# Industry Biosecurity Plan for the Grains Industry Threat Specific Contingency Plan

## **Cereal Leafminers**

# Agromyza ambigua, Agromyza megalopsis, Cerodontha denticornis, Chromatomyia fuscula, Chromatomyia nigra

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# 1 Purpose 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 a group of cereal-infesting leafminers (*Agromyza ambigua*, *A. megalopsis*, *Cerodontha denticornis*, *Chromatomyia fuscula* and *Ch. nigra*). 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 (Plant Health Australia, 2008) and be endorsed by the National Management Group prior to implementation.

## 2 Pest information/status

#### 2.1 Pest details

Scientific name	Common names
Agromyza ambigua (Fallén, 1823)	leafminer
Agromyza megalopsis (Hering, 1933)	barley leaf-mining fly, barley miner fly
Cerodontha denticornis (Panzer, 1806)	wheat sheath leafminer
Chromatomyia fuscula (Zetterstedt, 1838)	oat leafminer fly, grass leafminer
Chromatomyia nigra (Meigen, 1830)	grass leafminer, wheat leafminer, corn linear leafminer

#### 2.1.1 General information

Taxonomic position – Order: Diptera; Family Agromyzidae; Subfamily Agromyzinae (*Agromyza ambigua*, *A. megalopsis*) or Phytomyzinae (*Cerodontha denticornis*, *Chromatomyia fuscula*, *Ch. nigra*)

The Agromyzidae are a well-known group of small, morphologically similar flies whose larvae feed internally on plants, often as leaf and stem miners. A number of agromyzid leafminer species are oligophagous pests of cereal and grass species, attacking a number of host species within the Poaceae family. Table 1 lists 31 agromyzid species considered as pests of cereals worldwide [largely derived from Spencer (1973, 1990) and Darvas & Papp (1985)].

In all cases, the pest species are not considered to be of major importance to cereal production. Spencer (1973) suggested

"Leaf-mining damage by this group is normally not severe, but causes concern when very young plants are attacked or when populations become abnormally large."

Typically outbreaks of agromyzids on cereals are very sporadic. For example, large outbreaks of *Agromyza megalopsis* occurred in Germany in 1964 and 1965 but have since not been a problem there (Geigenmüller 1966; Spencer 1973). Similarly in 1965, a large outbreak of *A. nigrella*, very similar to *A. megalopsis*, was recorded in England (Duthoit 1968).

Spencer (1973) also gives the example of *Cerodontha (Poemyza) lateralis* which normally only occurs in Europe in low numbers. However, he cited two severe outbreaks – one near Moscow in 1886 and another in the Ukraine in 1929, but observed that

"...it has since not been reported as a pest of any consequence."

Table 1. Agromyzid pest of cereals	Distribution	Cereal Host	References
Agromyza albipennis	Europe, Japan, Canada, USA	Hordeum vulgare, Oryza sativa, Secale cereale, Triticum aestivium	Spencer (1973, 1990), Darvas & Papp (1985)
Agromyza ambigua	Europe, Canada, USA	Avena sativa, Hordeum vulgare, Secale cereale, Triticum aestivum	Spencer (1973, 1990), Darvas & Papp (1985)
Agromyza hordei	India	Hordeum vulgare	Spencer (1973, 1990)
Agromyza intermittens	Europe, North Africa, Japan	Hordeum vulgare, Secale cereale, Triticum aestivum	Spencer (1973, 1990), Darvas & Papp (1985)
Agromyza megalopsis	Europe, Iraq	Hordeum vulgare, Secale cereale	Spencer (1973, 1990), Darvas & Papp (1985)
Agromyza nigrella	Europe	Avena sativa, Hordeum vulgare, Secale cereale, Triticum aestivum	Spencer (1973, 1990), Darvas & Papp (1985), Duthoit (1968)
Agromyza nigrociliata	Europe	Secale cereale, Triticum aestivum	Spencer (1973, 1990), Darvas & Papp (1985),
Agromyza oryzae	Japan, Siberia	Oryza sativa	Spencer (1973, 1990)
Agromyza parvicornis	Canada, USA and South America	Zea mays	Spencer (1973, 1990)
Agromyza prespana	Europe	Triticum aestivum	Darvas & Papp (1985)
Agromyza rondensis	Europe	Hordeum vulgare, Secale cereale, Triticum aestivum	Spencer (1973, 1990), Darvas & Papp (1985)
Agromyza yanonis	Japan	Hordeum vulgare, Triticum aestivum	Spencer (1973, 1990)
Chromatomyia fuscula	Europe, Canada, Japan	Avena sativa, Hordeum vulgare, Secale cereale	Spencer (1973, 1990), Darvas & Papp (1985),

Table 1. Agromyzid pest of cereals	Distribution	Cereal Host	References
Chromatomyia nigra	Europe, Canada, USA, Japan	Avena sativa, Hordeum vulgare, Secale cereale, Triticum aestivum	Spencer (1973, 1990), Darvas & Papp (1985)
Cerodontha (Cerodontha) australis	<b>Australia</b> , New Zealand	Hordeum vulgare, Triticum aestivum	Spencer (1973, 1990)
Cerodontha (Cerodontha) denticornis	Europe, Egypt, Morocco, Japan	Avena sativa, Hordeum vulgare, Triticum aestivum	Hafez <i>et al.</i> (1970), Spencer (1973, 1990)
Cerodontha (Cerodontha) dorsalis	Canada, USA, Argentina, Brazil, Peru, Costa Rica, Mongolia	Avena sativa, Hordeum vulgare, Secale cereale, Triticum aestivum	Luginbill & Urbahns (1916), Spencer (1973, 1990)
Cerodontha (Cerodontha) milleri	Australia	unknown	Spencer (1973, 1990)
Cerodontha (Poemyza) incisa	Europe, Japan, Canada	Avena sativa, Hordeum vulgare, Secale cereale, Triticum aestivum	Spencer (1973, 1990), Darvas & Papp (1985)
Cerodontha (Poemyza) lateralis	Europe	Hordeum vulgare, Secale cereale, Triticum aestivum	Spencer (1973, 1990), Darvas & Papp (1985)
Cerodontha (Poemyza) orbitona	Ghana, South Africa	Oryza sativa	Spencer (1973, 1990)
Cerodontha (Poemyza) oryzivora	Malaysia	Oryza sativa	Spencer (1973, 1990)
Liriomyza flaveola	Europe	Avena sativa, Hordeum vulgare	Spencer (1973, 1990), Darvas & Papp (1985)
Liriomyza marginalis	USA, Caribbean, South America	Avena sativa	Spencer (1973, 1990)
Pseudonapomyza asiatica	Asia, Africa	Zea mays	Spencer (1973, 1990)
Pseudonapomyza atra	Europe	Avena sativa, Hordeum vulgare, Triticum aestivum	Spencer (1973, 1990), Darvas & Papp (1985)
Pseudonapomyza dilatata	Fiji, Samoa	Zea mays	Spencer (1973, 1990)

Table 1. Agromyzid pest of cereals	Distribution	Cereal Host	References	
Pseudonapomyza hispanica	Spain, Israel	Sorghum halepense	Spencer (1973, 1990)	
Pseudonapomyza spicata	Taiwan, Pacific, Iraq, Israel, Egypt, Sudan	Triticum aestivum, Zea mays	Spencer (1973, 1990)	
Pseudonapomyza spinosa	<b>Australia</b> , Egypt, Nigeria, South Africa, India, Micronesia, Samoa	Hordeum vulgare, Triticum aestivum	Spencer (1973, 1990)	
Pseudonapomyza zeae	Ghana	Zea mays	Spencer (1973, 1990)	

Damage to the plant is caused predominantly by the internal leaf mining of the larvae, but also by the leaf punctures made by females when they are feeding or laying eggs. Mines may be in the form of a blotch or may be more narrow and winding (serpentine). This damage results in a depressed level of photosynthesis in the plant, and can also facilitate entry of bacterial and fungal diseases.

