Industry Biosecurity Plan for the Grains Industry
Threat Specific Contingency Plan

American serpentine leafminer, *Liriomyza trifolii,* bundled with *L. cicerina, L. huidobrensis, L. sativae, L. bryoniae* and *Chromatomyia horticola*

Prepared by Dr Peter Ridland, Dr Mallik Malipatil
and Plant Health Australia

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1 Purpose of this Contingency Plan

This Contingency Plan provides background information on the biology and available control measures to assist with preparedness for an incursion into Australia of American serpentine leafminer (*Liriomyza trifolii*) and several other agromyzid pest leafminers (*L. cicerina, L. huidobrensis, L. sativae, L. bryoniae* and *Chromatomyia horticola*). It provides guidelines 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.

2 Pest information/status

2.1 Pest Details

Liriomyza trifolii (Burgess, 1880)	Common name; American serpentine leafminer
Liriomyza huidobrensis (Blanchard, 1926)	Common name; pea leafminer, South American leafminer, serpentine leafminer
Liriomyza sativae (Blanchard, 1938)	Common name; vegetable leafminer
Liriomyza bryoniae (Kaltenbach, 1858)	Common name; tomato leafminer
Chromatomyia horticola (Goureau, 1851)	Common name; pea leafminer
Liriomyza cicerina (Rondani, 1875)	Common name; chickpea leafminer

2.1.1 General information

Taxonomic position – Class: Insecta; Order: Diptera; Family: Agromyzidae

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.

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. In contrast, *L. cicerina* is likely to be detected first in broadacre cropping areas.

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 much crop loss and excessive use of broad-spectrum insecticides (Rauf *et al*. 2000). *Liriomyza huidobrensis* is the dominant agromyzid at higher elevations (>1000 m) in tropical Asia and has caused much damage to potato in particular (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 *Liriomyza 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. *Liriomyza trifolii* and *L. bryoniae* are also well established in East and Southeast Asia but are being displaced by *L. sativae* (Abe & Tokumaru 2008).

Griffiths (1967) split *Phytomyza atricornis* into two species, *P. horticola* and *P. syngenesiae*, which could only be distinguished by examination of the male genitalia. Subsequently, these species were placed in genus *Chromatomyia*. Hence, in the literature on *P. atricornis* before 1967, there is uncertainty which species is actually being studied. *Chromatomyia syngenesiae* is a very common agromyzid in Australia and is found on a range of Asteraceae (very common on sow thistle, *Sonchus oleraceus*) and so diagnosis of *C. horticola* will be difficult in the field. Saito (2004) indicated that recent large outbreaks of *C. horticola* on *Pisum sativum* in Japan were associated with a combination of low susceptibility to insecticides in the species and the destruction of the parasitoid complex due to frequent insecticide applications.

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 some 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).

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.

2.1.2 Life cycle

All agromyzid species included in this plan have a very similar lifecycle.

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 (but feeding punctures by *Chromatomyia horticola* and *C. syngenesiae* are distinctly larger), 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. The eggs are inserted just below the leaf surface and hatch in 2-5 days according to temperature. Many eggs may be laid on a single leaf (Parrella 1987).

Eggs are inserted just below the leaf surface. The number of eggs laid varies according to temperature and host plant. There are three larval stages that feed within the leaves. The larvae predominantly feed on the plant in which the eggs are laid. Although the larvae of some species can exit one leaf and enter another, this has not been reported for these species. The larvae of *Liriomyza* spp. leave the plant to pupate (Parrella & Bethke 1984) so pupae may be found in crop debris, in the soil or sometimes on the leaf surface. In contrast, the larvae of *Chromatomyia horticola* pupate inside the leaf at the end of the larval mine. The puparium of Agromyzidae (as with all higher Diptera) consists of the sclerotized integument of the third instar larva enclosing the pupa. Pupariation is adversely affected by high humidity and drought.

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).

2.1.2.1 Liriomyza trifolii (Burgess)

As with all insects, the rate of immature development of *L. trifolii* is dependent on temperature. At a uniform temperature of 28°C one generation cycle can be accomplished in 14-15 days, but at lower temperatures the time taken is progressively longer. In a detailed study of the effects of temperature on the development of *L. trifolii* in the United Kingdom, Miller & Isger (1985) observed that pupae could remain viable outdoors for several months and are able to withstand freezing temperatures. However, they did not detect any diapause. Suss *et al.* (1984) reported diapause in *L. trifolii* (threshold of 16°C) but this has not been substantiated in other studies. The adult can survive temperatures down to about 12°C but does not appear to feed or lay eggs at these lower temperatures. In heated glasshouses where suitable hosts may be grown throughout the year, the breeding and development of *L. trifolii* will be virtually continuous. In cool glasshouses generation rates will be different throughout the seasons, with fairly rapid development during the summer and pupae remaining undeveloped in the soil during the coldest periods (CABI 2006).

Feeding punctures and leaf mines are usually the first and most obvious sign of the presence of *Liriomyza*. They remain intact and relatively unchanged over a period of weeks. The duration of larval development also depends on temperature and probably host plant. Several generations can occur during the year, breeding only being restricted by the temperature and the availability of fresh plant growth in suitable hosts (Spencer 1973).

