# Review

# Biological control of *Liriomyza* leafminers: progress and perspective

## Tong-Xian Liu[1]*, Le Kang[2], Kevin M. Heinz[3] and John Trumble[4]

**Address:** [1] Department of Entomology, Texas AgriLife Research, Texas A&M University System, 2415 E. Highway 83, Weslaco, TX 78596, USA. [2] State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China. [3] Department of Entomology, Texas A&M University, College Station, TX, USA. [4] Department of Entomology, University of California, Riverside, CA, USA.

*Correspondence: Tong-Xian Liu. Fax. 01 956-968-0641. Email: tx-liu@tamu.edu

## Abstract

There are more than 330 *Liriomyza* species (Diptera: Agromyzidae) and many are economically important pests of field crops, ornamentals and vegetables. Given the substantial economic losses associated with various aspects of *Liriomyza* feeding as well as the ability of these insects to rapidly develop resistance to insecticides, researchers from many countries have attempted to use biological control to manage these pests. Unfortunately, progress on the science and implementation of effective *Liriomyza* biological control is hampered by the literature being scattered widely and in many different languages. A primary goal of this review is to consolidate the available information and provide an analysis of the published work. Investigations of natural enemies of *Liriomyza* have identified approximately 140 species of parasitoids, a few species of predators (including nematodes), and some entomopathogens. Some species of parasitoids and nematodes have been mass-reared and used for biological control of *Liriomyza* species under confined environmental conditions. While chemical control is still used extensively, conservation biological control and inoculative releases are being adopted as a primary strategy for *Liriomyza* suppression in select situations.

**Keywords:** Leafminer, Parasitoid, Predator, Entomopathogen, Entomopathogenic nematode, Biological control

## Introduction

There are more than 330 *Liriomyza* species described to date, and many are economically important pests of field crops, ornamentals and vegetables throughout the world [1–3]. More than 20 species of *Liriomyza* have been reported as being economically important, and at least six species are polyphagous: *Liriomyza sativae* (Branchard), *Liriomyza trifolii* (Burgess), *Liriomyza huidobrensis* (Branchard), *Liriomyza bryoniae* (Kaltenbach), *Liriomyza strigata* (Meigen) and *Liriomyza longei* Frick [4, 5]. Believed to be of Neotropic origin, the geographical distribution of *Liriomyza* species was restricted to the New World until the mid-1970s. As a result of anthropogenic activities, these species now occur in most of the temperate and tropical regions in the world [6].

The management of agromyzid leafminers continues to be a topic of extensive research and scientific debate. Synthetic and natural insecticides for leafminer control have been extensively researched and are commonly used by farmers and producers regardless of production scale and crop [7–9]. The effectiveness of these insecticides has been reduced by their indiscriminate use, which has adversely impacted natural enemies and resulted in the development of resistance to several groups of insecticides [7–10].

Integrated pest management (IPM) seeks to provide an effective and economical control strategy that minimizes the disturbance of anthropogenic control measures on the natural components of the agro-ecosystems. Asa result, biological control is often emphasized as an important remediation strategy to combat pest outbreaks.

IPM has been researched extensively for the management of *Liriomyza* on field vegetables and other field crops, thus providing the tools necessary for implementation in selected crops and regions of the world [5, 11–18].

Extensive, worldwide investigations of *Liriomyza* natural enemies report more than 140 species of parasitoids [1, 6, 19–38], but only a few species of predators (including entomopathogenic nematodes) and entomopathogens. Several of these species of parasitoids and nematodes have been mass-reared and used for biological control of *Liriomyza* species under confined environmental conditions. Here, we review some major natural enemy species of *Liriomyza* that have been successfully used or have excellent potential for commercialization and subsequent use in biological control programmes. We also review progress in conservation biological control that promotes natural control and the use of biorational insecticides in integrated leafminer management programmes. However, even a comprehensive review such as this cannot include all of the research available for a topic as broad as biological control of *Liriomyza*, and we have necessarily been selective.

## Natural Enemies

*Liriomyza* species are known to have rich natural enemy communities, particularly in their areas of origin [1, 6, 21, 26]. The following provides the key literature listing the natural enemies of *Liriomyza* species by guild.

### Parasitoids

Noyes [30] listed over 300 species of agromyzid parasitoids, and over 80 species that are known to attack *Liriomyza* species. Minkenberg and van Lenteren [6] discussed several European parasitoid species in detail in their review. La Salle and Parrella [22] listed 23 Nearctic species of parasitoids of *Liriomyza*. At least 14 parasitoid species are known from Florida alone [19, 20, 23, 24]. Vega [29] listed 72 species of parasitoids from various countries, with the majority coming from South America. There are several regional reviews for the leafminer parasitoids in Asia and the Pacific Islands [21, 27, 31], including 28 species in Japan [25], 14 species in China [26, 35, 37–39], 11 species in Indonesia [40], 8 species in Malaysia [26, 31] and 18 species in Vietnam [31, 32, 34]. Çikman and Uygun [28] and Çikman *et al.* [33] reviewed the parasitoid species in Turkey. Some parasitoid species are also hyperparasitoids such as *Neochrysocharis formosa*, which is an endohyperparasitoid of several parasitoids of *Liriomyza*. In one study, hyperparasitism by *N. formosa* was found to be as high as 100% for the 2 months immediately following the first inoculative release of *Diglyphus isaea* [41].

### Predators

Although several predatory species have been found feeding on *Liriomyza*, predators are not considered to be important as biological control agents as compared with parasitoids [5, 16–18]. The most common predators are mirids, including: *Cyrtopeltis modestus* (Dist.), *Dicyphus cerastii* Wagner, *Dicyphus tamaninii* Wagner and *Macrolophus caliginosus* Wagner [42–46]. The adults and nymphs are mobile and can prey on leafminer larvae or pupae. *D. tamaninii* may also damage tomato fruits when prey density is low [45, 47]. *M. caliginosus* originates from the Mediterranean, is an important predator of *Liriomyza*, and is able to survive even with low levels of food [48]. *M. caliginosus* used alone or in combination with parasitoids (*D. isaea*) has been used to control *L. bryoniae* in commercial tomato greenhouse situations [43, 48].

Several other predators of *Liriomyza* have been recorded. A ponerine ant (Formicidae: Ponerinae) has been recorded attacking *L. trifolii* larvae [49]. A cecidomyiid fly, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) has been recorded as a predator of *L. bryoniae* on tomato in greenhouses [50]. A number of predaceous flies have been reported preying on *Liriomyza*, including empidids (Diptera: Empididae) and muscid flies (Diptera: Muscidae) attacking adult *L. trifolii* in Israel [51] and some dolichopodids (Diptera: Dolichopodidae) attacking *Liriomyza* spp. in Indonesia [40]. The larva and adult of a predatory thrips, *Franklinothrips vespiformis* (Crawford) has been reported to attack *L. trifolii* larvae [52]. A lynx spider in the Oxyopidae (Arachnida) has been recorded attacking *L. trifolii* adults [49].

### Entomopathogenic Nematodes

A few species of entomopathogenic nematodes have been found infecting *Liriomyza* spp., and those nematodes include *Heterohabditis heliothidis*, *Heterohabditis megidis*, *Heterohabditis* sp. (strain UK 211), *Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* (Filipjev) (=*Neoaplectana feltiae*) [53–61].

Commercially available entomopathogenic nematodes of *Liriomyza* are often reared *in vitro* on an insect host (often the larvae of *Galleria mellonella* L.). The infective stages of nematodes are subsequently sprayed on *Liriomyza*-infested plants *Liriomyza* at crop- and environmentally specific application rates and frequencies depending on the crop [62]. The infective juveniles enter the leafmine via the punctures created by female flies during egg laying or host feeding. Upon contact with the host larva, nematodes are likely to infect the insect via the anus rather than the mouth, but not through spiracles. The nematodes can kill the larvae, the prepuparia and the early puparia. First- and second-instar larvae of *Liriomyza* die soon (0.25–0.66 h) after penetration by a nematode, whereas prepuparia die after an average of 15 h. LeBeck [62] discovered *S. carpocapsae*

to reproduce successfully in all immature stages of *L. trifolii* except for the first-instar and the early puparium. In addition, these nematodes can also attack early puparia of *Liriomyza* in soil. Entomopathogenic nematodes have been found to cause significant reductions in *Liriomyza* populations after application. However, efficacy varies depending on pest species, development stage of the pest, concentrations of nematodes and environmental conditions. Larval mortality of *Liriomyza* can be as low as 4% [54], or reach as high as 85–97% under laboratory or greenhouse conditions at high humidity [55, 63], thus presenting a substantial limitation to widespread use of entomopathogenic nematodes for control of leafminer larvae.

Environmental conditions are critical to the survival and virulence of nematodes. Evidence from various studies suggests a requisite ambient relative humidity of greater than 90% for nematodes to have sufficient time to enter the high humidity leaf mines before desiccating [54–56, 64]. Hara *et al.* [54] found that high levels of relative humidity (at least 92%) were needed to attain even moderately high (greater than 65%) levels of parasitism. Interestingly, temperature is not as critical as humidity. Williams and MacDonald [56] found that when *S. feltiae* was applied to secondinstar larvae, it was equally effective throughout the temperature range of 10–30°C. In some cases, special adjuvants may be required to make the nematodes more effective, such as glyceririne [55]. These apparently modify the microclimate to the benefit of the natural enemy.

An additional concern is that entomopathogenic nematodes do not appear to reliably become established; they frequently disappear quickly after application even following situations where high levels of mortality result. Although nematodes can provide suppression rapidly, and they can be applied through both commercial equipment or manually, biological control of *Liriomyza* using entomopathogenic nematodes has generally proven impractical because of the low mortality (<80%) and sensitivity to humidity [65]. Entomopathogenic nematodes are commercially available in many countries around the world. Currently, because of their high cost and variable effectiveness on *Liriomyza* in comparison with other control agents, their use is restricted to high-value crops in small niche markets. However, the wide range of susceptible *Liriomyza* instars, and the relatively rapid death after penetration by very few nematodes, should encourage continued research and use of entomopathogenic nematodes for leafminer control. From a practical standpoint, the fact that they have better storage ability and can be distributed using existing technologies is equally important. In addition, they may integrate well with other practices [60, 61].

