Training Workshop on Diagnostics of Leafminers of Agricultural Importance

29 February - 11 March, 2016

Research Center for Biology, Indonesian Institute of Science (LIPI), Cibinong, Bogor, Indonesia

Compilation of Lecture Notes by:

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2016

Liriomyza and *Chromatomyia* leafminers of economic significance (Diptera: Agromyzidae)

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What is a fly?

- Anatomical Atlas of Flies
- http://www.ces.csiro.au/biology/fly/fly.html#

Family Agromyzidae

- The following combination of characters will define the family Agromyzidae (Hennig 1958; Spencer 1987):
- vibrissae present
- 1-7 orbital bristles present
- wing with costal break present at the apex of Sc
- wing cell cup small; wing veins A_1+CuA_2 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.

Genus *Liriomyza*

- Adult flies of the genus *Liriomyza* have the following morphological characters (EPPO 2005; Spencer 1987):
- 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 M_{1+2}
- discal cell (dm) small
- second (outer) crossvein (dm-cu) 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).

Genera that may be confused with *Liriomyza*

- The closely related genera *Phytomyza* (6 species in Australia), *Chromatomyia* (1 species in Australia) and *Phytoliriomyza* (21 species in Australia) (Elliott 2006), can generally be separated from *Liriomyza* by their proclinate (forward pointing) fronto-orbital setulae (always reclinate or occasionally upright or missing in *Liriomyza*), scutellum generally grey or black but occasionally slightly yellowish centrally (entirely yellow in most *Liriomyza*), and a distinct male genitalia. *Phytoliriomyza* species are gall-forming (on stem or leaf) internal feeders whereas *Chromatomyia*, *Phytomyza* and *Liriomyza* species are typically leafminers. In *Phytomyza* and *Chromatomyia*, the costa extends only to R_{4+5} whereas in *Phytoliriomyza* and *Liriomyza* it extends to vein M (Spencer 1977).

Family Drosophilidae

The drosophilid, *Scaptomyza flava*, is recorded as a common leafminer on forage brassicas, vegetable brassicas and brassicaeous weeds in Tasmania (Osmelak 1983), Victoria (Malipatil MB & Ridland PM unpublished data) and New Zealand (Martin 2004). It can be distinguished from *Liriomyza* by the lack of incurved lower orbital bristles, uniformly pale body colour and characteristic male genitalia (*Liriomyza* with incurved (reclinate) lower orbital bristles present, body usually dark with scutellum yellow and characteristic male genitalia).

Liriomyza and *Chromatomyia* of SEAsia

Agromyzidae - >20 genera, >2450 species worldwide

***Liriomyza* - >300 described species worldwide**

Major pest species

- ***Liriomyza huidobrensis***
- ***Liriomyza sativae***
- ***Liriomyza trifolii***
- ***Liriomyza bryoniae***
- ***Liriomyza chinensis***
- ***Liriomyza brassicae***
- ***Chromatomyia horticola***
- ***Chromatomyia syngenesiae***

Liriomyza of Australia

Agromyzidae - 13 genera, 150 species

***Liriomyza* - 18 described species**

Common species

- ***Liriomyza brassicae***
- ***Liriomyza betae (=chenopodii)***
- ***Liriomyza caulophaga***
- ***Liriomyza meracula***
- ***Liriomyza helichrysi***
- ***Liriomyza obscurata***
- ***Liriomyza electa***

Liriomyza

Major pest species of exotic threat to Australia

- ***Liriomyza huidobrensis*** **- widely distributed**
- ***Liriomyza sativae*** **- widely distributed**
- ***Liriomyza trifolii*** **- widely distributed**
- ***Liriomyza bryoniae*** **- widely distributed**
- ***Liriomyza chinensis*** **- China, Japan, Malaysia, Indonesia**
- ***Liriomyza cicerina*** **- few countries in Africa, Asia and Europe**

Liriomyza

Major pest species of exotic threat to Australia

- ***Liriomyza huidobrensis*** **- highly polyphagous**
- ***Liriomyza sativae*** **- highly polyphagous (primarily but not exclusively Solanaceae, Fabaceae and Asteraceae)**
- ***Liriomyza trifolii*** **- highly polyphagous**
- ***Liriomyza bryoniae*** **- highly polyphagous**
- ***Liriomyza chinensis*** **-** ***Allium*** **(onions, etc)**
- ***Liriomyza cicerina*** **- Fabaceae (beans, peas, etc)**