L. trifolii pupation occurs outside the leaf, in the soil beneath the plant. Pupa development will vary according to season and temperature. Adult emergence occurs 7-14 days after pupation at temperatures between 20°C and 30°C (Leibee 1984). Peak emergence of adult *L. trifolii* occurs before midday (McGregor 1914). Males usually emerge before females and mating takes place from 24 hours after emergence. Adults of *L. trifolii* live between 15 and 30 days. On average, females live longer than males.

Populations of *L. trifolii* from locations outside of the Americas are considered to be the result of introductions (Spencer 1973). As expected, introduced populations contain only a fraction of the variation present in New World populations. Indeed, of the 73 flies sampled from introduced populations ranging across the globe, all were in the *trifolii*-W clade and in fact all but four carried haplotype T-9 (Scheffer & Lewis 2006). A pepper (*Capsicum annuum* L. (Solanaceae))-specialized population was recognized in California (Morgan *et al.* 2000, Reitz & Trumble 2002). Scheffer & Lewis (2006) extended

the known distribution of the pepper-specialized population to include Florida, Mexico, and Honduras. The pepper-feeding clade is nested within the *trifolii*-W clade.

2.1.2.2 Liriomyza huidobrensis (Blanchard)

The life cycle is typical for Agromyzidae and a useful summary is provided by Weintraub & Horowitz (1995).

In Peru, the life cycle 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).

Studies on *L. huidobrensis* developmental rates in lettuce at different constant temperatures (11 to 28±1°C) revealed a linear increase with temperature (Head *et al*. 2002). The theoretical lower threshold temperatures for development for each larval instar 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). They estimated 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.

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 its near relative, *L. sativae* (Chen & Kang 2005 a, 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. Interestingly, Martin *et al*. (2005) found that no *L. huidobrensis* survived the winter in southern Ontario, Canada.

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). The name *Liriomyza langei* has been applied to North American (USA & Hawaii) populations, and the name *L. huidobrensis* applied to Central and South American populations. 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*.

2.1.2.3 Liriomyza sativae (Blanchard)

Pettit *et al*. (1991) calculated a lower threshold for *L. sativae* infesting lima beans of 10°C and used the data to develop a phenology model useful for forecasting distribution of life stages in a glasshouse population. Detailed biological studies of *L. sativae* have also been reported by Haghani *et al.* (2006, 2007) on melon and by Tokumaru & Abe (2003) on kidney bean.

In the USA, *L. sativae* was displaced as the dominant agromyzid pest by *Liriomyza trifolii*. This was attributed, at least in part, to the reduced level of insecticide susceptibility of *L. trifolii* to a wide range of insecticides (Parrella & Keil 1984; Parrella 1987). However, in Japan, *L. trifolii* is being displaced by

L. sativae (Abe & Tokumaru 2008), which is attributed to the higher fecundity of *L. sativae* (Tokumaru & Abe 2003) and to the inability of *Dacnusa sibirica* Telenga, an effective braconid parasitoid of *L. trifolii*, to complete development on *L. sativae*.

Detailed studies of overwintering of *L. sativae* pupae in China found no indication of pupal diapause and suggested the -2°C isotherm of the minimum mean temperature of January was the overwintering range limit for *L. sativae* (Chen & Kang 2005 a, b).

Scheffer & Lewis (2005) used cytochrome oxidase 1 sequence variation to detect three major clades (A, L and W) within *L. sativae*. While further data are required before their suggestion of cryptic species can be substantiated, it was noteworthy that all invasive populations (i.e. samples from countries apart from the Americas) were from the sativae-W clade. Scheffer *et al.* (2006) did observe that invasive populations of *L. sativae* in the Philippines had greater genetic variation than invasive populations of *L. huidobrensis* and *L. trifolii*. It would be worthwhile determining the haplotype of any invasive flies detected in Australia. This may help to pinpoint their origin.

2.1.2.4 Liriomyza bryoniae (Kaltenbach)

A detailed study of the biology of *L. bryoniae* on tomatoes was conducted by Minkenberg & Heldermann (1990) who calculated a lower development threshold of 8.1°C for all the immature lifestages with a thermal constant of 296 day-degrees. These results were very similar to those obtained by Saito (1988) (on melons) (7.4°C with a thermal constant of 288 day-degrees), and by Nedstam (1985) (on tomatoes) (8.1°C with a thermal constant of 303 day-degrees). These data were used by Boot *et al.* (1992) to develop a simulation model describing the population dynamics of *L. bryoniae* and a eulophid parasitoid, *Diglyphus isaea* in glasshouses. In a comparative study, Tokumaru & Abe (2003) found the developmental threshold temperatures for total development of *L. sativae, L. trifolii*, and *L. bryoniae* on kidney bean to be 10.7, 9.8 and 8.1°C, respectively. The thermal constants for *L. sativae, L. trifolii* and *L. bryoniae* were 248, 251 and 317 day-degrees, respectively. The adult emergence rates for all three species were highest at 25°C. The total fecundity was highest for *L. sativae* among the three *Liriomyza* species. Adults of *L. sativae* lived significantly longer than those of *L. trifolii* and *L. bryoniae*. The intrinsic rates of natural increase for *L. sativae, L. trifolii* and *L. bryoniae* were 0.21, 0.17 and 0.12, respectively.

