INDUSTRY BIOSECURITY PLAN

FOR THE NURSERY & GARDEN INDUSTRY

Threat Specific Contingency Plan

Serpentine leaf miner

Liriomyza huidobrensis

Plant Health Australia

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Plant Health AUSTRALIA

Nursery & Garden Industry Australia

Disclaimer

Further information

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1 Purpose and background of this contingency plan

This contingency plan provides background information on the pest biology and available control measures to assist with preparedness for an incursion into Australia of Serpentine leaf miner (*Liriomyza huidobrensis*). While many species of leaf miners occur throughout the world, this document will refer only to this species unless otherwise stated.

It provides guidelines and options for steps to be undertaken and considered when developing a Response Plan to this pest. Any Response Plan developed using information in whole or in part from this contingency plan must follow procedures as set out in PLANTPLAN and be endorsed by the National Management Group prior to implementation.

This contingency plan was developed for the Nursery and Garden Industry Australia (NGIA), and therefore is focussed on production nurseries covered by this association. In the event of an incursion, operations not covered by the NGIA (e.g. retail nurseries) will not be eligible for Owner Reimbursement Costs, as defined in the Emergency Plant Pest Response Deed, if affected by actions carried out under the Response Plan.

The information for this plan has been primarily obtained from CABI Crop Compendium (**www.cabicompendium.org**), as well as the CD on Polyphagous Agromyzid Leafminers developed by Mallik Malipatil and Peter Ridland. Modifications and additions to the plan have been completed to make the information relevant to an incursion of Serpentine leaf miner in the nursery and garden industry.

Additional information on the management of a leaf miner incursion can be found in the "Leafminer pest-generic incursion management plan for the Australian vegetable industry" developed by Elio Jovicich in August 2009. This document contains supplementary information regarding leaf miner impact on vegetable production in Australia.

2 Australian nursery industry

The Australian nursery industry is a significant horticultural sector with a combined supply chain (production to retail/grower) valued at more than $6 billion dollars annually. The industry employs approximately 45,000 people spread over more than 20,000 small to medium sized businesses including production nurseries and retail outlets. The industry is located predominantly along the Australian coastline and in major inland regions servicing urban and production horticulture.

Nursery production adds value to Australia‟s primary industry‟s sector and in 2008/2009 is forecast to contribute more than $2 billion to the national economy. Nursery production is a highly diverse primary industry servicing the broader $14 billion horticultural sector within Australia (Table 1).

Table 1. *Nursery production supply sectors within Australian horticulture*

Production Nursery	Horticultural markets	Economic value
Container stock[1]	Ornamental/urban horticulture	$2 billion retail value
Foliage plants[1]	Interior-scapes	$87 million industry
Seedling stock[2]	Vegetable growers	$3.3 billion industry
Forestry stock[3]	Plantation timber	$1.7 billion industry
Fruit and nut tree stock[2]	Orchardists (citrus, mango, etc)	$5.2 billion industry
Landscape stock[1]	Domestic & commercial projects	$2 billion industry
Plug and tube stock[4]	Cut flower	$319 million industry
Revegetation stock[1]	Farmers, government, landcare	$109 million industry
Mine revegetation	Mine site rehabilitation	Value unknown
	Total horticultural market value	**$14.5 billion**

3 Eradication or containment determination

The decision to eradicate should be based both on the potential economic impact of host damage resulting from the Serpentine leaf miner infestation and on technical feasibility. Eradication costs must factor in long term surveys to prove the success of the eradication program. Two years with no detections of the pests will be necessary to confirm that no Serpentine leaf miner remain before pest free status can be declared.

No specific eradication matrix has been determined for Serpentine leaf miner, however the general decision process as outlined in Figure 1 should be followed in determining if an incursion of this pest will be eradicated or managed/contained. The final decision between eradication and management will be made through the National Management Group.

[1] Data sourced from Market Monitor

[2] Data sourced from Horticultural Handbook 2004

[3] Data sourced from ABARE 2005

[4] Data sourced from industry

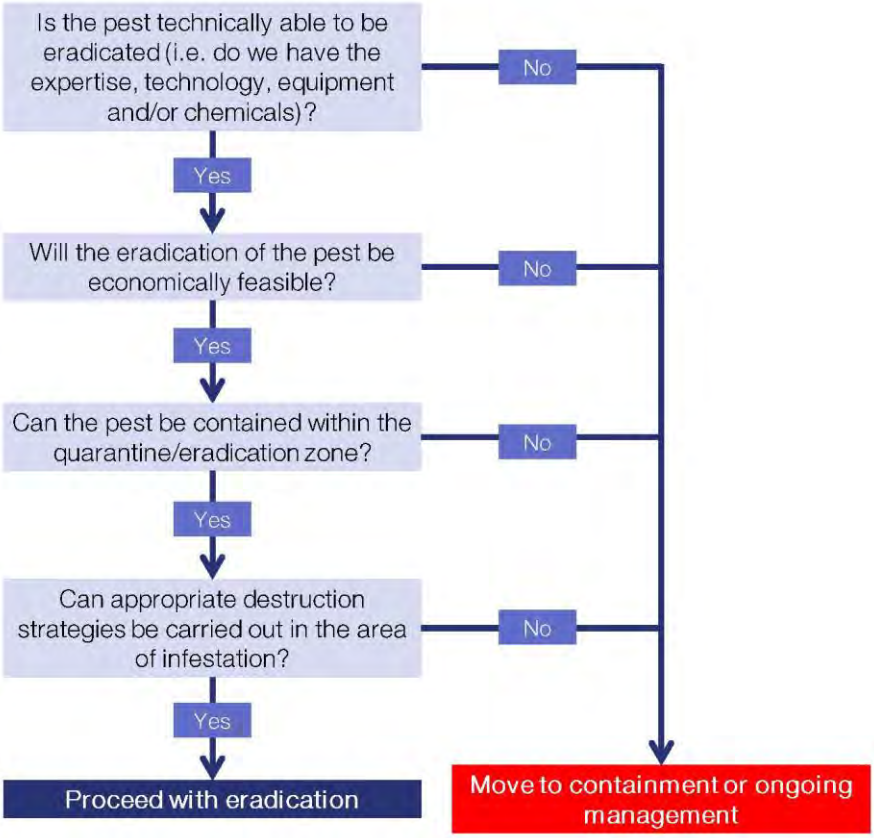

Figure 1. *Decision outline for the response to an exotic pest incursion*

4 Pest information/status

4.1 Pest details

Common names:	Serpentine leaf miner; pea leaf miner; South American leaf miner
Scientific name:	*Liriomyza huidobrenis*
Synonyms:	*Liriomyza cucumifoliae; Agromyza huidobrensis; Liriomyza dianthi; Liriomyza langei; Liriomyza decora*
Taxonomic position:	Kingdom, Animalia; Phylum, Arthropoda; Class, Insecta; Order, Diptera; Family, Agromyzidae

4.1.1 Background

The Agromyzidae are a well-known group of small, morphologically similar flies whose larvae feed internally on plants, often as leaf and stem miners. Nearly all species are very host-specific but a few highly polyphagous species have become important pests of agriculture and horticulture in many parts of the world. These key species: *Liriomyza bryoniae, L. huidobrensis, L. sativae, L. trifolii* and *Chromatomyia horticola* are not yet present in Australia and pose a significant quarantine threat to Australian agriculture and horticulture.

Liriomyza huidobrensis is now considered a complex of two cryptic species. This follows a study of specific sequences in mitochondrial and nuclear genomes (Scheffer, 2000; Scheffer & Lewis, 2001). *L. huidobrensis* has been separated into multiple subspecies using molecular techniques, after it was noted that populations in different areas appeared to prefer different plant hosts and exhibited varying levels of insecticide resistance. Flies from California and Hawaii formed one clade, whilst specimens from South and Central America formed another clade (Scheffer, 2000; Scheffer & Lewis, 2001).

Subsequently, the name *L. huidobrensis* (Blanchard) became restricted to flies belonging to the South American clade, which includes all *L. huidobrensis* in Central and South America and all introduced populations of *L. huidobrensis*. The name *L. langei* Frick has been resurrected for the Californian clade of *L. huidobrensis*-like flies found in California and Hawaii. It is estimated that the South American and Californian clades diverged approximately 2 million years ago. Although the two species have been separated using DNA sequence data, they unfortunately cannot be separated morphologically (Scheffer, 2000; Scheffer & Lewis, 2001).

All invasive populations in Africa, Asia, Canada and Europe were found to belong to *L. huidobrensis* as so defined (Scheffer *et al.,* 2001). *Liriomyza langei* and *L. huidobrensis* could not be separated morphologically, but a PCR-RFLP protocol for separating them has been published (Scheffer *et al*., 2001). Consequently the literature from USA on *L. huidobrensis* should in fact be considered as relating to *L. langei*.

L. huidobrensis, in South and Central America, was under natural biological control until it was secondarily subjected to massive amounts of insecticides in the 1970s that were directed at a lepidopterean pest in potatoes (Chavez & Raman, 1987). This exposure to insecticides bred resistance, causing the leafminer to develop into an economically important pest. At present, the only effective insecticides are translaminar insecticides (abamectin, cyromazine, neem and spinosad), which penetrate the leaves to affect the leafminer larvae (Weintraub, 2002).

Typically, these polyphagous leafminers are considered to have invaded countries via movement of infested plants (generally ornamentals such as chrysanthemum) (Minkenberg, 1988; Spencer, 1989). While fully-formed mines should be readily visible to quarantine officials, signs of early infestations are much less obvious and are easily overlooked (Spencer, 1989). It is highly likely that the initial incursions of these species will be in horticultural or urban areas, with subsequent spread into broadacre cropping regions.