### Entomopathogens

Entomopathogenic fungi that have been reported to infest *Liriomyza* include *Beauveria bassiana* (Bals.) Vuill,

*Paecilomyces fumosoroseus* (Wize) Brown and Smith, *Paecilomyces lilacinus* Vaginitis, *Metarhizium anisopliae* (Metsch.) Sorokin and *Verticillium lecanii* Viegas [66]. Most of these are commercially available for management of numerous pest insects in greenhouses. Research suggests that the efficacy of these materials is not always consistent. Bordat *et al.* [67] tested the susceptibility of *L. trifolii* and *L. sativae* puparia to 11 strains of entomopathogenic fungi in the laboratory. Puparia were placed in peat infected at a rate of about $10^8$ conidiospores/g with suspensions of *B. bassiana*, *M. anisopliae*, *Paecilomyces farinosus* and *P. fumosoroseus*. At 25°C, *L. trifolii* was susceptible to *P. farinosus* (23% adult emergence) and 2 strains of *P. fumosoroseus*. *Liriomyza sativae* was generally less susceptible than *L. trifolii* to the tested strains. One strain of *M. anisopliae* and one strain of *P. farinosus* were modestly efficient, as adults emerged from only 24 and 28% of puparia, respectively. Borisov and Ushchekov [68] tested six species of entomopathogenic fungi against *L. bryoniae*, and found that *P. lilacinus* and *M. anisopliae* were the most effective, reducing adult emergence from soil by 70–94 and 60–88%, respectively, as compared with the untreated control. We could find no information on the potential interactions between fungi and the parasitoid species that attack the puparia. Possibly as a result of inconsistent performance, the entomopathogenic fungi have not attracted attention as leafminer biological control agents, even in greenhouses where environmental conditions can be likely optimized. There appears to be considerable potential for research designed to (1) maximize performance (strain selection, timing and application strategies) of entomopathogens for leafminers, (2) evaluate the possible interactions of parasitoids and entomopathogenic fungi, and (3) develop environmental manipulation or application technologies that minimize the effects of environmental adversity.

### Biological Control Using Parasitoids

Biological control of *Liriomyza* under field conditions has achieved mixed results, and most researchers have targeted *L. trifolii* and *L. sativae* [26]. Biological control efforts have developed in two major agricultural areas: horticultural industries under protected environments [5, 16, 26, 69] and in commercial vegetable production [13]. In general, biological control efforts under greenhouse conditions have focused on inoculation and augmentation strategies. Augmentative releases of natural enemies have been successfully applied in protected environments for control of many vegetable pests [5, 70]. In comparison, successful cases of biological control for leafminers on ornamental crops are few [16]. In field crops, conservation biological control has been the most successful approach [15].

Yano [66] discussed some limiting factors in biological control programmes, including low tolerance of pest

damage by some crops, availability of a reliable supply of natural enemies, poor advisory service for growers and the need for registration and importation of some parasitoids. Many species of arthropod natural enemies are now registered for release in many countries with New Zealand as one notable exception [71], thus resulting in sufficient numbers of imported or indigenous natural enemies now supplied by private companies.

Heinz and Parrella [72] described the attack behaviour, host size selection and host feeding behaviour of the larval ectoparasitoid *Diglyphus begini* (Asmead) on *L. trifolii* in chrysanthemum; and this suite of behaviours has been subsequently verified in other parasitoids attacking *Liriomyza* spp. in various other crops [73–76]. After encountering a larval host, a female parasitoid may either reject a host completely (resulting in no probing with its ovipositor), probe the host with its ovipositor and feed on exuding haemolymph (termed host feeding), or it may probe the host and place one or more eggs in or adjacent to the host (oviposition). The effects of parasitoids on biological control of *Liriomyza* leafminers can vary significantly as the densities of indigenous parasitoids occurring within field grown crops is highly dependent upon a variety of biotic and abiotic parameters. Generally, parasitoid assemblages in natural, unmanaged habitats tend to be more species rich than assemblages of parasitoids on leafminers in agricultural habitats, although significant heterogeneity occurs among studies [77].

The literature suggests a general pattern of parasitism in field crops. Johnson *et al.* [11] found that parasitism of *L. sativae* on tomato by *Chrysocharis oscinidis* was usually low early in crop development and gradually increased as the crop matured, when insecticides were not used. In celery, *L. sativae* and *L. trifolii* populations reached peak levels early in the season, but declined to negligible levels for the last month of the growing season as parasitism reached 90% [78]. Heinz and Chaney [79] found *L. huidobrensis* populations in early season celery to be quite low, possibly because of the activity of indigenous parasitoids. However, biological control in late season celery was frequently lost because of a high influx of leafminer adults from neighbouring lettuce fields. Neuenschwander *et al.* [80] found that five indigenous eulophid species plus five other rare parasitoids frequently caused 90% parasitism on *L. trifolii*, and rates were highest in fields free from insecticides. In Victoria, Australia, Bjorksten *et al.* [81] found that natural control exerted by local parasitoids was high, with 100% control of *Liriomyza chenopodii* reached in beets within 1–3 weeks of mines appearing and 100% control of *Liriomyza brassicae* within 6 weeks.

Interestingly, invading leafminer populations have sometimes been observed to decline naturally after a few years and it has been hypothesized that this is the result of the action of local natural enemies [26]. For example, after the invasion of *L. trifolii* in Senegal in 1980, five indigenous and five another parasitoid species were found, and parasitism frequently reached 90% [80].

*Liriomyza huidobrensis* and *L. sativae* were first found in Indonesia in 1994, and eight species of parasitoids were found in 1998 [40], although only one has been found to be common currently. Similar parasitoid diversity has been found on *Liriomyza* species expanding their ranges into in Iran [82], Malaysia [83], Vietnam [32, 34], Japan [66], China [27, 84] and the USA [85]. Globally, the effort to determine the occurrence of leafminer parasitoids has resulted in an exceptionally large number of species of potentially useful parasitoids [32, 34, 84, 86–88].

This regional biodiversity clearly provides a rich community of indigenous parasitoids that contribute to the control of the invasive *Liriomyza* species. Despite the difficulties in quantifying the effects of indigenous parasitoids (often due to patterns of pesticide use), they should be treated as a resource and protected as much as possible. Although specialized to attacking hosts with a leafmining habit, there is little evidence that *Liriomyza* parasitoids belonging to Eulophidae display and high degree of specificity among leafmining hosts [26, 39]. This behaviour can be beneficial in terms of conservation biological control, provided none of the leafmining species are viewed as valuable non-target organisms.

### Conservation Biological Control in Field Crops

Successful biological suppression of *Liriomyza* species to non-economic levels has been effective in large-scale, commercial field crops. Typically, biological control is most effective in crops such as tomatoes, cucurbits and potatoes that produce fruit that are not directly attacked by leafminers. Solanaceous plants, in particular, have an excess of photosynthate production, thus permitting considerable foliar damage without appreciable yield losses [89, 90]. It has been found that *L. huidobrensis* infestations often look worse than the actual damage the leafminers did, and in contrast, low-level infestations of *Liriomyza* on solanaceous plants (tomatoes) will increase yield [91]. In lima beans, the leaves actually produce restitutive growth that fills in the mines with photosynthetically active mesophyll tissue, allowing very significant damage before yield losses occur [92]. Crops such as lettuce and spinach, where the leafminer-damaged portion of the plant is harvested, are most likely to benefit from biological control efforts early in the season before the harvestable portion develops. The value of biological control for other crops, such as celery, will vary depending on which *Liriomyza* species are present and when the populations occur. For example, *L. trifolii* feeds on the leaves of celery, and the leaves are removed at harvest. However, plants that are damaged early in the season, or that have 25% or more of the leaves mined late in the season, will suffer significant loss of photosynthate production that extends the time required for crop completion [93]. In contrast, *L. huidobrensis* often produce mines in the petioles, which are the marketed portion of

the plant [79]. For celery attacked by this species, early season biological control is most optimal. Regardless of the timing, the conservation and use of biological control agents provide a strategy that can help reduce pesticide resistance by delaying the need for insecticide applications and by removing individuals that could be developing resistance. Even with the crops that are most problematic, there are a number of strategies suggested to maximize control of leafminers.

Probably the most important strategy to maximize naturally occurring biological control has been the selection of pesticides, which have minimal impact on leafminer parasitoids. This strategy has been used to develop widely used IPM programmes on a variety of crops. In Mexico, an IPM programme for spring plantings of tomatoes produced a reduction in leafminer puparia in collection trays from 185.1/tray/day in a conventional pesticide programme to 4.1/tray/day [14]. This is below the 20 puparia/tray/day believed to cause economic losses, and net profits were substantially higher in the IPM plots [14]. Similar results were found in celery in California [15, 93, 94] and tomatoes in California [90] and Israel [95]. This latter tomato study also documented a dramatic reduction in adult parasite mortality in the IPM programme as compared with the conventional pesticide programme. In every case, these programmes reduced: (1) overall pesticide use, (2) leafminer populations, (3) potential environmental problems, and (4) worker health and safety concerns, while at the same time increasing net profits. However, all of these crops have a complex of herbivorous pests, and the increased profits undoubtedly resulted from a combination of maximizing leafminer parasitism and minimizing losses from other pests. Thus, for biological control of *Liriomyza* spp. to be cost-effective, developing IPM programmes that incorporate pesticides with minimal effects on leafminer parasitoids is a viable strategy.

