Older larvae require bulbous petioles to feed on. The petioles here show considerable damage and the larvae should be offered new petioles. This is done by placing new petioles on the older ones. The larvae will transfer themselves.

Photo: A. Wright

9. Emerging adults are sexed and each female placed with one or more males in an oviposition chamber.

Photo: A. Wright

Typical damage caused by larvae of *Niphograpta albiguttalis* in a rearing pool

Adults are collected from the laboratory culture or from the rearing pools. To collect from rearing pools, the likely time that adults will emerge is checked (28 or more days after oviposition or 7 days after pupation). Each morning and each evening, the pool is checked for moths which are then collected. Adults will sit on the underside of the laminae, so the laminae are gently brushed using hands and forearms while watching for moths. Moths are collected using a small insect net or directly into small containers. If only one sex is collected, they are held in containers until the other sex is available. Collection of adults continues over a number of days assuming that each female will provide several hundred larvae.

Grooved leaves are provided to paired moths in ventilated plastic containers with paper towelling on the base to absorb excess moisture. A plastic container 11 cm diameter × 11 cm deep can hold up to five females and five or more males, along with one or two grooved leaves per female. Ventilation can be provided by punching holes in the lid or by replacing the lid with a gauze cover. Alternatively, one female and one or more males can be held on one grooved lamina in a standard Petri dish.

As adults start to oviposit within 1 day of emergence and the majority of eggs are laid within 2 days, it is critical to pair adults and place them into oviposition chambers as soon as possible after emergence. In larger containers, females can be left until death or can be removed after 3 days and placed in a new container with freshly prepared laminae and moistened paper towelling. In Petri dishes, the moths should be moved to a new leaf each day. The adults usually live for about 5 days, so one to five transfers will be necessary depending on the size of the chambers.

Eggs are laid within the grooves of the damaged leaf tissue and are maintained in the containers in which they were laid. Larvae hatch within 3–5 days of oviposition and will start to feed on the leaf tissue, usually close to the site of oviposition. The leaf will begin to deteriorate after about 3 days and the young larvae should be encouraged to move to new leaf material. To do this, two young, undamaged laminae are collected, washed in tap water and dried with paper towel. These laminae are placed in a container with one old leaf (containing larvae) between the two new ones. Young larvae will disperse and commence feeding on the new leaves. After about 12 hours,

Photo: R. Chan

Young *Niphograpta albiguttalis* larvae are held for development on young tender leaves in chambers and new leaves are provided regularly

Table 9. *Niphograpta albiguttalis:* stages in establishing and maintaining an insect-rearing pool

Establish colony	
Add plants	▶ Collect field plants that are healthy and rapidly growing with tender, bulbous petioles. ▶ Add plants to the pool so that less than 75% of the pool surface is covered. ▶ Remove other plants or insects (if *Neochetina* spp. are present it may be necessary to spray with pyrethrin sprays. Allow 3 or 4 days before adding *Niphograpta* to sprayed plants). ▶ Allow plants to settle in for at least 3–4 days before adding insects.
Inoculate with insects	▶ Release mated females or pairs of adults into the cage. ▶ Place larvae onto the youngest petioles of plants, one larva per plant, maximum 200 larvae.
Pool maintenance	
Daily	
Check pools	▶ Remove any weeds, unwanted insects, or plants with foreign insect damage. This is particularly important if the pool is not held within a glass or shade house.
Check plant density	▶ Ensure that the plant cover does not exceed 75% of the pool area. If necessary remove plants, particularly older plants. Break up colonies of ramets to separate off-shoot plants from older plants. ▶ Removed plants can be field released, placed into a new pool or checked for the presence of larvae or pupae that can be removed and returned to the pool.
Fortnightly	
Fertilise	▶ Dilute 200 g soluble complete fertiliser in a bucket of water. ▶ Add iron supplement if available, e.g. 2 litres of chelated iron (EDTA). ▶ Add fertilisers to pool and stir through water column.
Harvesting	
15–18 days after adults present	▶ Check the size of larvae by opening a selection of petioles. ▶ When most larvae are about 3rd instar, remove half of the plant material from the pool and release in the field. ▶ Add new plants from the plant culture pool to the rearing pool to return plant density to approximately 75% cover. ▶ Remaining larvae will continue to develop through and eventually emerge as adults. ▶ Harvesting can occur again 15–18 days following emergence of adults.

any larvae that remain on the old leaf are carefully moved, using a fine brush, and the old leaf is discarded. As the larvae grow, they will need to be supplied with new leaves every 3 days. During the process of adding new leaves, some of the larvae should be moved to additional containers to reduce density. After the 9th day, when larvae have reached the late 2nd or 3rd instar and require greater quantities of food, leaf laminae will no longer be suitable and petioles should be provided.

To provide petioles, healthy plants are selected from the plant-growing pools, the young, bulbous petioles are cut from the crown, and the laminae removed. The petioles are placed in a plastic container and the laminae bearing larvae are laid over the petioles. Most of the larvae will move into the petioles over a period of 12 hours. Any remaining can be carefully moved with a fine brush. The laminae should then be discarded. The number of petioles and larvae per container will depend on container size. For example, a plastic container 26 cm long × 16 cm wide × 14 cm high can hold a single layer of about 30 petioles and initially 60 larvae. As the larvae grow

Photo: L. Hugo

Petioles selected and cut from healthy plants for use in rearing larger *Niphograpta albiguttalis* larvae

in size they will require a petiole each. After 3 days, two containers are each prepared with new petioles and half of the petioles containing larvae placed into each. After 12 hours, each of the older petioles are split open and any larvae that have not already moved to the new petioles are removed. The old petioles are discarded. The process of supplying new petioles should be repeated every 3 days until larvae have pupated inside the petioles.

Once pupation occurs, if the petioles remain in good condition they can be held until adults emerge. If the petioles deteriorate, they should be carefully opened and the pupae removed and placed onto moist paper in a container. When the adults emerge, they should be sexed and each female placed with one or more males in an oviposition chamber to mate and begin oviposition or released into the rearing pools if the populations there require a boost.

At all stages during the transfer of larvae, distribution of the frass and rotting leaf material should be avoided, as this can lead to fungal and bacterial problems in crowded rearing containers. This is difficult when the larvae are small and easier as they grow larger. Old material (laminae or petioles) should not be left in the containers once larvae have transferred to new material. Containers should be cleaned at each transfer. Larvae should be handled as little as possible and moved gently using a small, moistened soft brush. The larval integument is soft and can be easily pierced by brush hairs, leading to the death of the insect.

Larvae maintained on leaves in the laboratory can be collected in the 3rd instar for field release. In the field, young plants are selected to receive the larvae. A small section is cut out of each petiole, a small hollow made in the internal tissues, a larva placed inside and the cut piece of petiole replaced.

The Thai method of laboratory rearing

A modification to the above method was developed by Dr A. Winotai. Whole leaves (petiole and lamina) are used rather than excised laminae. This has the advantage that (compared to cut laminae) the quality of the plant material is maintained for longer, extending the time during which hatching larvae are able to feed before additional food is required. Consequently, the amount of handling required for small larvae is reduced. This method may result in lower mortality of early stages, as well as time and labour savings.

Leaves with soft, bulbous petioles — ideally the 2nd, 3rd or 4th open leaf of small plants — are selected. They are removed from the plant by cutting at the base of the petiole. The stalk close to the cut end is wrapped with a strip of foam plastic and then fitted into a small glass or plastic vial filled with water. The foam gives a firm seal to hold the petiole within the vial. Four or five grooves are made around the petiole, along the length of the petiole bulb, to encourage oviposition. These are made to a depth of approximately 1 mm, removing the epidermis and underlying tissue.

Round plastic containers measuring approximately 15 cm in diameter and 28 cm in height and having a press-fit lid are used for oviposition. Moistened tissue or paper towel is placed on the container base and the lid of each container is fitted with gauze. Five to six prepared leaves and their vials are placed into each container, before adding a pair of newly emerged adults.

The adults are left in the container until death. Females are kept alive for up to 1 week by providing a feeding station. This is a cotton plug in a glass tube filled with honey or sugar solution (approximately 10%) placed on the container floor or suspended from the lid gauze by fine wire. After 5–7 days, the leaves are removed from the vials and transferred to a plastic box with moist tissue in the base. Four or five new laminae washed in tap water are placed in the box and the young larvae transfer to these naturally. Larvae are kept in these containers for 7–10 days and additional leaves added daily or as required.