Since the early 1990s, there has been a rapid movement of *L. huidobrensis* and *L. sativae* eastward through tropical and sub-tropical areas of Asia, resulting in large crop losses and excessive use of broad-spectrum insecticides (Rauf *et al*., 2000). *L. huidobrensis* is the dominant agromyzid at higher elevations (>1000 m) in tropical Asia, with the potato crops receiving the greatest damage (Rauf *et al*., 2000; Shepard *et al*., 1998; Sivapragasam & Syed, 1999; Spencer, 1989), while *L. sativae* is the dominant pest in lowland areas (Andersen *et al.,* 2002; Rauf *et al*., 2000; Spencer, 1989). Although *L. huidobrensis* has been recorded in Lombok on potatoes in 2003 (I Wayan Supartha, personal communication), this species is considered less likely than *L. sativae* to establish in northern Australian areas (apart from the Atherton Tablelands) (Malipatil & Ridland, 2008). With *L. sativae* being recorded in Timor Leste in 2003 (PaDIL, 2005) and in Irian Jaya in 2005 (I Wayan Supartha, personal communication), this species is now within its natural dispersal range of northern Australia and so poses a direct threat to many horticultural crops grown there. Intensive surveillance of vegetable crops in Papua New Guinea and the Torres Strait should be undertaken as this would be the most likely natural pathway to Australia. *L. trifolii* and *L. bryoniae* are also well established in East and Southeast Asia but are being displaced by *L. sativae* (Abe & Tokumaru, 2008).

The biology and ecology of polyphagous *Liriomyza* spp. have been reviewed by Kang *et al.* (2008), Murphy & LaSalle (1999), Parrella (1982, 1987) and Waterhouse & Norris (1987).

Several generations may be produced during the year, with eggs being laid just beneath the surface of the leaf. On hatching, the larvae “mine” the leaf, hence the name leafminer. Damage to the plant is caused in several ways: (i) by the stippling that results from punctures made by females for feeding on sap and laying eggs; (ii) by the internal mining by the larvae; (iii) by allowing pathogenic fungi to enter the leaf through the feeding punctures (Deadman *et al.,* 2000; Matteoni & Broadbent, 1988) and (iv) mechanical transmission of plant viruses (Costa *et al.,* 1958; Zitter & Tsai, 1977). This damage results in a depressed level of photosynthesis in the plant. Extensive mining also causes premature leaf drop, which can result in sun scalding of fruit or reduced tuber filling of potatoes (CABI, 2006).

The larvae of *L. huidobrensis* tunnel in the chloroplast-containing spongy mesophyll layers, disrupting photosynthesis (Shephard *et al*., 1998). The larvae leave winding trails (mines) as they feed inside leaves and other plant parts. The mines are easily visible and when the larvae are in large numbers this feeding damage can cause substantial economic losses (Arnold, 2002).

4.1.2 Life cycle

The life cycle for Serpentine leaf miner is typical for Agromyzidae and a useful summary is provided by Weintraub & Horowitz (1995). In Peru, the life cycle timing is as follows: egg stage (3-4 days); first-instar larva (3-4 days); second-instar larva (2-3 days); third instar (3-4 days); pupal stage (12-18 days). Females had an average longevity of 3-28 days; male longevity was 2-6 days. The mean number of eggs laid per female in winter was 117 and in spring was 161 (Mujica & Cisneros, 1997).

Female flies use their ovipositor to puncture the leaves of the host plants causing wounds which serve as sites for feeding (by both male and female flies) or oviposition. Feeding punctures of *Liriomyza* species are rounded, usually about 0.2 mm in diameter, and appear as white speckles on the upper leaf surface. The appearance of the punctures does not differ between *Liriomyza* species, nor can the pattern of their distribution on the leaf be used to separate species. Feeding punctures cause the destruction of a large number of cells and are clearly visible to the naked eye. Typically about 15% of oviposition punctures made by *L. trifolii* contain viable eggs (Bethke & Parrella, 1985) but this percentage varies with host plant quality.

Eggs are inserted just below the leaf surface and hatch in 2-5 days depending on the temperature (Figure 2). The number of eggs laid varies according to temperature and host plant (Parrella, 1987). There are three larval stages, and all feed within the leaf or stem tissue. The larvae predominantly feed on the plant in which the eggs are laid, although some species can exit one leaf and enter another (not reported for *L. huidobrensis*). The larvae leave the plant to pupate (Parrella & Bethke, 1984), with pupae found in crop debris, in the soil or sometimes on the leaf surface. Pupariation is adversely affected by high humidity or drought.

Figure 2. *L. huidobrensis egg excised from under plant leaf surface (©UC Statewide IPM Project Jack Kelly Clark)*

Parthenogenic females have not been reported for these species, although *Phytomyza plantaginis*, a common leafminer on *Plantago* spp., is parthenogenic in Australia and USA. Adults are primarily active in early morning, shortly after sunrise, and again just before sunset (Weintraub & Horowitz, 1995).

Studies on *L. huidobrensis* developmental rates in lettuce at different temperatures (11 to 28°C) revealed a linear increase in developmental rates with temperature (Head *et al*., 2002). The theoretical lower threshold temperatures for development for the three larval instars and pupae were 5.4, 6.3, 6.2 and 5.7°C, respectively. The calculated degree-days for each stage were 84.3, 30.1, 58.9 and 143.7, respectively. Similar studies were performed on beans (15-30°C) (Lanzoni *et al*., 2002), estimating the minimum developmental temperatures for egg, larva and pupa at 8.1, 7.7 and 7.3°C, respectively. The upper thresholds for egg, larva and pupa were calculated to be 31.1, 35.3, and 27.9°C, respectively.

In Southern USA, the endemic leafminers" life cycle is most probably continuous throughout the year, although there is a noticeable first generation, which reaches a peak in April, with the lifecycle completed in 17-30 days during summer, and 50-65 days in winter. In the Netherlands and Czech Republic, the leafminer is mainly a glasshouse pest, but a proportion of the pupae can survive outdoors during an average Dutch winter, proving how adaptable this pest is (Vlk, 1999; WA, 2003).

Figure 3. *Leafminer pupa (© UC Statewide IPM Project Jack Kelly Clark)*

Liriomyza huidobrensis has been found up to 3,000 m above sea level and has been shown to be more cold-hardy (Chen & Kang, 2002; 2004) than *L. sativae* (Chen & Kang, 2005a, b), with a mean pupal supercooling point (transition temperature at which body fluids begin to form ice crystals) of -20.9°C compared to *L. sativae* with a mean pupal supercooling point of -11.5°C (Zhao & Kang, 2000). This behaviour gives *L. huidobrensis* a far greater climatic range for survival. Indeed, Chen & Kang (2004) evaluated populations in China from 25°N to 42°N and found increasing cold tolerance with latitude and suggested that the January isotherms between -4°C and -6°C was the critical area beyond which *L. huidobrensis* could not overwinter successfully in the field. Martin *et al*. (2005) found that no *L. huidobrensis* survived the winter in southern Ontario, Canada.

Figure 4. *Adult Liriomyza huidobrensis (© UC Statewide IPM Project Jack Kelly Clark)*

4.1.3 Dispersal

Agromyzid flies are considered as "moderate fliers" (Yoshimoto and Gressitt, 1964) and in agricultural situations, the flies tend to remain close to their target crops, often only moving very short distances between host plants (Zehnder & Trumble, 1984). However, they do have the capacity to move longer distances by wind dispersal. Spencer & Stegmaier (1973) suggested that substantial wind movements of agromyzids between islands have occurred in the Florida area. Yoshimoto and Gressitt (1964) reported that agromyzids were trapped at sea as far as 50 km from the coast near Korea. Glick (1939) recorded low numbers of agromyzids being trapped by nets at 1,500 m (towed by aircraft). In Australia, White (1970) recorded low numbers of the grass-feeding agromyzid, *Cerodontha australis*, being trapped as high as 600 m by airborne drogue-nets.

There is no information on the natural dispersal of *L. huidobrensis*, and very little information on dispersal in other species. Jones & Parrella (1986) measured the dispersal of *L. trifolii* in a chrysanthemum greenhouse and found that females flew significantly farther than males. Ozawa *et al.* (1999) measured the height at which *L. trifolii* fly in a greenhouse and found that they were primarily close to the plants. Nonetheless natural dispersion within the crop is by flight. This species is considered a weak flier, using flying in the day between 0800 and 1000 and at night between 1800 and 2000 (Wang *et al.,* 1998).

L. huidobrensis is not carried phoretically by any other organism. It can be wind-blown into crops from surrounding vegetation/fields.

Pathways can also be human assisted movement of infected plants or parts of plants (pathway for egges, larvae), soil or potting media (pathways for pupae) or packaging. *L. sativae* was probably introduced to Europe via imports for glasshouse cultivation (CABI, 2007).

Early outbreaks are often associated when material is moved from propagating nurseries to other commercial premises, and imported chrysanthemum and other ornamental cuttings pose a high risk (Cheek *et al*., 1993). Once in an area a high frequency and amount of trade traffic could lead to rapid dispersal. In South Africa virtually the whole of the country was infested within three years (Visser, pers com., 2001).

4.2 Affected hosts

4.2.1 Host range

Liriomyza huidobrensis, along with two other leafminers of economic importance, *L. sativae* and *L. trifolii*, is on the EPPO A1/A2 quarantine list (Shiao, 2004), in part due to the breadth of its plant host range. Unlike most species of Agromyzidae, *L. huidobrensis* is highly polyphagous and has been recorded on plants from 14 families (Spencer, 1973, 1990). This leafminer has no clear preference for any particular family; hosts include numerous vegetable and flower crops (refer to Appendix 1 for list) (Scheffer & Lewis, 2001; WA, 2003). The most economically important crops attacked are beet, spinach, peas, beans, potatoes and cut flowers (most commonly gypsophila, more rarely carnations and chrysanthemum) (Spencer, 1989), as well as lupins, field peas and faba beans.