Although not always possible, habitat diversification and management can be important for enhancement of *Liriomyza* parasitoids. For example, weed patches near crops can be important reservoirs for parasitoids [96]. Salvo *et al.* [97] analysed the parasitoid assemblages of *L. huidobrensis* in relation to natural, urban and cultivated habitats through experimental and comparative methodologies. Their results showed that overall parasitism and parasitoid species-richness were lower in simple as compared with complex habitats. In these tests, parasitism of *L. huidobrensis* on weeds increased in the sequence: cultivated systems > urban sites > natural ecosystems. Small leafminer populations attracted the highest total number of parasitoid species in cultivated habitats. Some degree of habitat specialization was detected in eulophid species, which were particularly scarce in cultivated habitats, with the reverse being found for braconids.

Even though many parasitoid species are polyphagous and attack several leafminer species, some are strongly influenced by host plants [26]. Parasitoid species and the proportion of each species reared from the leafminers vary with host plants, crop timing and geographic locations [34, 40]. Johnson and Hara [98] suggest that effective biological control may depend on matching the most effective natural enemies with a given *Liriomyza* species and crop. Crop monoculture has been considered one of the factors contributing to the disruption of parasitoids; at least some of the major parasitoids have particular crop 'preferences' and thus their impact may be reduced on 'non-preferred' crops. This variability in host-plant preference was evident on adjacent plantings of celery and tomatoes in California, where some parasitoids showed substantial crop preferences, while others did not [85]. Gratton and Welter [86] studied the population dynamics and parasitoid assemblages of *Liriomyza helianthi* Spencer over a 3-year period on the *Helianthus annus* L. and *Xanthium strumarium* L. in northern California. They found that the most common species, *Diglyphus* spp. and *Neochrysocharis arizonensis* (=*Closterocerus arizonensis*) showed no bias in association with a particular leafminer or plant species, but one parasitoid, *Closterocerus ainsliei* Crawford, was strongly associated with *H. annus*. This variability complicates our ability to predict which parasitoid species will provide the most useful leafminer control, particularly in crops or natural systems that have not been investigated in detail.

Although host location cues of parasitic wasps include visual, acoustic, and contact and taste cues, most evidence indicates that chemical information plays the most important role in plant–leafminer–parasitoid interactions [99]. For instance, Dicke and Minkenberg [100] found that volatile blend from leafminer-infested tomato leaves affected the behaviour of *Dacnusa sibirica* in the absence of visual cues. Petitt *et al.* [101] also reported that *Opius dissitus* preferentially landed on leafminer-infested rather than non-infested potted lima bean plants. Over the past 20 years, evidence has been accumulating that provides a physiological basis for host-plant discrimination by leafminer parasitoids [99]. Plant volatile compounds have been shown to elicit significant responses attractive to parasitoids in electrophysiological and behavioural experiments [102–104]. The olfactory sensibility of parasitoids only respond to a limited number of the compounds released by insect-damaged plants, thereby substantially reducing the number of compounds that require testing [102]. Using an electroantennogram (EAG), Zhao and Kang [105] found that neither the healthy host nor non-host plants of *L. sativae* elicited distinctive EAG responses in *D. isaea*; whereas odours of physically damaged leaves, whether from host or non-host plants, elicited strong EAG responses of the leafminer and its parasitoid. This study implied that physical damage can induce production of EAG-active compounds in host-plants which is distinct from that in healthy plants. Wei and Kang [102] and Wei *et al.* [103, 104] showed that (*Z*)-3-hexen-1-ol induced by the leafminer damage is the most important chemical that attracts leafminer

parasitoids, and TMTT and 3-methylbutanal oxime play important roles in distinguishing the host and non-host plants of *Liriomyza* species. Therefore, the infochemicals induced by leafminers from host and non-host plants could be employed as repellents to push leafminers away from crops and attractants to pull natural enemies.

### Classical Biological Control under Field Conditions

For the purposes of this review, classical biological control refers to the practice of importing and releasing non-indigenous parasitoids for leafminer suppression. Some notable successes in the control of *Liriomyza* spp. have been achieved on some of the Pacific Ocean islands – Hawaii, Tonga and Guam [98, 106]. In Hawaii, a particularly successful programme of parasitoid introductions against *L. trifolii* and *L. sativae* was achieved in the late 1970s and 1980s. *Ganaspidium utilis* Baerdsley was introduced in 1977 and had a major impact on *L. trifolii* and *L. sativae* on watermelons and may be important for suppression of *L. trifolii* on celery [106]. *Neochrysocharis diastatae* had a significant impact on both leafminers on several vegetable crops [106]. In Tonga, *G. utilis* and *C. oscinidis* were released in 1988 for the control of *L. trifolii* on watermelon, pumpkin, tomato, bean and Irish potato with great success [106]. Johnson and Hara [98] reported that the predominant parasites reared from the four major species of *Liriomyza* infesting 12 different host crops in North America and Hawaii. They found that although no single parasitoid species was the predominant biological control agent in most crops; however, *D. begini*, *Halticoptera circulus* and *Chrysonotomyia punctiventris* were either the first or second most reared species in 60.9, 26.1 and 21.7% of the studies, respectively. Because of uneven distribution of parasitoids among crops, Johnson and Hara [98] suggested that effective biological control may depend on matching the 'most effective' parasitoid species complex with a given *Liriomyza* species and crop.

### Host Feeding

Host feeding, a form of parasitoid predation, on leafminer larvae plays a significant and positive role in generating successful biological control [107–109]. In augmentation biological control, where natural enemies are commercially mass-produced and subsequently released into a target crop, host killing by host feeding may contribute significantly to leafminer suppression. However, host feeding is unprofitable in the mass-production step because it does not result directly in production of progeny. Hondo *et al.* [110] found that seven species of eulophid parasitoids (*Pnigalio katonis*, *Hemiptarsenus varicornis*, *D. isaea*, *Diglyphus minoeus*, *Diglyphus pusztensis*, *Chrysocharis pentheus* and *N. formosa*) are solitary and idiobiont parasitoids, and their adults kill leafminer larvae

directly through host feeding. Each female of an introduced and a native *D. isaea* could feed and kill up to 466 and 239 *L. trifolii* larvae at 20°C, respectively, in their lifespan. Parrella *et al.* [42] found that *Diglyphus intermedius*, *D. begini* and *Chrysocharis parksi* killed more *L. trifolii* larvae through host feeding than were parasitized. Chien and Ku [111] found the native parasitoids of *L. trifolii* from Taiwan, *H. varicornis*, *C. pentheus*, *Chrysonotomyia okazakii* and *N. formosa*, exhibited host-feeding behaviours. For purposes of host feeding or parasitization, all of these parasitoids preferred to oviposit on third instars.

### Augmentative Biological Control

As described by Yano [66], augmentation involves efforts to increase populations of natural enemies through releases of insectary-reared stock. Inoculative augmentation refers to the application of an indigenous agent early in the season to enhance subsequent buildup in the biological control agent population, whereas inundative augmentation refers to the mass application of an agent with the primary objective of high initial kill. Because parasitoids can provide effective suppression of leafminers in the field when disruptive insecticides are not used, there has been considerable interest in augmentative releases. This approach has been used principally in greenhouse-grown crops, but it may also be applicable to field crops in some circumstances. For example, in Western Sumatra, Indonesia, extension workers successfully taught farmers in areas of low pesticide usage to redistribute parasitized leafminers to cauliflower fields where parasitoids were absent or had low activity [112]. This approach was successful, in part, because of the farmers avoided the costs of a mass-rearing programme.

The ultimate success of augmentative biological control may depend on releases of biological control agents that maximize establishment, are released in synchrony with the host, and can be integrated into IPM programmes in conjunction with co-existing natural enemies [59, 60, 113] or insecticides. Thus, determining species complex as well as the optimal release schedules and rates that maximize the effectiveness of natural enemies can increase the effectiveness of augmentative biological control and increase potential economic benefits. The practical application of augmentative biological control has been hindered by the high cost of natural enemies, problems associated with the availability and quality of natural enemies, a lack of rigorous research on successful versus ineffective release rates, and in many cases, the lack of economic analyses.

### Mass-Rearing Parasitoids

Mass rearing sufficient host material is essential for mass-rearing parasitoids. To rear any *Liriomyza* species requires

quality host plants under suitable environmental and nutritional conditions. The ideal host plants should be easily propagated and maintained, be attractive to females for oviposition, and support high numbers of leafminer larvae. Various host plants have been used to rear *Liriomyza* spp., including lima bean [114, 115], tomato [116] and cowpea [117]. To date, no artificial diet has been reported for leafminers.

Many species of parasitoids of *Liriomyza* have been successfully mass-reared or at least have been the subjects of mass rearing attempts. Because the cost of parasitoid production is often rather high, reducing rearing costs is critical to implementation [118]. The commercial production of natural enemies is likely to be substantial until demand for inundative releases increases to allow mass production to achieve greater efficiency of scale. Until this occurs, inundative release against *Liriomyza* species in field crops is unlikely.

There are several factors that can interfere with efficient mass production of parasitoids. For example, over-production of males contributes to higher costs for biological control because only females directly kill pests. Ode and Heinz [73] found that host size positively affects both male and female wasps, and females produced more daughters in larger hosts and more sons in smaller hosts. They developed a technique of presenting female *D. isaea* with groups of sequentially larger leafminer hosts (*L. huidobrensis*) to attack; this generated progressively more female-biased sex ratios. After 3 days of providing increasingly larger hosts they were able to reduce the sex ratios produced by individual females from 57% male to 36% male. Sex ratios produced by groups of females dropped from 64% male to 45% male. Chow and Heinz [17, 117] developed a similar technique to create less male-biased sex ratios for *D. isaea*. Using a combination of small and large hosts produced slightly lower percentages of males (from 66% to 56%) as compared with using only large hosts. This could reduce costs of females by 23%, and reduce overproduction of males in *D. isaea* with no compromise in biological control efficacy.