Photo: A. Wright

In the Thai rearing method, the cut end of the petiole is inserted into a glass vial and several vials are held in each container

Infested laminae are released directly into the field or larvae are transferred to whole plants for field release. To maintain the colony, some larvae are transferred to whole plants and then placed in a rearing pool where they complete development and provide adults for the next rearing session.

The South African method of rearing

This method (M. Hill, pers. comm.) is a combination of laboratory and pool rearing and has the advantage of minimal handling of the insect and therefore reduced rates of mortality.

Five male and five female pupae are collected and held until the adults emerge. The new adults are placed into a cage 30 cm × 30 cm × 30 cm, with two bulbous plants floating on water. One-third of the upper epidermis on the two youngest leaves of both plants is scraped off using a scalpel to encourage oviposition. After 2 days, the plants are checked for eggs and, if eggs are present, the plants are removed and replaced with new plants. New adults are added to the cage as required.

The plants with eggs that were taken from the oviposition cage are held in an aquarium 40 cm × 40 cm × 30 cm until the eggs darken, i.e. until the head capsules of the larvae are visible. Five or six new bulbous plants are then placed around the plant that contains the eggs and all the plants are held in contact with each other with a piece of string. These clumps of plants are placed either in an outside pool or in an aquarium in the laboratory. The eggs hatch and the larvae move onto the plants and begin development. After a week, each clump of plants is split and each plant is surrounded with five or six new plants to form five or six new clumps. To provide pupae for the rearing process described here, the splitting and re-clumping is repeated two more times to allow larvae time to pupate. Then pupae are dissected from the plants and held to supply new adults for addition to the oviposition cage.

To provide material for field release, the process of splitting and re-clumping is repeated once only so that plants contain mature larvae. The clumps of plants are then placed in the field.

Harvesting for field release

N. albiguttalis is usually collected as eggs, larvae or pupae for release. Adults are fragile, short-lived and do not transport readily.

Releasing eggs can be done by allowing females to lay eggs on whole plants on which a third of the upper epidermis on the two youngest leaves has been scraped off. Once the eggs darken, pieces of leaf with eggs are cut off and placed inside the young curled leaf at the centre of a fresh bulbous plant and the plant is placed in the field (M. Hill and C. Cilliers, pers. comm.).

To release larvae, half of the plants in the rearing pools can be collected and taken into the field when the larvae they contain are half-grown (see above, *Rearing the moth in pools*). Similarly, clumps of plants infested with mature larvae, using the South African rearing method (described above), can be placed amongst bulbous plants in the field. These methods do not allow accurate counts of the larvae released to be made.

Larvae reared in the laboratory can be released directly into the field by carefully placing the larvae onto selected plants that have small bulbous petioles — preferably placing a larva into the curled leaf at the centre of each plant, or by inserting each larva into a bulbous petiole. To do this, a plant is selected, a small section of the youngest petiole is removed (say 1 cm^2) and a small portion of the petiole's internal tissues hollowed out, then a larva is gently inserted and the removed piece replaced. The plants are then placed into the field. These methods permit accurate counts of larvae placed in the field but mortality rates are likely to be higher due to handling.

Larvae reared in the laboratory can also be transferred to new bulbous plants in the laboratory or in pools, left for a few days, and the whole plants taken to the field. This allows the intense labour of transferring larvae to be done in the laboratory rather than in the field. It does, however, require large numbers of plants.

6.3. *Xubida infusellus* rearing

Receiving a newly-imported colony

Suitable plant material should be collected or grown before receiving a colony. Newly-imported colonies are likely to arrive as larvae, though some may have pupated, particularly if the colony was delayed.

If the authorities are satisfied that the insect is host specific (refer to Section 5.3 and Appendices 3 and 4) and can be released in your country and you have received a parasite- and disease-free colony, it may not be necessary to rear the insect in quarantine. However, the package should be opened in a quarantine facility or secure laboratory and all unhealthy insect material, all plant material and the packaging destroyed in the quarantine facility or secure laboratory.

Containers should be prepared with healthy, whole water hyacinth plants with slender petioles floating on water in dishes. The larvae are transferred carefully to the plants, no more than two larvae per plant. Once pupation occurs, pupae are sexed and held until adults emerge and can be paired and placed into oviposition containers (see below). Eggs are placed on plants held in rearing pools. Thereafter, the combined pool and container mass-rearing techniques described below should be followed.

If the colony received is not known to be free of parasites or diseases, larvae from different locations should have been packaged separately

for transportation, and they should be transferred and maintained in separate containers, inside a quarantine facility. All unhealthy insect material and all plant material imported with the colony and the packaging material must be destroyed in quarantine. Fresh plants with slender petioles should be prepared and larvae transferred carefully to them. Plants with larvae from the same location should be held away from other plants and larvae. Sterile techniques should be used when transferring larvae from different locations to minimise the possibility of cross-contamination.

It may be necessary to follow the container-rearing techniques, in the quarantine or secure laboratory, using either whole plants or excised petioles, until sufficient numbers are available to assess the presence or absence of disease. For this insect, such techniques are very time-consuming and require large numbers of plants. Techniques to manage a colony to determine and eliminate disease are outlined in Section 6.1.

Recommended rearing methods

The development of container-rearing techniques for *X. infusellus*, necessary for rearing in the confined space of quarantine, proved difficult and required a very large manpower commitment. Although modifications were made for mass-rearing, the requirement for a large supply of cut plants remained and frequent transfer of larvae meant that the labour requirement remained high. The method generally resulted in high mortalities, especially during the early instars (Julien and Stanley 1999).

Eventually a method was developed combining container rearing with rearing in larger pools. This permits larval development to occur on water hyacinth in pools. Pupae or adults are collected from the pool so that oviposition is managed in containers in the laboratory. This method eliminates the need for frequent transfers of larvae and dramatically reduces labour and consumable requirements. Once established, the rearing process can be maintained by one person working 1–2 hours daily (Julien and Stanley 1999), ensuring the continuous production of eggs or larvae for field release. Another advantage is that pool rearing allows the provision of better quality plant material for larval development and this results in larger insects than the container method. Therefore, the combined pool and container rearing method is recommended and described below.

In its native range, this moth can be heavily infected with a microsporidian disease (*Nosema* sp.). It is important that when biological control agents are released in a new country they are free of parasites and diseases. Procedures to eliminate disease from a culture of *X. infusellus* are outlined in Section 6.1.

Pool rearing

General methods for establishing and maintaining pools for rearing *X. infusellus* are outlined in Section 6.1. Details specific to *X. infusellus* are shown in Box 3a,b and Table 10.

Eggs or early instar larvae, obtained from the laboratory colony, are placed on water hyacinth plants in pools and allowed to develop through to the pupal stage. Petioles should be inspected daily for emergence windows, indicating that larvae have pupated. These are easily seen on good quality plants.

When emergence windows are observed, whole plants or single petioles can be removed and dissected. Pupae can be removed, sexed according to the diagrams shown in Figure 6, and transferred to smaller containers. As most pupae are removed before emergence, the process is not self-sustaining and pools will need to be re-inoculated with egg batches or larvae at intervals of about 6 weeks. Returning eggs to the pools also reduces the likelihood of causing a

Table 10. *Xubida infusellus:* stages in establishing and maintaining an insect-rearing pool

Establish colony	
Add plants	▶ Collect healthy and undamaged field plants. ▶ Loosely cover the pool surface with plants. ▶ Remove other plants or insects (if *Neochetina* spp. are present it may be necessary to spray with pyrethrin sprays. Allow 3 or 4 days before adding *Xubida* to sprayed plants). ▶ Allow plants to settle in for at least 3–4 days before adding insects.
Inoculate with insects	▶ Add approximately 2000 eggs from laboratory cultures. ▶ Egg batches on gauze can be stapled or pinned to the leaf surface. ▶ Distribute eggs evenly throughout pool. ▶ Alternatively, release moths into the cage.
Pool maintenance	
Daily	
Check pools	▶ Remove any weeds, unwanted insects, or plants with foreign insect damage. This is particularly important if the pool is not held within a glass or shade house.
Check plants	▶ Check water hyacinth petioles for pupal emergence windows, indicating time to harvest.
Fortnightly	
Fertilise	▶ Dilute 200 g soluble complete fertiliser in a bucket of water. ▶ Add iron supplement if available, e.g. 2 litres of chelated iron (EDTA). ▶ Add fertilisers to pool and stir through water column.
Check plant density	▶ If water is visible between the plants, add more plants from plant-growing pools.
Six weekly	
Reinoculate with insects	▶ Add approximately 2000 eggs from laboratory cultures. ▶ Egg batches on gauze can be stapled or pinned to the leaf surface. ▶ Distribute eggs evenly throughout pool.
Harvesting	
When emergence windows are visible	▶ Remove whole plants or petioles, dissect and remove pupae.

genetic bottleneck. Bottlenecks have the potential to reduce the quality of cultures though the accidental selection of poor genetic stock as might occur if only very low numbers of insects are relied on to sustain the pool cultures.