Liriomyza of Australia

Hosts

- ***Liriomyza brassicae*** **- Brassicaceae, Tropaeolaceae (*Tropaeolum majus*)**
- ***Liriomyza betae*** **- Chenopodiaceae, Caryophyllaceae, Brassicaceae**
- ***Liriomyza caulophaga - Beta*** **(Chenopodiaceae)**
- ***Liriomyza helichrysi*** **- *Helichrysum* (Asteraceae)**

Liriomyza

Australia's preparedness

(= development of diagnostic capability for these exotic species)

ACIAR project: *Liriomyza* leaf miner: developing effective pest management strategies for Indonesia and Australia

Liriomyza of Indonesia

- **All *Liriomyza* collected from Indonesia and on different hosts, including *Chrysanthemum,* identified**
- ***L. huidobrensis, L. sativae, L. brassicae* and *L. chinensis***
- ***L. katoi* and *L. yasumatsui,* both on *Chrysanthemum* hosts, are new records to Indonesia (North Sulawesi)**

Liriomyza in Indonesia

- ***L. huidobrensis* (introduced around 1994) and *L. sativae* (arrived around 1996), both damaging a wide range of vegetable crops, with catastrophic damage to potato crops by *L. huidobrensis***
- ***L. huidobrensis* occurs above 1000 m while *L. sativae* is restricted to elevations below 700 m**
- **Both species can occur at altitudes between 700 - 1000 m, sometimes in the same plot**

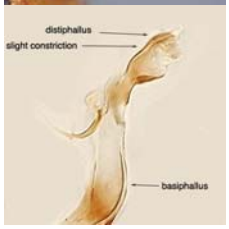
Liriomyza in Indonesia

- **A third serious leafminer pest, *L. chinensis* was introduced into Indonesia around 2000 and is a major pest of onions in lowland areas of central Java**
- **Two additional species, *Liriomyza katoi* and *L. yasumatsui* collected from North Sulawesi are the most recent introductions (about 2002) and appear to prefer C*hrysanthemum* and related Asteraceae**

Leafminers Identification

1. Morphological:
   - Diagnostic characters,
   - Keys, etc.
2. Molecular:
   - Protein variation (allozymes),
   - DNA variation (RFLP, DNA sequencing, DNA Barcoding).

Leafminers – Morphology



Malipatil & Ridland 2008 - Lucid Key

Reliable identification only possible from adult males

Leafminer Flies (Agromyzidae)

Vegetable Leafminer

- *Liriomyza sativae*
- Most serious Agromyzid pest.
- Larval & Pupal lifestages in leaves.
- *Highly Polyphagous:* Recorded from nine plant families
- Spread throughout tropical and subtropical Asia since the 1990's.

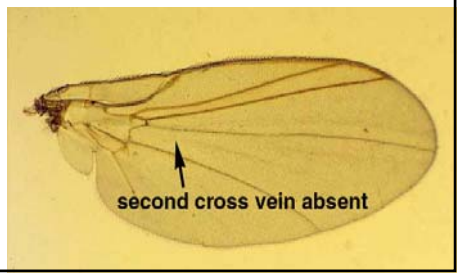
Source:
Lucid Key – Malipatil & Ridland 2008

Damage on bean (P. Ridland)

Liriomyza

Diagnostic protocols - Morphological

- Lucid identification keys for
  - exotic pest species
  - major Australian species



Liriomyza

External morphology similar

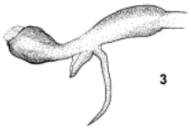
Male genitalia distinct

- ***L. katoi***
- ***L. yasumatsui***
- ***L. trifolii***
- other species from *Chrysanthemum* hosts

Liriomyza male genitalia:

L. katoi

L. yasumatsui



L. trifolii

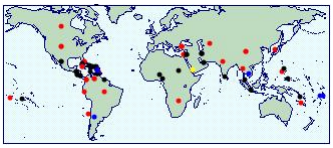
Liriomyza sativae - Distribution

North, Central and South America;

Europe; Africa; Middle east;