In unsprayed situations, populations of agromyzids are usually well-regulated by a range of generalist larval and larval-pupal parasitoids from the families Eulophidae, Pteromalidae, Eucoilidae and Braconidae (Darvas *et al.* 1999; Duthoit 1968; Murphy and LaSalle 1999). In Australia, the endemic parasitoid fauna associated with agromyzids (Bjorksten *et al.* 2005; Kleinschmidt 1970; Lambkin *et al.* 2008; Lardner 1991) would be capable of regulating invasive agromyzids provided that the parasitoids were not disrupted by application of broad-spectrum insecticides (Darvas & Andersen 1999).

<u>Agromyza spp.</u> Spencer (1990) listed 33 species of *Agromyza* reared from Poaceae with another 43 described species deduced to be grass feeders. The great majority of species are present in the northern hemisphere, particularly Europe and Canada. In Australia, only three *Agromyza* spp. are recorded (all endemic to Australia) and, while none of their hosts are known, their male genitalia are of the characteristic form of grass-feeders (Spencer 1963, 1977). There are no *Agromyza* spp. recorded in New Zealand (Spencer 1976*b*, 1990).

<u>Cerodontha spp.</u> Spencer (1990) lists 41 species reared from Poaceae with another 69 species deduced to be grass-feeders. Adults are often caught in large numbers (e.g. Lambkin *et al.* 2008) but larval feeding is difficult to detect, taking place inside the leaf sheath and not normally visible (Spencer 1990). There are nine *Cerodontha* spp. recorded in Australia, with two species, *Ce. milleri* and *Ce. australis* being closely related to *Ce. denticornis* which is considered the most serious *Cerodontha* pest of cereals (Darvas & Papp 1985). In New Zealand, Spencer (1973, 1976*b*) pointed out that early records of *Ce. australis* (Cumber 1962; Cumber & Harrison 1959; Hamilton 1947; May 1960; Morrison 1938) had been mis-identified as *Ce. denticornis*. *Cerodontha australis* is a very abundant agromyzid in grass pastures (Barker 1994; Barker *et al.* 1984). However, infestation levels in Waikato rarely exceeded 20% of tillers of *Lolium multiflorum* or *L. perenne* infested which Barker (1984) considered to be non-economic damage. In a detailed survey in New Zealand, Bejakovich *et al.* (1998) found *Ce. australis* in barley (62% of sites), rye (70% of sites), oats (35% of sites), wheat (59% of sites), ryegrass (72% of sites) and brome grass (44% of sites).

There is little information on the effect of *Ce. australis* on cereals (Cumber 1962). Spencer (1973) re-examined specimens identified as *Ce. australis* in Australia and found that most of them were in fact a

different species *Ce. milleri*. While there are no host records for *Ce. milleri*, Spencer (1973, 1977) considers that the species will be a grass-feeder. This species is frequently trapped (Lambkin et al. 2008; White 1970) [n.b. White (1970) nominated the trapped species in South Australia as *Ce. australis* but, on the basis of known distribution records in Australia, the specimens trapped in the drogue-net in South Australia would have almost certainly have been *Ce. milleri*].

The presence of *Ce. australis* and *Ce. milleri* in Australia will make detection of *Ce. denticornis* very difficult in any surveys of pasture grasses.

<u>Chromatomyia spp.</u> There are 19 species of grass-feeding <u>Chromatomyia</u> recorded from Europe, Canada, Japan, USA mainland and Alaska (Spencer 1990). Most of the species have a restricted host range, but <u>Ch. fuscula</u> and <u>Ch. nigra</u> have been recorded from 20 and 46 genera of Poaceae respectively (Spencer 1990). There are no known grass-feeding <u>Chromatomyia</u> or <u>Phytomyza</u> species in Australia so detection of <u>Ch. fuscula</u> and <u>Ch. nigra</u> will be relatively simple on the basis of the characteristic puparia embedded in the leaves, similar to <u>Ch. syngenesiae</u> puparia in sowthistle (<u>Sonchus oleraceus</u>). It should be noted that a recent phylogenetic PhD study of the genus <u>Phytomyza</u> (Winkler 2008) has shown that <u>Chromatomyia</u> is polyphyletic and nested within <u>Phytomyza</u>. This nomenclatural change from (<u>Chromatomyia</u> to <u>Phytomyza</u>) is likely to be published shortly.

# 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 that serve as sites for feeding (by both male and female flies) or oviposition. Feeding punctures cause the destruction of a large number of cells and are clearly visible to the naked eye. The punctures can also provide entry points for plant diseases. 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; Spencer 1973).

The number of eggs laid varies according to temperature and host plant. There are three larval stages (instars) that feed within the leaves. The larvae feed on the plant in which the eggs are laid. Leafminers are selective feeders, consuming those tissues high in nutritive value and avoiding tissues with detrimental compounds (Kimmerer & Potter 1987; Scheirs *et al.* 1997). Agromyzids feeding on Poaceae feed primarily on the mesophyll cells. They avoid feeding on the epidermis and also avoid major leaf veins (Scheirs *et al.* 1997).

The larvae of *Agromyza ambigua* and *A. megalopsis* usually leave the plant to pupate (Spencer 1973) so pupae may be found in crop debris, in the soil or sometimes on the leaf surface. In contrast, the larvae of *Chromatomyia fuscula* and *Ch. nigra* pupate inside the leaf at the end of the larval mine (Darvas *et al.* 2000a). *Cerodontha denticornis* also pupates internally although some *Cerodontha* spp. pupate externally, whether in the ground or stuck on the outside of the leaf. 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.

## 2.1.2.1 Agromyza ambigua

Adult flies emerge in spring and lay eggs on suitable grasses, including cereals. Spencer (1973) cites Italian work suggesting the species is univoltine (ie only one generation per year), with adults emerging in late spring but spending the summer and subsequent winter as pupae (inside the puparia).

# 2.1.2.2 Agromyza megalopsis

In Europe, oviposition occurs mainly in early summer and the females can lay from 20-30 eggs. Usually the pupariation occurs in the soil but occasionally puparia can be found in the mines. Most of the early summer generation of larvae then diapause as pupae but some will emerge in the late summer or autumn (Spencer 1973). In Bulgaria, Khristov (2000) found that *A. megalopsis* had one complete and a second incomplete generation, pupated over winter in the soil and emerged as an adult in early spring (the last 10 days of March).

## 2.1.2.3 Cerodontha denticornis

In northern Japan, Iwasaki (2000) observed 3 generations per year. This species is very common in Europe laying its eggs in the leaves of a number of species of forage grasses including meadow foxtail, couch, giant and meadow fescues, Yorkshire fog and canary reed-grass. Andersen *et al.* (2004) observed that *Ce. denticornis* was much more abundant in pastures (*Festuca pratensis* and *Phleum pratense*) than in spring barley. In Egypt, Hafez *et al.* (1970) noted that populations of *Ce. denticornis* were most abundant in February. In southern California, the closely-related species *Ce. dorsalis* is recorded as having as many as 8 generations per year (Spencer 1973).

#### 2.1.2.4 Chromatomyia fuscula

In Norway, the life cycle of *Ch. fuscula* populations in the vicinity of cultivated crops and pasture grasses was studied in detail by Andersen (1991). He found that *Ch. fuscula* overwinters as adult flies (instead of overwintering in puparia) in wooded areas adjacent to the cropping areas. This is a very uncommon overwintering strategy among agromyzid flies (Darvas *et al.* 2000*a*, 2000*b*). The flies begin to colonize grasses in the end of April (snow cover remains in these areas until mid-April). Once seedling barley became available at the end of May, this crop becomes the favoured host (Darvas & Andersen 1996). The next generation of leafminers is detected from midsummer until early autumn, with the emerging adults then overwintering until the next season.