Container rearing

The pupae collected from the rearing pools can be placed into a clean container or plastic tub of approximately 10 cm high and 10 cm diameter (850 mL capacity) with a tightly fitting lid. To maintain high humidity, the container should have dampened filter paper in the base, and need not be provided with ventilation.

Pupae can be maintained in groups of up to 10 of mixed sexes. The pupal period is approximately 8 days at 25°C. Because the actual time of pupation will not be known, only the time at which pupae were removed from the pool, it is necessary to check for adult emergence regularly. The first adults to emerge are likely to be males as they have a shorter development time than females. Although the emergence of the sexes will eventually overlap, the discrepancy in emergence times can lead to problems of insufficient adults of both sexes at the same time. Cooling the early pupating males at 22°C for several days will help synchronise emergence. Some idea of the age of pupae can be gained from their colour, with pupae darkening as they approach emergence. This can be used as a rough guide for placing similarly staged pupae together for emergence.

Box 3a. *Xubida infusellus* pool rearing

Photo: A. White

1. Plants with slender petioles should cover the pool surface so that no water is visible.

Photo: A. White

2. After larvae are placed on the plants and allowed to develop, plants should be inspected daily for emergence windows.

Photo: A. White

3. The presence of round emergence windows in the leaf petiole indicates that the larvae have pupated. (A hole in the window suggests that an adult has exited from the stem.)

Photo: A. White

4. Pupae can be removed by carefully splitting the plant along the petiole and through the roots to reveal the pupa within its tunnel.

Box 3b. *Xubida infusellus* container rearing

Photo: J. Stanley

1. Pupae are placed into clean, plastic containers, each with a tightly fitting lid and dampened filter paper in the base. Adults are collected and paired as they emerge.

Photo: J. Stanley

2. Paired adults are placed into oviposition chambers. Oviposition substrates are prepared by folding a 2 cm tuck into a strip of gauze that is stretched across the top of the oviposition container.

Photo: A. White

3. A small section of water hyacinth leaf placed in the fold of the gauze may encourage oviposition.

Photo: A. White

4. Eggs are laid in long lines onto the gauze substrate.

Photo: A. White

5. Strips of gauze containing lines of eggs are cut away and held in smaller containers.

Photo: A. White

6. Just before hatching, the strips of gauze with eggs are transferred to chambers with cut sections of petiole. Hatching larvae burrow into the petiole sections. The very small larvae tend to move upwards and may escape if lids are not well sealed. To prevent escapes, the chambers are inverted.

On emergence, a pair of moths is collected and placed into an oviposition container. This container is the same as that used for emergence but incorporating a fine gauze (mesh cloth) oviposition substrate stretched across the entire top of the container before the lid is fitted. The gauze is cut to a square approximately 20 cm × 20 cm and prepared for oviposition by folding a 2 cm tuck across the middle. The tuck in the gauze simulates the sort of substrate that the females seek for their eggs. The gauze is then placed over the entire opening of the container and pulled taut as the lid is replaced. A small section of water hyacinth lamina (approximately 5 cm^2) placed between the lid and gauze may encourage oviposition. The filter paper base should be kept moist with a light spray of water, as required, and replaced if it becomes obviously dirty.

Most females will mate on the first night following emergence, while males continue to mate for up to three nights. Within the oviposition containers, females will survive for an average of four nights and up to seven nights and lay about 200 eggs. Oviposition begins on the night following mating and continues nightly, declining over time. Eggs are yellow and laid in a mass consisting of several neat rows within a white foamy substance. Egg masses are 0.3–6.0 cm long, incorporating between 10 and 300 eggs, and are attached to folds and creases in the gauze. The gauze, with eggs attached, is removed daily and replaced with new gauze.

The gauze should not be unfolded as this can damage the eggs, but a small strip of gauze containing the eggs can be cut away. The strips of gauze are placed into a sealed container with moistened filter paper. The eggs will hatch in 6–7 days at about 25°C and will darken just before hatching. When hatching is anticipated, gauze with egg batches still attached can be stapled or pinned to the leaves of plants within the rearing pool.

Rearing for field release

Field releases can be made of eggs collected during container rearing, of pupae harvested directly from the rearing pools, or of larvae reared in petioles.

Before larvae can be released they should be established in cut sections of petioles that can be pinned to field plants. To do this, about ten sections of slender petioles, 5–8 cm in length, are placed upright into a container similar to those used in rearing. A piece of gauze with eggs attached or about 15 newly-hatched larvae are introduced to the container and the container is sealed. Neonates will move upwards, so if the container seal is not complete it may be wise to carefully invert the containers. Larvae will enter the petioles and begin feeding. Once within the petiole the larvae can be transferred to the field for release.

Older larvae can also be transported and released within cut sections of petiole. Each larva will require three to four sections of petiole, each 7–10 cm long, for its development. Because of their size, older larvae that have exited their cut petioles can be easily recovered. Their containers should be well ventilated during transit but with care taken not to let the larvae escape.

If necessary, storage can be prolonged by holding the larvae in petioles at 12–15°C. At these cooler temperatures, development is slowed, reducing the amount of frass and decaying plant material produced, and preventing fungal build-up. During storage, regular checks should be made and insects transferred to clean containers with fresh plant material when necessary.

Chapter 7
Release and Evaluation

7.1 General

Transport and release

The following points are relevant to the transport and release of *Niphograpta albiguttalis* and *Xubida infusellus*. More specific details are given for each species separately in Sections 7.2 and 7.3.

Transport

Insects should be transported and released as soon as possible after collecting and before the plant material deteriorates. It is very important to prevent overheating of the transportation containers. Holding them in direct sunlight should be avoided. Container lids should be taped to prevent accidental opening or escape of tiny larvae during transit.

Size of release population

For insects with limited dispersal abilities, for example, the *Neochetina* weevils, control is reached earlier if the insects are released over a large number of sites. The number of insects released at any site should exceed that necessary to achieve a self-perpetuating population and subsequent population expansion should provide the numbers required for control at each site. Unless repeated failures to establish occur, the size of the release population at any site is unlikely to reduce the time between release and control. For other agents that disperse readily, such as the moths, the number released at any site is far more important. The immediate decline in the population density as they disperse following release may be enough to cause the population to founder. However, the number of sites at which these insects are released can be relatively low, as natural dispersal may initiate control at new sites.

Selection of release site

Releases are likely to lead to establishment and 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 events flush the weed out to sea. Agents should be released at source infestations as high up the catchment as possible. In general, the release of biological control agents should be made away from locations where herbicidal or physical controls may occur. Such control measures generally prevent establishment or reduce the impact of control agents.

Timing of release

It is sensible to make field releases in the cool of the evening, particularly if larvae are being released by placement onto field plants. Hot, sunny conditions may lead to increased mortality through desiccation as the larvae transfer to the field plants.

Record keeping

Records should be kept of the estimated number and stages of insects released. An example of a consignment sheet to accompany a shipment of insects being released is shown in Box 4.

Transport over long distances

For transport over long distances or between countries, containers should be loosely packed in a strong, sealed box that will protect the insects from heat. The container 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.

Post-release 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 predictions to be made of the potential effectiveness of the agent if introduced to other regions. In addition, information on the interactions between agent and weed 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 level; and
- the progressive impact on the weed infestation and the eventual level of control achieved.

7.2 *Niphograpta albiguttalis*

Transport and release

N. albiguttalis is usually released in the larval or pupal stage but has been released in the egg and adult stage. Establishment has occurred following release of each of the different stages. In some areas, open bamboo enclosures have been constructed on the water surface to assist monitoring. Floating cages erected over water hyacinth plants have been used to concentrate emerging adults at the release site in an effort to assist establishment.

When releasing *N. albiguttalis*, the condition of the water hyacinth plants is critical. No amount of effort will establish insects on unsuitable plants (Center 1984). The most suitable plants are softer plants, either young, vigorously-growing plants with bulbous petioles (the same sort of plant required for rearing) or on particularly lush plants with slender petioles (Bennett and Zwölfer 1968; Harley 1990; Wright 1996). Plants with bulbous petioles are typical of rapidly expanding infestations or plants growing at the open water margins of dense infestations (see Section 2.1).