Non-commercial hosts: Because broadleaf weeds and senescent crops may serve as alternative hosts, destruction of weeds and deep ploughing of crop residues are recommended. Adults experience difficulty in emerging from puparia buried deeply in soil.

4.2.2 Current geographic distribution

Liriomyza huidobrensis is now widespread through Africa (Comoros, Kenya, Mauritius, Morocco, Réunion, Seychelles, South Africa); Asia (China, India, Indonesia, Israel, Jordan, Democratic People's Republic of Korea, Lebanon, Malaysia, Philippines, Singapore, Sri Lanka, Syrian Arab Republic, Taiwan, Thailand, Vietnam); Central America (Belize, Costa Rica, Dominican Republic, El Salvador, Guadeloupe, Guatemala, Honduras, Nicaragua, Panama); Europe (Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Finland, France, Germany, Greece, Hungary, Italy, Malta, Netherlands, Norway, Poland, Portugal, Spain, Switzerland, Turkey, United Kingdom [incursions eradicated]); North America: Canada, mainland USA (*Liriomyza langei*); Oceania: Guam, Hawaii (*Liriomyza langei*); South America (Argentina, Brazil, Chile, Colombia, Ecuador, French Guiana, Peru, Uruguay, Venezuela) (CABI, 2006).

Although endemic to South and Central America, *L. huidobrensis* is only considered a minor pest. In the coastal areas of Peru it is generally kept in check by a large complex of natural hymenopteran parasitoids.

It was first detected in Europe in 1987 in the Netherlands where it was found on glasshouse lettuces. It is presumed to have been imported directly from South America. It has since spread considerably in the European and Mediterranean region, but remains absent from a significant number of countries, particularly in central and eastern Europe. However, since the early 1980s there has been a dramatic increase in damage to vegetables, especially potatoes, due to over-use of insecticides, leading to the development of insecticide-resist leafminers, and the elimination of its natural enemies (Shephard *et al*., 1998).

From 1990 to 2000, *L. huidobrensis* became globally invasive and can now be found in many greenhouses and vegetable and flower growing areas of Europe, Asia, Africa, and the Middle East (Scheffer & Lewis, 2001). In North America it is confined to California and Hawaii (USA), and it has been a glasshouse pest in Florida and Virginia.

Insecticide-resistant *L. huidobrensis* was first detected in Europe in 1987 on glasshouse lettuces, grown in the Netherlands. The EPPO presumed that the leafminer was imported directly from South America. Within the EPPO region it has spread to Austria, Belgium, Cyprus, Czech Republic (Vlk, 1999), France, Israel, Italy (including Sicily), Malta, Netherlands, Portugal, Spain (including Canary Islands), and the UK (England, Northern Ireland, Scotland). *L. huidobrensis* has been intercepted, or has occurred and been eradicated, in Denmark, Finland, Germany, Ireland and Sweden. The leafminer remains absent from a significant number of countries in Central and Eastern Europe[5].

Subsequent to the leafminers" spread throughout Europe, it was introduced into Israel. The first outbreak in Israel occurred in February 1992 in the Jordan Valley, when chrysanthemum growers encountered a leafminer that could not be chemically controlled. It is thought that *L. huidobrensis* had probably entered Israel 1-2 years prior to this sighting (Weintraub and Horowitz, 1995).

L. huidobrensis has been reported in Asia, within India, Thailand[5], China (He *et al*., 2002), Taiwan (Shiao & Wu, 2000), and Indonesia (Shephard *et al*., 1998).

The pea leafminer was first found in Taiwan in 1999, most likely on smuggled in on plant material or entered due quarantine failure. It is now a widespread and dominant pest species on some vegetable crops (Cucurbitaceae, legumes and composites) (Shiao & Wu, 2000). Shortly thereafter, there were outbreaks of *L. huidobrensis* in China, which were proven to originate from the South American clade after phylogenetic analysis (He *et al*., 2002).

The greatest threat to the Australian potato industry comes from Indonesia. *L. huidobrensis* was first reported in potatoes, in Java, in 1994. Since then *L. huidobrensis* has rapidly spread and can be found on many vegetable and ornamental crops in Java, Sumatra and Sulawesi. It has become widely established on both cultivated and weed hosts. *L. huidobrensis* is thought to have been introduced into Indonesia on cut flowers from Europe or North America (Shephard *et al*., 1998).

L. huidobrensis has established because cooler temperatures, such as in the highland vegetable production areas of Indonesia, favour feeding and oviposition. Severe damage occurs on potato, shallots, tomato, beans, and other crops, ornamentals and weeds at high elevations in Sumatra and Java (Shephard *et al*., 1998). It should be noted "that potato was especially susceptible to attack by *L. huidobrensis*, and severely damaged fields were common. On occasion estimated yield losses were 75%. Farmers mentioned that no pesticide was effective against the leafminer in potato, and some growers were abandoning potato cultivation in favour of sweet potato. In the Alahan Panjang area of West Sumatra, it was estimated that about 40% of the usual potato-growing area was now planted to other crops due to devastating attacks by *L. huidobrensis* (Shephard *et al*., 1998).

It has been intercepted, but is not established in Australia.

4.2.2.1 POTENTIAL DISTRIBUTION IN AUSTRALIA

Based on climatic conditions, the Serpentine leaf miner has the potential to establish in most coastal areas, with the exception of Northern Territory, far north Queensland, and eastern and northern Western Australia. For a detailed CLIMEX model refer to Jovicich (2009).

4.2.3 Symptoms

Feeding punctures appear as white speckles on leaf surfaces between 0.13 and 0.15 mm in diameter. Oviposition punctures are smaller (0.05 mm), more uniformly round and difficult to detect with the naked eye. The larval mines are usually white with dampened black and dried brown areas, and are usually associated with the midrib and lateral leaf veins. Mines are typically serpentine, of irregular shape, increasing in width as larvae mature. Several larvae feeding on a single leaf may produce a secondary 'blotch' mine type and leaf wilt may occur (Spencer, 1973, 1989). The larvae feed primarily

[5] Source: **www.eppo.org/QUARANTINE/insects/Liriomyza_huidobrensis/LIRIHU_ds.pdf**

within the leaf tissues, with the exception of peas, where larvae may also feed on the outer surface of young seed-pods.

In potato, feeding punctures can often be seen all over the growing plant, giving the impression that a generalized outbreak of larval infestation is in progress. Larval damage on potato plants follows a fixed pattern, somewhat different from that of the adult fly population. The initial larval infestation and corresponding damage occur in the lower third of the plant, moving upwards to the top of the plant. At this time, the above ground parts of the plant become necrotic and die. Larval damage is consistently less severe during vegetative growth stages than when the plant is full grown. The occurrence of egg extrusion in the growing leaves might explain this phenomenon (Mujica & Cisneros, 1997).

Figure 5. *Leafminer mines on spinach leaf, making the produce unsaleable (© Ohio State University)*

Figure 6. *Damage to celery typical of Liriomyza huidobrensis (Source: Ministry of Agriculture and Food Ontario, Canada. © Queen's Printer for Ontario)*

Figure 7. *Mines on snap-beans caused by feeding larvae (© J. Lotz, Division of Plant Industry University of Florida)*

Figure 8. *Mines on potato caused by feeding larvae (© REDEPAPA-CORPOICA)*

4.3 Diagnostic information

For a series of guidelines for the diagnostic testing of *Liriomyza* species, see the „Diagnostic protocol for the detection of leafminers"[6] prepared by Mallik Malipatil and John Wainer. Additional diagnostic and other related information as well as illustrated LUCID identification keys and fact sheets for all agromyzid species included in this plan are to be found in Malipatil & Ridland (2008). Diagnostic protocols for *Liriomyza* spp. have also been developed as an approved EPPO Standard (OEPP/EPPO, 2005).

To distinguish adults of *L. huidobrensis* from other leaf miners of quarantine concern, the following simplified key can be used for initial identification (Figure 9; accurate identification requires dissection of male terminalia and all identifications made with this key should be confirmed by a specialist).