Storage of parasitoids can also be problematic. The effective duration of storage can be affected by temperature and host plants. Chien *et al.* [119] investigated the suitable life stages and conditions for storage of *N. formosa* and subsequent offspring production. They found that female wasps could be stored at 15°C for 10–40 days or at 25°C for 10 days and still maintain high fertility for mass production purposes. However, for inoculative field releases where host-feeding was also useful, *N. formosa* females could be stored at 15°C for 50 days or 25°C for 20 days, or 1-day-old pupae at 10°C for 2–3 weeks. In a similar study, Chien *et al.* [120] found that for field releases of *H. varicornis* without reducing host-feeding capacity, females could be stored at 15°C for 10–30 days or at 25°C for 10–15 days. *H. varicornis* females could also be stored at 15°C for 40 days or

1-day-old pupae at 10°C for 1–4 weeks. Under storage conditions of 15°C for 10 days, female *H. varicornis* showed a comparable daily oviposition pattern to that of the control. Mass-rearing and while maintaining a high fertility of the wasps, *H. varicornis* females could be stored at 15°C up to 30 days, 25°C for 10–15 days, or 1-day-old pupae at 10°C for 1–4 weeks.

## Inundative Augmentation of Parasitoids in Greenhouses

Inundative mass-releases of various species of parasitoids have achieved mixed success. Because of the costs of mass-rearing programmes for inundative releases, most of the research to date has been conducted in greenhouses with relatively high-value crops. Unlike some of the vegetable crops that can tolerate damage, commercially produced ornamental crops must meet very high aesthetic standards that may limit the usefulness of biocontrol agents once marketable foliage is produced. Several parasitoid species, including *D. sibirica* [121], *D. begini* [122–124] and *D. isaea* [118] have been inundatively released for controlling *Liriomyza* spp. in various crop systems. These are discussed individually in the following text.

### D. isaea

*D. isaea* is one of the few species of leafminer parasitoids that are commercially available [16, 125]. Prices in early 2008 ranged from approximately US$86–112 for 250 adults. Inundative releases of *D. isaea* against *L. trifolii* have been tested as part of IPM programmes in greenhouse vegetables [126–128]. *D. isaea* has proven effective against *L. trifolii* on tomatoes [127]. Ozawa *et al.* [127] released *D. isaea* at 0.13 females/plant for 5 releases, 0.19 females/plant for 8 releases and 0.15 females/plant for 3 releases from spring through summer, and the mortalities of leafminer larvae increased by 94, 98 and 100%, respectively. The numbers of empty mines were 1.3, 2.2 and 3.4 per leaf at the end of the experiment, respectively. The maximum parasitism levels, based on the number of adult parasitoids emerging from samples collected in the release greenhouses, were 92, 87 and 95%, respectively. Boot *et al.* [129] reported nearly 100% parasitism of *L. bryoniae* following an inoculative release of *D. isaea*. Cabitza *et al.* [130] released mass-reared individuals of *D. isaea* to control *L. trifolii*, and documented 100% suppression despite high levels of infestation of the pest (which reached 74 mines/plant). Similarly, Ushchekov [116] reported that *L. bryoniae* in the summer–autumn rotation could be effectively reduced by a single release of 1 female/15 larvae of *Liriomyza*. Ulubilir and Sekeroglu [131] found that with the release rate of *D. isaea* at 100 adults/100 m$^2$ (at 10 different locations within a greenhouse, each 10 adults/10 m$^2$), larval populations of *L. trifolii* decreased to <1 larva/leaf, which was similar to the larval

densities of the cyromazine-treated plots. However, while *Liriomyza* control can be achieved through inundative releases, the cost:benefit ratios were not reported, and the economic viability of the approach could not be documented.

Sampson and Walker [132] used commercial tomato nurseries to test whether a simple pest density threshold for *D. isaea* release could be used to improve early parasitoid establishment. They used an action threshold of one new mine per plant per week to trigger releases of *D. isaea*. As compared with controls, three releases at 7-day intervals allowed *D. isaea* to establish faster, numbers of parasitoids used were reduced, and control costs were reduced. *Liriomyza bryoniae* populations were controlled without economic damage and no chemical treatments were required.

Recently, Sha *et al.* [133] generated nuclear ribosomal internal transcribed spacer 1 sequences that suggested that *D. isaea* is a complex of cryptic species. China was believed to have at least four species. This could explain, in part, the variability in successful suppression of leafminers at different locations. However, the authors did not conduct cross-breeding studies to determine if the genetic differences were enough to stop breeding and prevent the production of viable offspring. Regardless, this is an exciting area that requires more study. At the very least, the genetic variation might allow selective production of parasitoids better adapted to specific environmental conditions.

D. begini

*D. begini* is a species that has been extensively studied but is not commercially available. Heinz *et al.* [134] found that twice-weekly releases of the parasitoid over a period of 3 weeks in July–September in 1986 and 1987, effectively controlled *L. trifolii* on marigolds for 6 weeks after the first release and maintained the pest at a very low level for a further 8 weeks. Parasitoid numbers steadily increased until the first week of September, and then declined, probably as a result of a decrease in the host population caused by parasitism. In a subsequent study, Heinz and Parrella [108] inundatively released *D. begini* on marigolds grown for seed. The release reduced *L. trifolii* populations to nearly zero within 8 weeks of the first release, and the leafminers remained at that level for the duration of the crop. In addition, the numbers of viable seed per plant were greater in marigolds grown under biological control compared with those treated with insecticides [135]. This parasitoid has also been successfully released for biological control of *L. trifolii* on greenhouse-grown chrysanthemums [107]. Rathman *et al.* [124] reported that the recurring cost of producing 1000 parasitoids/day was estimated as US$19.20 for *D. begini*. Similarly, Parrella *et al.* [122] reported that the daily cost (recurring costs only) to produce 1000 parasitoids was US$19.40. However, these costs do not include the worker benefits, facilities lease or depreciation, profit margins required

by commercial producers, utility costs or the costs of shipping. As a result, the costs to growers would be substantially higher if purchases were made from a commercial operation.

D. isaea *and* D. sibirica

*Liriomyza trifolii* has been successfully controlled by releasing *D. isaea* and *D. sibirica* on tomato in greenhouses with >90% parasitism [126, 136, 137]. At least in the study by Matsumura *et al.* [137], the release of 250 wasps per 7.5 acres was economically feasible. Abd-Rabou [69] reared and released 90 000 *D. isaea* and *D. sibirica* on cucumber and tomato in greenhouses to control *L. trifolii*. The parasitism rates of *D. sibirica* reached 11.6 and 7.2% at the 11th week from the release date, on cucumber and tomato, respectively. *D. sibirica* can be purchased independently or in combination with *D. isaea* from many different commercial sources. In bulk shipments (1250 or more wasps), costs can be as little as approximately US$40 per 250 wasps (www.rinconvitova.com). Thus, cost-effective control would not be likely in the Abd-Rabou [69] study if parasitoid costs were similar to current commercial prices.

H. varicornis

Ozawa *et al.* [138] determined the effectiveness of biological control against *L. trifolii* by using five releases of *H. varicornis* on cherry tomatoes in greenhouses at rates of 0.33 and 0.16 females per plant over 2 months. The density of leafminer larvae at the peak of the occurrence in the 0.33 and 0.16 females per plant treatments and the control treatment were 2.8, 15.9 and 23.6 per leaf, respectively. The density of mines in the 0.33 and 0.16 females per plant greenhouses and the control greenhouses were 6.2, 32.2 and 38.0 per leaf, respectively. The percentage of parasitism in 0.33 and 0.16 females per plant greenhouses were 43.7 and 4.8% at 3 weeks after the first release and were 80.3 and 73.1% at 4 weeks after the first release, respectively. These results suggest that biological control by *H. varicornis* against *L. trifolii* could be effective on tomatoes in greenhouses at a release rate of 0.33/plant. However, potential yield losses associated with more than six mines per leaf were not evaluated, as the authors did not have a leafminer-free treatment.

*Other species*

*N. formosa* and *Opius pallipes* have also been mass-produced for control of *Liriomyza*. Hondo *et al.* [110] developed a strategy for mass production of *N. formosa*, but the costs of production were not described. However, releases of *N. formosa* on eggplants in greenhouses timed to coincide with the first mines of *L. trifolii* did suppress leafminer damage to non-damaging levels [139]. Similarly, the use of multiple releases of *O. pallipes* improved control of *L. bryoniae* [140]. Again, the economics of parasitoid releases were not presented, and the costs of biocontrol

strategies could not be compared with conventional control approaches.

### Inoculative Releases

Minkenberg and van Lentern [5] and Boot *et al.* [129] provided a detailed review of the literature on inoculative releases for *Liriomyza* spp. suppression in Europe, and this work will not be repeated here. Inoculative parasitoid releases were generally considered to be economically viable. In other countries, the results were not always consistently positive. Neuenschwander *et al.* [80] released nine species of parasitoids to control *L. trifolii* in Senegal in 1982 and 1983. Although many were recovered shortly after release, only *O. dissitus* was recovered in later samples and became relatively abundant. In Japan, Ozawa *et al.* [136] determined the effectiveness of the inoculative releases of *D. isaea* and *D. sibirica* at 47–125 wasps per ha for controlling *L. trifolii* in greenhouse experiments. Selective insecticides were applied in biologically controlled greenhouses while various insecticides, including non-selective insecticides, were applied in the 'chemical control' greenhouses. The density of *L. trifolii* larvae in biological-control greenhouses was maintained at the same level as that in chemical-control greenhouses, and the mortality of the leafminer increased up to 100%. The damage to tomato plants was not severe, and fewer insecticides were applied. Thus, this approach does have considerable potential for commercial use, but successful application of parasitoids appears to require a comprehensive IPM approach that includes controlling other pests using strategies that have minimal impact on the biological control agents.

### Factors Influencing Biological Control of *Liriomyza*

Although some notable successes in the control of *Liriomyza* species have been achieved, many parasitoid introductions have simply failed, proven ineffective, or not been adopted commercially because of poor economic returns. The problems with high costs of rearing and the low tolerance to pest damage for some crops (particularly leafy vegetables and ornamentals) are the main reasons hindering the adoption of more intensive biological control strategies. A few other concerns have also affected the use of parasitoids as the primary control strategy for leafminers, and these are discussed below.