2.1.2.5 Chromatomyia horticola (Goureau)

Wang & Yan (1986) studied the biology of *C. horticola* on pea at 21°C. The egg stage lasted 1-3 days with a hatching rate of 97%. The duration of the 1st instar averaged 2.5 days (mortality rate 65%), the 2nd instar duration was 1.7 days (mortality rate 0%) and the 3rd instar duration was 1.8 days (0% mortality rate). The pupal stage lasted 7.5 days with 83% of pupae giving rise to adults. Oviposition began 1-3 days after emergence and females laid 475 eggs. Adult females lived for up to 35 days and males up to 5 days. Sharma *et al.* (1997) studied the biology of *C. horticola* on pea at 16-22°C. The maximum longevity of females was 56 days. The maximum average production of progeny was 10 female eggs per female on the 32nd day of life span and the gross reproductive rate (GRR) was 186 female eggs per female. Mizukoshi & Togawa (1999) calculated the lower developmental threshold and the thermal constant in the stage from egg to adult emergence to be 6.0°C and 270 day-degrees, respectively. Mitsunaga *et al.* (2006) showed that multiple inseminations of the females were needed to maximise fecundity. They also showed that longevity of males and females was greatly extended when they had access to leaf sap.

2.1.2.6 Liriomyza cicerina (Rondani)

Weigand & Tahhan (1990) noted that in Syria, the first leafminer generation emerged from diapause in late March and the second generation reached peak numbers in mid May. When the host chickpea plants mature the leafminers disappear and it is suspected that they survive the summer and winter as pupae in diapause. In Turkey, Çikman & Civelek (2006) reported that the adults of *L. cicerina* emerged in the second half of April and the first half of May when average temperature was 9.0-14.3°C and the ground temperature was 19.2-21.2°C. The larvae of *L. cicerina* appeared after 3-20 days of adult emergence when the plants were 5-10 cm high. The population densities of adults and larvae reached the maximum level two times in the season, one of them at the end of May, the second at the end of June.

2.2 Affected Hosts

2.2.1 Host range

Liriomyza trifolii is highly polyphagous and has been recorded from 25 families (Spencer 1990). The most important crops attacked are beans, celery, chrysanthemum, cucumber, gerbera, gypsophila, lettuce, onion, potato and tomato (Spencer 1989), as well as peanuts, soybeans, lentils, lupins, faba beans and chickpeas.

Liriomyza huidobrensis is highly polyphagous and has been recorded from 14 families (Spencer 1973, 1990). The most 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.

Liriomyza sativae is a polyphagous pest of many vegetable and flower crops (Spencer 1973, 1990). It has been recorded from nine plant families, although its preferred hosts tend to be in the Cucurbitaceae, Fabaceae and Solanaceae families (Spencer 1973, 1990), as well as peanuts.

Liriomyza bryoniae is highly polyphagous and has been recorded from 16 plant families (Spencer 1990). It is an important pest of tomatoes, cucurbits (particularly melons, watermelon and cucumber) and glasshouse-grown lettuce, beans and lupins (Spencer 1989, 1990).

Chromatomyia horticola is a highly polyphagous species. It has been recorded from 268 genera from 36 families but is most commonly recorded on the Brassicaceae, Fabaceae and Asteraceae families (Spencer 1989, 1990), as well as sunflower, field peas, canola, lentils, lupins and chickpeas.

The extreme degree of polyphagy seen in the *Liriomyza* species described above is unusual; only five of the more than 300 species of the genus are considered to be truly polyphagous (Spencer 1973).

In contrast, *Liriomyza cicerina* has only been recorded from Fabaceae: *Cicer arietinum* (chickpea), *Hymenocarpus circinnatus* (disk trefoil), *Melilotus alba* (white sweetclover), *M. officinalis* (yellow sweetclover), *Ononis* species, including *O. arvensis* (field restharrow), *O. repens* (common restharrow), *O. spinosa* (spiny restharrow). *Liriomyza cicerina* occurs commonly on *Ononis* species in Western Europe and it seems probable that this is the primary host, from which it colonised *Cicer arietinum* when this plant was introduced into Southern Europe from India in classical times (Dempewolf 2004; Spencer 1973, 1990).

Non-commercial hosts:

Because broadleaf weeds and senescent crops may serve as sources of inoculum, destruction of weeds and deep ploughing of crop residues are recommended. Adults experience difficulty in emerging if they attempt to emerge from puparia buried deeply in soil.

2.2.2 Geographic distribution

Liriomyza trifolii is present in many areas of Europe, Asia, Africa, Central America and the Caribbean, North and South America and Oceania. *L. trifolii* is widespread in Cyprus, France, Italy, Spain, Israel, Senegal and Martinique. It has been intercepted, but is not established in Australia. (CABI 2006; EPPO 1997).