[6] Available for download from **www.planthealthaustralia.com.au/pidd**

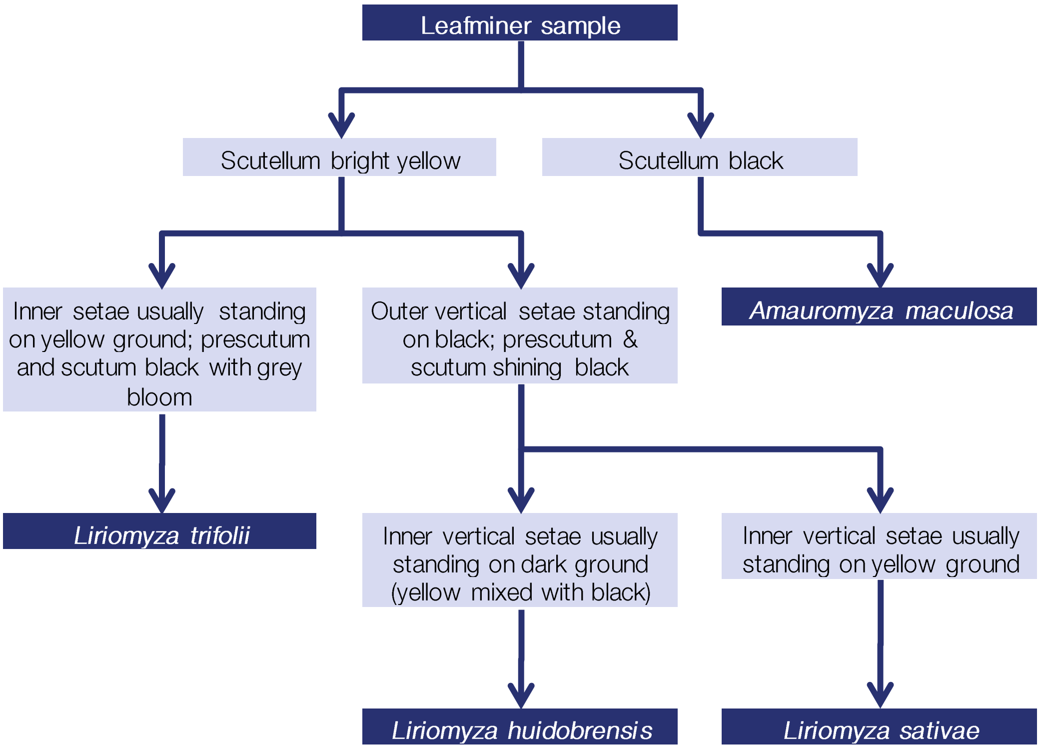

Figure 9. *Simplified diagnostic key for leafminer identification*

Other morphological differences are described in Spencer (1973) and Knodel-Montz & Poe (1982). Menken & Ulenberg (1986) have described a method to distinguish between four species of *Liriomyza* (*L. bryoniae* and the three species in the above key), using starch gel electrophoresis and enzyme staining (see also OEPP/EPPO, 1992). This method can be used on single individuals. An improved version has been published by Oudman *et al.* (1995).

An expert with a good knowledge and understanding leaf miners would be required to identify the pest. Care must be taken in distinguishing leaf miners from Serpentine leaf miners as a number of species share some features.

The State Chief Plant Health Manager will select the diagnostic faculties to be used during a response to a serpentine leaf miner incursion. Contact details for a number of diagnostic facilities can be found in Appendix 2.

4.4 Pest risk ratings and potential impacts

A pest risk analysis has been carried out on this insect, taking into account the entry, establishment and spread potentials, together with the economic and environmental impact of establishment. A summary of these ratings are shown in Table 2. Based on this information, Serpentine leaf miner is considered a **medium** overall risk to Australia.

Table 2. *Pest risk ratings for Serpentine leaf miner as determined in the IBP for the Nursery and Garden Industry (Plant Health Australia, 2008)*

Potential or impact	Rating
Entry potential	Medium
Establishment potential	High
Spread potential	High
Economic impact	High
Overall risk	**High**

4.4.1 Phytosanitary risk

L. huidobrensis is a major quarantine pest and is officially listed as such in many areas, for example, EPPO (OEPP/EPPO, 1984). It is primarily a tropical and warm temperate species, but in some parts of Europe it has shown an ability to become a major pest of a wide variety of ornamental or vegetable crops grown under glasshouse conditions. The action protocols for the UK (Cheek *et al.*, 1993) provide an example of the contingency plans that should be in place in countries not yet invaded by this pest.

All stages are killed within a few weeks by cold storage at 0°C. Newly-laid eggs are the most resistant and it is recommended that cuttings of infested ornamental plants be maintained under normal glasshouse conditions for 3-4 days after lifting, to allow eggs to hatch. Subsequent storage of the plants at 0°C for 1-2 weeks should then kill leafminer larvae (Webb and Smith 1970).

Cold storage (0°C) treatment is not a viable option for the importation of fresh vegetables and cut flowers and therefore produce inspections are required. To avoid the introduction of *L. huidobrensis* (and other leafminer species) into further European countries, EPPO (OEPP/EPPO, 1990) recommended that propagating material (except seeds) of capsicum, carnations, celery, chrysanthemums, *Cucumis, Gerbera*, *Gypsophila*, lettuces, *Senecio hybridus* and tomatoes, from countries where the pests occur, must have been inspected at least every month during the previous 3 months and found to be pest-free. A phytosanitary certificate is required for cut flowers and for vegetables with leaves.

Although there are phytosanitary measures in place, intercepts of insects and other invertebrates occur, mostly on cutflower imports from African countries (South Africa, Zimbabwe and Kenya), India, Singapore and to a lesser extent China. These intercepts are a cause of concern to Australia, as the climate and wide host range provides ideal conditions for establishment of *L. huidobrensis*. Once established, the economic impact would be high, and spread rapid, due to the frequent movement of produce between nurseries and markets in the cutflower and other horticultural industries (WA, 2003).

4.4.2 Entry potential

Rating: MEDIUM

Leaf miners may likely enter Australia via imported plant material containing leaves, particularly seedlings or material for propagation, where the eggs and larvae are borne internally. An increase in international movement of ornamental flowers has been suggested as the basis for many of the incursions of *L. trifolli* reported globally (Spencer, 1989). The pests are now widely distributed throughout the world‟s tropical and subtropical regions, with Australia remaining one of the few land masses free of its presence.

Unhatched eggs pose the highest risk, as they are contained within the host tissue, and no mines are evident until the larvae hatch and begin to feed. With some treatments used at the point of entry, the leaf miner eggs and larvae are not destroyed.

4.4.3 Establishment potential

Rating: HIGH

L. huidobrensis has a high reproductive rate with the potential to undergo many generations in a season. Together with a wide range of potential hosts, this gives *L. huidobrensis* a high establishment following entry. Northern areas of Australia within the sub-tropical/ tropical zones would be most at risk.

Our climate and the wide range of host plants both in glasshouses and outside provides ideal conditions for establishment.

4.4.4 Spread potential

Rating: HIGH

L. huidobrensis attacks a wide range of ornamental and vegetable crops and transportation of these plants could contribute to its spread. In crops showing active mining, the flies may be seen walking rapidly over the leaves with only short, jerky flights to adjacent leaves. The cut flower and other horticultural industries involve the frequent movement of produce between nurseries and markets, which would assist in the spread of multi-host species such as leaf miners.

4.4.5 Economic impact

Rating: HIGH

L. huidobrensis is a serious pest of potato, vegetables and ornamental plants in the field and glasshouses in many parts of the world (Lange *et al.*, 1957). It has proved a much more serious pest than *L. trifolii* in Israel (Weintraub and Horowitz, 1995).

Damage is caused by larval mining in the leaves and petioles. The photosynthetic ability of the plants is often greatly reduced as the chlorophyll-containing cells are destroyed. Severely infested leaves may fall, exposing plant stems to wind action, and flower buds and developing fruit to scald (Musgrave *et al.*, 1975). The presence of unsightly larval mines and adult punctures in the leaf palisade of ornamental plants can further reduce crop value (Smith *et al.*, 1962; Musgrave *et al.*, 1975). In young plants and seedlings, mining may cause considerable delay in plant development, leading to plant loss.

As control pesticides are ineffective at controlling *L. huidobrensis* (eggs and larvae protected within host tissue), additional pest management measures would have to be put in place to control this pest if it became established.

4.4.6 Environmental impact

Rating: MEDIUM

L. huidobrensis is polyphagous, and would attack a wide range of native *Solanum* as well as some *Amaranthus, Gossypium, Abelmoschus* and *Cassia* species. It is unlikely that populations of these plants would be threatened to any significant extent.

The chemical control of leafminers is difficult, and the systemic and translaminar products effective in their control may not be needed for use against any other pests. This may cause additional environmental pollution.

In endemic environments, populations of *Liriomyza* species are balanced by natural enemies such as small parasitic wasps, predatory muscid flies, entomophagous nematodes and fungal pathogens (Murphy & La Salle, 1999). However, natural enemies are more susceptible than *Liriomyza* pests to the broad-spectrum insecticides that have been extensively used in agriculture over the last 50 years. The survival of resistant strains of Agromyzidae in the absence of natural enemies has resulted in very large and damaging populations of *Liriomyza* pest species.

4.4.7 Human health impact

Rating: LOW

If used inappropriately, some of the products used exclusively in the control of *L. huidobrensis* may pose a minor health risk to applicators and consumers.

4.4.8 Overall risk

Rating: HIGH

Based on the individual ratings above, the combined overall risk is considered **high**.

5 Pest management

5.1 Response checklist

The following checklist (Table 3) provides a summary of generic requirements to be identified and implemented within a Response Plan.

Table 3. *Checklist of requirements to be identified in a Response Plan*

Checklist item	Further information
Destruction methods for plant material, soil and disposable items	Sections 6.1.1 and 6.1.2
Disposal procedures	Section 6.1.5
Quarantine restrictions and movement controls	Section 6.3
Decontamination and property cleanup procedures	Section 6.5
Diagnostic protocols and laboratories	Sections 4.3 and 9.2
Trace back and trace forward procedures	Section 6.6
Protocols for delimiting, intensive and ongoing surveillance	Section 5.2
Zoning	Section 6.4
Reporting and communication strategy	See PLANTPLAN

A range of specifically designed procedures for the emergency response to a pest incursion and a general communication strategy refer to PLANTPLAN (Plant Health Australia, 2009). Additional information is provided by Merriman and McKirdy (2005)[7] in the Technical Guidelines for Development of Pest Specific Response Plans.

5.2 Surveys and epidemiology studies

Information provided in Section 5.2.1 to 5.2.3 provides a framework for the development of early detection and delimiting surveys for Serpentine leaf miner in Australia.

Where Serpentine leaf miner is found in a production nursery that is in close proximity to potential host trees and shrubs, periodically inspect nearby hosts for signs of Serpentine leaf miner infestation. Infested sources within the nursery may provide an opportunity for Serpentine leaf miner to spread to trees and shrubs outside the nursery.