Temperature controls the developmental rate of many organisms, including insects. The thermal constant (TC), expressed in degree-days, is the amount of heat that each species requires to complete its life cycle or part of it, regardless of the temperature to which it is exposed (Chiang 1985). The thermal constant provides a valuable tool for insect pest control; in forecasting infestations monitoring and timing insecticide applications (Zalom *et al.*, 1983). Information about calculating degree-days can be found at UC Davis IPM website http://www.ipm.ucdavis.edu/WEATHER/ddconcepts.html.

The development of egg, larvae and pupae of *Ch. fuscula* was studied at 5 temperatures by Andersen & Fugleberg (1997). The estimated lower developmental threshold (LDT) for immature development (egg-adult emergence) for *Ch. fuscula* was 3.0°C with a thermal constant (TC) of 400 degree-days above 3.0°C. This means that the species needs to accumulate 400 degree-days above 3.0°C to develop from egg to adult. Andersen & Fugleberg (1997) pointed out that in south-eastern Norway, the

summer generation flies emerged at the end of July and that typically there was sufficient warm weather in late summer and early autumn (August averaged 390 degree-days above 3.0°C and September averaged 235 degree-days above 3.0°C) to allow a second generation to develop. However, the population is univoltine with the emerging flies in late summer not having eggs developing until after winter. They suggested the diapause mechanism was related to length of photoperiod. Similarly in Hokkaido, Japan, Iwasaki (1995) observed *Ch. fuscula* to be univoltine. The lower developmental threshold is much lower than polyphagous pest species such as *L. huidobrensis* (LDT = 8.1°C, TC = 280 degree-days above LDT), *L. trifolii* (LDT = 10.5°C, TC = 234 degree-days above LDT) (Lanzoni et al. 2002) and *L. sativae* (LDT = 10.2°C, TC = 250 degree days above LDT) (Haghani *et al.* 2007).

When overwintering females were caged on barley seedlings, they made 3-4 feeding punctures per hour and laid one egg per hour (Darvas & Andersen 1996). The vast majority of feeding punctures and eggs were found on the upper leaf surface. They chose the tips of the leaves for feeding, but laid their eggs in the middle part of the leaf blade. Leaf sheaths were never utilized by *Ch. fuscula* (Darvas & Andersen 1996).

*Ch. fuscula* is only considered to be a pest species in the high latitudes of Scandinavia (Andersen 1989). It is present, but not considered to be a problem, in other parts of Europe, which suggests that it is unlikely to be a significant problem in Australian cereal-growing areas.

# 2.1.2.5 Chromatomyia nigra

In Oregon, Kamm (1977) observed that the reproductive season of *Ch. nigra* infesting pasture grass seed crops begins in October and ends in June. Reproduction increased when adults were exposed in the laboratory to 15°C and short days (10 h light, 14 h dark); reproduction was reduced but did not cease when adults were exposed to 15°C and long days (16 h light, 8 h dark). However, at 21°C, long days (16 h light, 8 h dark) drastically reduced reproduction of F<sub>1</sub> adults and completely inhibited reproduction of F<sub>2</sub> progeny. These findings suggest that *Ch. nigra* would be best suited to autumn, winter and early spring in Australian cereal growing areas. Parasitism of the natural field population was observed to be less than 15% during autumn and winter but increased to 77% in spring.

In Japan, Sasakawa (1953) studied the ecology of *Ch. nigra* and observed that flies emerged from the overwintering puparia early in the spring (at the end of March to the beginning of April). The eggs were usually deposited beneath the upper or lower epidermis near the top and near the margin of the leaf. Usually one or two eggs are found on a single leaf, but sometimes he found more than three eggs. Initially, the larva mined towards the base of the leaf, before it turned towards the apical part of the leaf. The mine was of the linear type through its entire life cycle. The larvae pupated near the end of the mine and a pair of anterior spiracles of the pupa projected on the surface of the leaf. The flies of the second generation emerged at the beginning to the middle of May. A third generation of flies emerged in the autumn and oviposited on other Poaceae.

#### 2.1.3 Dispersal

Agromyzid flies are considered as "moderate fliers" (Yoshimoto & 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 & 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* (but in fact most likely to be *Ce. milleri* which Spencer (1973) described), being trapped as high as 600 m by airborne drogue-nets. Recent work in Japan (Iwasaki *et al.* 2008) demonstrated that *Chromatomyia horticola* puparia did not diapause and would not emerge when held for 75 days at 0°C. This indicated that *Ch. horticola* could not survive in winter in Hokkaido, Northern Japan (3 months of snow). They also provided trapping data evidence to support their hypothesis that *Ch. horticola* migrated into Hokkaido (43°N) each spring on low-level jet-streams. This study is the most convincing example to date of long distance natural movements of an agromyzid species.

The puparia of the cereal-infesting agromyzids could potentially disperse over long distances by the movement of infested hay. The puparia of *Agromyza megalopsis* and *A. ambigua* could also survive for long periods in dry soil and can therefore potentially be spread in seed, machinery or equipment with soil contamination.

## 2.2 Affected hosts

#### 2.2.1 Host range

As cereal-infesting leafminers generally have a wide host range with members of the Poaceae family, they are considered a potential risk to the Australian grains industry.

# 2.2.1.1 Agromyza ambigua

Host plants affected include Agropyron sp., Avena sativa (oats), Hordeum vulgare (barley), Secale cereale (rye) and Triticum aestivum (wheat) (Spencer 1973, 1990).

## 2.2.1.2 Agromyza megalopsis

Cereal host plants affected include *Hordeum vulgare* and *Secale cereale*. No other grass hosts have been recorded (Spencer 1990) although Dempewolf (2004) suspects that some other wild grasses could be hosts. This species is very similar to, and can be confused with, *A. nigrella*, which infests a range of wild grass hosts (Spencer 1973). In Europe, *A. megalopsis* is one of the important *Agromyza* species frequently occurring in cereals with heavy outbreaks occurring in the mid 1960s in southern Germany (Spencer 1973, 1989). D'Aguilar *et al.* (1976) studied four species of *Agromyza* attacking cereals in the Paris area. During their study, they found that *A. megalopsis* caused serious damage to young barley plants in spring, *A. nigrella* primarily attacked wheat, while *A. intermittens* and *A. luteitarsis* were found on both hosts. Chemical control was sometimes required.

#### 2.2.1.3 Cerodontha denticomis

Cerodontha denticornis has been recorded from 14 genera of Poaceae [Agropyron, Alopecurus, Arundo, Calamagrostis, Dactylis, Festuca, Holcus, Hordeum, Lolium, Phalaris, Phleum, Poa, Secale and Triticum<sub>I</sub> (Benavent-Corai et al. 2004; Spencer 1990). Cereal hosts are Avena sativa, Hordeum vulgare and Triticum aestivum.

# 2.2.1.4 Chromatomyia fuscula

Chromatomyia fuscula has been recorded from 20 genera of Poaceae [Alopecurus, Arrhenatherum, Avena, Beckmannia, Bromus, Cinna, Dactylis, Deschampsia, Helictotrichon, Hordeum, Lolium, Melica, Milium, Phalaris, Phleum, Poa, Secale, Sesleria, Trisetum and Triticum] (Spencer 1990). Important cereal hosts are Avena sativa, Hordeum vulgare and Secale cereale. Across central Europe C. fuscula normally occurs in low numbers below the threshold of economic significance whereas in northern Europe, high levels of infestation can result in yield losses (Andersen 1989; Spencer 1973).

#### 2.2.1.5 Chromatomyia nigra

Chromatomyia nigra has been recorded from 46 genera of Poaceae [Aegilops, Agropyron, Agrostis, Aira, Alopecurus, Ammophila, Anthoxanthum, Apera, Arrhenatherum, Avena, Brachypodium, Briza, Bromus, Calamagrostis, Catapodium, Chaetopogon, Cynosurus, Dactylis, Deschampsia, Desmazeria, Eleusine, Festuca, Gaudinia, Helictotrichon, Hierochloe, Hordeum, Lagurus, Lamarckia, Lepturus, Lolium, Melica, Milium, Molinia, Nardus, Phalaris, Phleum, Pholiurus, Poa, Polypogon, Secale, Sesleria, Setaria, Trisetum, Triticum, Vulpia and Zea] (Scheirs et al. 2003; Spencer 1973, 1990). Important cereal hosts are Avena sativa, Hordeum vulgare, Secale cereale and Triticum aestivum.

