

National Diagnostic Protocol

Liriomyza trifolii

American serpentine leaf miner



NDP 27 V1

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National Diagnostic Protocols (NDPs) are diagnostic protocols for the unambiguous taxonomic identification of plant pests. NDPs:

- are a verified information resource for plant health diagnosticians
- are consistent with ISPM No. 27 – Diagnostic Protocols for Regulated Pests
- provide a nationally consistent approach to the identification of plant pests enabling transparency when comparing diagnostic results between laboratories; and,
- are endorsed by regulatory jurisdictions for use (either within their own facilities or when commissioning from others) in a pest incursion.

Where an International Plant Protection Convention (IPPC) diagnostic protocol exists it should be used in preference to NDPs although NDPs may contain additional information to aid diagnosis. IPPC protocols are available on the IPPC website:

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Process

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Document status

This version of the National Diagnostic Protocol (NDP) for *Liriomyza trifolii* is current as at the date contained in the version control box below.

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Further information

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1 INTRODUCTION

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.

Typically, 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, signs of early infestations are much less obvious and are easily overlooked (Spencer, 1989).

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 and Trumble, 1984). However, they do have the capacity to move longer distances by wind dispersal (Spencer and Stegmaier (1973), Yoshimoto and Gressitt (1964), Glick (1939) and White (1970)).

The biology and ecology of polyphagous *Liriomyza* spp. have been reviewed by Kang *et al.*, (2008), Murphy and LaSalle (1999), Parrella (1982, 1987) and Waterhouse and 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 and Broadbent, 1988) and (iv) mechanical transmission of some plant viruses (Costa *et al.*, 1958; Zitter and 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).

1.1 Primary host range

Liriomyza trifolii attacks a wide range of ornamental and vegetable crops. The host range encompasses over 400 species of plants in 24 families (CABI, 2003; Reitz and Trumble, 2002, see Appendix 1). The extreme degree of polyphagy is unusual among species of *Liriomyza*; only five of the more than 300 species of the genus are considered to be truly polyphagous (Spencer, 1973).

2 TAXONOMIC INFORMATION

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Family: Agromyzidae
Genus: *Liriomyza*
Species: *trifolii*

Scientific Name: *Liriomyza trifolii* (Burgess), 1880

Synonyms

Oscinis trifolii Burgess, 1880
Agromyza phaseolunata Frost, 1943
Liriomyza alliovora Frick, 1955
Liriomyza phaseolunata Frost 1943
Liriomyza trifolii de Meijere, 1925
Liriomyza alliivora

Common names

American serpentine leafminer (English)
Celery leafminer (English)
Chrysanthemum leafminer (English)
Serpentine leafminer (English)
Mineuse du gerbera (French)
Floridaminierfliege (German)
Minador pequeño del frijol (Spanish)

3 DETECTION

3.1 Sites of infestation

Adult feeding punctures and larval feeding mines occur only on leaves. The adults however can be found on any part of the plant.

Eggs are inserted under the leaf surface and the number of eggs laid varies according to host and environmental conditions.

Pupation occurs outside the leaf, in the soil beneath the plant. In contrast, the larvae of *Chromatomyia horticola* pupate inside the leaf at the end of the larval mine.

3.2 Symptom description

Feeding punctures and leaf mines are usually the first and most obvious sign of the presence of *Liriomyza*. Adult feeding punctures of *Liriomyza* species 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. They remain intact and relatively unchanged over a period of weeks.

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 (Figure 1). 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).



Figure 1. Mine in tomato leaf caused by *Liriomyza* leafminer, USA. Photograph by: J. Castner, University of Florida.

In very small leaves the limited area for feeding results in the formation of a secondary blotch at the end of the mine, before pupation. Spencer (1985) noted the growth of many *L. trifolii* from mines which began with a conspicuous spiral, however the shape of the leaf mine can vary and should not be used as a diagnostic feature.

Fungal destruction of the leaf may also occur as a result of infection introduced by *L. trifolii* from other sources during breeding activity. Wilt may occur, especially in seedlings.