Liriomyza bryoniae is present in Europe (Albania, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Malta, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russian Federation, Slovenia, Spain, Sweden, Ukraine, United Kingdom), Asia (China, India, Israel, Japan, Republic of Korea, Nepal, Taiwan, Turkey, Turkmenistan, Vietnam) and North Africa (Egypt, Morocco) (CABI 2006).

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).

Liriomyza sativae is found in Asia (China, India, Indonesia, Iran, Israel, Japan, Jordan, Malaysia, Oman, Philippines, Saudi Arabia, Sri Lanka, Thailand, Timor Leste, Turkey, Uzbekistan, Vietnam, Yemen); Europe (Estonia, Finland, United Kingdom [incursions eradicated]); Africa: Cameroon, Egypt, Nigeria, South Africa, Sudan, Zimbabwe; North America (Canada, Mexico, USA); Central America (Bahamas, Barbados, Costa Rica, Cuba, Dominica, Dominican Republic, Guadeloupe, Guatemala, Honduras, Jamaica, Martinique, Montserrat, Netherlands Antilles, Nicaragua, Panama, Puerto Rico, Saint Kitts and Nevis, Saint Lucia, Trinidad and Tobago); South America (Argentina, Brazil, Chile, Colombia, French Guiana, Peru, Venezuela); Oceania (American Samoa, Cook Islands, Federated States of Micronesia, French Polynesia, Guam, New Caledonia, Northern Mariana Islands, Samoa, Vanuatu) (CABI 2006).

Chromatomyia horticola is widespread in Africa (Cameroon, Cape Verde, Central African Republic, Congo Democratic Republic, Egypt, Eritrea, Ethiopia, Gabon, Gambia, Kenya, Libya, Madagascar, Morocco, Rwanda, Senegal, South Africa, Uganda, Zimbabwe); Asia (China, India, Indonesia, Iraq, Israel, Japan, Democratic People's Republic of Korea, Republic of Korea, Kuwait, Malaysia, Mongolia, Nepal, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam); Europe (Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Lithuania, Macedonia, Malta, Montenegro, Netherlands, Poland, Portugal, Russian Federation, Serbia, Spain, Sweden, Switzerland, Ukraine, United Kingdom) (CABI 2006).

Liriomyza cicerina is found in Africa: Algeria, Egypt, Libya, Morocco, Tunisia; Asia: Afghanistan, India, Iran, Iraq, Jordan, Kazakhstan, Lebanon, Syria, Turkey, Turkmenistan, Uzbekistan; Europe: Albania, Azerbaijan, Bulgaria, Denmark, England, Estonia, Germany, Italy, Portugal, Romania, Slovakia, Spain, Sweden, Ukraine, Yugoslavia (CABI 2006).

2.2.3 Symptoms

2.2.3.1 Liriomyza trifolii (Burgess)

Feeding punctures appear as white speckles between 0.13 and 0.15 mm in diameter. Oviposition punctures are usually smaller (0.05 mm) and are more uniformly round.

The larval mine is on the upper surface of the leaf, in the leaf mesophyll tissue, and is linear, shallow, at first greenish, then later whitish, winding irregularly and frequently forming a secondary blotch. The trails of frass are distinctive in being deposited in black strips alternately at either side of the mine (like *L. sativae*), but becomes more granular towards the end of the mine (unlike *L. sativae*) (Spencer 1973).

In very small leaves the limited area for feeding results in the formation of a secondary blotch at the end of the mine, before pupariation. In Kenya, Spencer (1985) notes the growth of many *L. trifolii* from mines which began with a conspicuous spiral. This is not a characteristic associated with *L. trifolii* on other continents.

2.2.3.2 Liriomyza huidobrensis (Blanchard)

Feeding punctures appear as white speckles between 0.13 and 0.15 mm in diameter. Oviposition punctures are smaller (0.05 mm) and are more uniformly round. 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).

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. The development of the larval damage follows a rather fixed pattern, somewhat different from that of the adult fly population. First, 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, practically the whole above ground part of the plant becomes necrotic and dies. 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).

2.2.3.3 Liriomyza sativae (Blanchard)

The larva forms a narrow, upper surface linear mine, essentially as in *L. trifolii (*Spencer 1989). Populations on a leaf can be very large; Spencer (1989) has recorded 80 larvae on a single leaf of *Ricinus* (Euphorbiaceae).

2.2.3.4 Liriomyza bryoniae (Kaltenbach)

The patterns of the larval mines of *L. bryoniae* and *L. trifolii* are virtually identical. The larvae form narrow, linear mines and avoid the main veins (Spencer 1989).

2.2.3.5 Chromatomyia horticola (Goureau)

The larvae form narrow, linear mines, pupating in the leaf at the end of the mine, with the anterior spiracles projecting through the leaf epidermis. The endemic *C. syngenesiae* is found exclusively on

Asteraceae in Australia (often on *Sonchus*) and so it will be very difficult to distinguish between *C. horticola* and *C. syngenesiae* on the basis of symptoms on Asteraceae (molecular test of larvae or microscopic examination of the male genitalia is required).