Young larvae are unable to commence feeding and survival is poor on leaves with a hard cuticle (Wright and Bourne 1986). Cuticle hardness is influenced by a number of factors, including the following.

- *Physiological leaf age*. Center and Durden (1981) found *N. albiguttalis* established readily where plants appeared healthy and robust but failed to establish on less vigorous plants. They speculated that the age of the plant tissue was more important than petiole form in influencing establishment.
- *Application of herbicides* (Wright and Bourne 1986, 1990). Rapid growth of moth populations has often occurred following

Box 4. 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)

.....

Photo: W. Orapa

The most suitable release sites for *Niphograpta albiguttalis* include small plants growing at open water margins of infestations

Photo: M. Julien

Bamboo or timber enclosures on the water surface hold small bulbous plants favoured by the moth at a release site. Releasing into floating cages may help concentrate insects at the release site

treatment of water hyacinth infestations with the herbicide 2,4–D (Wright 1984). Experiments revealed that the application of the herbicide softens leaf petioles, which may allow more young larvae to enter the plant (Wright and Bourne 1990). It may be possible to manipulate plant quality through the judicious application of low levels of herbicide to improve the establishment and impact of the insects (Wright and Bourne 1990).

- *Season*. In some cooler areas, cuticle hardness of water hyacinth may be greater in winter than in summer and thus leaf penetration may be expected to be poorer during this time. This may restrict development of populations in the cooler periods.

Plant condition is not only critical to the success of the feeding larvae, but also to subsequent increases in population. Ovipositing moths have been shown to discriminate between different growth forms of water hyacinth (Center 1984; Wright 1984). Adults disperse from less suitable sites and concentrate in areas where plant growth is more favourable. If the release site is of poor quality, dispersal of adults will prevent population numbers building up sufficiently to ensure establishment.

The rapid establishment and ready dispersal of *N. albiguttalis* reduces the need for restocking or field redistribution of infested material (Center 1984; Wright 1996).

Post-release evaluation

Close observation is required to monitor the establishment and spread of *N. albiguttalis* and assess its effectiveness as a control agent. Adults may disperse and select oviposition sites away from the release points so wide searches may be required during monitoring (see Center 1984). The use of cages in which to release individuals theoretically enables easier monitoring of the success of establishment. However, this moth failed to establish in Papua New Guinea, either inside or out of cages (M. Julien and W. Orapa, unpublished observations).

Attack by *N. albiguttalis* can be heavy but sporadic. An awareness of the presence and requirements of the insects during the post-release management of sites is important to prevent the elimination of viable populations. Management practices that cause a sudden

and dramatic decline in plant populations, particularly the smaller, tender plants that grow along the edges of mats, may also result in the loss of the insects' food supply and consequently the insects themselves from an area.

The impact of *N. albiguttalis* on water hyacinth is often not readily apparent because the effect is to reduce the rate of growth and expansion of water hyacinth populations. In Australia, impact was observed at most sites within 5 years of release (Wright 1984) but has not been quantified.

Center (1987) evaluated the effects of *N. albiguttalis* on water hyacinth at one site over 1 year in Florida, USA, using the following methods. Individual ramets were tagged and, at regular intervals, plant size, condition of the plants and damage caused by the moth and by *Neochetina* weevils was assessed. Other samples of mats were collected and the number of ramets per unit area was counted and damage assessed. Four ramets from each sample were randomly selected for detailed examination of injury or disease, and the cause diagnosed.

With these measurements, Center (1987) determined the following. Leaf condition declined in response to increasing damage by *N. albiguttalis*. However, as the abundance of the moth declined, new apical growth displaced older damaged growth and plant condition improved. For plants tagged in December (winter), the moth killed 31% of ramets but only when insect abundance was high in spring. Of the remaining ramets, the *Neochetina* weevils killed 43% over a prolonged period and 26% survived the 12-month study. None of the plants that were tagged later in the study were killed by the moth. The percentages of leaves and ramets damaged by *N. albiguttalis* were highest in spring and very low or zero at other times. This correlated with declined abundance which was associated with a change in the morphology of the plants, i.e. from the short, bulbous form to the taller, elongated form.

The general conclusion is that *N. albiguttalis* is limited by preferred plant condition (Center 1987; A. Wright, pers. comm.) and possibly by damage caused by other herbivores (Wright 1984). In most situations, this insect will not be able to bring water hyacinth under control but could complement the action of other biological control agents and other control methods.

Photo: M. Julien

Release cages may assist monitoring of post-release insect development and oviposition

7.3 *Xubida infusellus*

Transport and release

X. infusellus has been released in the egg, larval and pupal stages (Julien and Stanley 1999).

- Eggs are placed in the field still attached to the gauze strips on which they were laid in the oviposition containers. The gauze can be stapled or pinned to leaves and petioles. The water hyacinth lamina can be folded over the egg mass to provide protection from desiccation and predators. Eggs should be located so that hatchlings can move directly onto the plant or can lower themselves on silken threads to water hyacinth plant material below.

Photo: M. Julien

Releasing *Xubida infusellus* into large field cages limits the dispersal of emerging adults

- Larvae can be released immediately after hatching, as neonates still on the gauze where the eggs were laid — the gauze being attached to water hyacinth as for the eggs

Photos: A. White

Pieces of petiole containing larvae can be pinned (top) or rolled and pinned to laminae (bottom)

(above). Larvae can also be released in cut petioles. Establishing them in petiole pieces is described in Section 6.3. The sections of petiole can be attached directly to petioles of field plants using a pin, or rolled within a water hyacinth leaf and pinned.

- Pupae can be placed in small pupation boxes that allow the emerging adults to escape but exclude rain and protect the pupae from direct sunlight. These boxes can be hung in trees over water hyacinth mats. It is important that both male and female pupae emerge together, so synchronising emergence by slowing the development of male pupae may be necessary (see Section 6.3).

In view of the delayed emergence of female moths compared with male moths, it is important to continue releasing insects at each site over a long enough period to ensure that males and females coincide. This will improve the likelihood that males and females will emerge at the same time and successfully mate. Large field cages have been used to limit the dispersal of emerging adults in order to maximise the chances of mating and establishment.

The condition of the water hyacinth plants where the insects are released is important. Cage experiments have shown that plant nutrient levels can have a significant impact on development rates and fecundity of insects (J. Stanley and M. Julien, unpublished data). Consequently, sites with higher nutrient plants are likely to provide better opportunities for establishment and performance of the agent.

Post-release evaluation

Three methods have been developed to assess the establishment of *X. infusellus*:

- Visual assessment — scanning the release site and surrounding area for characteristic damage followed by destructive sampling of

Photo: J. Stanley

The release area can be assessed visually for characteristic *Xubida infusellus* damage

damaged plants for larvae and pupae. The damage sought is girdling of petioles at about mid-height with the portion of petiole above wilted, yellow or missing.

A set time for such searches can be used. This method of assessment can verify whether eggs and larvae released into a water hyacinth infestation have survived and successfully developed into adults but does not provide reliable estimates of population density. This method has proved ineffective in localities infested with *Neochetina* weevils to such an extent that the characteristic 'ring-barking' damage could not be found during general scanning.

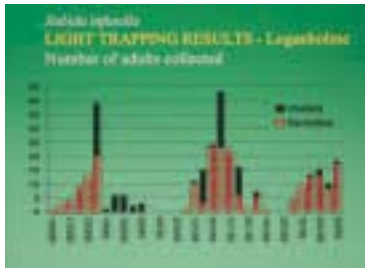
- Light trapping. A halogen, mercury vapour, ultraviolet or incandescent light set in front of a white sheet attracts adults at dusk. The trapping of adults at lights over an extended period in Australia indicated that the insect was established. Catches were large enough to indicate fluctuations in populations. Live male and female moths can be collected from the sheet into small vials to provide adults for rearing purposes. Virtually all females collected at lights have already mated.

Photo: J. Stanley

A light trap set in front of a white sheet attracts *Xubida infusellus* adults at dusk

Photo: J. Stanley

Moths are collected from the sheet into small containers



Light trap data can indicate establishment of populations and changes in abundance over time (data: J. Stanley and M. Julien, unpublished)

Photo: M. Julien

Delta traps can be constructed from available materials

Photo: M. Julien

A Delta trap with the pheromone bait hanging from the apex and 'stickem' (a sticky paste that traps visiting male moths) on the floor

Photo: M. Julien

A Delta trap placed in the canopy of water hyacinth by tying it to petioles

- Pheromone trapping. The components of the *X. infusellus* female pheromone have been identified (Stanley et al. 2000) and an artificial attractant developed. This pheromone blend is used as bait within Delta traps set amongst the water hyacinth canopy. These traps can be easily constructed from readily available materials. Pheromone traps allow sampling of male moths over large areas and for extended periods. The current pheromone blend can indicate the presence or absence of the insect in an area, but catches are very small relative to light trapping and so give little indication of population size. The trapping of adult males at a site several months after the release of insects indicates that breeding has occurred in the field. Trap catches 1 year after release suggest that the insect has established in the area. Although the artificial pheromone is not available commercially, its components are. These components and their ratios are given in Stanley et al. (2000).