### Multiple Pests

Most natural enemies are effective against only a small group of pest species. Therefore, a critical technical factor in commercializing natural enemies is integrating the use of natural enemies with other control practices that are intended to control other pests on a crop. Since it is usually impossible to control all pest species with natural enemies, most successful programmes integrate the use of natural enemies with cultural control strategies, resistant plant cultivars and selective pesticides.

### Thermal Tolerance

The roles of thermal adaptation in *Liriomyza* distribution and control have been extensively reviewed by Kang *et al.* [99]. Biological control agents used in greenhouses are most effective if they are highly adaptable to extreme temperatures [70]. Therefore, when selecting among leafminer parasitoids, it is desirable to evaluate their thermal tolerance [141], particularly in relation to development and reproduction throughout their lifetimes [110]. While there are many publications that document thermal tolerances in *Liriomyza* species [142], few publications provide this information for leafminer parasitoids. However, Hondo *et al.* [110] do provide thermal tolerances for seven common species. They concluded that *N. formosa* would be best during warm or hot seasons, and *D. isaea* is more effective during cooler seasons. Generally, *Liriomyza* species of tropical origin cannot successfully overwinter under natural conditions in the temperate areas [99]. The natural enemies should coincide with the distribution of their prey or hosts, and those for consideration should be able to adapt similar thermal requirements to their host or prey.

### Interactions among Natural Enemies

When multiple species of natural enemies are released into a crop, or if a mass-reared species is introduced into a system containing existing natural enemy populations, competitive interactions are likely to occur. Although competitive interactions between natural enemies have been studied extensively in other systems for over 100 years, and a few studies on leafminer parasitoid interactions were published in the 1980s, most of the research on leafminer natural enemy competition has been published in the past 10 years. Examples of both positive and negative interactions are provided below.

### Parasitoid–Parasitoid Interactions

*Liriomyza* parasitoids may interfere with each other when larval ectoparasitoids (e.g. *Diglyphus* spp.) and larval–pupal endoparasitoids (e.g. *C. oscinidis* and *G. utilis*) are present in a cropping system. Larval ectoparasitoids are able to parasitize leafminers that already contain a living endoparasitoid larva, resulting in the death of the endoparasitoid. Endoparasitoids do not parasitize leafminers with ectoparasitoids because the parasitized hosts will not pupate so the endoparasitoids can complete their life cycles [137].

Bader *et al.* [113] assessed the influence of two commercially available parasitoids *D. isaea* and *D. sibirica* attacking *L. longei* on chrysanthemum. They concluded that levels of interspecific competition among parasitoid species were undetectable at leafminer densities typical of field-grown ornamental crops (low densities), and thus, the efficacy of one species released into a backdrop of potentially competing parasitoids did not negatively or positively affect the outcome of the augmentative biological control.

### Parasitoid–Nematode Interactions

A combination of entomopathogenic nematodes and parasitoids has been found to be more effective than either natural enemy used alone. Sher and Parrella [57] found adult females of *D. begini* are able to detect and avoid ovipositing on nematode-infected hosts, and paralysed *L. trifolii* larvae. However, Head *et al.* [59] found that 98% of eggs laid by the female wasps were deposited alongside healthy larvae, although adult *D. isaea* did not discriminate between healthy and *S. feltiae* nematode-infected leafminer larvae of *L. huidobrensis* for host feeding, indicating a synergetic effect when the entomopathogenic nematodes and the parasitoids are used together. Negative interferences are also seen, however, with nematodes decreasing the likelihood of *D. begini* developing to adults and nematodes directly infecting and killing *D. begini* larvae [57]. Head *et al.* [59] also found that in intact leaf mines of *L. huidobrensis*, larvae already parasitized by *D. isaea* that had developed to the larval or pupal stage, and *D. sibirica* in the larval stage were subsequently also infected by *S. feltiae* following foliar application. This reduced the potential of the wasps to survive until the adult stage. In the same study, they also found that a soil drench of imidacloprid did not cause a significant reduction in the number of *D. sibirica*, which survived the treatment and developed to adult emergence.

### Nematodes–Insecticide Interactions

Head *et al.* [58, 59, 63] studied the compatibility of *S. feltiae* with different insecticides against *L. huidobrensis*. They found that some insecticides (abamectin, deltamethrin and heptenophos) exhibited detrimental effects on the infective juveniles. However, exposure of the infective juveniles to dry pesticide residues on foliage did not have significant detrimental effects on the level of control of *L. huidobrensis* when compared with the effect of nematodes applied to residue-free foliage.

### Parasitoid–Pesticide Interactions

Modern pesticides have provided a potent means of suppressing *Liriomyza* and numerous other pests.

However, a biologically based IPM strategy would seek to conserve or enhance natural enemies, particularly parasitoids, because the overuse and misuse of insecticides results in numerous problems including increased costs, pesticide resistance, contamination of the environment, toxicity to humans and non-target organisms. The differential destruction of natural enemies of *Liriomyza* through insecticide use was first reported in the early 1950s during the first leafminer outbreaks in North America. Hills and Taylor [143] found that repeated applications of DDT against *L. sativae* reduced the parasitoid population, which resulted in a pest outbreak. Many subsequent studies with chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, etc., have confirmed this conclusion [13–15].

There is an extensive recorded literature concerning the compatibility of using biological control agents and selective insecticides for management of *Liriomyza* in various cropping systems [97]. The most commonly used insecticides for management of *Liriomyza* species include abamectin, cyromazine and spinosad, but because of the diversity of co-occurring pests in most crops, leafminer parasitoids can be exposed to many different compounds that are not specific to leafminer control. Some of these materials have been tested for toxicity to parasitoids, and a partial list has been presented by Salvo and Valladares [97].

The potential effects of herbicides and fungicides on leafminer parasitoids have been largely overlooked. Mancozeb, one of the most commonly used fungicides, was the only fungicide we found tested in the literature. This compound had no negative effects on larvae and pupae of *H. varicornis* and *D. isaea*, progeny production and longevity of *H. varicornis* adults, or leaf residence time for *H. varicornis* [88, 144]. No papers were found examining effects of herbicides.

## Conclusion

Biological control of *Liriomyza* has featured significantly in the management of invasive *Liriomyza* in both field and protected culture systems. However, we feel that more effort should be made to understand, conserve and enhance local natural enemies before the introduction of exotic parasitoids is considered. In particular, gaps should be identified in local parasitoid guilds such that ecologically compatible exotic agents can be identified. However, cross-commodity patterns of excessive pesticide application may be a larger threat to local biodiversity than importing non-specialist leafminer parasitoids. Although there are some exciting examples of successful control with natural enemies, replacement of chemical pesticides with biological agents will not happen without major efforts to develop economically viable IPM programmes and lower costs of mass rearing parasitoids. At present, a wide range of insecticides are used to manage *Liriomyza*

species, but insecticide resistance can develop rapidly [9] and this has increased the value of implementing effective biological control strategies. Many newer chemical insecticides and biopesticides have minimal impact on non-target organisms and should be compatible with natural enemies. These compounds will provide an opportunity to develop new IPM programmes that maximize the benefits of biological control agents.

## Acknowledgements

## References

1. Spencer KA. Agromyzidae (Diptera) of Economic Importance. The Hague, Dr. W. Junk B.V., Netherlands; 1973.

2. Parrella MP. Biology of *Liriomyza*. Annual Review of Entomology 1987;32:201–24.

3. Kang L. Ecology and Sustainable Control of Serpentine Leafminers. Science Press, Beijing, China; 1996. 254 pp.

4. Morgan DJW, Reitz SR, Atkinson PW, Trumble JT. The resolution of California populations of *Liriomyza huidobrensis* and *Liriomyza trifolii* (Diptera: Agromyzidae) using PCR. Heredity 2000;35:53–61.

5. van der Linden A. Biological control of leafminers on vegetable crops. In: Heinz KM, van Driesche RG, Parrella MP, editors. Biocontrol in Protected Culture. B Ball Publishing, Batavia, IL; 2004. p. 235–51.

6. Minkenberg OPJM, van Lenteren JC. The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and host plants: a review. Agriculture University of Wageningen Papers 1986;86–2:1–50.

7. Parrella MP, Keil CB, Morse JG. Insecticide resistance in *Liriomyza trifolii*. California Agriculture 1984;38(1/2):22–3.

8. Sanderson JP, Parrella MP, Trumble JT. Monitoring insecticide resistance in *Liriomyza trifolii* (Diptera: Agromyzidae) with yellow sticky cards. Journal of Economic Entomology 1989;82:1011–8.

9. Ferguson JS. Development and stability of insecticide resistance in the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to cyromazine, abamectin, and spinosad. Journal of Economic Entomology 2004;97:112–9.

10. Lange WH, Agosta GG, Goh KS, Kishiyama JS. Field effect of insecticides on chrysanthemum leafminer and a primary parasitoid, *Chrysocharis ainsliei* (Crawford), on artichokes in California. Environmental Entomology 1980;9:561–2.

11. Johnson MW, Oatman ER, Wyman JA. Effects of insecticides on populations of the vegetable leafminer and associated parasites on summer pole tomatoes. Journal of Economic Entomology 1980;73:61–6.

12. Johnson MW, Oatman ER, Wyman JA. Natural control of *Liriomyza sativae* (Dip: Agromyzidae) in pole tomatoes in Southern California. Entomophaga 1980;25:193–8.

13. Trumble JT. Vegetable insect control with minimal use of insecticides. HortScience 1990;25:159–64.

14. Trumble JT, Alvarado–Rodriguez B. Development and economic evaluation of an IPM program for fresh market tomato production in Mexico. Agriculture, Ecosystem and Environment 1993;43:267–84.

15. Reitz SR, Kund GS, Carson WG, Phillips PA, Trumble JT. Economics of reducing insecticide use on celery through low input management strategies. Agriculture, Ecosystem and Environment 1999;73:185–97.