## 2.2.2 Geographic distribution

## 2.2.2.1 Agromyza ambigua

This species is distributed across Europe, [Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Italy, Poland, Serbia, Slovakia, Spain (mainland and Canary Is.), Sweden, Switzerland, The Netherlands] (Martinez 2007), USA and Canada (Spencer 1973).

#### 2.2.2.2 Agromyza megalopsis

This species has been recorded in Europe [Bulgaria, Czech Republic, France, Germany, Hungary, Slovakia and Serbia] (Martinez 2007) and Iraq (Spencer 1973).

#### 2.2.2.3 Cerodontha denticomis

This species is widely distributed throughout Europe [Albania, Austria, Azores, Belgium, Bulgaria, Canary Islands, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Japan, Lithuania, Madeira, Norway, Poland, Serbia, Slovakia, Spain, Sweden, Switzerland, The Netherlands] (Martinez 2007), as well as being recorded from Egypt (Hafez *et al.* 1970), Morocco, Russia, Afghanistan, Iran, Turkey and Japan (Spencer 1973).

#### 2.2.2.4 Chromatomyia fuscula

This species is distributed across Europe [Czech Republic, Denmark, Finland, France, Germany, Hungary, Italy, Norway, Poland, Slovakia, Sweden, The Netherlands, Serbia] (Martinez 2007) and is considered a significant pest of spring barley in Scandinavia (Andersen 1989; Hågvar *et al.* 1998). It has also been reported in Japan (Iwasaki 1995), parts of Canada (Andersen & McNeil 1995) and Greenland (Spencer 1973).

#### 2.2.2.5 Chromatomyia nigra

This species has a widespread distribution throughout Europe [Belarus, Belgium, Great Britain, Czech Republic, Denmark, Estonia, Faroe Is., Finland, France, Germany, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Madeira, Norway, Poland, Serbia, Slovakia, Sweden, Switzerland, The Netherlands] (Martinez 2007). It has also been reported in Japan, India and parts of Canada and the USA (Kamm 1977; Sandhu & Deol 1975; Spencer 1973).

#### 2.2.3 Symptoms

# 2.2.3.1 Agromyza ambigua

The larval mine is found on the upper youngest leaves of the plant and is normally short and broad (Spencer 1976a). The shallow whitish mine begins as a fine ascending corridor that is overrun when the direction changes and the mine quickly widens. The final mine is characteristically short with trails of frass appearing as big black grains along their length (Spencer 1973). Initially the larva does not feed towards the apex of the leaf. The fully-grown larva exits the leaf and pupates in the soil.

Spencer (1973) records that

"...A. ambigua, although widespread in Europe, is a fairly uncommon species and no records are known of its occurrence in large numbers."

On the other hand, Dempewolf (2004) records that

"Together with other grass mining agromyzids, *Agromyza ambigua* can reach higher infestation levels and may cause yield loss. However, the significance of *Agromyza ambigua* within the grass feeding leafminer complex is not fully understood."

#### 2.2.3.2 Agromyza megalopsis

Eggs are deposited in a transverse row near the leaf margin and close to the leaf base. The hatching larvae then form a mine runnning towards the leaf tip. Neighbouring mines can merge resulting in large blotch mines with several larvae being found in the same mine. The blotchy pseudo-polylarval mine moves towards the tip and usually reaches the end of the lamina (Darvas *et al.* 2000a). The larvae completely consume the mesophyll and the affected leaves often wilt or die. The sheath is never attacked and so even when there is total lamina destruction, there is still a significant assimilation capacity remaining in the unattacked sheaths (Darvas & Andersen 1996). In Bulgaria, Khristov (2000) found that *A. megalopsis* caused a 27% yield decrease in barley.

#### 2.2.3.3 Cerodontha denticomis

The larvae mine in the leaf sheath of grasses and pupate inside the mine, in contrast to *Agromyza* spp. that pupate outside the leaf. Normally the mine in the sheath can be almost invisible from outside, making detection difficult. Like most other *Cerodontha* spp., the mine is extremely thin, corresponding with the laterally flattened first segment and the narrow mandibles of the larva. Larvae initially make straight, slender leaf-mines toward the leaf base, then enter the leaf sheath, where the majority of infestations occur, followed by pupation. Mines on a leaf are visible only as very narrow white lines, and as scattered inconspicuous white patches on leaf sheath. In Japan, spring wheat is more susceptible than winter wheat to infestation. Stems infested by first-generation larvae accounted for only about 2%

loss in yield due to decreased grain weight (Iwasaki 2000). In Egypt, Hafez *et al.* (1970) noted that *Ce. denticornis* larvae attack the lower parts of the leaf-blade with the mine extending to the stem, where the larvae usually pupate. With severe infestations, the plants gradually dry and finally die, but when infestations are slight, the stems only blister and become calloused. Newly-formed mines are visible to the naked eye. They are linear and light green in colour. The mine increases in length from 0.5 cm when first formed to 5.4 cm when the larva becomes fully-grown. Most commonly, there are 2 larvae found in a mine, although as many as 9 larvae have been observed in a mine. The mines are usually formed at the base of the leaf or near its tip at a distance from the midrib.

In a study of the closely-related species, *Ce. australis* on *Lolium perenne* in New Zealand, Barker *et al.* (1984) noted that

"Oviposition occurs in the leaf blades of grasses. The larvae mine down the leaf into the sheath and stem, where pupation takes place, Damaged leaves turn pale yellow in colour and eventually die back from the tip. Sheath mining by the second and third instar larvae causes progressive reduction in tiller vigour, but is often not apparent until after completion of larval development and pupation. In some instances larvae penetrate the tiller, cause destruction of the innermost leaves and growing point or, destroy secondary tillers at the base of the originally infested tiller."

## 2.2.3.4 Chromatomyia fuscula

Females prefer the youngest two barley leaves for feeding and oviposition (Darvas & Andersen 1996). Feeding punctures are mainly at the tip of the leaves, whereas eggs are mainly deposited in the middle and base of the young leaves. Nearly all eggs (96%) are deposited on the upper surface of the leaf (Darvas & Andersen 1996). The larval mine is whitish and on the upper surface of the leaf with a descending corridor half way along the leaf blade. Within the mine, distinct black frass grains are evident. Pupation occurs within the mine. Anterior spiracles of the puparium penetrate the plant epidermis. In a detailed study in Norway with spring barley, Andersen (1989) estimated a loss of yield of 10 kg for each per cent of the total leaf area mined (for a 4 t/ha crop yield). The economic injury level was estimated as 16.3% of the total area mined. Andersen (1989) suggested that the relatively low impact of *Ch. fuscula* on yield could be explained by the barley plants growing past the agromyzid infestation. New leaves are being formed while the agromyzid larvae mine in the older leaves.

## 2.2.3.5 Chromatomyia nigra

The larvae form narrow, white linear mines on the upper surface of the leaf. The mine contains large frass grains that are deposited further apart than their diameter. Feeding larvae consume only a relatively small portion of the leaf. Often the remaining portion of the injured leaves prematurely turn yellow and deteriorate from the point of entry to the tip of the leaf (Kamm 1977). Pupation occurs within the mine. The anterior spiracles of the orange-brown puparium penetrate the epidermis.

# 2.3 Entry, establishment and spread

When the Grains Industry Biosecurity Plan was prepared in 2004 cereal leafminers were given an overall risk rating as medium. As a consequence of a medium risk rating in the Threat Summary Table this contingency plan was commissioned. During preparation of the contingency plan, literature searches undertaken by the author have shown the majority of the target cereal leafminer species were

well-adapted to the cold environments in the northern hemisphere and unlikely to establish and flourish in the warmer Australian climate. As a consequence, the rating in the Threat Summary Tables has been changed to a low risk for cereal leafminers (*Agromyza ambigua*, *A. megalopsis*, *Cerodontha denticornis*, *Chromatomyia fuscula*, *Ch. nigra*).