2.2.3.6 Liriomyza cicerina (Rondani)

The larva forms a serpentine mine in the mesophyll tissue. In heavy infestations, leaves desiccate and fall prematurely. Yield losses of up to 30% are commonly recorded (Weigand & Tahhan 1990).

2.3 Entry, establishment and spread

Aside from chance entry through incoming imports and arrivals, the invasive agromyzids (apart from *L. cicerina*) 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. trifolii* reported globally (Spencer 1989). The pests are now widely distributed throughout the world's tropical and sub-tropical regions, with Australia remaining one of the few land masses free of its presence.

In terms of the grains industry, it is highly likely that the initial incursions will be in horticultural areas and the grains industries will be facing a secondary attack from these incursions into horticulture if eradication is not achieved.

Entry potential: Low

The likelihood of entry for these *Liriomyza* species is low via pulses as they attacks only leaves and are not associated with seeds. However, on other hosts, particularly ornamentals, the likelihood is much higher as they have a very broad host range (except for *L. cicerina*). The greatest risk lies in eggs which have been deposited in the leaves yet have not hatched and therefore do not show signs of mining. Depending upon the type of treatment at the port of entry, the eggs and larvae may survive treatment.

Establishment potential: High

L. trifolii has a high reproductive rate (15 day life cycle in optimum conditions) with the potential to produce many generations in a season, and a wide range of potential hosts, the likelihood of establishment is high. Particularly northern areas of Australia within the sub-tropical/tropical climate zones are at risk.

Spread potential: Medium

L. trifolii attacks a wide range of ornamental and vegetable crops and transportation of these plants could potentially contribute to its spread. As these leafminers are not active fliers, dispersing within, but not often between the crop(s) by flight, the spread potential is medium. In crops showing active mining, the flies may be seen walking rapidly over the leaves with only short, jerky flights to adjacent leaves.

Economic impact: High

The host range affected by quarantine *Liriomyza* species is extensive. Damage in severe infestations from both leaf-puncturing and larval-mining can lead to total crop losses. Control on edible crops is difficult due to the limited availability of effective treatments and the potential for rapid and explosive development of the pest population (Parrella 1987). Worldwide, outbreaks of *Liriomyza* leafminers are associated with excessive use of broad-spectrum insecticides (Murphy & La Salle 1999). Insecticide resistance in the invasive species makes control difficult. Chemical insecticides are incompatible with the increasing trend to biological control in glasshouses (CABI 2006) and in broad-acre crops. Larvae are susceptible to cold storage (2 weeks at 0°C but pupae may remain viable in soil at cold temperatures (EPPO 1997). *Liriomyza* species have been reported to vector some non-persistent plant viruses (Costa *et al.* 1958; Zitter & Tsai 1977).

Environmental impact: High

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.

Overall risk: High

Liriomyza pest species have been documented as readily establishing after introduction and rapidly spreading. Control is difficult. Economic impacts could be highly significant in most crops and across most cropping areas. Environmental impacts both directly and as reservoirs for crop infection are also likely to be significant. Excluding entry of *Liriomyza* pest species is the preferred scenario.

2.4 Diagnostic information

2.4.1 Diagnostic protocol

For a series of guidelines for the diagnostic testing of *Liriomyza* species, including *L. trifolii,* see the Grains Industry Biosecurity Plan's 'Diagnostic protocol for the detection of leafminers' 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).

2.5 Response checklist

2.5.1 Checklist

Guidelines for Response Checklists are still to be endorsed. The following checklist provides a summary of generic requirements to be identified and implemented within a Response Plan:

- Destruction methods for plant material, soil and disposable items
- Disposal procedures

- Quarantine restrictions and movement controls
- Decontamination and farm cleanup procedures
- Diagnostic protocols and laboratories
- Trace back and trace forward procedures
- Protocols for delimiting, intensive and ongoing surveillance
- Zoning
- Reporting and communication strategy

Additional information is provided by Merriman & McKirdy (2005) in the Technical Guidelines for Development of Pest Specific Response Plans and by Malipatil & Wainer (2006) in National Diagnostic Protocols for the detection of leafminers.

2.6 Delimiting survey and epidemiology study

Delimiting surveys should comprise local surveys around the area of initial detection concentrating on areas of poor growth.

2.6.1 Sampling method

Sampling method taken from the Grains Industry Biosecurity Plan 'Diagnostic protocol for the detection of leafminers' prepared by Malipatil & Wainer (2006). Any personnel collecting insect or leaf samples for assessment should notify the diagnostic laboratory prior to submitting samples to ensure expertise is available to undertake the diagnosis. General protocols for collecting and dispatching samples are available within PLANTPLAN, Appendix 3 (Plant Health Australia 2008a).

Number of specimens to be collected

A large sample of specimens would be preferable. The aim is to obtain an adult male. Adult females are identifiable with certainty only to genus level; therefore males are needed to examine genitalia details to confirm species identification.

Preferred stage to be collected

Of the four life stages (egg, larva, pupa and adult) only adults are identifiable to species using morphological features. Larvae and pupae are identifiable to species using electrophoretic and molecular tests only.

How to collect

Adult flies can be hand collected into glass vials or vacuum collected either with vacuum sampler, or swept from foliage with a hand net. Adult flies 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 large jar for rearing in the laboratory for obtaining adult flies.