Asia (China, India, Indonesia, Japan, Malaysia, Thailand, Sri Lanka, Vietnam);

Oceania:
American Samoa,
Cook Islands,
Federated 80 States of Micronesia,
French Polynesia,
Guam,
New Caledonia,
Northern Mariana Islands,
Samoa,
Vanuatu



But to date not been recorded from Papua New Guinea (PNG) or from the Australian mainland

Source: CABI 2013

Leafminers – NAQS Surveys

Left: NAQS Director of Plant Health Surveillance James Walker (right) and DAFF Senior Entomologist Anthony Rice inspect Citrus plants in the lush Daintree region, Queensland.
Photo: Jennifer Eddie

Liriomyza sativae initially detected by NAQS in Torres Strait in 2008 from a single tomato plant

Leafminers – NAQS Surveys

Specimen

FTA Cards Samples

FTA Cards:

Advantages:
Storage at ambient temperature.
No Ethanol or freezing required
In the field.

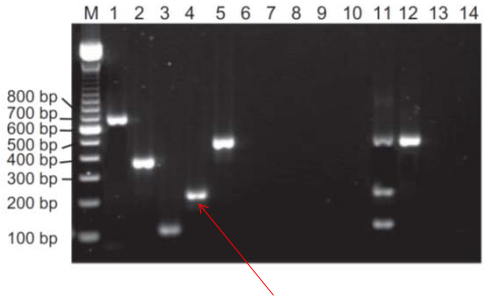
Partially process DNA extraction, reduced biosecurity / biohazard risks.

Easy to transport.

Disadvantage: Morphological destruction of specimens

DNA extractions:
DNeasy® Blood and Tissue Kit; Qiagen

Leafminers – Molecular Identification



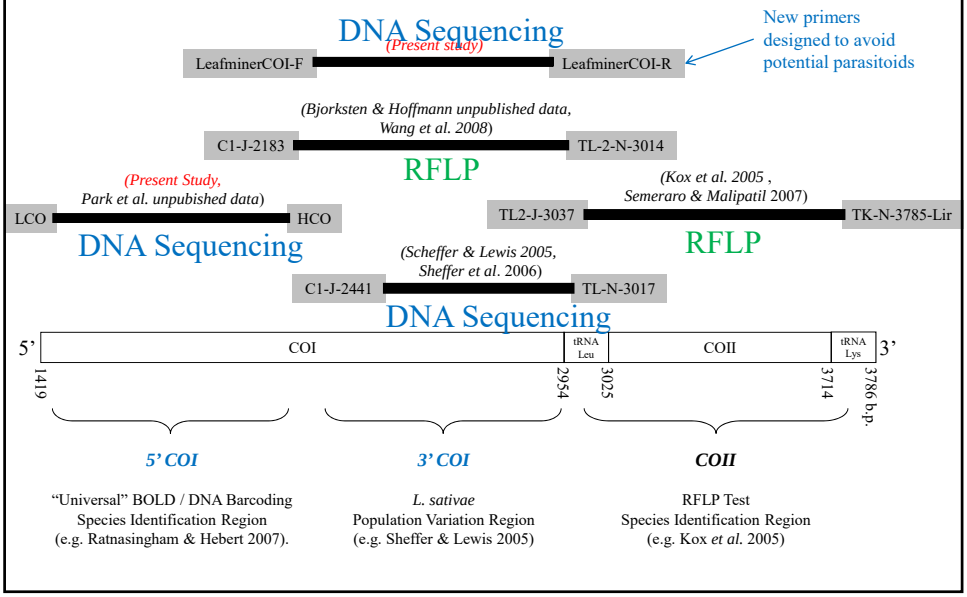
L. sativae

MOLECULAR ECOLOGY RESOURCES
Molecular Ecology Resources (2012) doi: 10.1111/1755-0998.12025