Apart from chance entry through incoming imports and arrivals, cereal-infesting leafminers (*Agromyza ambigua, A. megalopsis, Cerodontha denticornis, Chromatomyia fuscula* and *Ch. nigra*) may likely enter Australia via imported plant material containing leaves where the eggs and larvae (and possibly puparia) are borne internally. Unlike the polyphagous *Liriomyza* spp., there have been very few examples of successful incursions of cereal-infesting agromyzids internationally in the last 100 years. Certainly *Agromyza* spp. are largely restricted to the Northern Hemisphere (Spencer 1990).

## **Entry potential: Low**

The likelihood of entry by infested members of the Poaceae family is low as the cereal-infesting leafminers attack only leaves, and the puparia of those species pupating externally would be most unlikely to be transported successfully with seed.

There is a possibility of transportation of eggs, larvae and puparia (particularly where pupation is within the leaves) in inadequately fumigated hay or as a contaminant (i.e. living Poaceae) in imported products. Certainly the alfalfa blotch miner (*Agromyza frontella*) has been observed to move, on average, 93 km per year in USA, primarily through transport of infested hay, trucking, wind dispersal or adult flight (Hutchison *et al.* 2007; Venette *et al.* 1999). This species originated from Europe and was first recorded in USA in 1968 (Venette *et al.* 1999).

There are no known infestations of these cereal-infesting leafminers in Indonesia, Timor-Leste or Papua New Guinea. This means that it would not be possible for natural emigrations of the leafminers to Australia to occur, unlike the current situation in Indonesia and Timor-Leste that would potentially allow *Liriomyza sativae* and *L. huidobrensis* to move naturally into northern Australia (Malipatil & Ridland 2008).

#### **Establishment potential: Medium**

Establishment potential is medium. There is a wide distribution of suitable hosts in the grain growing regions of Australia but the climate in the major areas would be sub-optimum for some of the species, specifically *Chromatomyia fuscula* and *Ch. nigra*. There may be other cereal-infesting species not dealt with specifically in this plan, which may be better adapted to higher temperatures and so more likely to cause problems in the Australian cereal industries, e.g. an unidentified *Agromyza* sp. [possibly *A. nigrella*, which is an important cereal pest in Egypt (El-Serwy (2003)] caused significant problems to wheat in Saudi Arabia (El-Hag & El-Meleigi 1991a, 1991b).

#### **Spread potential: Medium**

Cereal-infesting leafminers attack a wide range of cereals, pasture grasses and weedy grasses. As these leafminers are "moderate" fliers, mainly dispersing within the crop by flight, the spread potential is lessened. In crops showing active mining, the flies may be seen walking rapidly over the leaves with only short jerky flights to adjacent leaves. The wide host range of Poaceae will mean that the agromyzid flies will have many alternative hosts and will be able to spread rapidly along natural

corridors of grasses. Transportation of cereal and pasture hay could also potentially contribute to the spread of cereal-infesting leafminers. However, natural populations of leafminers are likely to be restricted by the dry, hot summers in the cereal growing areas. Unless these leafminer species can oversummer by entering a facultative diapause or quiescence, the species will struggle to survive in areas with hot, dry summers. This will restrict their potential to spread.

#### **Economic impact: Low**

Arrival and establishment is expected to cause losses in grain production and increased expenditure on insecticides and other management practices. However, most damage is caused to young plants which would be able to compensate for the damage. The host range affected by these leafminers is extensive. However, it appears most unlikely that significant crop losses would occur regularly. In Europe these species only cause sporadic problems to crops. The only exception appears to be in Norway, where *Ch. fuscula* regularly causes economic damage (Andersen 1989). However, this species appears to be poorly adapted to conditions in Australian cereal-growing areas.

# **Environmental impact: Rating = Very Low**

There is negligible potential to degrade the environment or otherwise alter ecosystems by affecting species composition or reducing the longevity or competitiveness of wild hosts. There could be some potential damage to alpine areas where snow grasses, *Poa* spp., could be attacked by *Ch. fuscula* or *Ch. nigra*.

Overall risk: Low

# 2.4 Diagnostic information

#### 2.4.1 Diagnostic protocol

Accurate identification is an issue with this group because many of the species are closely related and are likely to have a similar host range in the Poaceae. Any field collection, particularly on non-cereals, may well have specimens of the endemic *Cerodontha milleri* or *Ce. australis* so care will be needed with identifications. Definitive identification relies on examination of the male genitalia. In addition, many species in the genera *Agromyza* and *Cerodontha* do not have accurate host records since they have been described from flies caught in traps or sweep nets and were not reared from mines. Since Australia does not have any grass-feeding *Chromatomyia* or *Phytomyza* spp., the presence of the distinctive puparia in the leaves of infested leaves will be a simple indicator of *Ch. fuscula* or *Ch. nigra*.

Initial identification of field-collected agromyzids in Australia will require use of the key to Australian Agromyzidae (Spencer 1977). This will enable determination of the agromyzid specimen to genus level. Keys to the exotic cereal-infesting agromyzids covered in this plan are given in the key to European agromyzid species attacking cereals by Darvas & Papp (1985). Further detailed information is given by Dempewolf (2004), Griffiths (1980) and Spencer (1973, 1990). It would be useful for a working key for exotic and endemic agromyzid species infesting Poaceae to be constructed in preparation of an incursion into Australia.

Dempewolf (2004) gives the following summary of the important diagnostic characters of the 5 species (hyperlinked below) being considered in this plan. Details of morphological characters are given in Dempewolf (2004) and Spencer (1973, 1987).

## 2.4.1.1 Agromyza ambigua Dempewolf (2004)

Wing length: 2.5-3.0 mm. Genae light brown, concolorous with frons between dorsocentral bristles. Frons clearly dark at sides and perhaps in alcohol specimens light brown in the central region. Third antennal segment angulate. 3-4 dorsocentral bristles, gradually increasing in size; the first one is only slightly longer than acrostichals. As in most grass feeding *Agromyza*, posterior spiracles of the larva and puparium both have three openings. There are two mouth hooks on each mandible (de Meijere 1925).

## 2.4.1.2 Agromyza megalopsis Dempewolf (2004)

Wing length: about 2.5-2.7 mm. Body fine pubescent and shining, mesonotum green metallic. 4-5 dorsocentral bristles present, of these, 1-2 can be presutural. The first, presutural dorsocentral bristles are about half as long as the hind ones. Posterior spiracles of larva and puparium each with three bulbs. The position of the two spiracles can be used to separate *A. megalopsis* from the closely related *A. nigrella*. In *A. megalopsis*, the two posterior spiracles are situated close together whereas those of *A. nigrella* are more separated (Spencer 1973). Pupation occurs outside the leaf.

## 2.4.1.3 Cerodontha (Cerodontha) denticornis Dempewolf (2004)

The subgenus *Cerodontha* is characterisd by by the presence of a conspicuous spine or projection at the dorso-apical corner of the first antennal flagellomere, and only two (apical) scutellar bristles.

Cerodontha denticornis is notable for its colour variation. In the Northern Hemisphere, the darkest flies are normally found in the spring and early summer from April to June, while the palest are normally found from June to September. In the majority of specimens, the coloration is intermediate (Spencer 1973). A complete description of this species is given by Sasakawa (1961). Mesonotum greyish, abdominal tergites rather brown, both subshining. Frontorbital bristles only slightly inwards directed. Frontorbital setulae sparse, situated in one row together with the bristles.

Wing length: 1.8-2.6 mm. Third antennal segment with strong spine. Cephalopharyngeal skeleton of the larva is thin with mouth hooks alternating regularly. The large number of bulbs of anterior posterior spiracles are arranged tree-like.