Agricultural inspectors and other nursery visitors should avoid moving contaminated plant material between nurseries. Shoes, tools and vehicle tyres should be thoroughly washed of soil and then sanitised with a registered disinfectant. Extra precaution should be taken when working in areas known to be infested, including disposable overboots that may be used and disposed of on-site.

[7] Available on the PHA website (**www.planthealthaustralia.com.au/go/phau/biosecurity/general-biosecurity-information**)

5.2.1 Technical information for planning surveys

When developing surveys for *L. huidobrensis* presence and/or distribution, the following characteristics of the pest provide the basic biological knowledge that informs the survey strategy:

- The Agromyzidae are a well-known group of small, morphologically similar flies whose larvae feed internally on plants as leaf and stem miners. Some highly polyphagous species have become important pests of agriculture and horticulture in many parts of the world. The key species are not present in Australia and pose a significant quarantine threat to Australian agriculture and horticulture.
- *L. huidobrensis* is similar to a number of *Liriomyza* spp. which need to be considered in diagnosing samples.
- Endemic host species in Australia are likely to be numerous and widely dispersed.
- The risk of pest movement on machinery, equipment and personal effects is high.
- Significant proportions of Australia have favourable climatic conditions for *L. huidobrensis* spread and establishment.

5.2.2 Surveys for early detection of an incursion in a nursery

Effective ways to monitor *L. huidobrensis* in commercial nurseries are:

- The small black and yellow flies may be detected where they fly closely around host plants or move erratically and rapidly upon the leaf surfaces.
- Sweep nets should be used to collect adults.
- Yellow sticky traps are effective for ongoing targeted surveillance, especially ones covered with 3-phenylopriionaldehyde that are placed at plant height (Weintraub & Horowitz, 1996). Yellow sticky traps can be used for ongoing surveillance by placing at least one trap per 900 m^2, a rate as used in an Insect Pest Management (IPM) strategy against established populations (Heinz and Chaney, 1995).
- Visual inspections – examine the leaf surface for punctures of the epidermis and the obvious greenish-white mines with linear grains of frass along their length (see Figure 5 to Figure 8). For accurate identification, examination of the leaf mine and all stages of development are crucial. The larvae will be found feeding at the end of the mine, or the mine will end with a small convex slit in the epidermis where the larva has left the mine to pupate on the ground. Sometimes the pupa may be found adhering to the leaf surface, although in most cases the fully fed larva will have found its way to the ground beneath the plant to pupate. This is especially true in hot, dry conditions where the larva/pupa would quickly desiccate if exposed on the leaf surface. Empty pupal cases can be found on the ground, and are split at the anterior end, but the head capsule is not usually separated from the rest of the case.

Targeted surveillance should be focussed on high-risk areas. These are commercial propagators and production nurseries involved with the import and export of cutflowers and other nursery produce.

If an incursion of *L. huidobrensis* is to be eradicated in a nursery, it must be detected early, before the insect has had the opportunity to disperse to a large extent. It is therefore necessary to consider pathways and plan surveys accordingly. Important points to consider when developing early detection surveys are:

- The greatest entry risk currently comes from importations of host plants or other goods. Therefore surveys at importing nurseries and ports are required.
- Awareness information should be targeted at people who are in regular close contact with potential hosts in high risk areas or movement vectors (e.g. production nursery operators).
- Systematic and careful inspection of nursery crops and propagative plant material is essential to prevent introduction of *L. huidobrensis* and limit its spread within and from infested nurseries. Early detection of the pest, while at very low levels, will provide the best chance of eradication.
- An inspector must be trained to recognize the basic identification of all stages of *L. huidobrensis*, including egg masses, nymphs and adults as well as other endemic leafminers for comparison (see Section 4.3). A nursery layout map that includes approximate locations of known host species will be required to develop a strategy for surveys. A survey map should include species and cultivar names, locations, approximate quantity and sources of targeted plants within the area. During the survey walkthrough, record the date, observations, and sampling information directly onto the survey map. The recorded information should be reviewed and used to develop the most efficient survey strategy each time the nursery is inspected.
- Begin the inspection with an overview of the area from the crop perimeter or with a quick walk-through. If suspicious symptoms or stages of *L. huidobrensis* are apparent, immediately examine them more closely and collect samples if required. If no symptoms are apparent from the overview inspection, start the complete inspection by walking a systematic path through the crop. A common survey technique is to move relatively quickly down a walkway and scan both sides of adjacent production beds, back and forth. If suspicious symptoms are seen, inspect plants more closely. A good-quality 10x magnification hand lens can help identify many pest symptoms. If plants are found with suspicious symptoms or stages of *L. huidobrensis* are apparent, a sample should be taken and the plant marked with plastic tape or a flag with the location noted on the survey map. Also, a few plants can be selected at random to closely inspect for *L. huidobrensis*, particularly cryptic stages, such as eggs. Surveys can be prioritised to highest risk stock.
- Stock or cuttings of hosts from outside sources should be monitored closely. Note outside sourced plants on survey maps for weekly examination.

5.2.3 Delimiting surveys in the event of an incursion

- In the event of an incursion, delimiting surveys will be required to inform the decision-making process.
- The size of the survey area will depend on the size of the known infested area and the insect population size, as well as prevailing winds and movement of plant material during the period prior to detection.
- Initial surveys should be carried out in a 1.5 km radius of the initial detection.
    - The survey area to 1.5 km radius around each new detection.
- All potential host species (refer to Section 4.2 and 9.1) should be surveyed, with particular attention paid to the species in which the pest was initially detected.

- Depending on the time of year as well as the crop area within the survey radius, soil and litter as well as other likely overwintering sites should be included in samples. If more than 20 infestation sites are found in a specific geographic area, a serious infestation is suggested.
- If the incursion is in a populated area, publication and distribution of information sheets and appeals for public assistance may assist.

5.2.4 Collection and treatment of samples

Protocols for the collection, transport and diagnosis of suspect Emergency Plant Pests (EPPs) must follow PLANTPLAN (Plant Health Australia, 2009). Any personnel collecting samples for assessment should notify the diagnostic laboratory prior to submitting samples to ensure expertise is available to undertake the diagnosis. Sampling techniques for *L. huidobrensis* have been taken from the „Diagnostic protocol for the detection of leaf miners"[8] prepared by Malipatil and Wainer (2006).

All sample containers should be clearly labelled with the name, address and contact phone number of both the sending and receiving officers. In addition containers should be clearly labelled in accordance with the requirements of PLANTPLAN (Plant Health Australia, 2009). Containers should be carefully sealed to prevent loss, contamination or tampering of samples. The Chief Plant Health Manager will select the preferred laboratory. Additional labelling includes the identification of plant species/parts affected, location of affected plant (where available include GPS reading) as well as symptoms and an image if available.

Refer to PLANTPLAN for packing instructions under IATA 650.

5.2.4.1 COLLECTION OF SPECIMENS

Sampling procedures

Samples can be collected by hand, using a vacuum sampler, using yellow sticky traps or swept from foliage with a hand net. Adults are normally found on the foliage. However the most practical and reliable method is the collection of leaves with mines containing pupae or mature larvae in a jar for rearing in the laboratory to obtain adult flies.

Yellow sticky traps

Adults are attracted to the yellow colour of sticky traps, and detections via this method can be used as a indicator of leaf miner presence but not of parasitoid activity. Traps should be placed between plant rows at either 30 cm off the ground or 20-30 cm above the plant canopy height. In nurseries, trap densities should be set at one trap per 100 m^2.

Traps should be monitored weekly, and their condition checked as they can be ruined by rain, dust and other insects. Peak detection of Serpentine leaf miner using sticky traps overseas occurs just after sunrise in warm weather.

Number of specimens to be collected

Where possible, collect multiple specimens representative of all life stages of the population available. It is important for at least one of the specimens to be an adult male. Adult females are identifiable as

[8] Available from the Pest Information Document Database (**www.planthealthaustralia.com.au/pidd**)

with certainty only to genus level, and males are needed to examine genitalia details to confirm species identification.

Record the identity of the host plant where the fly was collected. Record the location, preferably as GPS co-ordinates, or alternatively, a map reference or distance and direction from a suitable landmark. If the land is privately owned, record the owner‟s details including contact telephone numbers.

How to preserve L. huidobrensis

Adults and nymphs can be placed in 70% ethanol and stored indefinitely. Adults can also be stored dried but will become brittle which can result in damage in transit. Specimens required for molecular diagnostic work should be killed and preserved in absolute ethanol or frozen (-80°C).

How to transport L. huidobrensis

Vials of ethanol should be sealed to avoid leakage and packed with cushioning material in a strong box. Dried specimens can be packed in tissue in an uncrushable vial, which is then packed with cushioning material in a strong box.

How to collect and send plant samples with eggs, larvae or pupae

Winged adults are the preferred stage for identification. However, if adult *L. huidobrensis* specimens are not available, eggs, larvae and pupae should be collected and would need to be reared to the adult stage for species identification in an appropriate quarantine facility.

Leaves with suspect egg masses or mines with larvae present should be picked and refrigerated (not frozen). Leaves should be removed and placed in a sealed plastic bag between moist sheets of newspaper. Each sealed bag should be placed in a second bag along with additional paper to absorb excess moisture. Bagged samples should then be placed in a non-crushable container with paper/bubble/foam to fill the remaining space and protect samples during transit.

Precaution

Overheating or desiccation of samples prior to despatch should be prevented. Samples may be stored in a fridge (4-10°C) for a few days if necessary.

Receipt

On receipt of the samples the diagnostic laboratory should follow strict quarantine and processing guidelines. In keeping with ISO 17025 refer to PLANTPLAN (Plant Health Australia, 2009).