16. Chow A, Heinz KM. Biological control of leafminers on ornamental crops. In: Heinz KM, van Driesche RG, Parrella MP, editors. Biocontrol in Protected Culture. B Ball Publishing, Batavia, IL; 2004. p. 221–38.

17. Chow A, Heinz KM. Using hosts of mixed sizes to reduce male–biased sex ratio in the parasitoid wasp, *Diglyphus isaea*. Entomologia Experimentalis et Applicata 2005;117:193–9.

18. Chow A, Heinz KM. Manipulation of sex ratios in mass rearing of *Diglyphus isaea* (Walker), an ectoparasitoid of agromyzid leafminers. Bulletin of International Organization for Biological Control of Noxious Animals and Plants 2005;28(1):63–6.

19. Stegmaier CE. Host plants and parasites of *Liriomyza trifolii* in Florida (Diptera: Agromyzidae). Florida Entomologist 1966;49:75–80.

20. Stegmaier CE. Parasitic Hymenoptera bred from the family Agromyzidae (Diptera) with special reference to South Florida. Florida Entomologist 1972;55:273–82.

21. Waterhouse DF, Norris KR. Biological Control: Pacific Prospects. Inkata Press, Melbourne; 1987.

22. La Salle J, Parrella MP. The chalcidoid parasites (Hymenoptera: Chalcidoidea) of economically important *Liriomyza* species (Diptera: Agromyzidae) in North America. Proceeding of Entomological Society of Washington 1991;93:571–91.

23. Schuster DJ, Gilreath JP, Wharton RA, Seymour PR. Agromyzidae (Diptera) leafminers and their parasitoids in weeds associated with tomato in Florida. Environmental Entomology 1991;20:720–3.

24. Schuster DJ, Wharton RA. Hymenopterous parasitoids of leaf–mining *Liriomyza* spp. (Diptera: Agromyzidae) on tomato in Florida. Environmental Entomology 1993;22:1188–91.

25. Konishi K. An illustrated key to the hymenopterous parasitoids of *Liriomyza trifolii* in Japan. Miscellaneous Publication of National Institute for Agro-Environmental Sciences 1998;22:27–76.

26. Murphy ST, La Salle J. Balancing biological control strategies in the IPM of New World invasive *Liriomyza* leafminers in field vegetable crops. Biocontrol News and Information 1999;20:91N–104N.

27. Xu Z, Gao Z, Chen X, Hou R, Zeng L. Hymenopterous parasitoids of *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in Guangdong Province, China. Natural Enemies of Insects 1999;21:126–32.

28. Çikman E, Uygun N. The determination of leafminers (Diptera: Agromyzidae) and their parasitoids in cultivated and non-cultivated areas in Sanlurfa province, southern Turkey. Turkish Journal of Entomology 2003;27:305–18.

29. Vega PB. Dípteros de interés agronómico. Agromícidos plaga de cultivos hortícolas intensivos. ARACNET 11 – Boletín Sociedad Entomológica Aragonesa 2003;33:293–307.

30. Noyes J. Universal Chalcidoidea Database. The Natural History Museum; 2004. Available from: URL: http://www.nhm.ac.uk/research-curation/projects/chalcidoids/.

31. Fisher N, Ubaidillah R, Reina P, La Salle J. *Liriomyza* parasitoids of Southeast Asia 2008. Available from: URL: http://www.ento.csiro.au/science/Liriomyza_ver3/index.html.

32. Tran TTA, Tran DH, Konishi K, Takagi M. The vegetable leafminer *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) and its parasitoids on cucumber in the Hochiminh Region of Vietnam. Journal of Faculty of Agriculture, Kyushu University 2005;50:119–24.

33. Çikman E, Beyarslan A, Civerlek HS. Parasitoids of leafminers (Diptera: Agrimyzidae) from Southeast Turkey with 3 new records. Turkish Journal of Zoology 2006;30:167–73.

34. Tran DH, Tran TTA, Konishi K, Takagi M. Abundance of the parasitoid complex associated with *Liriomyza* spp. (Diptera: Agromyzidae) on vegetable crops in Central and Southern Vietnam. Journal of Faculty of Agriculture, Kyushu University 2006;51:115–20.

35. Zeng L, Zhang WQ, Wu JJ. Preliminary studies on the parasitoids of *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in Guangdong. Natural Enemies of Insects 1999;21:113–6.

36. Griffiths GCD. The Alysiinae (Hym., Braconidae) parasites of the Agromyzidae (Diptera). Beitraege zur Entomologie 1968;18:5–62.

37. Zhu CD, La Salle J, Huang DW. A review of Chinese *Diglyphus* Walker (Hymenoptera: Eulophidae). Oriental Insects 2000;34:263–88.

38. Zhu CD, La Salle J, Huang DW. A study of Chinese *Cirrospilus* Westwood (Hymenoptera: Eulophidae). Zoological Studies 2002;41:23–46.

39. Chen XX, Lang FY, Xu ZH, He JH, Ma Y. The occurrence of leafminers and their parasitoids on vegetables and weeds in Hangzhou area, Southeast China. BioControl 2003;48:515–27.

40. Rauf A, Shepard BM, Johnson MW. Leafminers in vegetables, ornamental plants and weeds in Indonesia: surveys of host plants, species composition and parasitoids. International Journal of Pest Management 2000;46:257–66.

41. Ozawa A, Ota M, Kobayashi H. Hyperparasitism of *Neochrysocharis formosa* (Westwood) on the primary parasitoid, *Diglyphus isaea* Walker, of the American serpentine leafminer, *Liriomyza trifolii* (Burgess). Annual Report of Kanto–Tosan Plant Protection Society, Tsukuba, Japan 2002;49:109–112.

42. Parrella MP, Christie GD, Robb KL, Bethke JA. Control of *Liriomyza trifolii* with biological agents and insect growth regulators. California Agriculture 1982;36(11/12):17–9.

43. Nedstam B, Johansson-Kron M. *Diglyphus isaea* (Walker) and *Macrolophus caliginosus* Wagner for biological control of *Liriomyza bryoniae* (Kaltenbach) in tomato. Bulletin of International Organization for Biological Control of Noxious Animals and Plants 1999;22(1):261–3.

44. Carvalho P, Mexia A. First approach on the potential role of *Dicyphus cerastii* Wagner (Hemiptera: Miridae), as natural control agent in Portuguese greenhouses. Bulletin of International Organization for Biological Control of Noxious Animals and Plants 2000;23(1):261–4.

45. Lucas E, Alomar O. Impact of *Macrolophus caliginosus* presence on damage production by *Dicyphus tamaninii* (Heteroptera: Miridae) on tomato fruits. Journal of Economic Entomology 2002;95:1123–9.

46. Castañé C, Alomar O, Goula M, Gabarra R. Colonization of tomato greenhouses by the predatory mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii*. Biological Control 2004;30:591–7.

47. Salamero A, Gabarra R, Albajes R. Observations on the predatory and phytophagous habits of *Dicyphus tamaninii* Wagner (Heteroptera: Miridae). Bulletin of International Organization of Biological Control 1987;10(2):165–9.

48. Arnó J, Alonso E, Gabarra R. Role of the parasitoid *Diglyphus isaea* (Walker) and the predator *Macrolophus caliginosus* Wagner in the control of leafminers. Bulletin of International Organization of Biological Control 1987;10(10):79–84.

49. Prieto MAJ. Biology and ecology of the chrysanthemum miner *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) in the Department of Valle del Cauca. Revista Colombiana de Entomología 1982;6:77–84.

50. Van Lenteren JC, Ramakers PMJ, Woets J. The biological control situation in Dutch glasshouses; problems with *Trialeurodes vaporariorum* (Westwood), *Liriomyza bryoniae* Kalt. and *Myzus persicae* Sulz. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Gent, Belgium: Universiteit Gent 1979;44:117–25.

51. Freidberg A, Gijswijt MJ. A list and preliminary observations on natural enemies of the leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) in Israel. Israel Journal of Entomology 1984;17:115–6.

52. Arakaki N, Okajima S. Notes on the biology and morphology of a predatory thrips, *Franklinothrips vespiformis* (Crawford) (Thysanoptera: Aeolothripidae): first record from Japan. Entomological Science 1998;1:359–63.

53. Harris MA, Begley JW, Warkentin DL. *Liriomyza trifolii* (Diptera: Agromyzidae) suppression with foliar applications of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) and abamectin. Journal of Economic Entomology 1990;83:2380–4.

54. Hara AH, Kaya HK, Gaugler R, Lebeck LM, Mello CL. Entomopathogenic nematodes for biological control of the leafminer, *Liriomyza trifolii* (Dipt.: Agromyzidae). Entomophaga 1993;38:359–69.

55. Broadbent AB, Olthof THA. Foliar application of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) to control *Liriomyza trifolii* (Diptera: Agromyzidae) larvae in chrysanthemums. Environmental Entomology 1995;24:431–5.

56. Williams EC, Macdonald OC. Critical factors required by the nematode *Steinernema feltiae* for the control of the leafminers *Liriomyza huidobrensis*, *Liriomyza bryoniae* and *Chromatomyia syngenesiae*. Annals of Applied Biology 1995;127:329–41.

57. Sher RB, Parrella MP. Biological control of the leafminer, *Liriomyza trifolii*, in chrysanthemums: implications for intraguild predation between *Diglyphus begini* and *Steinernema carpocapsae*. Bulletin of International Organization for Biological Control of Noxious Animals and Plants 1999;22(1):221–4.

58. Head J, Walters KFA, Langton S. The compatibility of the entomopathogenic nematode, *Steinernema feltiae*, and chemical insecticides for the control of the South American leafminer, *Liriomyza huidobrensis*. BioControl 2000;45:345–53.

59. Head J, Palmer LF, Walters KFA. The compatibility of control agents used for the control of the South American leafminer, *Liriomyza huidobrensis*. Biocontrol Science and Technology 2003;13:77–86.