3.3 Confusion with other organisms in Australia

3.3.1 Family Agromyzidae

The family Agromyzidae, of which the world fauna has about 1900 species in 26 genera, is represented in Australia by 13 genera and about 150 species. Of the 376 species in the genus *Liriomyza*, only 18 species occur in Australia (Spencer, 1977).

The following combination of characters will define the family Agromyzidae:

Vibrissae present, 1-7 frontal bristles present; wing with costal break present at the apex of *Sc*, cell *cup* small, *A1* not reaching wing margin; male with pregenital sclerites with a fused tergal complex of tergites 6-8, with only two spiracles between tergite 5 and genital segment; female anterior part of abdominal segment 7 forming an oviscape.

In practice, agromyzids are reasonably easy to recognize because the larvae of all species feed in the living plant tissue and approximately three-quarters of the species are leaf-miners. Generally the larvae are cylindrical in shape, tapering anteriorly, with projections bearing the anterior and posterior spiracles, the former located on the dorsal surface of prothorax, the latter posteriorly directed at the rear; strongly sclerotised mouthparts, the mandibles with its longitudinal axis at about right angles to the rest of the cephalopharyngeal skeleton and usually bearing two or more pairs of equal sized anteriorly directed teeth.

Common Australian genera of Agromyzidae that may be confused with *Liriomyza* are the very close genera *Chromatomyia* and *Phytoliriomyza*.

Chromatomyia (common species *C. syngenesiae*) and *Phytoliriomyza*, (of which Australia has 21 species), can be separated from *Liriomyza* by their proclinate (forward pointing) orbital setulae (always reclinate or occasionally upright or missing in *Liriomyza*), scutellum generally grey or black but occasionally slightly yellowish centrally (bright yellow in *L. trifolii*), and a distinct male genitalia. *Phytoliriomyza* species can be further differentiated from *Liriomyza* species by the costa which extends only to R4+5 (Spencer 1977). In addition *Phytoliriomyza* species are gall-forming (on stem or leaf) internal feeders whereas *Chromatomyia* and *Liriomyza* species are typically leafminers.

3.3.2 Drosophilidae

The drosophilid *Scaptomyza flava*, recorded as a common leafminer on cultivated brassicas in Tasmania (Osmelak, 1983) and in Victoria, can be distinguished from *Liriomyza* by the lack of incurved lower fronto-orbital bristles, uniformly pale body colour and characteristic male genitalia (incurved lower fronto-orbital bristles present, body usually dark with scutellum yellow and characteristic male genitalia in *Liriomyza*).

3.3.3 Genus *Liriomyza*

Adult flies of the genus *Liriomyza* have the following morphological characters:

small flies, 1-3 mm in length; fronto-orbital setulae reclinate (backward pointing); usually with a dark pre-scutellar area concolorous with the scutum, rarely yellow; scutellum yellow in most species, rarely dark; costa extends to vein M1+2; discal cell small; second crossvein present in most species; stridulating organ present in males (a “scraper”, a chitinized ridge on the hind-femora, and a “file”, a line of low chitinized scales on the connecting membrane between the abdominal tergites and sternites).

Morphological differences between many closely related species of *Liriomyza* are slight, but the minor colour differences which are detectable, are remarkably constant. However, a positive identification of many such species is only possible from examination of the male genitalia (Spencer, 1973).

Australian *Liriomyza*

All Australian species of *Liriomyza* could be potentially confused with *L. trifolii*. However, to distinguish adult *L. trifolii* from these, mostly non-pest, Australian species, the following combination of characters can be used:

frons bright yellow colour of head under outer and inner vertical bristles yellow; colour of hind margin of eyes mostly yellow; third antennal segment yellow; scutellum yellow; mesonotum greyish mat; femora yellow with occasional slight brownish striations; wings with costa extending to apex of vein M1+2, second cross vein present, length of last section of M3+4 3 to 4 times length of penultimate; male distiphallus with one distal bulb with a marked constriction between lower and upper halves.