Chapter 8
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 use of the water body.

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.

The importance of the *Neochetina* weevils, *N. eichhorniae* and *N. bruchi*, for water hyacinth control is already known (Julien et al. 1999). Control of large monospecific mats can be achieved in 2–6 years, the variation being due largely to the nutrient status of the weed and hydrological factors. However, the *Neochetina* weevils are not suitable for all infestations of the weed. They are less effective in cooler areas, and in swamps, marshlands and other shallow waters. It is thought that additional agents, such as the moths described here, might complement the actions of the weevils and provide accelerated or better control in some situations.

The limitations to biological control mean that 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 may not be required. 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.

There is limited understanding of the interactions between the different biological control agents. Heard and Winterton (2000) demonstrated the different requirements and

hence potential for the damage caused by the two *Neochetina* weevils to be complementary. However, relationships with the moths are less clear. *N. albiguttalis* laid more eggs on water hyacinth plants where the leaf surface was damaged by *N. eichhorniae* (DeLoach and Cordo 1978) but, according to Wright (1984) and Wright and Bourne (1986), *N. albiguttalis* is often less abundant in areas where there are large populations of *N. eichhorniae*.

In Australia, *N. albiguttalis* was released over the same range as the two *Neochetina* species and now often occurs in areas where one or both *Neochetina* species are present (Wright 1981, 1996). Water hyacinth plants may be attacked simultaneously by both weevils and this moth. Although it has been suggested that there may be a synergistic relationship between *N. eichhorniae* and *N. albiguttalis* (DeLoach and Cordo 1978), others have found no evidence to support this (Wright 1981), and Wright (1984) suggests that heavy attack by *N. eichhorniae* may limit attack by *N. albiguttalis*. Both *N. eichhorniae* and *N. albiguttalis* prefer lush water hyacinth (Wright 1984). *N. eichhorniae* appears more effective in northern Australia than in cooler, southern regions whereas damage by *N. albiguttalis* is higher during the cooler months (Wright 1984). Seasonal fluctuations occur in both the plant and insect populations. Typically, the water hyacinth grows rapidly from spring until mid-summer, when insect populations build and reduce the mass of water hyacinth from mid-summer onwards.

The presence of a number of biological control agents in the same area can make it difficult to quantify the impact of each. For example, although *N. albiguttalis* has been established in the White Nile since 1980, its impact on water hyacinth remains unknown. *N. eichhorniae*, which was present for a long time on its own, is believed to have been responsible for the reduction in water hyacinth in the Sudan (Beshir and Bennett 1985).

X. infusellus is established at a subtropical site in Australia where winter temperatures approach zero and where *Neochetina* weevils cause heavy damage in summer. In addition, the site has been regularly subjected to earth works to improve drainage and application of herbicides to reduce the weed. Despite these activities, the population of the moth has prevailed. In tropical Papua New Guinea, *X. infusellus* is established on the remaining mat of water hyacinth after both *Neochetina* species successfully reduced its size from 70% cover of a eutrophic lake to about 20% cover (M. Julien and W. Orapa, unpublished data).

Chapter 9 Summary

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. However, because of the known limitations of the weevils, additional agents can be used to improve control. Because of its known safety and its potential to reduce the spread of water hyacinth, we recommend that the moth *N. albiguttalis* should be the third insect considered for release.

N. albiguttalis is unlikely to provide overall control of water hyacinth in the same way that the *Neochetina* weevils have. This insect has specific host-plant requirements, preferring young, tender plants. In the field, it reduces the occurrence of the small, fast-growing form of the weed. Thus it reduces the colonisation rate of water hyacinth across the water's surface. It also reduces the downstream spread of the weed because it is usually the small plants at the edge of mats that are washed away. The moth also attacks the fast-growing young plants following germination of seeds. Overall, the damage caused by *N. albiguttalis* complements other controls that target the larger, tougher form of the weed comprising most mats of water hyacinth.

In contrast, *X. infusellus* larvae thrive on tall water hyacinth plants that make up the stable mats of this weed. The larvae of this moth are large, tunnel in the lower petioles and in the crown and cause a great deal of damage. It was considered by DeLoach (1975) to be the most damaging of the insects found attacking the weed in its native range. The damage by this moth will complement damage by the other control agents if it has a slightly different climate and habitat preference. For example, if it can tolerate cooler conditions than the water hyacinth weevils or can build high populations in shallow and swampy areas.

The impact of *X. infusellus* remains to be seen. It has proven a difficult insect to establish and despite many and varied releases in Papua New Guinea and Australia is known to be established at one site only in each country. This should not preclude the use of *X. infusellus* in other countries. It is often the case that control agents do well in some countries and not in others.

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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.

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 of a single plant.

epidermis
Outermost layer of cells in a plant or animal.

eradicate
Remove or destroy completely.

eutrophic
Rich in nutrients.

herbaceous
Non-woody seed-bearing plant.

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.

morphology
The form or appearance of an organism.

necrosis
Death of a piece of tissue.

neonate
A newly-hatched larva.

nocturnal
Active by night.

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

perennial
Persisting for a number of years.

petiole
Stalk joining a lamina (leaf blade) to a 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.

spike
An unbranched inflorescence of sessile (without a stalk) flowers.

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 *Niphograpta albiguttalis*, 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 — no choice; 3. Oviposition — unknown design; 4. Placement of eggs. 5. Placement of neonates — multiple choice; 6. Placement of neonates — no choice; 7. Larval feeding — details unknown; 8. Unknown design.

Alismataceae

Echinodorus grandiflorus Mich. [burhead]
- Argentina (1)

Limnocharis flava Buch. [limnocharis]
- Malaysia (7)
- Thailand (7)

Sagittaria graminea Michx. [sagittaria]
- Australia (1)

Sagittaria lancifolia L.
- USA (Florida) (6)

Sagittaria montevidensis Cham. & Schltdl. [arrowhead]
- Argentina (1,4,6)

Amaranthaceae

Alternanthera philoxeroides (Martius) Griseb. [alligator weed]
- Argentina (1,4,6)
- Thailand (7)

Anacardiaceae

Mangifera indica L. [mango]
- Malaysia (7)
- Thailand (7)
- Vietnam (7)

Annonaceae

Annona squamosa L. [custard apple, sugar apple, sweet sop]
- Thailand (7)

Apiaceae

Eryngium eburneum Decne
- Argentina (1)

Hydrocotyle ranunculoides L.f. [water pennywort]
- Argentina (1,4,6)

Apocynaceae

Nerium oleander L. [oleander]
- Thailand (7)

Araceae

Colocasia esculenta (L.) Schott. [taro]
- India (2,6)
- USA (Florida) (6)

Colocasia sp.
- Malaysia (7)

Pistia stratiotes L. [water lettuce]
- Argentina (1,4,6)
- Thailand (7)
- Vietnam (7)

Zantedeschia aethiopica (L.) Spreng. [calla]
- Argentina (1)

Arecaceae

Cocos nucifera L. [coconut]
- Malaysia (7)
- Thailand (7)

Asteraceae

Chrysanthemum indicum L. [garden chrysanthemum]
- Thailand (7)

Helianthus annuus L. [sunflower]
- Argentina (1)
- Australia (1)

Lactuca sativa L. [lettuce]
Argentina (1,4,6)
Australia (1)
Vietnam (7)

Azollaceae

Azolla pinnata R. Br.
[ferny azolla, green azolla]
Thailand (7)

Bombacaceae

Durio zibethinus Murray [durian]
Malaysia (7)

Brassicaceae

Brassica caulorapa (DC.) Pasq.
[kohlrabi]
India (2)
Brassica chinensis L.
[Chinese white cabbage]
Thailand (7)
Vietnam (7)
Brassica oleracea var. *capitata* (L.) Alef. [cabbage]
Argentina (1,4,6a)
Vietnam (7)
Brassica oleracea L. [Chinese kale]
Australia (1)
India (2)
Vietnam (7)
Rorippa nasturtium-aquaticum (L.) Hayek = *Nasturtium officinale* L. [watercress]
Australia (1)
Malaysia (7)
USA (Florida) (4,6)