5.2.5 Epidemiological study

The extent of infestation in a nursery, on a property or within a region will depend on the initial population size and whether conditions have been favourable for the pest to spread from the initial location. Sampling should be based upon the origins of the initial suspect sample(s). Factors to consider will be:

- The proximity of other susceptible plants to the initial infestation source, including both current and previous crops. This will include crops in the nursery or on the property with the initial detection and those on neighbouring properties.
- Machinery or vehicles that have been into the infested area or in close proximity to the infestation source.
- The extent of human movements into and around the infested area. A possible link to the recent importation of plant material from other regions should also be considered.
- The source of any nursery stock propagation material.
- If any other crops have been propagated from the same source and/or distributed from the affected nurseries.
- Serpentine leaf miner does not tolerate hot (<30°C) temperatures.
- Cold temperatures reduce leaf miner activity, but Serpentine leaf miner will survive Australian winters in diapause.
- Leaf miners have a high rate of fecundity under warm conditions.
- Distribution of insects is generally aggregated in crops.
- Adults are moderate-weak fliers, normally travelling less than 1 km by flight, with a maximum distance of less than 10 km, unless assisted by wind.

5.2.6 Models of spread potential

Modelling data is limited but there has been some work done looking at the spread of *Agromyza frontella* (alfalfa blotch leafminer) which has been estimated to be spreading at a rate of 96 km per year in Minnesota (Hutchison *et al.* 2007; Venette et al 1999). Milla & Reitz (2005) used the biological data on *L. huidobrensis* derived by Lanzoni *et al.* (2002) to develop a simple spatial/temporal model for *L. huidobrensis* in Florida. Similar models can be easily developed for all species. Jones & Parrella (1986) studied movement and dispersal of *L. trifolii* in a chrysanthemum greenhouse. They found that female flies flew further on average (21.5 m) than male flies (18.0 m). However, both sexes were also caught, albeit in low numbers at the extremity of the house (102 m) (Taylor 1978).

5.2.7 Pest Free Area guidelines

Determination of Pest Free Areas (PFAs) should be completed in accordance with the International Standards for Phytosanitary Measures (ISPMs) 8 and 10 (IPPC, 1998a, 1999).

General points to consider are:

- Design of a statistical delimiting field survey for the presence of the insect on host plants (see Section 5.2 for points to consider in the design).

- Surveys should be completed as described in the BioSecure HACCP manual (Nursery and Garden Industry Australia, 2008), including monitoring processes (summarised in Table 4 and Table 5), indicator plants and weed monitoring.
- Surveys should also consider alternative hosts (see Sections 4.2.1 and 9.1) and not be limited to the primary infested host.
- Information (including absence of the pest) should be recorded.

Table 4. *Summary of monitoring processes for protected production areas as described in BioSecure HACCP Guidelines*

Wear protective clothing when handling suspect samples
Walk at random through the area in a zigzag pattern
Take at least 10 minutes to inspect 10-20 plants or plug trays per 100 m^2 of production area
Inspect the tops and bottoms or leaves, looking for any direct evidence of insects
Inspect the entire plant if it has less than 6 leaves, or from larger plants select six leaves from all parts of the plant (upper, lower, middle) and examine them individually
Inspect the length of all stems and branches for insects and symptoms
During individual plant inspection, strike the foliage over a white sheet of paper or a paper plate to dislodge small insects for easier viewing
If any plants show suspect symptoms or evidence of eggs or nymphs (refer to Section 4.2.3) take a sample (refer to Section 5.2.4) to be formally diagnosed (refer to Section 4.3)
Check for a problem that have occurred regularly in the past, until you are certain it is not present
Record on the „Crop Monitoring Record" sheet the presence or absence of the pest
Routinely inspect growing areas and remove alternate hosts and reservoirs of the pest, including weeds, crop residues and old plants that will not be marketed

Additional information is provided by the IPPC (1995) in Requirements for the Establishment of Pest Free Areas. This standard describes the requirements for the establishment and use of PFAs as a risk management option for phytosanitary certification of plants and plant products. Establishment and maintenance of a PFA can vary according to the biology of the pest, pest survival potential, means of dispersal, availability of host plants, restrictions on movement of produce, as well as PFA characteristics (size, degree of isolation and ecological conditions).

Table 5. *Summary of monitoring processes for field production areas as described in BioSecure HACCP Guidelines*

Wear protective clothing when handling suspect samples
Pay particular attention to areas on the windward side, the sides bordering ditches, canals or other uncultivated areas and growing block centres
Place a flag or other marker at the entrance to the block or sampling area at the beginning of each inspection
Vary the entrance point in the sampling area (1m to 3m) for each subsequent sampling so that the same plants are not inspected each time
Walk at random through the area in a zigzag pattern
The scout should follow the same general pattern at each sampling
Make an effort to select those plants that appear less healthy for visual inspection
Take at least 10 minutes to inspect 10-20 plants or plug trays per 100 m^2 of production area
Inspect the tops and bottoms or leaves, looking for any direct evidence of insects
Inspect the entire plant if it has less than 6 leaves, or from larger plants select six leaves from all parts of the plant (upper, lower, middle) and examine them individually
Inspect the length of all stems and branches for insects and symptoms
During individual plant inspection, strike the foliage over a white sheet of paper or a paper plate to dislodge small insects for easier viewing
If any plants show suspect symptoms or evidence of eggs or nymphs (refer to Section 4.2.3) take a sample (refer to Section 5.2.4) to be formally diagnosed (refer to Section 4.3)
Check for a problem that have occurred regularly in the past, until you are certain it is not present
Record on the „Crop Monitoring Record" sheet the presence or absence of the pest
Routinely inspect growing areas and remove alternate hosts and reservoirs of the pest, including weeds, crop residues and old plants that will not be marketed

5.3 Availability of control methods

5.3.1 General procedures for control

- Keep traffic out of affected areas and minimize movement in adjacent areas.
- Adopt best-practice property hygiene procedures to retard the spread of the pest between fields and adjacent properties.
- After surveys are completed, destruction of the infested plant material is an effective control.
- On-going surveillance of infested areas to ensure the pest is eradicated.
- Do not use any material from infested plants for propagation.

5.3.2 Chemical control

Some insecticides, particularly pyrethroids (abamectin) and also cyromazine (Van der Staay, 1992; Leuprecht, 1993) are effective, but leaf miner resistance can sometimes make control difficult (Ferguson 2004; Parella and Keil 1984; Parrella *et al*., 1984; Macdonald, 1991). Application of ineffective insecticides often results in a larger leafminer problem as the pesticide reduces field densities of leafminer parasitoids. The insecticides cyromazine (Trigard®), abamectin (Avid®) and spinosad (Success®) have been shown to be effective against these leafminer pests since they target larvae inside the leaves.

Any chemicals used for the eradication or control of *L. huidobrensis* in Australia must be registered for use through the Australian Pesticides and Veterinary Medicines Authority (APVMA). For information regarding this process visit the APVMA website (**www.apvma.gov.au**).

5.3.3 Biological control

A review of natural enemies and biological control of *L. huidobrensis* is provided by Waterhouse and Norris (1987). Natural enemies periodically suppress leaf miner populations (Spencer, 1973). *Dacnusa sibirica* (Van de Veire, 1991; Leuprecht, 1992), *Opius pallipes* and *Diglyphus isaea* (Van der Linden, 1991; Benuzzi & Raboni, 1992) are under consideration for use as natural enemies of the pest in European glasshouses.

Natural enemies include the following parasitoids: A*grostocynips clavatus, Chrysocharis* spp., *Diglyphus* spp., *Ganaspidium utilis, Halticoptera circulus, Oenogastra* and *Opius* spp.

Dacnusa sibirica is used as a biocontrol agent in glasshouses in Germany, but requires three to four releases per week (Leuprecht, 1992). In Dutch glasshouses, successful control was achieved using releases of *D. sibirica* in combination with *Opius pallipes*, in conjunction with the naturally present *Diglyphus isaea* (Linden, 1991). In Austria it was found that these parasitoids could be used in combination with cyromazine (Stolz and Lenteren, 1996).

Control using nematodes has been shown to be successful; Williams and Macdonald (1995) used foliar applications of *Steinernema feltiae* and species of *Heterorhabditis* (strain UK 211).

Salvo and Valladares (1995) reviewed the parasite complex of *L. huidobrensis* on faba beans (*Vicia faba*) in Colombia (part of its native range) and found that larval/pupal parasitoids caused a higher level of parasitism than purely larval parasitoids and that *Opius scabriventris* was the dominant species (52% parasitism). In Costa Rica, Hidalgo and Carballo (1991) found that weeds acted as natural reservoirs for parasitoids and in the same area Carballo *et al.* (1990) found that *Diglyphus* sp. was of similar importance to *Opius* spp.

5.4 Market access impacts

Within the AQIS PHYTO database (**www.aqis.gov.au/phyto**), export of plants (nursery stock, bulbs, cuttings or tissue culture) or fresh fruit or vegetables for a range of countries requires an additional declaration regarding freedom from *L. huidobrensis* (as at September 2009). Should *L. huidobrensis* be detected or become established in Australia, additional countries may require a specific declaration or supplementary measures upon export. Latest information can be found within PHYTO, using an Advanced search "Search all text" for *Liriomyza huidobrensis.*

6 Course of action

Additional information is provided by the IPPC (1998b) in Guidelines for Pest Eradication Programmes. This standard describes the components of a pest eradication programme which can lead to the establishment or re-establishment of pest absence in an area. A pest eradication programme may be developed as an emergency measure to prevent establishment and/or spread of a pest following its recent entry (re-establish a PFA) or a measure to eliminate an established pest (establish a PFA). The eradication process involves three main activities: surveillance, containment, and treatment and/or control measures.

The course of action to a *L. huidobrensis* incursion outlined in this document relates only to *L. huidobrensis* and not other exotic leaf miner species. Should the incursion be identified as another *Liriomyza* spp., alternative response strategies may be needed.