Other exotic *Liriomyza*

For many years *L. trifolii* was confused with the vegetable leafminer, *L. sativae* (not yet found in Australia), as both have much the same distribution and host range. But they are readily distinguishable from each other: *L. trifolii* has both vertical bristles on yellow ground (outer vertical bristle on black ground in *L. sativae*), a matt greyish mesonotum (shining black in *L. sativae*), yellow occasional slight brownish striations on femora (bright yellow femora in *L. sativae*), and male distiphallus with one distal bulb with a marked constriction between lower and upper halves (one distal bulb with a slight constriction between upper and lower halves in *L. sativae*).

Comparative images can be found in Padil (<http://www.padil.gov.au/> accessed 17.02.2016).

4 IDENTIFICATION

4.1 Stages of Development

4.1.1 Eggs

Eggs of *L. trifolii* are 0.2-0.3 mm X 0.1-0.15 mm, off white and slightly translucent.

4.1.2 Larva

This is a legless maggot with no distinct or separate head capsule (Figure 2). It is transparent when newly hatched but turns to yellow-orange in later instars, and is up to 3 mm long. There are three larval stages. The two posterior spiracles each have three pores, the outer two elongate similar to as in other species such as *L. sativae*.

Spencer (1973) describes distinguishing features of the larvae. Petitt (1990) describes a method of identifying the different instars of the larvae of *L. sativae*, which can be adapted for use with the other *Liriomyza* species, including *L. trifolii*. While this will identify the instars, it will not differentiate between species.



Figure 2. Larva of the American serpentine leafminer, *Liriomyza trifolii* (Burgess)
Photograph by: Lyle J. Buss, University of Florida.

4.1.3 Pupa

This is oval, distinctly segmented, and slightly flattened ventrally, 1.3-2.3 mm X 0.5-0.75 mm, with variable colour, pale yellow-orange darkening to golden-brown (Figure 3). The two posterior spiracles are on a pronounced conical projection, and each has three distinct bulbs, two of which are elongate.



Figure 3. Pupa of the American serpentine leafminer, *Liriomyza trifolii* (Burgess). Photograph by: Lyle J. Buss, University of Florida.

4.1.4 Adult

Liriomyza trifolii is a very small species (1.0 -1.3 mm body length, and up to 1.7 mm in females) with conspicuously matt greyish mesonotum although the ventral surface and legs are yellow (Figures 4 and 5). The thorax and abdomen are mostly gray and black. All antennal segments, face and frons are bright yellow. The hind margin of the eye is largely yellow. The third antennal segment is noticeably but finely pubescent. The orbital setulae are sparse and reclinate. The mesonotum has conspicuous yellow patches at each hind-corner adjoining the scutellum, which is bright yellow. The mesopleura are predominantly yellow with a small blackish-grey patch on the lower margin, and the sternopleura are largely black with the upper margin yellow. The coxae are yellow, the femora are largely yellow but with slight, variable brownish striation, and the tibiae and tarsi are darker, brown. The abdomen is largely black but the tergites are variably yellow, particularly at the sides. The squamae are yellowish, with a dark margin and fringe. The wings are 1.3-1.7 mm in length. Male and female *L. trifolii* are generally similar in appearance. In the male the distiphallus is distinctly constricted apically, the neck behind adjoining and relatively long, a little shorter than the distiphallus proper (Figures 7 and 8).

A detailed description of the species is found in Spencer (1973).



Figure 4. Adult American serpentine leafminer, *Liriomyza trifolii* (Burgess). Photograph by: Lyle J. Buss, University of Florida.

4.2 Specimen collection, handling and preservation

Specimens to be collected

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

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 (e.g., see Fig. 7).

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 and manage 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. They can be stored and transported in the newspaper. For laboratory rearing of adult flies, mined leaves containing larvae can be collected in a large jar and kept in a constant temperature room for regular checking. Pupae are outside the mines, on the leaf material or in soil underneath, and can be collected in a jar for rearing.