Bromeliaceae

Ananas comosus (L.) Merr. [pineapple]
Argentina (1)

Cannaceae

Canna indica L. [canna, Indian shoot]
Thailand (7)
Canna orientalis Rosc.
India (2,6)

Caricaceae

Carica papaya L. [papaya, pawpaw]
Malaysia (7)
Thailand (7)
Vietnam (7)

Chenopodiaceae

Atriplex nummularia Lindley [old man saltbush]
Australia (1)
Beta vulgaris L. [beetroot]
Argentina (1,4,6)
Australia (1)

Commelinaceae

Commelina benghalensis L. [day flower]
India (2)
Commelina coelestis Willdenow = *C. tuberosa*
Argentina (1)
Commelina virginica L. [day flower]
Argentina (1)
Tradescantia crassifolia Cavanilles [spiderwort]
Argentina (1)
Tripogandra elongata (G.F.W. Mey) Woodson
Argentina (1)
Zebrina pendula Schnizlein [wandering Jew]
Argentina (1,6)

Convolvulaceae

Ipomea aquatica Forsk. [potato vine, morning glory]
Malaysia (7)
Thailand (7)
Vietnam (7)
Ipomea batatas (L.) Lam. [sweet potato]
Argentina (1)
Australia (1)
Malaysia (7)
Vietnam (7)

Cucurbitaceae

Cucumis melo L. [musk melon]
Argentina (1)
Cucumis sativus L. [cucumber]
Thailand (7)
Cucurbita maxima Duchesne ex Lam. [pumpkin]
Australia (1)
India (2,6)
Vietnam (7)
Cucurbita pepo L. s.lat. [marrow]
Argentina (1,4)

Cyperaceae

Eleocharis haumaniana Barros
Argentina (1)
Scirpus californicus (C.A. Mey.) Steud.
Argentina (1)
Scirpus sp.
USA (Florida) (6)

Euphorbiaceae

Hevea brasiliensis Muell. Arg. [rubber]
Malaysia (7)
Phyllanthus acidus Skeels [star gooseberry]
Thailand (7)
Sauropus androgynus L.
Vietnam (7)

Fabaceae

Glycine max (L.) Merr. [soybean]
Argentina (1)
Thailand (7)
Vietnam (7)
Medicago sativa L. [lucerne]
Argentina (1)
Australia (1)
Pachyrhizus erosus L. [yam bean]
Vietnam (7)
Phaseolus vulgaris L. [bean]
Thailand (7)
Trifolium subterraneum L. [subterranean clover]
Australia (1)

Haloragaceae

Myriophyllum brasiliense Cambess [parrot feather]
Argentina (1)

Hydrocharitaceae

Limnobium stoloniferum (G.F.W. Meyer) Grisb. [frogbit]
Argentina (1,4,6)

Juncaceae

Juncus sp.
USA (Florida) (6)

Lamiaceae

Mentha cordifolia Opiz [kitchen mint]
Thailand (7)

Lemnaceae

Lemna trisulca L. [narrow-leaved duck weed]
Thailand (7)

Liliaceae

Agapanthus africanus Lam. [African lily]
Argentina (1)
Allium ascalonicum L. [shallot]
Thailand (7)
Allium cepa L. [onion]
Argentina (1)
Australia (1)
India (2,6)
Asparagus officinalis L. [asparagus]
Argentina (1)
Australia (1)
Crinum asiaticum L. [giant lily]
Thailand (7)

Malvaceae

Gossypium hirsutum L. [cotton]
Argentina (1)
Gossypium sp. [cotton]
Australia (1)
Thailand (7)

Marantaceae

Phrynium parviflorum
Vietnam (7)

Mimosaceae

Mimosa pigra L. [giant sensitive plant]
Thailand (7)
Neptunia oleraceae Lour [watercress]
Thailand (7)

Moraceae

Morus alba L. [white mulberry]
Vietnam (7)

Musaceae

Musa paradisiaca (L.) [banana]
India (2,6)
Musa sapientum L. [banana]
Thailand (7)
Musa sp. [banana]
Malaysia (7)
Vietnam (7)

Myrtaceae

Eucalyptus saligna Sm. [Sydney blue gum]
Australia (1)
Eucalyptus tereticornis Sm. [forest red gum]
Australia (1)
Psidium guajava L. [guava]
Thailand (7)

Nelumbonaceae

Nelumbo nucifera Gaertner [Indian lotus, sacred lotus]
Thailand (7)

Nymphaeaceae

Nuphar advena (Aiton) Aiton f. [waterlilly, spatterdock]
USA (Florida) (6)
Nymphaea odorata Aiton
USA (Florida) (6)

Oleaceae

Jasminum sambac Ait. [Arabian jasmine]
Thailand (7)

Onagraceae

Jussiaea repens L. = *Ludwigia adscendens* (L.) Hara [creeping water primrose]
Thailand (7)
Ludwigia peploides (Kunth) Raven [water primrose]
Argentina (1,4,6)
Australia (1)
Vietnam (7)

Orchidaceae

Dendrobium sp. [orchid]
Thailand (7)
Vanda sp. [orchid]
Malaysia (7)

Oxalidaceae

Averrhoa carambola L. [starfruit]
Malaysia (7)

Palmae

Elaeis guineensis Jacq. [oil palm]
Malaysia (7)

Poaceae

Eleusine coracana (L.) Gaertn. [ragi, Indian millet]
India (2,6)
Oryza sativa L. [rice]
Argentina (1,4,6)
Australia (1,8)
Malaysia (7)
Thailand (7)
Vietnam (7)
Panicum elephantipes Nees
Argentina (1,4,6)
Paspalum repens L. [water paspalum]
Argentina (1,4)
Saccharum officinarum L. [sugarcane]
Argentina (1,4,6)
India (3)
Thailand (7)
Triticum aestivum L. = *T. vulgare* [wheat]
Australia (1)
Zea mays L. [maize]
Argentina (1,4,6)
Australia (1)
Thailand (7)
Vietnam (7)

Polygonaceae

Polygonum lapathifolium L. = *Persicaria lapathifolium minus* Hudson [knotweed, smartweed]
Australia (1,8)
Polygonum minus Hudson [kesum]
Malaysia (7)
Polygonum sp.
Argentina (1)
USA (Florida) (6)
Polygonum stelligerum Cham. [smart weed]
Argentina (1)
Rumex brownii Campdera [swamp dock]
Australia (1)

Pontederiaceae

Eichhornia azurea (Sw.) Kunth [anchored water hyacinth]
Argentina (1,4,6)
Eichhornia natans
Republic of South Africa (8)
Monochoria africana
Republic of South Africa (8)
Monochoria cyanea (F. Mueller) F. Mueller [water hyacinth]
Australia (1)
Monochoria hastata (L.) Solms-Laub. [phak top thai]
Thailand (7)
Vietnam (7)
Monochoria vaginalis (Burman f.) C.Presl ex Kunth [water hyacinth]
Thailand (7)
Pontederia cordata L. [pickerel weed]
Argentina (1,4,6)
Australia (1,2,8)
USA (Florida) (4,6)

Punicaceae

Punica granatum L. [pomegranate]
Thailand (7)

Rhamnaceae

Ziziphus mauritiana Lamk. [Indian jujube]
Thailand (7)

Rosaceae

Fragaria x *ananassa* Duchesne [strawberry]
Australia (1,8)
Malus domestica Borkh. = *Pyrus malus* [apple]
Australia (1)

Rubiaceae

Morinda citrifolia L. [Indian mulberry, rotten cheesefruit]
Thailand (7)

Rutaceae

Citrus aurantifolia (Christm.) Swingle [lime]
Thailand (7)
Citrus limon (L.) Burm. f. [lemon]
Australia (1,8)
Citrus reticulata Blanco [mandarin]
Australia (1,8)
Citrus sinensis (L.) Osbeck [sweet orange]
Australia (1)
Citrus spp.
Malaysia (7)

Salviniaceae

Salvinia auriculata Aubl. [salvinia]
Argentina (1)
Salvinia molesta Mitchell [salvinia]
Australia (1,8)

Sapindaceae

Dimocarpus longan Lour. [longan]
Thailand (7)
Litchi chinensis Sonn. [lychee]
Vietnam (7)
Nephelium lappaceum L. [rambutan]
Malaysia (7)

Sapotaceae

Achras zapota (L.) van Royen = *Manilkara apota* [sapota]
Vietnam (7)
Mimusops elengi L. [bullet wood]
Thailand (7)