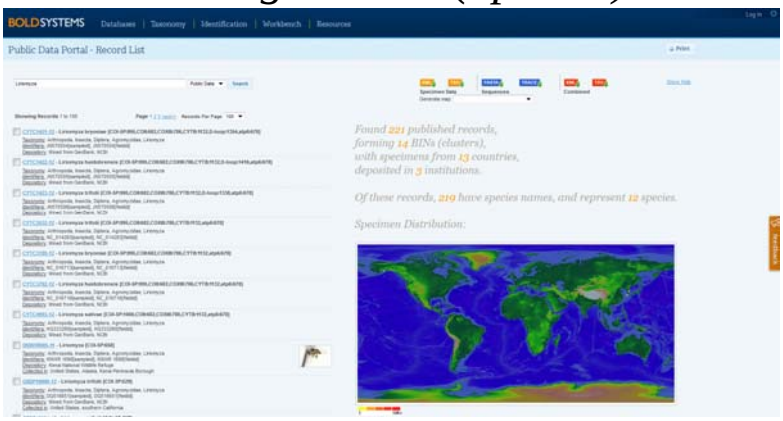
Primer design for identifying economically important *Liriomyza* species (Diptera: Agromyzidae) by multiplex PCR

SHIGEO NAKAMURA,* TOSHIO MASUDA,* ATSUSHI MOCHIZUKI,† KAZUHIKO KONISHI,‡ SUSUMU TOKUMARU,§ KEIICHIRO UENO¶ and TAKUHIRO YAMAGUCHI¶

Leafminers – Molecular Identification



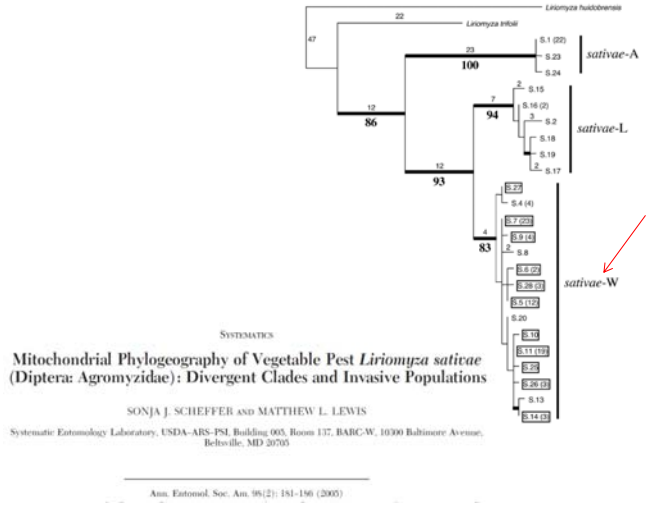
Leafminers – Molecular Identification
5' region COI (Species)



Species Identification, BOLD (2014):

- Agromyzidae, 323 x species, (212 x species sequences accessible)
- *Liriomyza*, 31 x species (17 x species sequences accessible)

Leafminers – Molecular Identification
3' region COI (Populations)



SYSTEMATICS

Mitochondrial Phylogeography of Vegetable Pest *Liriomyza sativae* (Diptera: Agromyzidae): Divergent Clades and Invasive Populations

SONJA J. SCHEFFER AND MATTHEW L. LEWIS

Systematic Entomology Laboratory, USDA–ARS–PSI, Building 005, Room 137, BARC-W, 10300 Baltimore Avenue, Beltsville, MD 20705

Ann. Entomol. Soc. Am. 98(2): 181–196 (2005)

Population Identification:

Three genetic groups of *L. sativae*

All *L. sativae* populations outside the Americas "W" clade.

To date hundreds of *L. sativae* screened: (Scheffer *et al.* 2005 & 2006)

Leafminers – Molecular Identification

- Verified new location records for *L. sativae* from multiple hosts (tomato, pumpkin, castor oil plant, & weeds).
- Useful identification technique for larval / pupal life-stages. New primers used avoid potential parasitoids.
- Found it useful to screen both COI-5' (species ID) & COI-3' (population ID).
- Revealed multiple introductions into PNG and Torres Strait.
- Surrounding region (SE Asia) not screened genetically yet.
- Not detected from Australian mainland, to date.

Acknowledgements

Biology and economic importance of leafminers

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Agriculture Victoria, Melbourne Australia

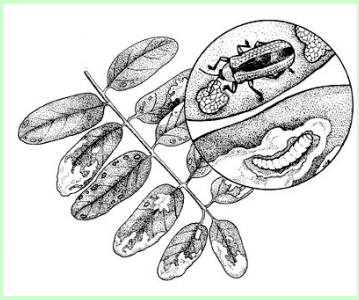
Leaf miner

Definition: A leaf miner is a species, the larva of which lives and feeds for a part of all of its time between the epidermal layers of a leaf.