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 100% ethanol or frozen (-80°C).

4.3 Morphological or morphometric characteristics

4.3.1 Morphological identification of adult flies

Definitive diagnosis of *Liriomyza* species requires examination of male genitalia and comparison with validated voucher specimens. Experience with identification of *Liriomyza* and other related Agromyzidae and the ability to interpret and relate structures, particularly those of male genitalia, should be demonstrated.

a. Proposed methodology for diagnosis:

1. Examine adult fly specimens under dissecting microscope to assess whether or not they belong to genus *Liriomyza* using external morphological characters.
2. If they are *Liriomyza*, then key through the identification key given in the key below to distinguish them from the Australian species of this genus.
3. If the specimens key to *L. trifolii* or *L. cicerina*, select male specimen and carefully detach abdomen from specimen.
4. Soak abdomen in 10% KOH for several hours until it becomes soft and cleared. Wash in water.
5. Carefully dissect out the genitalia and mount in Hoyer's media on a microscope slide. Remainder of the abdomen may also be mounted on the same slide but under a separate cover slip.
6. Cross label the slide with the data on the remainder of the specimen left in alcohol.
7. Now the slide is ready for examination. Place slide in an oven (incubator) at 40-45°C for drying for a few days before storing in the collection.

A comprehensive illustrated lucid interactive key to distinguish *L. trifolii* from other major exotic *Liriomyza* species, as well as common Australian leafminer species is available on a CD-Rom, as well as on the web [<http://www.lucidcentral.org/keys/v3/leafminers>] (Malipatil and Ridland, 2008).

Following is a simplified key to distinguish the exotic *Liriomyza trifolii*, *L. cicerina*, *L. huidobrensis* and *L. sativae* from the Australian leafminer species of the genus *Liriomyza*.

1. Scutellum largely black, sometimes brownish or greyish, never yellow
 *L. helichrysi*, *L. primitiva*, *L. scaevolae* and non-*Liriomyza* leafminers
 Scutellum distinctly yellow, at least centrally (e.g., Fig. 5) 2
2. Second cross-vein in wing lacking
 *L. caulophaga*, *L. chenopodii*, *L. lepida*, *L. meracula*, *L. oleariana*, *L. tenera*
 Second cross-vein in wing present (e.g., Fig. 6) 3
3. Femora yellow, without dark or brownish striations 4
 Femora yellow, with dark or brownish striations 5
4. Male aedeagus with distiphallus with one distal bulb with slight constriction between lower and upper halves; [vertex of head black under outer vertical bristles and yellow under inner bristles; shining black mesonotum *L. sativae*

- Male aedeagus with distiphallus not as above; vertex of head black under inner and outer vertical bristles or yellow under inner bristles
*L. brassicae*, *L. cassiniae*, *L. compositella*, *L. electa*, *L. languida*, *L. obscurata*
5. Vertex of head yellow under outer vertical bristles *L. trifolii*
 Vertex of head black under outer vertical bristles 6
6. Third antennal segment yellow; last section of wing vein $M_{3+4} < 2$ times length of penultimate
*L. inopinata*
 Third antennal segment dark, at least partially; [last section of wing vein $M_{3+4} \geq 2$ times length of
 penultimate 7
7. Male aedeagus with single lobed distiphallus; scutellum bright yellow centrally, black at sides;
 mesonotum largely matt *L. impolita*
 Male aedeagus with bilobed distiphallus; scutellum yellow; mesonotum shining black 8
8. Entire 3rd antennal segment variably black; male distiphallus with distal lobes meeting from
 their rims to their bases; smaller species, wing length 1.3 - 1.5 mm; Leguminosae only
*L. cicerina*
 Only distal 1/3 antennal segment dark; male distiphallus with distal lobes meeting only at their
 rims; larger species, wing length 1.7 - 2.2 mm; polyphagous *L. huidobrensis*



Figure 5a & b. Adult of American serpentine leafminer, *Liriomyza trifolii* (Burgess).
 (Photograph by: A. Ames, Victorian State Government Department of Environment and Primary
 Industries, Australia).