How to collect plant samples if required

Leaves with suspect feeding punctures or leaf mines should be picked and placed between sheets of newspaper to permit slow drying. For laboratory rearing of adult flies, mined leaves containing pupae or mature larvae can be collected in a large jar and kept in a constant temperature room for regular checking.

How to preserve plant samples

Leaves with suspect feeding punctures or leaf mines can be stored between sheets of dry newspaper.

How to preserve leafminers

Adults and larvae can be placed in 70% ethanol and stored indefinitely, although their colour fades gradually with time. Specimens required for molecular diagnostic work should be killed and preserved in absolute ethanol or frozen (-80°C).

How to transport leafminers

Vials of ethanol should be sealed to avoid leakage and packed with cushioning material in a strong box.

How to transport plant sample

Leaves with suspect feeding punctures or leaf mines should be mailed as a flat package between sheets of dry newspaper.

2.6.2 Epidemiological study

Points to consider within the epidemiological study of leafminers are provided in Section 2.6.4.

2.6.3 Models of spread potential

No modelling data are available for spread of leafminers in broadacre cropping. 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). Milla & Reitz (2005) used the biological data on *L. huidobrensis* derived by Lanzoni *et al.* 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). They fitted the data to a generalised distance dispersal model (Taylor 1978).

2.6.4 Pest Free Area (PFA) guidelines

Pest free area guidelines relevant to this pest. Points to consider are:

- Statistical field survey for symptoms on host plants: consignments of pot plants, cut flowers and produce left on the premises should be inspected. Inspect 200 stems or units from each consignment.
- Statistical field survey for adult *Liriomyza* flies: install sticky traps at a minimum spacing of 25 traps per hectare (1 per 20m X 20 m). Count and record the number of *Liriomyza* flies on monitoring traps (maintain the same number of traps per unit area in each field to facilitate accurate analysis of pest levels over the outbreak period).
- Plant or soil sampling using appropriate diagnostic tests.
- Survey around irrigation systems, waterways, refuge habitats etc.

2.7 Availability of control methods

The cryptic nature of the pest, hidden and protected within the leaf tissue, makes treatment difficult and therefore a combination of control measures are needed for successful eradication. Good hygiene methods are encouraged, including isolation of newly imported material to prevent any pest associated with a consignment from spreading to other crops.

2.7.1 General procedures for control

- Keep traffic out of affected areas and minimize movement in adjacent areas.
- Adopt best-practice farm hygiene procedures to retard the spread of the pest between fields and adjacent farms.

2.7.2 Control if small areas are affected

Infested area should be sprayed with abamectin or cyromazine (a larvicide currently registered only for sheep blow fly) and plant material then destroyed by burning or deep burial.

2.7.3 Control if large areas are affected

If large areas are affected, it will be very difficult to eradicate, since these polyphagous pests have a wide host range and will certainly infest a range of weeds.

2.7.4 Cultural control

Crop debris should be removed immediately from cropping area and disposed of carefully. *Liriomyza* can complete their life cycle on both cut and unrooted plants, so crop debris remains a source of infestation. Physical removal and careful disposal of plant material (see Section 3.1.4) with visible signs of infestation is the most effective and cheapest control option.

2.7.5 Host plant resistance

Host plant resistance is the most likely tool for managing *Liriomyza cicerina*. Resistant germplasm has been identified (Malhotra *et al.* 2007).

2.7.6 Chemical control

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. However, *L. trifolii* and *L. huidobrensis* commonly are highly resistant to most other insecticide groups such as organophosphates, carbamates and pyrethroids. Application of ineffective insecticides to control the American serpentine leafminer is futile. It usually results in a larger leafminer problem as the pesticide reduces field densities of leafminer parasitoids.

2.7.7 Mechanical control

Destruction of host plants and deep ploughing of crop residues can assist with control as adults experience difficulty in emerging if they attempt to emerge from puparia buried deeply in soil.

2.7.8 Biological control

Parasitoids often provide effective suppression in the field when disruptive insecticides are not used. Leafminer parasitoids tend not to be very host-specific so there will be great difficulty in importing exotic parasitoids. Fortunately, there is a diverse fauna of eulophid and braconid parasitoids already present in Australia (some native, others inadvertent introductions). In southern Australia, common agromyzids, such as *L. chenopodii, L. brassicae*, *C. syngenesiae* (usually on *Sonchus oleraceus*) and *Phytomyza plantaginis* on weeds and other non-crop plants, would act as important reservoirs for populations of parasitoids (e.g. *Diglyphus isaea*) of invasive polyphagous agromyzids (Bjorksten *et al*. 2005; Lambkin *et al.* 2008). However, in the dryland cropping areas of Australia, the existing parasitoid complex would also need to enter into summer diapause or aestivation in order to oversummer successfully because the weed reservoirs would not be present in these areas in summer. In Japan, *Chrysocharis pubicornis* (Zetterstedt), a eulophid parasitoid attacking *Chromatomyia horticola*, has been shown to diapause in summer (Baeza Larios & Ohno 2007). This species is present in Australia but further research is needed in Australia to establish whether key parasitoids, such as *Diglyphus isaea*, exhibit a facultative summer diapause.