6.1 Destruction strategy

6.1.1 Destruction protocols

- General protocols:
    - Disposable equipment, infested plant material or growing media/soil should be disposed of by autoclaving, high temperature incineration or deep burial.
    - Any equipment removed from the site for disposal should be double-bagged.
    - Machinery used in destruction processes need to be thoroughly washed, preferably using a detergent or farm degreaser.
- Leaf miner destruction strategy:
    - Knock down adult populations with a surface insecticide and egg, larvae and pupae populations with a translaminar insecticide.
    - Infested plants can then be destroyed by enclosed incineration or deep burial.
        - Cold treatment (0°C) is effective (1-2 weeks for larvae or 3 weeks for eggs).
    - Methyl bromide is effective at killing eggs, larvae and pupae.
    - Planting media should be sterilised by autoclaving or methyl bromide, or disposed of through deep burial.
        - Covering soil with fabric will impede the emergence of adults, but will not destroy the pest.

6.1.2 Decontamination protocols

Machinery, equipment and vehicles in contact with infested plant material or growing media/soil, or present within the Quarantine Area, should be washed to remove plant material and growing media/soil using high pressure water or scrubbing with products such as a degreaser or a bleach solution (1% available chlorine) in a designated wash down area. When using high pressure water, care should be taken not to spread plant material. High pressure water should be used in wash down areas which meet the following guidelines:

- Located away from crops or sensitive vegetation.
- Readily accessible with clear signage.
- Access to fresh water and power.
- Mud free, including entry and exit points (e.g. gravel, concrete or rubber matting).

- Gently sloped to drain effluent away.
- Effluent must not enter water courses or water bodies.
- Allow adequate space to move larger vehicles.
- Away from hazards such as power lines.
- Waste water, growing media/soil or plant residues should be contained (see Appendix 18 of PLANTPLAN [Plant Health Australia, 2009]).
- Disposable overalls and rubber boots should be worn when handling infested plant material or growing media/soil in the field. Boots, clothes and shoes in contact with infested plant material or growing media/soil should be disinfected at the site or double-bagged to remove for cleaning.
- Skin and hair in contact with infested plant material or growing media/soil should be washed.

Procedures for the sterilisation of plant containers and growing media are provided within the BioSecure *HACCP* Guidelines, however, in the event of a *L. huidobrensis* incursion, procedures outlined in the BioSecure *HACCP* Guidelines may not be effective for the destruction of the pest. Any sterilisation procedure must be approved for use in the endorsed Response Plan.

6.1.3 Priorities

- Confirm the presence of the pest.
- Prevent movement of vehicles and equipment through affected areas.
- Stop the movement of any plant material that may be infested with the pest.
- Determine the strategy for the eradication/decontamination of the pest and infested host material.
- Determine the extent of infestation through survey and plant material trace back.

6.1.4 Plants, by-products and waste processing

- Any growing media/soil or infested plant material removed from the site should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial.
- As the pest can be mechanically transmitted, plant debris from the destruction zone must be carefully handled and transported for destruction.
- Infested areas or nursery yards should remain free of susceptible host plants until the area has been shown to be free from the pest.

6.1.5 Disposal issues

- Particular care must be taken to minimize the transfer of infested plant material or insects from the area.
- Host material, including leaf litter, should be collected and incinerated or double bagged and deep buried in an approved site.

6.2 Containment strategies

For some exotic pest incursions where eradication is considered impractical, containment of the pest may be attempted to prevent or slow its spread and to limit its impact on other parts of the state or country. Containment is currently being considered for inclusion within the EPPRD. The decision on whether to eradicate or contain the pest will be made by the National Management Group, based on scientific and economic advice (see Section 3).

6.3 Quarantine and movement controls

Consult PLANTPLAN (Plant Health Australia, 2009) for administrative details and procedures.

6.3.1 Quarantine priorities

- Plant material and growing media/soil at the site of infestation to be subject to movement restrictions.
- Machinery, equipment, vehicles and disposable equipment in contact with infested plant material or growing media/soil, or present in close proximity to the site of infestation to be subject to movement restrictions.

6.3.2 Movement controls

Movement controls need to be put in place to minimise the potential for translocation of the pest as a contaminant of plant material, growing media of other articles.

Movement of people, vehicles, equipment and plant material, from and to affected properties or areas, must be controlled to ensure that the pest is not moved off-property. Movement controls can be achieved through the following, however specific measures must be endorsed in the Response Plan:

- Signage to indicate quarantine area and restricted movement into and within these zones.
- Fenced, barricaded or locked entry to quarantine areas.
- Movement of equipment, machinery, plant material or growing media/soil by permit only. Therefore, all non-essential operations in the area or on the property should cease.
- Where no dwellings are located within these areas, strong movement controls should be enforced.
- Where dwellings and places of business are included within the Restricted and Control Areas movement restrictions are more difficult to enforce, however limitation of contact with infested plants should be enforced.
- If a production nursery is situated within the Restricted Area, all nursery operations must cease and no material may be removed without permission, due to the high likelihood of pest spread. Movement restrictions would be imposed on both host and non-host material.
- Residents should be advised on measures to minimise the inadvertent transport of *L. huidobrensis* from the infested area to unaffected areas.

- Clothing and footwear worn at the infested site should either be double-bagged prior to removal for decontamination or should not leave the site until thoroughly disinfected, washed and cleaned.
- Plant material or plant products must not be removed from the site.
- All machinery and equipment should be thoroughly cleaned down with a high pressure cleaner (see Section 6.1.2) or scrubbing with products such as a farm degreaser or a 1% bleach (available chlorine) solution, prior to leaving the affected area. Machinery should be inspected for the presence of insects and if found treatment with insecticide may be required. The clean down procedure should be carried out on a hard surface, preferably a designated wash-down area, to avoid mud being re-collected from the affected site onto the machine. When using high pressure water, care should be taken to contain all plant material and mud dislodged during the cleaning process.

6.4 Zoning

The size of each quarantine area will be determined by a number of factors, including the location of the incursion, biology of the pest, climatic conditions and the proximity of the infested property to other infested properties. This will be determined by the National Management Group during the production of the Response Plan. Further information on quarantine zones in an EPP incursion can be found in Appendix 10 of PLANTPLAN (Plant Health Australia, 2009). These zones are outlined below and in Figure 10.

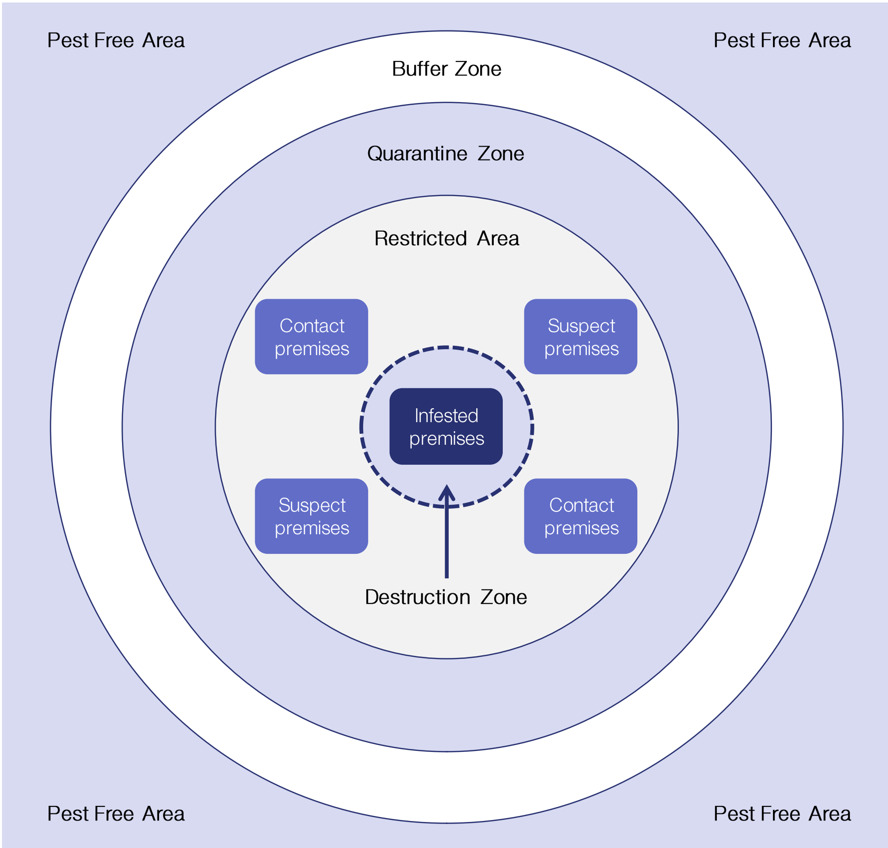

Figure 10. *Schematic diagram of quarantine zones used during an EPP incursion (not drawn to scale)*

6.4.1 Destruction Zone

The size of the destruction zone (i.e. zone in which the pest and all host material is destroyed) will depend on the ability of the pest to spread, distribution of the pest (as determined by delimiting surveys), time of season (and part of the pest life cycle being targeted) and factors which may contribute to the pest spreading.

All host plants should be destroyed after the level of infestation has been established. The delimiting survey will determine whether or not neighbouring plants are infested and need to be destroyed. Non-host plant material within this zone may be destroyed, based on recommendations in the Response Plan. The Destruction Zone may be defined as contiguous areas associated with the same management practices as, or in contact with, the infested area (i.e. the entire nursery, property or forest area if spread could have occurred prior to the infection being identified).

Particular care needs to be taken to ensure that plant material (including non-hosts) is not moved into surrounding areas.