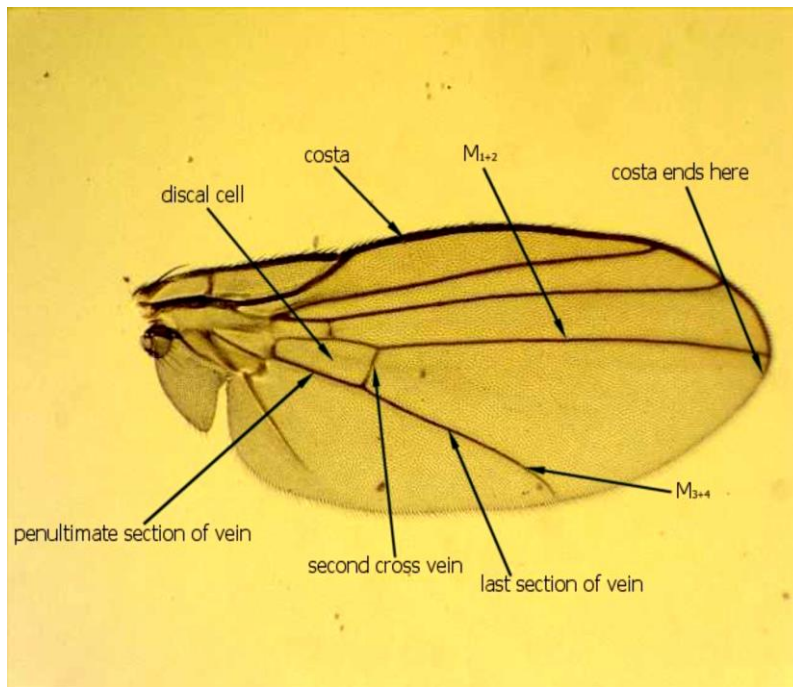


Figure 6. Wing of *Liriomyza* sp. (Photograph by: Victorian State Government Department of Environment and Primary Industries, Australia).

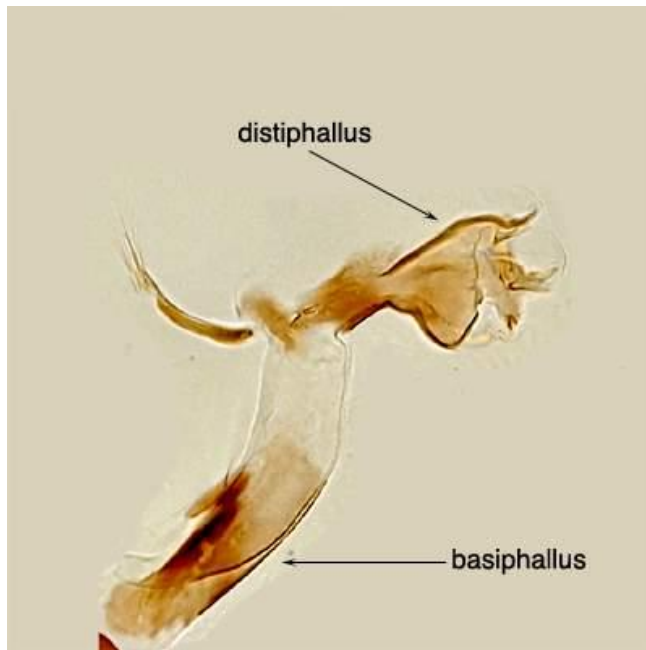


Figure 7. Aedeagus of American serpentine leafminer, *Liriomyza trifolii* (Burgess). (Photograph by: A. Ames, Victorian State Government Department of Environment and Primary Industries, Australia).