Already in Australia, there are suitable parasitoid species for mass-rearing and release in glasshouses, as is currently done in parts of Europe. However, it will be impracticable to mass-release on broad acre crops. It will be far more important to ensure that endemic populations of parasitoids (Bjorksten *et al.* 2005; Lambkin *et al.* 2008) are maintained and promoted by avoiding use of broad-spectrum insecticides against leafminers and other pests. There has been interest in applying entomophagous nematodes in glasshouse situations (Harris *et al*. 1990) but this will be impractical for broad-acre cropping.

3 Course of Action – Eradication Methods

3.1 Destruction strategy

3.1.1 Destruction protocols

- Disposable equipment, infected plant material or 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.

3.1.2 Decontamination protocols

Machinery, equipment, vehicles in contact with infected plant material or soil or present within the Quarantine Area, should be washed to remove soil and plant material using high pressure water or scrubbing with products such as a farm degreaser or a 1% bleach solution in a designated wash down. General guidelines for wash down areas are as follows:

- 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, soil or plant residues should be contained (see PLANTPLAN 2008 Appendix 18) (Plant Heath Australia 2008b).
- Disposable overalls and rubber boots should be worn when handling infected soil or plant material in the field. Boots, clothes and shoes in contact with infected soil or plant material should be disinfected at the site or double-bagged to remove for cleaning.
- Skin and hair in contact with infested plant material or soil should be washed.
- Decon 90 is a suitable detergent for using to decontaminate equipment or personnel.

3.1.3 Priorities

- Confirm the presence of the pest.
- Prevent movement of vehicles and equipment through affected areas.
- Priority of eradication/decontamination of infected host material.

3.1.4 Plants, by-products and waste processing

- Infected plant material should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial.
- All straw from susceptible hosts should be destroyed by burning as pupae will survive for long periods in dry straw.

3.1.5 Disposal issues

- Particular care must be taken to minimize the transfer of infected soil or plant material from the area as pupae may be present.

– Raking and burning infested crops could result in spreading the pest greater distances during the raking phase. Flies would be disturbed and would tend to disperse from the cut material. One option would be to spray the infested crops with an adulticide (e.g. maldison (Martin *et al.* 2006)) before cutting and raking.

3.2 Quarantine and movement controls

3.2.1 Quarantine priorities

– Plant material and soil at the site of infection to be subject to movement restrictions.

– Machinery, equipment, vehicles and disposable equipment in contact with infected plant material or soil to be subject to movement restrictions.

– Agromyzid flies usually only move short distances, and movement or quarantine controls may be effective providing the pests have not been established for a long period of time over a wide area. Most species covered by this Contingency Plan have a wide host range and this must be considered if establishing movement or quarantine controls.

3.2.2 Movement control for people, plant material and machinery

Differing responses have been observed to killing of eggs and larvae using cold treatments. In chrysanthemum cuttings, *L. trifolii* survived cold storage at 1.7°C for at least 10 days. Newly laid eggs of *L. trifolii* in chrysanthemums survived for up to 3 weeks in cold storage at 0°C. Eggs incubated for 36-48 h were killed after 1 week under the same conditions. All stages of larvae were killed after 1-2 weeks at 0°C (Webb & Smith 1970). EPPO (1990) recommendations propose that chrysanthemum cuttings be maintained under normal glasshouse conditions for 3-4 days after lifting to allow eggs to hatch. Subsequent storage of plants at 0°C for 1-2 weeks should then kill the larvae.

Movement of people, vehicle and machinery, from and to affected farms, must be controlled to ensure that infected soil or plant debris is not moved off-farm on clothing, footwear, vehicles or machinery.

- Signage to indicate quarantine area and/or restricted movement in these zones.
- Fenced, barricaded or locked entry to quarantine areas.
- Movement of equipment, machinery, plant material or soil by permit only
- Clothing and footwear worn at the infected site should either be double-bagged prior to removal for decontamination or should not leave the farm until thoroughly disinfected, washed and cleaned.
- Hay, stubble or trash must not be removed from the site.
- All machinery and equipment should be thoroughly cleaned down with a pressure cleaner prior to leaving the affected farm. 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.

3.3 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 infected property to other infected properties.

3.3.1 Destruction zone

Destruction Zone may be defined as contiguous areas associated with the same management practices as the infected area (i.e. the entire trial, paddock or farm if spread could have occurred prior to the infection being identified).

3.3.2 Quarantine zone

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

3.3.3 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.

3.3.4 Restricted Area

The Restricted Area is defined as the zone immediately around the infected premises and suspected infected 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.

3.3.5 Control Area

The Control Area is defined as all areas affected within the incursion. The Control Area comprises the Restricted Area, all infected premises and all suspected infected 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.