Leaf miner (e.g. Agromyzidae, Gracillaridae)
Borer (e.g. Tortricidae)
Intergrade between leaf mining and gall making (e.g. Cecidomyiidae)

Leaf miners

Coleoptera - Scattered among three families
Buprestidae, Chrysomelidae and Curculionidae



Leaf miners

Hymenoptera - leaf miners in this order are nearly all in the family Tenthredinidae or sawflies.

Leaf miners - Lepidoptera

The moth and butterfly order has about 400 species in about 20 families that mine leaves. The majority of the species occur in the families Nepticulidae, Tischeriidae, Gracilariidae, and Gelechiidae.

Gracillaridae: Leaf blotch miners, a large group of small to minute moths with pointed wings, often fold leaves. Examples are oak leaf miners, citrus leaf miner.Larvae are flattened with rudimentary legs and prothoracic segment enlarged laterally, pupate in mine.

Gelechiidae: Very large family, small moths, some leaf miners, others gall makers or leaf.

Leaf miners - Diptera

Two families most prominent, Agromyzidae and Anthomyiidae, also Cecidomyiidae (leaf miners or gall makers)

Blotch mines or linear mines

Genus *Liriomyza*

Genus *Liriomyza* contains more than 300 species.
distributed widely but most common in temperate areas; relatively few species in the tropics.
Within this genus 23 species are economically important, causing damage to agricultural and ornamental plants by their leaf mining activity.
Many of these damaging species are polyphagous, which is uncommon among the Agromyzidae; of 2450 described species in this family only 11 are considered to be truly polyphagous, and 5 of these are in the genus *Liriomyza*.

Feeding

"Serpentine leaf miner" was proposed as a common name for any member of this genus because of the wide distribution, polyphagous nature, and morphological similarity of many of the species
The mines are not always serpentine in all host plants. In addition, mine location in leaves may vary considerably, and either the upper or lower leaf mesophyll may be mined. Some species have larval stages that feed in potato tubers, bore through stems, and feed within seed heads.

Larvae

Agromyzidae larvae are phytophagous, feeding as leaf miners, less frequently as stem miners or stem borers. A few live on developing seeds, or produce galls.

Sometimes larvae in roots or under bark. The biology of many species is as yet unknown. There is a high degree of host specificity, an example being *Phytomyza ilicis*, the holly leaf miner that feeds on no other species.

Quarantine species

Some Agromyzidae are quarantine species in many countries. *Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii* are examples.

Economic importance

A number of species attack plants of agricultural or ornamental value, so are considered pests. These insects are very important to agronomy by the direct damage that they cause, particularly on young plants, the leaf of which may, for example, be completely destroyed. By their nutritional bites females of some species are able to inoculate pathogenic fungi, or to transmit viruses.

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Economic importance

About 10% of the species of Agromyzidae are considered pests. The most important pest genera are *Agromyza*, *Melanagromyza*, *Ophiomyia*, *Liriomyza*, *Napomyza* and *Phytomyza*.
For examples of pest species see Asparagus miner (*Ophiomyia simplex*),*Chromatomyia horticola*, Serpentine leaf miner (*Liriomyza brassicae*).
Some 110 species are known to occur on cultivated plants. A number of species are of particular importance, especially *Liriomyza* and *Ophiomyia* species. Species belonging to the genera *Liriomyza* or *Phytomyza* larvae are extremely polyphagous.

Economic importance

The economic impact of *Liriomyza* leafminers in the United States and throughout the world has been considerable; in California alone it was estimated that the chrysanthemum industry lost approximately 93 million dollars to *L. trifolii* from 1981 through 1985.
Liriomyza leafminers can impact crops in at least six ways: (a) by vectoring disease, (b) by destroying young seedlings, (c) by causing reductions in crop yields, (d) by accelerating leaf drop above developing tomatoes, thus causing "sunburning" of the fruit, (e) by reducing aesthetic value of ornamental plants, and (f) by causing some plant species to be quarantined.