60. Sher RB, Parrella MP, Kaya HK. Biological control of the leafminer *Liriomyza trifolii* (Burgess): implications for intraguild predation between *Diglyphyus begini* Ashmead and *Steinernema carpocapsae* (Weiser). Biological Control 2000;17:155–63.

61. Williams EC, Walters KFA. Foliar application of the entomopathogenic nematode *Steinernema feltiae* against leafminers on vegetables. Biocontrol Science and Technology 2000;10:61–70.

62. LeBeck LM, Gaugler R, Kaya HK, Hara AH, Johnson MW. Host stage suitability of the leafminer, *Liriomyza trifolii* (Diptera: Agromyzidae) to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Journal of Invertebrate Pathology 1993;62:58–63.

63. Head J, Palmer LF, Walters KFA. Development of an integrated control strategy for leafminers in leafy salads with potential for extrapolation to other cropping systems. Bulletin of International Organization for Biological Control of Noxious Animals and Plants 2002;25(1):97–100.

64. Williams EC. Entomopathogenic nematodes for leafminer control. Bulletin of International Organization of Biological Control 1993;16:58–162.

65. Colombo M, Locatelli DP. Laboratory evaluation of the activity of *Steinernema feltiae* Filip. and *Heterorhabditis* spp. on *Liriomyza trifolii* (Burgess) and *Opogona sacchari* (Bojer) infesting cultivated flowering plants. La Difesa delle Piante 1985;8:263–9.

66. Yano E. Recent development of biological control and IPM in greenhouses in Japan. Journal of Asia-Pacific Entomology 2004;7:5–11.

67. Bordat D, Robert P, Renand M. Susceptibility of *Liriomyza trifolii* (Burgess) and *L sativae* Blanchard (Diptera: Agromyzidae) to eleven strains of entomopathogenic fungi. Agronomia Tropical 1988;43(11):68–73.

68. Borisov BA, Ushchekov AT. Entomogenous fungi – *Hyphomycetes* against the nightshade leaf miner. Zashchita i Karantin Rastenii 1997;5:10–11.

69. Abd–Rabou S. Biological control of the leafminer, *Liriomyza trifolii* by introduction, releasing, evaluation of the parasitoids *Diglyphus isaea* and *Dacnusa sibirica* on vegetables crops in greenhouses in Egypt. Archives of Phytopathology and Plant Protection 2006;39:439–43.

70. Van Lenteren JC. Parasitoids in the greenhouse: Successes with seasonal inoculative release systems. In: Waage J, Greathead D, editors. Insect Parasitoids. Academic Press, London; 1986. p. 341–74.

71. Harrison L, Moeed A, Sheppard AW. Regulation of the release of biological control agents of arthropods in New Zealand and Australia. In: Hoddle M, editor. Second International Symposium on Biological Control of Arthropods. CABI Bioscience, Wallingford, Oxfordshire, UK; 2005. p. 715–25.

72. Heinz KM, Parrella MP. Attack behavior and host size selection by *Diglyphus begini* on *Liriomyza trifolii* in chrysanthemum. Entomologia Experminatlis et Applicata 1989;53:147–56.

73. Ode PJ, Heinz KM. Host-size-dependent sex ratio theory and improving mass-reared parasitoid sex ratios. Biological Control 2002;24:31–41.

74. Patel KJ, Schuster DJ, Smerge GH. Density dependent parasitism and host–killing of *Liriomyza trifolii* (Diptera: Agromyzidae) by *Diglyphus intermedius* (Hymenoptera: Eulophidae). Florida Entomologist 2003;86:8–14.

75. Martin AD, Stanley-Horn D, Hallett R. Adult host preference and larval performance of *Liriomyza huidobrensis* (Diptera: Agromyzidae) on selected hosts. Environmental Entomology 2005;34:1170–7.

76. Haghani M, Fathipour Y, Talebi AA, Baniameri V. Temperature-dependent development of *Diglyphus isaea* (Hymenoptera: Eulophidae) on *Liriomyza sativae* (Diptera: Agromyzidae) on cucumber. Journal of Plant Science 2007;80(2):71–7.

77. Salvo A, Fenoglio MS, Videla M. Parasitism of a leafminer in managed versus natural habitats. Agriculture, Ecosystem and Environment 2005;109:213–20.

78. Trumble JT, Nakakihara H. Occurrence, parasitization, and sampling of *Liriomyza* species (Diptera: Agromyzidae) infesting celery in California. Environmental Entomology 1983;12:810–4.

79. Heinz KM, Chaney WE. Sampling for *Liriomyza huidobrensis* (Diptera: Agromyzidae) larvae and damage in celery. Environmental Entomology 1995;24:204–11.

80. Neuenschwander P, Murphy SP, Coly EV. Introduction of exotic parasitic wasps for the control of *Liriomyza trifolii* (Dipt.: Agromyzidae) in Senegal. Tropical Pest Management 1987;33:290–7, 386, 390.

81. Bjorksten T, Robinson AM, La Salle J. Species composition and population dynamics of leafmining flies and their parasitoids in Victoria. Australian Journal of Entomology 2005;44:186–91.

82. Talebi AA, Asadi R, Fathipour Y, Kamali K, Moharramipour S, Rakhshani E. Eulophid parasitoids of agromyzid leafminers genus *Liriomyza* (Dip.: Agromyzidae) in Tehran, Iran. Bulletin of International Organization for Biological Control of Noxious Animals and Plants 2005;28:263–6.

83. Sivapragasam A, Syed AR, La Salle J, Ruwaida M. Parasitoids of invasive agromyzid leafminers on vegetables in Peninsular Malaysia. In: Proceedings of Symposium on Biological Control in the Tropics, 8–19 March 1999, MARDI Training Center, Serdang, Malaysia; 1999. p. 127–32.

84. Wen JZ, Lei ZR, Wang Y. Opiinae parasitoids of the leafminer *Liriomyza* spp. in China. Entomological Knowledge 2002;39:14–16.

85. Zehnder GW, Trumble JT. Host selection of *Liriomyza* species (Diptera: Agromyzidae) and associated parasites in adjacent plantings of tomatoes and celery. Environmental Entomology 1984;13:492–6.

86. Gratton C, Welter SC. Parasitism of natural populations of *Liriomyza helianthi* Spencer and *Calycomyza platyptera* (Thomson) (Diptera: Agromyzidae). Biological Control 2001;22:81–97.

87. Niranjana RF, Wijeyagunesekara HNP, Raveendranath S. Parasitoids of *Liriomyza sativae* in farmer fields in the Batticaloa District. Tropical Agriculture Research (Sri Lanka) 2005;17:214–20.

88. Bjorksten TA, Robinson M. Juvenile and sublethal effects of selected pesticides on the leafminer parasitoids *Hemiptarsenus varicornis* and *Diglyphus isaea* (Hymenoptera: Eulophidae) from Australia. Journal of Economic Entomology 2005;98:1831–8.

89. Johnson MW, Welter SC, Toscano NC, Ting IP, Trumble JT. Reduction of tomato leaflet photosynthesis rates by mining activity of *Liriomyza sativae* (Diptera: Agromyzidae). Journal of Economic Entomology 1983;76:1061–3.

90. Trumble JT, Kolodny-Hirsch DM, Ting IP. Plant compensation for arthropod herbivory. Annual Review of Entomology 1983;38:93–119.

91. Kotze DJ, Dennill GB. The effect of *Liriomyza trifolii* (Burgess) (Dipt., Agromyzidae) on fruit production and growth of tomatoes, *Lycopersicon esculentum* (Mill) (Solanaceae). Journal of Applied Entomology 1996;120:231–5.

92. Martens B, Trumble JT. Structural and photosynthetic compensation for leafminer (Diptera: Agromyzidae) injury in lima beans. Environmental Entomology 1987;16:374–8.

93. Trumble JT, Ting IP, Bates L. Analysis of physiological, growth, and yield responses of celery to *Liriomyza trifolii*. Entomologia Experimentalis et Applicata 1985;38:15–21.

94. Trumble JT, Carson WG, Kund G. Economics and environmental impact of a sustainable integrated pest management program in celery. Journal of Economic Entomology 1997;90:139–46.

95. Weintraub PG, Horowitz AR. Effects of translaminar versus conventional insecticides on *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) and *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) populations in celery. Journal of Economical Entomology 1998;91:1180–5.

96. Schuster DJ, Zoebisch TG, Gllreath P. Oviposition preference and larval development of *Liriomyza trifolii* on selected weeds. In: Proceedings of 3rd Annual Industry Conference on the Leafminer, Society of American Florists, Alexandria, VA; 1982. p. 137–45.

97. Salvo A, Valladares GR. Leafminer parasitoids and pest management. Ciencia e Investigación Agraria 2007;34:125–42.

98. Johnson MW, Hara AH. Influence of host crop on parasitoids (Hymenoptera) of *Liriomyza* spp. (Diptera: Agromyzidae). Environmental Entomology 1987;16:339–44.

99. Kang L, Chen B, Wei JN, Liu T-X. The roles of thermal adaptation and chemical ecology in *Liriomyza* distribution and control. Annual Review of Entomology 2009;54:127–45.

100. Dicke M, Minkenberg OPJM. Role of volatile infochemicals in foraging behavior of the leafminer parasitoid *Dacnusa sibirica* Telenga. Journal of Insect Behavior 1991;4:489–500.

101. Petitt FL, Turlings TCJ, Wolf SP. Adult experience modifies attraction of the leafminer parasitoid *Opius dissitus* (Hymenoptera: Braconidae) to volatile semiochemicals. Journal of Insect Behavior 1992;5:623–34.

102. Wei JN, Kang L. Eletrophysiological and behavioral response of a parasitoid to plant volatiles induced by two leafminer species. Chemical Senses 2006;31:467–77.