#### 2.4.1.4 Chromatomyia fuscula Dempewolf (2004)

Wing length: 2.3-2.6 mm. Very similar to *Ch. nigra*. Basal section of arista long, about as long as apical section. Frontal region and a part of the genae (cheeks) yellow or orange, occiput and posterior part of genae clear brown. Three main frontorbital bristles, of these only one inwards directed. One additional inwards-directed small hair-like bristle is situated before the first frontorbital bristle (the reduced 4th). The second dorsocentral bristle is located near the transverse suture. It may be difficult to interpret it as pre- or postsutural. Third antennal segment rounded.

Males have sparse pubescence on the eyes (Spencer 1973; Iwasaki 1995). The genitalia are distinct, especially between the three grass-mining *Chromatomyia* species: *fuscula*, *milii* and *nigra*. The anterior spiracles of the immature stages each have 8 bulbs on rather short stalks (Spencer 1973), shorter than in *Ch. nigra* (Spencer 1973). The mine is linear whitish, with frass in distinct black pellets. Pupation takes place within the mine.

A very detailed morphological study of *Ch. fuscula* was conducted by Darvas et al. (2000a).

## 2.4.1.5 Chromatomyia nigra Dempewolf (2004)

Male genitalia are distinct but, without dissection of the male genitalia, confusion with the other grass mining species *Ch. fuscula* may occur.

Wing length: 2.0-2.7 mm. Third antennal segment much shorter than broad. Frons variable either yellow or dark. Frontorbital setulae rather long. Arista of third antennal segment gradually tapering. Shape of third antennal segment important. Usually three large frontorbital setulae present, of these the anterior one is inwards directed. Sometimes a fourth smaller one may be found. Acrostichal setulae present. Pubescence of the male's eye is denser than in *Ch. fuscula* (Iwasaki 1995). Anterior stigma with rather long stalks distinctly longer than *Ch. horticola* and *Ch. fuscula* (Spencer 1973). Pupation takes place within the mine.

# 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 and McKirdy (2005) in the Technical Guidelines for Development of Pest Specific Response Plans.

# 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. It will be important to examine surrounding non-crop Poaceae as well as the cereals. After the initial detection, samples should be taken in the area within a 1.5 km radius of the initial detection, and the survey area expanded as further detections are made. Because of the small size of the pests and the wide range of Poaceae attacked, it is very likely that the incursion will not be detected until it is in fact very well established.

# 2.6.1 Sampling method

Visual examinations of plants to check for feeding punctures and mines will be the most suitable approach. As well, sweep netting should be used to check for adults. These samples will need to be assessed in the laboratory because of the wide range of specimens that will be caught. A complicating factor will be the likely presence of the endemic agromyzids, *Cerodontha milleri* or *Ce. australis*, in sweep net samples from pasture grasses. In a heavy infestation, it is likely that the numbers of adult agromyzids in sweep net samples would be very high (e.g. Lundgren *et al.* (1999) sampled *Agromyza frontella*, alfalfa blotch leafminer, in Manitoba and found numbers averaging as high as 117 adults per 10 sweeps).

Infestations of grass-infesting *Chromatomyia* spp. will be relatively easy to assess from symptoms (feeding punctures and larval mines) and the presence of puparia in the leaves. *Cerodontha* spp. will be more difficult to assess by symptoms as they tend to mine the leaf sheath rather than the leaf blade. *Agromyza* spp. will also be relatively easy to assess by leaf symptoms.

Yellow sticky traps have been used successfully to monitor *Agromyza* sp. (El-Hag 1992) but yellow water traps were not very useful for detecting *Chromatomyia fuscula* (Andersen 1991; Darvas & Andersen 1996). Mines by *Cerodontha* spp. are often difficult to detect because they are in the leaf sheath. Barker *et al.* (1984) assessed *Ce. australis* infestations by examining samples of 200 live and 200 dead tillers of ryegrass per pasture (they surveyed 63 ryegrass pastures) and by collecting adults with a suction sampler from 3 m<sup>2</sup> of pasture.

Bejakovich *et al.* (1998) surveyed pests and diseases in cereal and grass seed crops in New Zealand. Their sampling strategy was designed to detect pests infesting greater than 1% of plants at a particular site with 90% confidence. To achieve this aim, they examined at least 256 plants at each site from a minimum of 10 points per site. There are no published studies on the spatial distribution of the five targeted cereal-infesting agromyzids, so it is impossible to determine the precision of any proposed sampling strategy. The required data on the spatial distribution of the target species (mean and variance) would quickly be collected during the course of the surveys, so the precision of any subsequent survey could be determined fairly rapidly (Ruesink 1980). McMaugh (2005) provides much information on designing surveys and should be consulted. The most complete study of sampling of an agromyzid pest in a broad-acre crop is for *A. frontella* in North America (Harcourt 1982; Harcourt & Binns 1980*a*, 1980*b*; Harcourt *et al.* 1987). Eggs and larvae were assessed by examining leaves; prepupae were collected as they dropped from plants in pans containing ethylene glycol; puparia were sieved from soil and adults were collected in emergence traps as they emerged from the soil following eclosion. These studies provide a blueprint for any ecological studies to be conducted on the target species.

In USA, there is much routine surveying for cereal leaf beetle and, as a starting point, it is suggested that the Cereal Leaf Beetle Standardized Survey Methods designed by the Western Cereal Leaf Beetle

Working Group be followed. <a href="http://www.spokane-county.wsu.edu/smallfarms/CLB%20Archives/Oregon/Cereal%20Leaf%20Beetle%20Survey%20Methods%20-%20Brown.pdf">http://www.spokane-county.wsu.edu/smallfarms/CLB%20Archives/Oregon/Cereal%20Leaf%20Beetle%20Survey%20Methods%20-%20Brown.pdf</a>

## Sweeping

- Equipment: 38 cm (15") sweep net.
- One sweep = one swath from left to right.
- Execute a full 180 degrees to get a complete sweep.
- Target the top one third of the plant.
- Number of sweeps/field: 120 sweeps/crop (4 sets of 30 sweeps in different areas of the crop).
- Sweep only after dew has dried

#### Visual survey

- Visually search at least 10-15 minutes while sweeping (less-experienced surveyors search visually for additional 10 minutes).
- Search along boundaries, and in young host crops that are too low to sweep.

General protocols for collecting and dispatching samples are available within PLANTPLAN, Appendix 3 (Plant Health Australia 2008).

In surveys of *A. frontella* (Hutchison *et al.* 1997; Lundgren *et al.* 1999), sweep net samples (in this case either 10 or 20 sweeps per sample) were placed in plastic bags in the field, returned to the laboratory and held in a freezer prior to identification of *A. frontella* adults. It is recommended that a similar bagging process be done when sweeping for cereal-infesting leafminers. Similarly, infested plants should be bagged and reared through in a quarantine facility to confirm the species, as well as determine any natural enemies present.

# Number of specimens to be collected

A large number of specimens should be collected. The aim is to obtain adult males. 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. However, no tests are currently available for these cereal-infesting species. However, it would be possible to use a number of molecular markers already used for a range of agromyzid species (Scheffer *et al.* 2007).

#### How to collect

Adult flies can be hand-collected into glass vials, collected with a vacuum sampler or swept from foliage with a sweep 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 to obtain adult flies.

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. More detailed rearing methods are outlined in Fisher *et al.* (2005) and Lambkin *et al.* (2008).

## How to preserve cereal leafminers

Adults and larvae should be placed into vials containing 70% ethanol and then stored in the dark. The specimens can be stored indefinitely, although their colour fades gradually with time. Specimens required for molecular diagnostic work should be killed and preserved in absolute ethanol (and stored at -4°C) or else stored frozen (- 80°C).

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

## How to transport cereal leafminers

Leaves with suspect feeding punctures or leaf mines should be placed into large plastic bags with some tissue paper to absorb excess moisture. The bags can then be mailed (in a secure parcel) to the rearing laboratory.