3.4 Decontamination and farm clean up

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

3.4.1 Decontamination procedures

General guidelines for decontamination and clean up

- Refer to PLANTPLAN (Plant Health Australia 2008 a,b) for further information.
- Keep traffic out of affected area and minimize it in adjacent areas.
- Adopt best-practice farm hygiene procedures to retard the spread of the pest between fields and adjacent farms.
- Machinery, equipment, vehicles in contact with infected plant material or soil or present within the Quarantine Area, should be washed to remove soil and plant material using high pressure water or scrubbing with products such as Decon 90 detergent, a farm degreaser or a 1% bleach solution in a designated wash down area as described in 3.1.2.
- Only recommended materials should be used when conducting decontamination procedures, and should be applied according to the product label.

3.4.2 Decontamination if pest is identified in a small or large areas

Where crops are left in situ to dry out, kill any remaining adult leafminers with a weekly treatment. The final treatment should be carried out on the night preceding the removal of the crop. The residual crop debris must be removed quickly and efficiently. If material is in a nursery or glasshouse, it should be kept in a covered container, under a polythene sheet, or in sealed bags, to prevent adult flies from escaping. Alternatively, crop debris may be disposed of by incineration or by deep burial (at a depth of at least 1 m).

3.4.3 General safety precautions

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

3.5 Surveillance and tracing

3.5.1 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 in the pest quarantine area;
- surveying all properties identified in trace forward or traceback analysis as being at risk;
- surveying all host growing properties that are reliant on trade with interstate or international markets which may be sensitive to *Liriomyza* spp. presence;
- surveying commercial nurseries selling at risk host plants; and
- surveying other host growing properties and backyards.

3.5.2 Survey regions

Establish survey regions around the surveillance priorities identified above. These regions will be generated based on the zoning requirements (section 3.3), 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.

Steps outlined below 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.

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 infected plants. Contact premises may be determined through tracking movement of materials from the property that may provide a viable pathway for spread of the disease. 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 infected material if this is suspected to be the source of the outbreak;
- Labour and other personnel that have moved from infected, contact and suspect premises to unaffected properties (other growers, tradesmen, visitors, salesmen, crop scouts, harvesters and possibly beekeepers);
- Movement of plant material and soil from controlled and restricted areas; and
- Storm and rain events and the direction of prevailing winds that result in air-born dispersal of the pathogen during these weather events.

Phase 5:

Surveillance of nurseries, gardens and public land where plants known to be hosts of leafminers are being grown.

Phase 6:

Agreed area freedom maintenance, post control and containment.

3.5.3 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 the life cycle duration of the leafminer species concerned (in relation to temperature), cropping conditions, the previous level of infestation and the control measures applied. As a guide, the period of pest freedom required to confirm eradication should be no less than two generations of the pest.

- Establishment of sentinel plants at the site of infection (see Section 2.6.4).
- Maintain good sanitation and hygiene practices throughout the year.
- The monitoring traps or sentinel plants should remain in place and inspected on a fortnightly basis for a further 6 weeks and then on a monthly basis.
- Surveys comprising plant sampling for *Liriomyza* spp. or *C. horticola* to be undertaken for a minimum of 12 months after eradication has been achieved.

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5 Appendices

Appendix 1. Standard diagnostic protocols

Refer to the Grains Industry Biosecurity Plan, produced by Plant Health Australia, for Mallik Malipatil and John Wainer's 'Diagnostic protocols for the detection of leafminers'.

The recently published "Malipatil, M. & Ridland, P. (2008). Polyphagous Agromyzid Leafminers – identifying polyphagous agromyzid leafminers (Diptera: Agromyzidae) threatening Australian primary industries" both as a CD Rom and live online version provides up-to-date diagnostic keys and other related information on all species included in this plan.

For a range of specifically designed procedures for the emergency response to a pest incursion refer to Plant Health Australia's PLANTPLAN.

Appendix 2. Experts, resources and facilities

The following table lists the experts who can be contacted for professional diagnostics and advisory services in the case of an incursion.

Expert	State	Details
Dr Mallik Malipatil	Vic	DPI Victoria PMB 15, Ferntree Gully DC Vic 3156 Ph: (03) 9210 9222; Fax: (03) 9800 3521
Dr Peter Ridland	Vic	Consulting Entomologist 44 Gladstone Avenue, Northcote Vic 3070 Ph (03) 9486 3679; M 0437 885 116

The following table lists the facilities available for diagnostic services 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

DPI New South Wales Elizabeth Macarthur Agricultural Institute	NSW	Woodbridge Road Menangle NSW 2568 PMB 8 Camden NSW 2570 Ph: (02) 4640 6327; Fax: (02) 4640 6428
DPI New South Wales Tamworth Agricultural Institute	NSW	4 Marsden Park Road Calala NSW 2340 Ph: (02) 6763 1100; Fax: (02) 6763 1222
DPI New South Wales 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 5064 South Australia 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

Appendix 3. Communications strategy

A general Communications Strategy is provided in PLANTPLAN

Appendix 4. Market access impacts

Due to widespread worldwide distribution of some of these leafminer species such *as L. trifolii*, *L. huidobrensis* and *C. horticola* there are few phytosanitary requirements on the export of grains. As of August 2008, restrictions are listed within the AQIS PHYTO database for export of cut flowers, nursery stock and bulbs to many countries. Latest information can be found within PHYTO, using an Advanced search “Search all text” for *Liriomyza.*