103. Wei JN, Zhu J, Kang L. Volatiles released from bean plants in response to agromyzid files. Planta 2006;224:279–87.

104. Wei JN, Wang L, Zhu J, Zhang S, Nandi OI, Kang L. Plants attract parasitic wasps to defense themselves against insect pests by releasing hexenol. PLoS ONE 2007;2(9):e852.

105. Zhao YX, Kang L. The role of plant odours in the leafminer *Liriomyza sativae* (Diptera: Agromyzidae) and its parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae): orientation towards the host habitat. European Journal of Entomology 2002;99:445–50.

106. Johnson MW. Biological control of Liriomyza leafminers in the Pacific Basin. Micronesica Supplement 1993;4:81–92.

107. Parrella MP, Heinz KM, Nunney L. Biological control through augmentattion release of natural enemies: a strategy whose time has come. American Entomologist 1992;38:172–9.

108. Heinz KM, Parrella MP. Biological control of insect pests on greenhouse marigolds. Environmental Entomology 1990;19:825–35.

109. Heinz KM, Nunney L, Parrella MP. Toward predictable biological control of *Liriomyza trifolii* (Diptera: Agromyzidae) infesting greenhouse cut chrysanthemums. Environmental Entomology 1993;22:1217–33.

110. Hondo T, Kandori I, Sugimoto T. Mass production process of *Neochrysocharis formosa* as the biological control agent against *Liriomyza trifolii*. Memoirs of the Faculty of Agriculture of Kinki University 2006;39:41–54.

111. Chien CC, Ku SC. Instar preference of five species of parasitoids of *Liriomyza trifolii* (Hymenoptera: Eulophidae, Braconidae). Formosan Entomology 2001;21:89–97.

112. Zamzami. Augmentation of parasitoids (*Hemiptarsenus* spp. and braconids) to control *Liriomyza* spp. in Alaha Panjang District, West Sumatra Province, Indonesia. In: Lim GS, Soetikno SS, Loke WH, editors. Proceedings of a Workshop on Leafminers of Vegetables in Southeast Asia. CAB International Southeast Asia Regional Center, Serdang, Malaysia; 1999. p. 54–6.

113. Bader AE, Heinz KM, Wharton RA, Bográn CE. Assessment of interspecific interactions among parasitoids on the outcome of inoculative biological control of leafminers attacking chrysanthemum. Biological Control 2006;39:441–52.

114. Webb RE, Smith FF. Rearing a leaf miner, *Liriomyza munda*. Journal of Economic Entomology 1970;63:2009–10.

115. Petitt FL, Wietlisbach DO. Laboratory rearing and life history of *Liriomyza sativae* (Diptera: Agromyzidae) on lima bean. Environmental Entomology 1994;23:1416–21.

116. Ushchekov AT. *Diglyphus* as an efficient parasitoid of mining flies. Zashchita i Karantin Rastenii 1994;3:56–7.

117. Jeyakumar P, Uthamasamy S. Mass rearing of American serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae). Entomon 1997;22:243–5.

118. Chow A, Heinz KM. Control of *Liriomyza langei* on chrysanthemum by *Diglyphus isaea* produced with a standard or modified parasitoid rearing technique. Journal of Applied Entomology 2006;130:113–21.

119. Chien CC, Ku SC, Chang SC. Study of the storage and oviposition-regulating capability of *Neochrysocharis formosa* (Hymenoptera: Eulophidae). Plant Protection Bulletin (Taipei) 2005;47:213–27.

120. Chien CC, Ku SC, Chang SC. Study of the storage and oviposition–regulating capability of *Hemiptarsenus varicornis* (Hymenoptera: Eulophidae). Formosan Entomologist 2005;25:9–21.

121. Hendrikse A. A method for mass rearing two braconid parasites (*Dacnusa sibirica* and *Opius pallipes*) of the tomato leafminer (*Liriomyza bryoniae*). Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Gent, Belgium: Universiteit Gent 1980;45:563–71.

122. Parrella MP, Yost JT, Heinz KM, Ferrentino GW. Mass rearing of *Diglyphus begini* (Hymenoptera: Eulophidae) for biological control of *Liriomyza trifolii* (Diptera: Agromyzidae). Journal of Economic Entomology 1989;82:420–5.

123. Del Bene G. *Diglyphus isaea* (Wlk.) in commercial greenhouses for the biological control of the leafminers *Liriomyza trifolii* (Burgess), *Chromatomyia horticola* (Goureau) and *Chromatomyia syngenesiae* (Hardy) on chrysanthemum and gerbera. Redia 1990;73:63–78.

124. Rathman RJ, Johnson MW, Tabashnik BE. Production of *Ganaspidium utilis* (Hymenoptera: Eucoilidae) for biological control of *Liriomyza* spp. (Diptera: Agromyzidae). Biological Control 1991;1:256–60.

125. Lenteren JC, Roskam MM, Timmer R. 1997 Commercial mass production and pricing of organisms for biological control of pests in Europe. Biological Control 1997;10:143–9.

126. Ozawa A, Kobayasi H, Amano T, Ikari T, Saito T. Evaluation of imported parasitic wasps as biological control agents of the legume leaf miner, *Liriomyza trifolii* Burgess, in Japan. II. A field test on cherry tomatoes in a plastic greenhouse, in Shizuoka Prefecture. Proceedings of Kanto-Tosan Plant Protection Society 1993;40:239–41.

127. Ozawa A, Saito T, Ota M. Biological control of American serpentine leafminer, *Liriomyza trifolii* (Burgess), on tomato in greenhouses by parasitoids. I Evaluation of biological control by release of *Diglyphus isaea* (Walker) in experimental greenhouses. Japanese Journal of Applied Entomology and Zoology 1999;43:161–8.

128. Rodriguez JM, Rodriguez R, Florido A, Hernandez R. Integrated pest management on tomatoes in Gran Canaria (Canary Islands). Bulletin of International Organization for Biological Control of Noxious Animals and Plants 1997;20(4):39–44.

129. Boot WT, Minkenberg OPJM, Rabbinge R, De Moed GH. Biological control of the leafminer *Liriomyza bryoniae* by seasonal inoculative releases of *Diglyphus isaea*: simulation of a parasitoid-host system. European Journal of Plant Pathology 1992;98:203–12.

130. Cabitza F, Cubeddu M, Ballore S. Two years of observations on the application of biological control techniques against tomato pests on spring crops in greenhouses. Informatore Agrario 1993;49:103–6.

131. Ulubilir A, Sekeroglu E. Biological control of *Liriomyza trifolii* by *Diglyphus isaea* on unheated greenhouse tomatoes in Adana, Turkey. Bulletin of International Organization for Biological Control of Noxious Animals and Plants 1997;20:232–5.

132. Sampson C, Walker P. Improved control of *Liriomyza bryoniae* using an action threshold for the release of *Diglyphus isaea* in protected tomato crops. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Gent, Belgium: Universiteit Gent 1998;63(2b):415–22.

133. Sha C-D, Zhu R, Murphy W, Huang DW. *Diglyphus isaea* (Hymenoptera: Eulophidae): a probable complex of cryptic species that forms an important biological control agent of agromyzid leaf miners. Journal of Zoological Systematics and Evolutionary Research 2006;45:128–35.

134. Heinz KM, Newman JP, Parrella MP. Biological control of leafminers on greenhouse marigolds. California Agriculture 1988;42(2):10–12.

135. Heinz KM, Parrella MP. The effect of leaf-mining by *Liriomyza trifolii* on seed set in greenhouse marigolds. Ecological Applications 1992;2:139–46.

136. Ozawa A, Saito T, Ota M. Biological control of the American serpentine leafminer, *Liriomyza trifolii* (Burgess), on tomato in greenhouses by parasitoids. II. Evaluation of biological control by *Diglyphus isaea* (Walker) and *Dacnusa sibirica* Telenga in commercial greenhouses. Japanese Journal of Applied Entomology and Zoology 2001;45:61–74.

137. Mitsunaga T, Yano E. The effect of multiple parasitisms by an endoparasitoid on several life history traits of leafminer ectoparasitoids. Applied Entomology and Zoology 2004;39:315–20.

138. Ozawa A, Ota M, Saito T. Biological control of the American serpentine leafminer, *Liriomyza trifolii* (Burgess), on cherry tomato in greenhouses by the parasitoids, *Hemiptarsenus varicornis* (Girault). Annual Report of Kanto-Tosan Plant Protection Society, Tsukuba, Japan 2004;51:123–8.

139. Shimomoto M. Control of *Liriomyza trifolii* (Burgess) by *Neochrysocharis formosa* (Westwood), indigenous parasitoid, on eggplant in forcing culture. Bulletin of the Kochi Agricultural Research Center 2005;14:19–24.

140. Van Schelt J, Altena K. Growing tomatoes without insecticides. Proceedings of Section of Experimental and Applied Entomology, Netherlands Entomological Society 1997;8:151–7.

141. Klapwijk J, Martinez ES, Hoogerbrugge H, Boogert M, Den Bolckmans K. The potential of the parasitoid *Chrysonotomyia formosa* for controlling the tomato leafminer *Liriomyza bryoniae* in Dutch tomato greenhouses in winter. Bulletin of International organization of Biological Control 2005;28:155–8.

142. Huang LH, Chen B, Kang L. Impact of mild temperature hardening on thermotolerance, fecundity, and Hsp gene expression in *Liriomyza huidobrensis*. Journal of Insect Physiology 2007;53:1199–205.

143. Hills OA, Taylor EA. Parasitism of dipteran leafminers in cantaloupe and lettuce in Salt River Valley, Arizona. Journal of Economic Entomology 1951;44:759–62.

144. Prijono D, Robinson M, Rauf A, Bjorksten TA, Hoffmann AA. Toxicity of chemicals commonly used in Indonesian vegetable crops to *Liriomyza huidobrensis* populations and the Indonesian parasitoids *Hemiptarsenus varicornis*, *Opius* sp., and *Gronotoma micromorpha*, as well as the Australian parasitoids *Hemiptarsenus varicornis* and *Diglyphus isaea*. Journal of Economic Entomology 2004;97:1191–7.