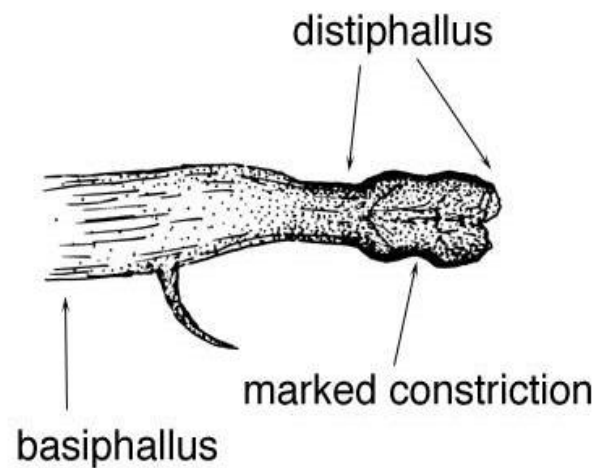


Figure 8. Distiphallus of American serpentine leafminer, *Liriomyza trifolii* (Burgess). (Drawing source: Malipatil & Ridland 2008).

4.4 Molecular diagnostic tests for larvae and pupae

Various PCR based molecular methods have been employed to identify *Liriomyza* species, including: PCR-RFLP, species specific PCR primers and DNA Barcoding (outlined further below). PCR methods are available in the EPP0 protocol (EPP0, 2005), however these specific techniques have not been validated for distinguishing Australian *Liriomyza* species from *L. trifolii*.

In Australia, the Department of Environment and Primary Industries, AgriBio, Bundoora has recently completed the development of this capability that is now available for testing future samples (Blackett *et al.* 2015). Dr Sonja Scheffer of Systematic Entomology Laboratory, USDA also has the capability to undertake molecular diagnostic tests for a number of *Liriomyza* species including *L. trifolii* (Scheffer, 2000).

Restriction Fragment Length Polymorphism (RFLP) tests have been used for identification of some *Liriomyza* species (summarized in Nakamura *et al.* 2013). In Australia, two molecular tests, have been previously adopted (summarized in Semeraro and Malipatil 2007) to potentially identify three exotic (to Australia) *Liriomyza* species - *L. huidobrensis*, *L. sativae* and *L. trifolii* - that have recently spread throughout Southeast Asia (Andersen *et al.* 2008). These tests each apply PCR-RFLP methods to distinguish these species from each other and from a limited number of Australian endemic or currently established *Liriomyza* species. The first test (Bjorksten & Hoffmann 2005) was developed to examine the Cytochrome Oxidase I (COI) gene region to distinguish between eight *Liriomyza* species, while Kox *et al.* (2005) developed an RFLP test for the Cytochrome Oxidase II (COII) gene region to distinguish eight *Liriomyza* species of economic concern.

Additional alternative molecular identification PCR-based protocols employing species-specific primers (for the COI gene) have also been developed for a limited number of *Liriomyza* species, including *L. trifolii* (Miura *et al.* 2004, Nakamura *et al.* 2013).

DNA barcoding (DNA sequence species identification) is potentially a much more informative method of molecular identification, as it directly characterizes a greater number of variable sites than the other molecular methods outlined above (e.g. Armstrong & Ball 2005), and can utilise the large number of reference specimens that are now present on DNA sequence databases. In common with other invasive species (Boykin *et al.* 2012) a variety of DNA regions have previously been utilised for DNA sequence identification of leafminers. The 3' region of the COI gene has been used to identify agromyzid species (Scheffer *et al.* 2006), while the 5' end of COI, generally considered the "Universal" DNA sequence identification region employed in DNA barcoding (Ratnasingham & Hebert 2007), is now also beginning to be employed for leafminer identification (Bhuiya 2011, Blackett *et al.* 2015). There are currently 26 species of *Liriomyza* that have DNA barcode reference sequences available on the Barcode of life database (BOLD, <http://www.boldsystems.org>, Accessed Oct 2013), including *L. trifolii*.

4.4.1 Electrophoretic identification of larvae and pupae

Electrophoresis methods are available in the EPP0 protocol (EPP0, 2005). However these techniques have not been validated for distinguishing Australian *Liriomyza* species from *L. trifolii*.