Solanaceae

Capsicum annuum L. [chilli]
Argentina (4,6)
Capsicum frutescens L. [bird chilli pepper]
Thailand (7)
Lycopersicon esculentum Miller [tomato]
Argentina (1,4,6)
Australia (1,8)
Solanum melongena L. [brinjal, eggplant]
Thailand (7)
Vietnam (7)

Sparganiaceae

Sparganium americanum Nutt.
USA (Florida) (6)

Sterculiaceae

Theobroma cacao L. [cocoa]
Malaysia (7)

Trapaceae

Trapa bicornis Osb. [water caltrops]
Thailand (7)

Typhaceae

Typha domingensis Pers. = *T. angustifolia* [lesser reedmace]
Thailand (7)
Typha latifolia L. [cattail]
Argentina (1,4,6)
USA (Florida) (6)
Typha orientalis C. Presl. [bullrush, broad leaved cumbungi]
Australia (1)

Umbelliferae

Daucus carota L. [carrot]
Argentina (1)
Australia (1)

Unknown

Angenica taiwaniana
Vietnam (7)

Vitaceae

Vitis vinifera L. [grape]
Thailand (7)

Appendix 2

Summary results of host-specificity tests carried out on *Niphograpta albiguttalis* 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 — no choice; 3. Oviposition — unknown design; 4. Placement of eggs. 5. Placement of neonates — multiple choice; 6. Placement of neonates — no choice; 7. Larval feeding — details unknown; 8. Unknown design. An 'a' following the type of test indicates that the tests were done on excised foliage.

Alismataceae

Limnocharis flava
Malaysia
7 1 larva survived for 48 hours following release of 15 larvae in 3 separate trials but larva dead after 72 hours, 10 of 15 larvae alive after 48 hours on water hyacinth, 2–4 pupae emerged

Sagittaria montevidensis
Argentina
1 45 eggs laid vs 7386 on water hyacinth

Amaranthaceae

Alternanthera philoxeroides
Argentina
1 6 eggs laid vs 7386 on water hyacinth

Apiaceae

Eryngium eburneum
Argentina
1 113 eggs laid vs 7237 on water hyacinth

Araceae

Pistia stratiotes
Argentina
1 7 eggs laid vs 8238 on water hyacinth

Asteraceae

Lactuca sativa
Vietnam
7 5 larvae surviving after 10 days, resulting in 2 pupae but no adults vs 2 larvae surviving after 10 days resulting in 1 pupa which emerged as adult on water hyacinth

Brassicaceae

Brassica oleracea
Argentina
1 86 eggs laid vs 8238 on water hyacinth

Rorippa nasturtium-aquaticum
Florida USA
4,6 3% survival of hatched eggs after 12 days and 22% survival of early instars after 3 days. Poor feeding response compared with water hyacinth

Commelinaceae

Tradescantia crassifolia
Argentina
1a 1 egg laid vs 8238 on water hyacinth (excised plant material)

Hydrocharitaceae

Limnobium stoloniferum
Argentina
1 15 eggs laid vs 4553 on water hyacinth
6 1 larva of 45 placed reached 2nd instar but then died vs 31 adults emerged from waterhyacinth

Liliaceae

Agapanthus africanus
Argentina
1a 1 egg laid vs 1064 on water hyacinth (excised plant material)

Allium cepa
India
3 eggs laid but larvae died within 48 hours
6 slight feeding, all larvae dead within 48 hours, placement of 1st instars

Poaceae

Eleusine coracana
India
3 eggs laid but did not hatch

Panicum elephantipes
Argentina
1 17 eggs laid vs 7174 on water hyacinth

Saccharum officinarum
Argentina
1 32 eggs laid vs 8238 on water

Polygonaceae

Polygonum sp.
Florida, USA
6 8% survival after 4 days but poor feeding response compared with water hyacinth

Pontederiaceae

Eichhornia azurea
Argentina
1 2012 eggs laid vs 8238 on water hyacinth
4 1 egg of 30 placed developed through to adult vs 7 adults and 1 pupa developed through on water hyacinth
6 5 larvae developed through to adult and 2 through to pupae of 45 placed vs 31 adults emerged on water hyacinth

Monochoria africana
Republic of South Africa
8 54% of petioles damaged following exposure — resulting in 3 pupae and 2 adults vs 100% damage to water hyacinth petioles resulting in 9 larvae and 7 pupae

Monochoria hastata
Thailand
7 feeding but could not complete life cycle
Vietnam
7 2 larvae surviving after 10 days but no pupae vs 2 larvae surviving after 10 days resulting in 1 pupa which emerged as adult from water hyacinth

Monochoria vaginalis
Thailand
7 feeding but could not complete lifecycle

Pontederia cordata
Argentina
1 961 eggs laid vs 8238 on water hyacinth
6 2 larvae of 45 placed developed through to adult vs 31 adults emerged from water hyacinth
Florida USA
6 6% survival after 8 days but no survival of hatched eggs after 14 days. Poor feeding response compared with water hyacinth
Australia
1 eggs laid, larval feeding extensive, resulting in one adult
2 eggs laid, larval feeding extensive but none completed development

Reussia rotundifolia
Argentina
1a 1576 eggs laid vs 8238 on water hyacinth (excised plant material)

Rutaceae

Citrus spp.
Malaysia
7 Feeding observed on 1 leaf following release of 15 larvae in 3 separate trials but no larvae alive after 48 hours, 10 of 15 larvae alive after 48 hours on water hyacinth, 2–4 pupae emerged

Salviniaceae

Salvinia auriculata
Argentina
1 112 eggs laid vs 8089 on water hyacinth

Sparganiaceae

Sparganium americanum
Florida, USA
6 28% survival after 3 days but low feeding response compared with water hyacinth

Typhaceae

Typha latifolia
Argentina
1 432 eggs laid vs 7174 on water hyacinth
6 1 larva of 15 placed reached 4th instar but then died vs 6 adults emerged from water hyacinth

Appendix 3

Plants tested in studies of the host-specificity of *Xubida infusellus*, 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 — no choice; 3. Oviposition — unknown design; 4. Placement of eggs. 5. Placement of neonates — multiple choice; 6. Placement of neonates — no choice; 7. Larval feeding — details unknown; 8. Unknown design. An 'a' following the type of test indicates that the tests were done on excised foliage. A 'b' following the type of test indicates that the tests were done on potted plants.

Alismataceae

Echinodorus grandiflorus Mich. [burhead]
Argentina (1,6a)
Sagittaria graminea Michx. [sagittaria]
Australia (5a)
Sagittaria montevidensis Cham. & Schltdl. [arrowhead]
Argentina (1,6a,6b)
Sagittaria sp. [arrowhead]
Argentina (6b)
Amaranthaceae
Alternanthera philoxeroides (Martius) Griseb. [alligator weed]
Thailand (6)

Araceae

Pistia stratiotes L. [water lettuce]
Argentina (1,6a,6b)
Thailand (6)

Asteraceae

Helianthus annuus L. [sunflower]
Argentina (1,6a)
Australia (5a)
Lactuca sativa L. [lettuce]
Australia (5a,6b)
Trinidad (6)
Uruguay (5,6)

Brassicaceae

Brassica oleracea var. *botrytis* (L.) Alef. [cauliflower]
Australia (5a)
Brassica oleracea L. [Chinese kale]
Uruguay (5,6)
Brassica sp. [sukuma wiki]
Argentina (6a)
Nasturtium sp.
Trinidad (6)
Rorippa nasturtium-aquaticum (L.) Hayek = *Nasturtium officinale* L. [watercress]
Australia (5a)

Bromeliaceae

Ananas comosus (L.) Merr. [pineapple]
Uruguay (6)

Cannaceae

Canna indica L. [canna, Indian shoot]
Thailand (6)
Canna orientalis Rosc.
India (7)

Chenopodiaceae

Beta vulgaris L. [beetroot]
Australia (5a)

Commelinaceae

Commelina elegans
Trinidad (6)
Commelina virginica L. [day flower]
Argentina (1,6b)
Uruguay (5,6)
Dichorisandra elongata (G.F.W. Mey)
Uruguay (5,6)
Tradescantia virginiana L.
Argentina (6b)
Trinidad (6)
Uruguay (5,6)
Zebrina pendula Schnizlein [wandering Jew]
Trinidad (6)
Uruguay (5,6)

Convolvulaceae

Ipomea aquatica Forsk. [potato vine, morning glory]
Thailand (6)
Ipomea batatas (L.) Lam. [sweet potato]
Australia (5a)