Economic importance

While the results of most of these six types of damage are obvious [e.g. heavy mining and stippling in young seedlings or transplants] can kill a plant and/or dramatically slow growth, it has been difficult to accurately associate specific levels of mining activity with reductions in crop yield. Reductions in photosynthesis and other physiological parameters have been measured in vegetable crops but have not been correlated to yield loss. Studies have shown that greenhouse raised tomatoes can tolerate high levels of damage by *L. sativae* without suffering appreciable losses in yield.

Economic importance

However in a recent study, *bryoniae* on tomato caused yield loss, which was greatest when mining occurred in the leaves close to a young, developing fruit. Predetermined damage thresholds have been incorporated into sequential sampling plans for tomato, but these plans have not received wide acceptance.

Experimental threshold on *L. sativae* in southern coastal pole-tomato fields in California calls for treatment when an average of 10 pupae per sampling tray per day accumulate 6ver a 3-4 day period.

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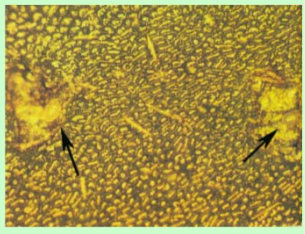
Damage symptoms

Life cycle



Invasive Polyphagous *Liriomyza* spp.

- highly polyphagous
- resistant to many groups of insecticides
- spreading rapidly worldwide in 90s - China, Japan, Israel, Indonesia, Malaysia, Philippines, Sri Lanka, South Africa, Taiwan
- **Papua New Guinea, Australia and/or New Zealand next?**

Polyphagous agromyzid leafminers in Asia

Species	Indonesia	Vietnam	Japan
Liriomyza huidobrensis	✓	✓	✓
Liriomyza sativae	✓	✓	✓
Liriomyza trifolii	✖	✓	✓
Liriomyza bryoniae	✖	✓	✓
Liriomyza chinensis	✓	✓	✓

Polyphagous *Liriomyza* spp. in Indonesia

- *L. huidobrensis* (first detected in 1994) and *L. sativae* (first recorded in 1996) damage a wide range of vegetable crops
  - Potato
  - Bean
  - Tomato
  - Cucumber
  - Onions
- Catastrophic damage to potato crops by *L. huidobrensis* (crops can defoliate 4 weeks prematurely)
- Excessive spraying of potatoes with insecticides (2-3 sprays per week)

Polyphagous *Liriomyza* spp. in Indonesia

- *L. huidobrensis* generally occurs above 1000 m
- *L. sativae* is largely restricted to elevations below 700 m
- Both species can occur at altitudes between 700–1000 m
- Ecological interactions have been investigated at CESAR (Warsito)

Liriomyza huidobrensis in Indonesia

Will these exotic polyphagous agromyzid species establish in Australia?

- Peacock et al. (2006) predict *L. trifolii* and *L. sativae* as likely invaders of New Zealand on basis of climatic variables
- Worner & Gevrey (2006) predict *L. trifolii* and *L. huidobrensis* as likely invaders of New Zealand using SOM (self-organising map) analysis based on CABI Crop Protection Compendium data
- Can we learn from those agromyzid species which have already colonized Australia?

Suspected *Liriomyza trifolii* in Indonesia

- Suspect *Liriomyza trifolii* on chrysanthemum in North Sulawesi
- Females keyed out as *L. trifolii*
- In reality, 2 new records for Indonesia (when males finally submitted)
  - *Liriomyza katoi*
  - *Liriomyza yasumatsui*

Response to insecticides

An important part of the biology of *Liriomyza* is the ability to develop resistance to insecticides. Insecticide resistance has been responsible for failure to control these leafminers for many years. However, definitive studies documenting insecticide resistance have only been done with *Liriomyza trifolii*, and more work with this and other species needed.

Insecticide control - lessons from Indonesia

- broad-spectrum insecticides (pyrethroids, organophosphates) are notorious for eliminating parasitoids and exacerbating leafminer problems
- **cyromazine** and **abamectin** are effective against larvae and are relatively safe against parasitoids (2007)

Agromyzid parasitoids in southern Australia

- Non-pest agromyzid species will provide a reservoir of parasitoids for cropping areas
  - *Chromatomyia syngenesiae*
  - *Liriomyza brassicae*
  - *Liriomyza betae* (syn. *L. chenopodii*)
- Key reservoir plants for parasitoids of agromyzids in southern Australia
  - sowthistle (*Sonchus oleraceus*)
  - chickweed (*Stellaria media*)
  - fat hen (*Chenopodium album*)
  - some brassicaceous weeds
- Augmentative biological control could be initiated relatively quickly since appropriate parasitoid species are already present in Australia (in particular *Diglyphus isaea*)