[Specimen handling information taken from the Grains IBP 'Diagnostic protocol for the detection of American serpentine leafminer, *Liriomyza trifolii* (Diptera: Agromyzidae)' (Malipatil & Wainer (2006)].

#### 2.6.2 Ecological study

Once an incursion of a cereal-infesting leafminer is detected, a number of important questions will need to be addressed to ensure that the subsequent actions are as effective as possible.

Pragmatically, the first challenge will be to assess the most cost-effective and appropriate insecticide for control of the pests. The next question to be answered is the spatial distribution of the pests, which will be needed to confirm the optimum sampling strategy (including sensitivity of various sampling options). Complementing this work, it will be important to determine the preferred hosts in the Poaceae in order to streamline surveying of non-crop hosts.

As soon as possible, a season-long study is needed of the population dynamics of the pest using an array of sampling methods (sweeping, plant inspection, pre-pupal counts (for *Agromyza* spp.) and emergence of adults (yellow sticky traps or water traps; emergence traps (for *Agromyza* spp.)). This study will also enable the role of endemic parasitoids to be determined.

#### 2.6.3 Models of spread potential

No modelling data are available for spread of leafminers in broadacre cropping. However, in the USA, the spread of *Agromyza frontella* (alfalfa blotch leafminer) has been estimated to be spreading at a rate of 93 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). They fitted the data to a generalised distance dispersal model (Taylor 1978).

## 2.6.4 Pest Free Area (PFA) guidelines

Points to consider are:

- Statistical field survey for symptoms on host plants and sweeping for adult flies. The survey will
  need to be stratified to ensure full coverage of the area, and include crops, pastures and
  endemic grasses.
- All suspect larvae or pupae to be reared out in the laboratory to confirm the identification.
- Aerial inspection or remote sensing should also be investigated as a tool, with suspect patches inspected and sampled to confirm or deny the presence of the pest.

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 pest free areas 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).

# 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. Do not move soil from infested paddocks to non-infested paddocks.
- To minimise the build up of cereal leafminer populations, paddocks should be ploughed after crop harvest and shortly before sowing, and crop residues should be collected and destroyed before the onset of rains to reduce carry over from one season to the next.

#### 2.7.2 Control if small areas are affected

Infested areas should be sprayed with esfenvalerate (adulticide), dimethoate (adulticide), abamectin (larvicide) or cyromazine (larvicide - currently registered only for control of sheep blow fly) and plant material then destroyed by burning or deep burial. If *Agromyza* sp. are present, then soil should be ploughed since the puparia of *Agromyza* sp. will be found in the top 5 cm of the soil.

# 2.7.3 Control if large areas are affected

If large areas are affected, the outbreak will be very difficult to eradicate, since these oligophagous pests have a wide host range within the Poaceae and will certainly infest a range of non-crop grasses. It will not be feasible to clear a large area of all species of Poaceae.

#### 2.7.4 Cultural control

Crop debris should be removed immediately from cropping area and disposed of carefully. Agromyzids 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. Following an infestation with an *Agromyza* sp. (where puparia oversummer in the soil), a non-host crop should be grown in the subsequent cropping season.

## 2.7.5 Host plant resistance

There has been very little use made of host plant resistance for control of cereal-infesting cereals. Sasakawa (1954) investigating the effect of barley cultivar on the biology of *Chromatomyia nigra*, found that the number of eggs laid on leaves was strongly correlated with the vigour of the cultivar. He also observed that the early maturing cultivars were more heavily infested than the late-maturing cultivars and that the mortality of larvae was lower on cultivars with higher crude protein levels.

Detailed studies by Scheirs *et al.* (2000, 2001*a*, 2001*b*) showed that ovipositional preference was correlated with adult feeding preference and that nearly all variation in adult performance of *Ch. nigra* was explained by foliar protein content. They also found that C<sub>4</sub> grasses were unsuitable as hosts because the ovipositor and eggs of *Ch. nigra* were too wide to fit between two adjacent veins of C<sub>4</sub> grasses (Scheirs *et al.* 2003).

#### 2.7.6 Chemical control

Unlike the polyphagous *Liriomyza* spp. (Ferguson 2004; Parrella & Keil 1984), the cereal-infesting agromyzids are not known to have developed resistance to any insecticide groups. A range of insecticide groups are effective in reducing populations of cereal-infesting leafminers. Darvas & Andersen (1996) noted that *Chromatomyia fuscula* was vulnerable to foliar insecticides because the flies prefered to oviposit on the youngest leaves. They showed that cyromazine and dimethoate both controlled *Ch. fuscula* but that dimethoate was more destructive of natural enemies than cyromazine. Andersen (1989) noted that Norwegian barley growers frequently sprayed their crops against *Ch. fuscula* with dimethoate even if the effect of an attack upon the yield was questionable. Andersen (1993) found that lambda-cyhalothrin and fenitrothion were effective against *Ch. fuscula*. If the infestations were restricted to the lower leaves then spraying had no significant effect on size or quality of the yield. However, in those fields where the infestation spread to the upper leaves, yields were affected. Andersen (1993) concluded that spraying at the 3-4 leaf stage or just before heading increased yields, and the latter treatment also increased quality. To achieve the best yield with least amount of insecticide, the best strategy was to spray just before heading if infestation was high.

In a European study the application of deltamethrin on winter barley decreased Agromyza megalopsis populations (Kazda 1997). In western Sweden, applications of esfenvalerate reduced *Ch. fuscula* 

populations and increased oat yields (Hedene 1992). Similar studies in France showed that applications of deltamethrin, dimethoate or phosalone reduced populations of *Ch. nigra* (Fischer & Chambon 1987). Procedures for evaluation of insecticides against dipteran leafminers have been documented in Europe (EPPO 2005). However, the danger of pesticide-induced outbreaks because of destruction of natural enemies should be considered when using broad-spectrum insecticides.

#### 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 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 (Murphy & LaSalle 1999; Spencer 1973). The initial outbreaks of A. megalopsis in Germany in 1964 and 1965 led to a big upsurge in parasitism, which was believed to have been the major reason for the subsequent observed population decline of A. megalopsis (Spencer 1973). Leafminer parasitoids tend not to be very host-specific so there will be great difficulty in importing exotic parasitoids into Australia. Fortunately, there is a diverse fauna of eulophid and braconid parasitoids already present in Australia (some native, others inadvertent introductions) (Bjorksten et al. 2005; Fisher et al. 2005; Lambkin et al. 2008). In southern Australia, common agromyzids, such as Liriomyza chenopodii, L. brassicae, Chromatomyia 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 cereal-infesting 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 to establish whether key parasitoids, such as Diglyphus isaea, exhibit a facultative summer diapauses under Australian conditions.

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.

#### 3 Course of action – Eradication methods

Additional information is provided by the IPPC (1998) 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 pest free area) or a measure to eliminate an established pest (establish a pest free area). The eradication process involves three main activities: surveillance, containment, and treatment and/or control measures.

# 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.
- Infected crops or pastures could be ploughed in.
- Insecticides could be used to destroy the pest.
- Farm machinery used in destruction processes need to be thoroughly washed, preferably using a detergent such as Decon 90.

## 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 Appendix 18 (PHA 2008)).
- 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

Specific priorities for eradication

- Confirm the presence of the pest.
- Prevent movement of vehicles and equipment through affected areas.

- Priority of eradication/decontamination of infected host material.
- Control cereal leafminer populations to prevent further spread.
- Inform all groups within the industry.
- Determine the extent of infection through survey.

## 3.1.4 Plants, by-products and waste processing

- Infected plant material removed from the infected site should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial (in a non-cropping area).
- All straw from susceptible hosts should be destroyed by burning as pupae can 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.
- No particular issues with resistance of disease to chemicals or physical treatments are known to exist.