Cucurbitaceae

Cucurbita maxima Duchesne ex Lam. [pumpkin]
Australia (5a)
Cucurbita pepo L. s.lat. [marrow]
Argentina (1,6a)

Fabaceae

Medicago sativa L. [lucerne]
Australia (5a)
Trifolium subterraneum L. [subterranean clover]
Australia (5a)

Hydrocharitaceae

Hydrilla verticillata (L.f.) Royle [hydrilla]
Thailand (6)

Liliaceae

Agapanthus africanus Lam. [African lily]
Uruguay (5,6)
Allium cepa L. [onion]
Australia (5a)
Trinidad (6)
Uruguay (5,6)
Asparagus officinalis L. [asparagus]
Australia (5a)
Uruguay (5,6)

Malvaceae

Gossypium hirsutum L. [cotton]
Australia (5a)

Mimosaceae

Neptunia natans [neptunia]
Thailand (6)

Musaceae

Musa paradisiaca (L.) [banana]
Australia (5a,6b)
India (7)

Myrtaceae

Eucalyptus maculata Hook. [spotted gum]
Australia (5a)
Eucalyptus tereticornis Sm. [forest red gum]
Australia (5a)

Nelumbonaceae

Nelumbo nucifera Gaertner [Indian lotus, sacred lotus]
Thailand (6)

Onagraceae

Ludwigia peploides (Kunth) Raven [water primrose]
Australia (5a,6b)

Poaceae

Oryza sativa L. [rice]
Argentina (1,6a)
Australia (5a)
Thailand (6)
Trinidad (6)
Uruguay (6)
Saccharum officinarum L. [sugarcane]
Argentina (1,6a,6b)
Australia (5a)
India (7)
Thailand (6)
Trinidad (6)
Uruguay (5,6)
Triticum aestivum L. = *T. vulgare* [wheat]
Australia (5a)
Zea mays L. [maize]
Argentina (6a)
Australia (5a)
Thailand (6)

Polygonaceae

Polygonum ferrugineum Wedd. [smart weed]
Argentina (1,6a)
Polygonum hydropiper L. [water pepper]
Australia (5a)
Polygonum lapathifolium L. = *Persicaria lapathifolium minus* Hudson [knotweed, smartweed]
Australia (5a)
Polygonum sp.
Australia (5a)
Polygonum stelligerum Cham. [smart weed]
Argentina (1,6a)
Rumex brownii Campdera [swamp dock]
Australia (5a)
Rumex crispus L. [curled dock]
Australia (5a)

Pontederiaceae

Eichhornia azurea (Sw.) Kunth [anchored water hyacinth]
Uruguay (5,6)
Monochoria hastata (L.) Solms-Laub. [phak top thai]
Thailand (6)
Monochoria vaginalis (Burman f.) C.Presl ex Kunth [water hyacinth]
Thailand (6)
Pontederia cordata L. [pickerel weed]
Argentina (1,6a)
Australia (5a,6b)
Uruguay (5,6)

Rosaceae

Fragaria x *ananassa* Duchesne [strawberry]
Australia (5a)
Malus pumila Mill. [apple]
Australia (5a)

Rutaceae

Citrus limon (L.) Burm. f. [lemon]
Australia (5a)
Citrus reticulata Blanco [mandarin]
Australia (5a)
Citrus sinensis (L.) Osbeck [sweet orange]
Australia (5a)

Salviniaceae

Salvinia molesta Mitchell [salvinia]
Australia (5a)

Solanaceae

Lycopersicon esculentum Miller [tomato]
Argentina (1,6a)
Australia (5a)

Trapaceae

Trapa bicornis Osb. [water caltrops]
Thailand (6)

Typhaceae

Typha domingensis Pers. = *T. angustifolia* [lesser reedmace]
Thailand (6)
Typha orientalis C. Presl. [bullrush, broad leaved cumbungi]
Australia (5a,6b)

Umbelliferae

Daucus carota L. [carrot]
Australia (5a)

Zingiberaceae

Zingiber officinale Rosc. [ginger]
Australia (5a,6b)

Appendix 4

Summary results of host-specificity tests carried out on *Xubida infusellus* 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 — no choice; 3. Oviposition — unknown design; 4. Placement of eggs. 5. Placement of neonates — multiple choice; 6. Placement of neonates — no choice; 7. Larval feeding — details unknown; 8. Unknown design. An 'a' following the type of test indicates that the tests were done on excised foliage. A 'b' following the type of test indicates that the tests were done on potted plants.

Alismataceae

Echinodorus grandiflorus
Argentina
6a actual outcome unknown but no larvae survived beyond day 12

Sagittaria sp.
Argentina
6a actual outcome unknown but no larvae survived beyond day 12

Araceae

Pistia stratiotes
Argentina
1 9 egg masses comprising 228 eggs laid vs 6 egg masses on water hyacinth
6a actual outcome unknown but no larvae survived beyond day 12

Asteraceae

Helianthus annuus
Argentina
6a larvae survived to day 20

Lactuca sativa
Australia
5a,b leaf feeding and stem tunnelling on excised plant material with survival to 4th and 5th instar; leaf feeding only on whole potted plants and no survival beyond 2nd instar

Brassicaceae

Brassica sp.
Argentina
6a actual outcome unknown but no larvae survived beyond day 12

Cannaceae

Canna orientalis
India
7 larval feeding but unable to complete development

Commelinaceae

Commelina virginica
Argentina
6b moderate feeding

Dichorisandra elongata
Uruguay
5 leaves with 2–10 small areas of feeding in one test; no damage in the second test
6 feeding damage score of 2.0 vs 5.0 for water hyacinth; no survival after 5 days

Tradescantia virginiana
Argentina
6b moderate feeding
Uruguay
5 plants with some leaves partially or completely dead in one test; no damage in the second test
6 feeding damage score of <2.0 vs 5.0 for water hyacinth; no survival after 5 days

Cucurbitaceae

Cucurbita pepo
Argentina
6a actual outcome unknown but no larvae survived beyond day 12

Musaceae

Musa paradisiaca
Australia
5a,b leaf feeding and stem tunnelling on excised plant material with survival to 4th and 5th instar; leaf feeding and stem tunnelling on whole potted plants with no survival beyond 1st instar
India
7 larval feeding but unable to complete development
Musa sp.
Trinidad
5 larvae fed for 1 month and then died

Onagraceae

Ludwigia peploides
Australia
5a,b leaf feeding only with no survival beyond 1st instar on both excised plant material and whole potted plants

Poaceae

Oryza sativa
Argentina
1 1 egg mass comprising 22 eggs laid vs 6 egg masses on water hyacinth
6a 3% of larvae produced pupae vs 14% on water hyacinth
Paspalum repens
Argentina
1 3 egg masses laid vs none on water hyacinth
Saccharum officinarum
Argentina
6a actual outcome unknown but no larvae survived beyond day 12
6b very slight feeding
India
7 larval feeding, one larvae completed lifecycle
Uruguay
6 feeding damage score of 0.5 vs 5.0 for water hyacinth
Zea mays
Argentina
6a 6.7% of larvae produced pupae vs 14% on water hyacinth

Polygonaceae

Polygonum ferrugineum
Argentina
1 1 egg mass laid vs none on water hyacinth
6a actual outcome unknown but no larvae survived beyond day 12
Polygonum stelligerum
Argentina
1 1 egg mass laid vs none on water hyacinth
6a actual outcome unknown but no larvae survived beyond day 12

Pontederiaceae

Eichhornia azurea
Argentina
1 14 egg masses comprising 185 eggs laid vs 6 egg masses on water hyacinth
Uruguay
5 serious damage to plants similar to that occurring on water hyacinth
6 feeding damage score of 5.0 as for water hyacinth
Monochoria hastata
Thailand
6 fed and developed from 1st to 5th instar larvae
Monochoria vaginalis
Thailand
6 fed and developed from 1st to 5th instar larvae
Pontederia cordata
Argentina
6a actual outcome unknown but no larvae survived beyond day 12
Australia
5a feeding and development duration as for water hyacinth
Uruguay
5 plants with some leaves partially or completely dead
6 feeding damage score of 5.0 as for water hyacinth

Solanaceae

Lycopersicon esculentum
Argentina
6a actual outcome unknown but no larvae survived beyond day 12

Typhaceae

Typha orientalis
Australia
5a,b leaf feeding and stem tunnelling with no survival beyond 1st instar on both excised plant material and whole potted plants

Zingiberaceae

Zingiber officinale
Australia
5a leaf feeding only with no survival beyond 1st instar on both excised plant material and whole potted plants