Leafminer parasitoids of South East Asia LUCID®

- CSIRO Entomology have prepared a Lucid Key **"Leafminer parasitoids of South East Asia"**
- Lucid® 2 & 3 versions
- Now available on WWW

www.ento.csiro.au/science/Liriomyza_ver3/index.html

Response to parasites and predators

Forty parasite species have been found to use members of the genus *Liriomyza* as hosts. Only larval and pupal parasites have been found, and under natural conditions parasitism is usually low early in crop development and gradually increases as the crop matures.
Under greenhouse conditions, inoculation and augmentation of parasites are needed for effective control. In such situations both larval and pupal parasites have been used. Many studies involving these leafminers and their parasites have been concentrated on identifying the parasite complex, estimating its ability to effect control, and determiningt he impact of insecticides on the parasites.

Workshop on the Diagnostics of Leaf miner Insects of Agricultural Importance

Introductions

- Your name
- Your country and organization (employer)
- Your job
- What you do
- Have you brought any specimens?
- Do you intend to collect and study leaf miners after this workshop?... from what crops?

Country Presentations

- **Last session on last day of workshop**
- **One presentation per country – participants to work in country teams**
- **Five minutes**
- **To include anything you did & experienced during this workshop – field observations / collecting, lab work, what leaf miner species you found in your material, basically what you learnt, also include social events, etc.**
- **Prepare power point presentation or other form of presentation with photos, etc**

Some Information Resources on Leafminers

A. Websites

The following links give access to a wide range of information about leafminers and their parasitoids, including images.

(i) Leafminers

Polyphagous Agromyzid Leafminers [M.Malipatil and P. Ridland]

http://keys.lucidcentral.org/keys/v3/leafminers/index.htm

Crop Protection Compendium

http://www.cabicompendium.org/cpc

Martin Dempewolf's agromyzid website

http://wbd.etibioinformatics.nl/bis/agromyzidae.php

Diagnostic photomicrographs of some pest leafminers

http://www.padil.gov.au/browsePestSpecies.aspx?id=16&o=1

EPPO Diagnostic protocols, data sheets, maps & images

http://www.eppo.org/QUARANTINE/listA2.htm

Anatomical Atlas of Flies (needs Broadband)

http://www.ento.csiro.au/biology/fly/fly.html

The Anatomical Atlas was created by CSIRO Entomology to accompany an ABRS-funded identification key to fly families of Australia (on the Fly CD) and US NSF-funded research into the evolutionary history of flies.

However, the Atlas can be used as a standalone resource to accompany any fly key or as an aid for teaching fly anatomy. The atlas works both ways: users can either click on a part to discover its name, or click on a name to discover the location and shape of a part. Common synonyms for anatomical terms are available from the information button that appears when terms and structures are highlighted.

(ii) Leafminer Parasitoids

Lucid key to *Liriomyza* Parasitoids of Southeast Asia

by N. Fisher, R. Ubaidillah, P. Reina and J. La Salle

http://www.ento.csiro.au/science/Liriomyza_ver3/index.html

Lucid Key to the World Genera of Eulophidae Parasitoids (Hymenoptera) of Leafmining Agromyzidae (Diptera) by Placido Reina and John La Salle

http://www.ento.csiro.au/science/eulophid_key/eulophids.html

B. Books

Agromyzidae (Diptera) of Economic Importance.

K.A. Spencer 1973. Series Entomologica Volume 9 Dr W Junk B.V. – The Hague

Handbook for the identification of British Insects Diptera Agromyzidae Volume X, Part 5 (g)

K.A. Spencer 1972 Royal Entomological Society of London

C. CD ROM's

Arthropods of Economic Importance, Agromyzidae of the World

By Martin Dempewolf

ip30.eti.uva.nl/bis/agromyzidae.php

On The Fly, The Interactive Atlas and Key to Australian Fly Families

Published by Australian Biological Resources Study (ABRS) and Centre for Biological Information Technology (CBIT) 2006

(M. Malipatil February 2016)