6.4.2 Restricted Area

The Restricted Area is defined as the zone immediately around the infested premises and suspected infested premises. The Restricted Area is established following initial surveys that confirm the presence of the pest. The Restricted Area will be subject to intense surveillance and movement control with movement out of the Restricted Area to be prohibited and movement into the Restricted Area to occur by permit only. Multiple Restricted Areas may be required within a Control Area.

6.4.3 Quarantine Zone

The Quarantine Zone is defined as the area where voluntary or compulsory restraints are in place for the affected property or properties. These restraints may include restrictions or movement control for removal of plants, people, growing media/soil or contaminated equipment from an infested property.

6.4.4 Buffer Zone

A Buffer Zone may or may not be required depending on the incident. It is defined as the area in which the pest does not occur but where movement controls or restrictions for removal of plants, people, soil or equipment from this area are still deemed necessary. The Buffer Zone may enclose an infested area (and is therefore part of the Control Area) or may be adjacent to an infested area.

6.4.5 Control Area

The Control Area is defined as all areas affected within the incursion. The Control Area comprises the Restricted Area, all infested premises and all suspected infested premises and will be defined as the minimum area necessary to prevent spread of the pest from the Quarantine Zone. The Control Area will also be used to regulate movement of all susceptible plant species to allow trace back, trace forward and epidemiological studies to be completed.

6.5 Decontamination and property clean up

Decontaminant practices are aimed at eliminating the pest thus preventing its spread to other areas.

6.5.1 Decontamination procedures

General guidelines for decontamination and clean up:

- Refer to PLANTPLAN (Plant Health Australia, 2009) for further information.
- Keep traffic out of affected area and minimize it in adjacent areas.
- Adopt best-practice property hygiene procedures to retard the spread of the pest between growing areas/fields and adjacent properties.
- Machinery, equipment, vehicles in contact with infested plant material or growing media/soil present within the Quarantine Zone, should be washed to remove growing media/soil and plant material using high pressure water or scrubbing with products such as a degreaser or a bleach solution in a designated wash down area as described in Section 6.1.2.
- Only recommended materials are to be used when conducting decontamination procedures, and should be applied according to the product label.
- Infested plant material should be disposed of by autoclaving, high temperature (enclosed) incineration or deep burial.

6.5.2 General safety precautions

For any chemicals used in the decontamination, follow all safety procedures listed within each MSDS.

6.6 Surveillance and tracing

6.6.1 Surveillance

Detection and delimiting surveys are required to delimit the extent of the outbreak, ensuring areas free of the pest retain market access and appropriate quarantine zones are established.

Initial surveillance priorities include the following:

- Surveying all host growing properties and businesses in the pest quarantine area.
- Surveying all properties and businesses identified in trace-forward or trace-back analysis as being at risk.
- Surveying all host growing properties and businesses that are reliant on trade with interstate or international markets which may be sensitive to *L. huidobrensis.*
- Surveying production nurseries selling at risk host plants.
- Surveying other host growing properties and backyards.

6.6.2 Survey regions

Establish survey regions around the surveillance priorities identified above. These regions will be generated based on the zoning requirements (see Section 6.4), and prioritised based on their potential likelihood to currently have or receive an incursion of this pest. Surveillance activities within these regions will either allow for the area to be declared pest free and maintain market access requirements or establish the impact and spread of the incursion to allow for effective control and containment measures to be carried out. Detailed information regarding surveys for *L. huidobrensis* have been outlined elsewhere in this plan (refer to Section 5.2).

Steps outlined in Table 6 form a basis for a survey plan. Although categorised in stages, some stages may be undertaken concurrently based on available skill sets, resources and priorities.

Table 6. *Phases to be covered in a survey plan*

Phase 1	• Identify properties that fall within the buffer zone around the infested premise • Complete preliminary surveillance to determine ownership, property details, production dynamics and tracings information (this may be an ongoing action)
Phase 2	Preliminary survey of host crops in properties in buffer zone establishing points of pest detection
Phase 3	Surveillance of an intensive nature, to support control and containment activities around points of pest detection
Phase 4	Surveillance of contact premises. A contact premise is a property containing susceptible host plants, which are known to have been in direct or indirect contact with an infested premises or the pest. Contact premises may be determined through tracking movement of materials from the property that may provide a viable pathway for spread of the pest. Pathways to be considered are: • Items of equipment and machinery which have been shared between properties including bins, containers, irrigation lines, vehicles and equipment • The producer and retailer of infested material if this is suspected to be the source of the outbreak • Labour and other personnel that have moved from infested, contact and suspect premises to unaffected properties (other growers, tradesmen, visitors, salesmen, crop scouts, harvesters and possibly beekeepers) • Movement of plant material and growing media/soil from controlled and restricted areas
Phase 5	Surveillance of production and retail nurseries, gardens and public land where plants known to be hosts of the pest are being grown
Phase 6	Agreed area freedom maintenance, post control and containment

6.6.3 Post-eradication surveillance

The period of pest freedom sufficient to indicate that eradication of the pest has been achieved will be determined by a number of factors, including cropping conditions, the previous level of infestation, the control measures applied and the pest biology.

Specific methods to confirm eradication of *L. huidobrensis* may include:

- Monitoring of sentinel plants that have been grown at the affected sites. Plants are to be grown *in situ* under quarantine conditions and monitored for symptoms or other indications of *L. huidobrensis* presence.
- If symptoms or suspect insects are detected, samples are to be collected and stored and plants destroyed.
- Targeted surveys for *L. huidobrensis* should be undertaken within the Quarantine Zone to demonstrate pest absence.
- Alternate non-host crops should be grown on the site and any self-sown plants sprayed out with a selective herbicide.

7 Technical debrief and analysis for stand down

Refer to PLANTPLAN (Plant Health Australia, 2009) for further details.

The emergency response is considered to be ended when either:

- Eradication has been deemed successful by the lead agency, with agreement by the Consultative Committee on Emergency Plant Pests and the Domestic Quarantine and Market Access Working Group.
- Eradication has been deemed impractical and procedures for long-term management of the pest risk have been implemented.

A final report should be completed by the lead agency and the handling of the incident reviewed.

Eradication will be deemed impractical if, at any stage, the results of the delimiting surveys lead to a decision to move to containment/control.

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8.1 Related Websites

Australian Pesticide and Veterinary Medicine Authority (**www.apvma.gov.au**)

CAB Compendium (**www.cabicompendium.org**)

Pest and Disease Image Library (**www.padil.gov.au**)

9 Appendices

9.1 Appendix 1: Extended host list of *Liriomyza huidobrensis*.

Botanical Name	Common Name	Botanical Name	Common Name
Primary hosts			
Allium cepa	onion	*Allium sativa*	garlic
Apium graveolens	celery	*Chrysanthemum x morifolium*	(chrysanthemum (flortists"))
Cucurbita peo	ornamental gourd	*Lactuca sativa*	(lettuce)
Phaseolus vulgaris	bean		
Secondary hosts			
Amaranthus	grain amaranth	*Amaranthus retroflexus*	redroot, Prince of Wales
Aster		*Beta vulgaris*	beetroot
Calendula	marigolds	*Capsicum annum*	bell pepper
Cucumis melo	melon	*Cucumis sativa*	cucumber
Datura		*Galinsoga*	
Gerbera	Baberton"s daisy	*Gypsophila paniculata*	babysbreath
Lathyrus	vetchling	*Linum*	
Lycopersicum esculentum	tomato	*Medicago sativa*	lucerne, alfalfa
Melilotus	melilots	*Petunia*	Petunia
Pisum sativum var. *arvense*	Austrian winter pea	*Solanum melongena*	aubergine
Solanum tuberosum	potato	*Spinacia oleracea*	spinach
Tagetes	marigold	*Tropaeolum*	nasturtium
Vicia faba	broad bean		
Wild hosts			
Bidens pilosa	spanish needle	*Emilia sonchifolia*	consumption weed
Galinsoga parviflora	gallant soldier	*Portulaca oleracea*	pigweed
Sonchus	sowthistle	Oxalis	wood sorrels

9.2 Appendix 2: Resources and facilities

Table 7 provides a list of diagnostic facilities for use in professional diagnosis and advisory services in the case of an incursion.

Table 7. *Diagnostic service facilities in Australia*

Facility	State	Details
DPI Victoria – Knoxfield Centre	Vic	621 Burwood Highway Knoxfield VIC 3684 Ph: (03) 9210 9222; Fax: (03) 9800 3521
DPI Victoria – Horsham Centre	Vic	Natimuk Rd Horsham VIC 3400 Ph: (03) 5362 2111; Fax: (03) 5362 2187
I&I NSW – Elizabeth Macarthur Agricultural Institute	NSW	Woodbridge Road Menangle NSW 2568 PMB 8 Camden NSW 2570 Ph: (02) 4640 6327; Fax: (02) 4640 6428
I&I NSW – Tamworth Agricultural Institute	NSW	4 Marsden Park Road Calala NSW 2340 Ph: (02) 6763 1100; Fax: (02) 6763 1222
I&I NSW – Wagga Wagga Agricultural Institute	NSW	PMB Wagga Wagga NSW 2650 Ph: (02) 6938 1999; Fax: (02) 6938 1809
SARDI Plant Research Centre – Waite Main Building, Waite Research Precinct	SA	Hartley Grove Urrbrae SA 5064 Ph: (08) 8303 9400; Fax: (08) 8303 9403
Grow Help Australia	QLD	Entomology Building 80 Meiers Road Indooroopilly QLD 4068 Ph: (07) 3896 9668; Fax: (07) 3896 9446
Department of Agriculture and Food, Western Australia (AGWEST) Plant Laboratories	WA	3 Baron-Hay Court South Perth WA 6151 Ph: (08) 9368 3721; Fax: (08) 9474 2658