5 CONTACTS FOR FURTHER INFORMATION

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

Expert	Details
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The National Diagnostic Protocol for *Liriomyza trifolii*, American serpentine leaf miner was peer reviewed and verified by Diane C Jones (Entomologist), Plant Health and Environment Laboratory, Ministry for Primary Industries, 14 Sir William Pickering Drive, Christchurch 8053, New Zealand.

7 REFERENCES

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8 APPENDICES

8.1 Host list

Host list of *Liriomyza trifolii*. Please note that this list should not be considered complete or definitive.

Primary hosts

Basellaceae: *Basella*

Caryophyllaceae: *Dianthus* (carnation), *Gypsophila* (baby's breath)

Chenopodiaceae: *Beta vulgaris* var. *saccharifera* (sugarbeet), *Spinacia oleracea* (spinach)

Compositae: *Ageratum*, *Ambrosia* (ragweed), *Artemisia* (wormwoods), *Aster*, *Baccharis*, *Bidens* (burmarigold), *Callistephus*, *Carthamus*, *Chrysanthemum* (daisy), *Dahlia*, *Eupatorium*, *Gaillardia*, *Galinsoga*, *Gerbera* (Barbeton daisy), *Helianthus*, *Lactuca sativae* (lettuce), *Senecio* (groundsel), *Sonchus* (milkthistle), *Tagetes*, *Taraxacum* (dandelion), *Tithonia*, *Tragopogon*, *Zinnia*

Convolvulaceae: *Ipomoea* (morning glory)

Cruciferae: *Brassica rapa* subsp. *chinensis* (Chinese cabbage)

Cucurbitaceae: *Citrullus*, *Cucumis melo* (melon), *Cucumis sativus* (cucumber), *Cucurbita pepo* (ornamental gourd)

Euphorbiaceae: *Ricinus*

Gramineae: *Avena sativa* (oats), *Hordeum* (barleys)

Labiatae: *Moluccella*, *Ocimum*, *Salvia*

Leguminosae: *Arachis hypogaea* (groundnut), *Cassia* (sennas), *Crotalaria*, *Glycine max* (soybean), *Lathyrus* (vetchling), *Macrotyloma*, *Medicago sativae* (lucerne), *Melilotus* (melilots), *Phaseolus* (beans), *Phaseolus lunatus* (lima bean), *Phaseolus vulgaris* (common bean, kidney bean), *Pisum sativum* (pea), *Trifolium* (clovers), *Trifolium repens* (white clover), *Trigonella*, *Vicia* (vetch), *Vigna unguiculata* (cowpea)

Liliaceae: *Allium sativum* (garlic), *Allium schoenoprasum* (chives), *Allium* spp., *Alstroemeria* (Inca lily)

Malvaceae: *Abelmoschus esculentus* (okra), *Gossypium* (cotton), *Malva* (mallow)

Polemomiaceae: *Phlox*

Rosaceae: *Crataegus* (hawthorns)

Scrophulariaceae: *Antirrhinum* (snapdragon)

Solanaceae: *Capsicum annuum* (bell pepper), *Cestrum*, *Solanum lycopersicon* (tomato), *Physalis* (groundcherry), *Solanum melongena* (aubergine), *Solanum tuberosum* (potato)

Tropaeolaceae: *Tropaeolum*

Typhaceae: *Typha*

Verbenaceae: *Verbena*

Zygophyllaceae: *Tribulus*, *Mollucella*

Secondary hosts

Chenopodiaceae: *Chenopodium* (goosefoot)

Compositae: *Bellis*, *Centaurea* (knapweed), *Erigeron* (fleabane), *Gazania*, *Xanthium* (cocklebur)

Iridaceae: *Gladiolus* hybrids (sword lily)

Primulaceae: *Primula* (primrose)

Leguminosae: *Glycine*, *Medicago* (medic)

Liliaceae: *Allium cepa* (onion)

Scrophulariaceae: *Linaria* (toadflax)

Umbelliferae: *Apium graveolens* var. *dulce* (bleached celery), *Daucus carota* (carrot)