#### 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, so movement or quarantine controls may be
  effective, providing the pests have not been established for a long period of time over a wide
  area. However, they have the capacity to move longer distances by wind dispersal. The
  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

Hay or stubble must not be removed from the site or used for feeding stock due to the risk of moving adults, larvae or pupae. Alicandro & Peters (1983) investigated the survival of *Agromyza frontella*, alfalfa blotch leafminer, when infested lucerne was cut for hay. Simulated windrow experiments revealed that 32% of 3<sup>rd</sup> instar larvae completed development and formed puparia in lucerne hay during field curing. However, 1<sup>st</sup> and 2<sup>nd</sup> instar larvae suffered almost complete mortality. They also noted that

adult emergence was 45% higher under windrows compared with areas where the harvested lucerne was raked immediately. While this study can only be used as a rough guide to the situation with cereal-infesting leafminers, it suggests that if infested cereals or pasture grasses are cut for hay, many 3<sup>rd</sup> instar larvae are likely to survive through to pupation. The study reinforces the dangers of moving hay from the infested area.

Movement of people, vehicle and machinery, from and to affected farms, must be controlled to ensure that infested soil (if an *Agromyza* sp. is the target pest) or plant debris is not moved off-farm on clothing, footwear, vehicles or machinery.

As described in Section 2.1.3, agromyzid flies are considered as "moderate fliers" (Yoshimoto & Gressitt 1964) but in agricultural situations will often only moving very short distances between host plants (Zehnder & Trumble 1984). However, the flies will be infesting non-crop Poaceae as well as the cereal crops. Additionally, they have the capacity to move longer distances by wind dispersal. This will make the establishment of guarantine areas of limited use in the longer term.

If Restricted or Quarantine Areas are required, movement of equipment or machinery is to be restricted and movement into the Area is to occur by permit only. The industry affected will need to be informed of the location and extent of the pest occurrence. People, vehicle and machinery movements, from and to affected farms, will need to be controlled to ensure that infested soil or plant debris is not moved offfarm on clothing, footwear, vehicles or machinery. Clothing and footwear worn at the infested site should not leave the farm or they must be thoroughly disinfested, washed and cleaned before wearing off-farm.

# 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

As described in Sections 2.1.3, 2.3 and 3.2.2, agromyzid flies are considered as "moderate fliers" (Yoshimoto & Gressitt 1964) but in agricultural situations will often only move very short distances between host plants (Zehnder & Trumble 1984). However, the flies will be infesting non-crop Poaceae as well as the cereal crops. Additionally, they have the capacity to move longer distances by wind dispersal. This means that the establishment of a destruction zone should only occur if the infestation is believed to be very restricted. 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.

If destruction zones are established, all host plants within initial site of infestation should be destroyed to reduce food source and/or refuge for leafminers. In addition or alternatively, the Destruction Zone may be defined as contiguous areas associated with the same management practices as the infested area (i.e. the entire trial, paddock or farm if spread could have occurred prior to the infestation 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 pathogen thus preventing its spread to other areas.

#### 3.4.1 Decontamination procedures

General guidelines for decontamination and clean up:

- 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 infested 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 are to be used when conducting decontamination procedures, and should be applied according to the product label.

Where crops are left *in situ*, kill any remaining adult leafminers with a weekly treatment of adulticide. 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. Crop debris may be disposed of by incineration or by deep burial (at a depth of at least 0.3 m).

Refer to PLANTPLAN (Plant Health Australia, 2008) for further information and Technical guidelines for the development of pest specific response plans (Merriman & McKirdy 2005).

## 3.4.2 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 properties/land with suitable hosts (Poaceae) in the pest quarantine area.
- Surveying all properties identified in trace-forward or trace-back analysis as being at risk.
- Surveying other host growing properties and areas growing non-crop Poaceae.
- Surveying host growing properties that are reliant on trade with interstate or international markets which are sensitive to leafminer presence.

#### 3.5.2 Survey regions

Establish survey regions around the surveillance priorities identified above. These regions will be generated based on the zoning requirements (see 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 and pastures 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 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 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-borne dispersal of the pest during these weather events.

#### Phase 5:

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

#### Phase 6:

Agreed area freedom maintenance, pest control and containment.

#### 3.5.3 Post-eradication surveillance

Specific methods to confirm eradication of leafminers may include:

- Surveys comprising plant sampling for immature leafminers and net sweeping for adults to be undertaken for a minimum of 12 months after eradication has been achieved. Surveys to be conducted weekly for 6 weeks after detection and treatment, then monthly.
- Deployment of yellow sticky traps in a 50 m grid in the zone. Traps should be inspected (and trap changed) on a weekly basis for 6 weeks and then traps should be deployed on a monthly basis (i.e. trap in field for 1 week each month).
- During summer, sentinel plants could be used at the site of infestation. The suggestion would be to use 10 pots of barley seedlings (in self-watering pots) put out each month for 1 week.

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#### 4.1.1 Agromyza ambigua

http://www.bladmineerders.nl/minersf/dipteramin/agromyza/ambigua/ambigua.htm

http://nlbif.eti.uva.nl/bis/agromyzidae.php?selected=beschrijving&menuentry=soorten&id=27

http://www.nhm.ac.uk/research-curation/research/projects/british-insect-mines/database/Checklist.do?pg=cl&flyId=7

http://www.ukflymines.co.uk/Flies/Agromyza\_ambigua.html

http://www.inra.fr/hyppz/RAVAGEUR/6agrspp.htm

## 4.1.2 Agromyza megalopsis

http://www.bladmineerders.nl/minersf/dipteramin/agromyza/megalopsis/megalopsis.htm
http://nlbif.eti.uva.nl/bis/agromyzidae.php?selected=beschrijving&menuentry=soorten&id=34

#### 4.1.3 Cerodontha denticornis

http://www.bladmineerders.nl/minersf/dipteramin/cerodontha/denticornis/denticornis.htm http://nlbif.eti.uva.nl/bis/agromyzidae.php?menuentry=soorten&selected=beschrijving&id=47

# 4.1.4 Chromatomyia fuscula

http://nlbif.eti.uva.nl/bis/agromyzidae.php?selected=beschrijving&menuentry=soorten&id=53

# 4.1.5 Chromatomyia nigra

http://www.bladmineerders.nl/minersf/dipteramin/chromatomyia/nigra/nigra.htm
http://nlbif.eti.uva.nl/bis/agromyzidae.php?selected=beschrijving&menuentry=soorten&id=56

# 5 Appendices

# Appendix 1. Standard diagnostic protocols

Initial identification of field-collected agromyzids in Australia will require use of the key to Australian Agromyzidae (Spencer 1977). This will enable determination of the agromyzid specimen to genus level. Keys to the exotic cereal-infesting agromyzids covered in this plan are given in the key to European agromyzid species attacking cereals by Darvas & Papp (1985). Further detailed information is given by Dempewolf (2004), Griffiths (1980) and Spencer (1973, 1990).

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	Principal Research Scientist (Biosystematics) and Curator of Victorian Agricultural Insect Collection, 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
Dr John La Salle	ACT	Head of the Australian National Insect Collection, and Theme Leader, Invertebrate Biodiversity Assets and Informatics CSIRO Entomology GPO Box 1700, Canberra ACT 2601
		Ph: (02) 6246 4262 ; Fax: (02) 6246 4264

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

Facility	State	Details
		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	SA	Hartley Grove Urrbrae SA 5064
Research Precinct		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	WA	3 Baron-Hay Court South Perth WA 6151
(AGWEST) Plant Laboratories		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**

Within the AQIS PHYTO database, no countries appear to have a specific statement regarding area freedom from cereal-infesting leafminers (*Agromza ambigua, A. megalopsis, Cerodontha denticornis, Chromatomyia fuscula* and *Ch. nigra*) (January 2009). Should cereal-infesting leafminers be detected or become established in Australia, some countries may require specific declarations. Latest information can be found within PHYTO (<a href="www.aqis.gov.au/phyo">www.aqis.gov.au/phyo</a>), using an Advanced search "Search all text" for species names.