Seasonality and community composition of parasitoid wasps of four agromyzid leafminer species (Diptera: Agromyzidae) in Victoria

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THESIS ABSTRACT

The vegetable leafminer, *Liriomyza sativae* Blanchard (Diptera: Agromyzidae), was first detected in the Torres Straits in 2008, crossed over to the tip of Cape York in 2015 and now poses a threat to Australian crops. This species is an economically important secondary pest in many parts of the world; populations readily evolve resistance to insecticides and benefit from the use of chemical controls against other pests, which kill their natural enemies. *Liriomyza sativae* is expected to spread into Australia's agricultural production areas and it is therefore valuable to investigate the local biological agents that could contribute to future integrated pest management (IPM). I assessed the abundance and diversity of the hymenopteran parasitoid wasps of four common agromyzid flies in southern Victoria (*Liriomyza brassicae* (Riley), *Liriomyza chenopodii* (Watt), *Phytomyza plantaginis* Goureau, and *Phytomyza syngenesiae* (Hardy)) to evaluate their potential as candidates for control of *L. sativae.* I monitored six sites in Melbourne over a period of 18 months by collecting mined leaves from a range of plant species and rearing adult flies and their parasitoids. Additional sites around Victoria were also sampled to offer a snapshot of the agromyzid presence across the state.

Eleven wasp species were identified in total, two of which were only classified to morphospecies level, including known parasitoids of *L. sativae* overseas and species reared from *L. sativae* populations from far north Queensland. Ninety percent of the parasitoids were eulophids (Hymenoptera: Eulophidae), with the rest consisting of opiines (Braconidae) and Pteromalinae (Pteromalidae). The adventive *Chrysocharis pubicornis* (Zetterstedt) was the most abundant parasitoid, reared almost entirely from *Phytomyza* hosts, followed by an *Asecodes* sp. and the introduced *Diglyphus isaea* (Walker). We recorded the first male *Ph. plantaginis* for Australia. Males were only found at Melbourne locations and these populations tended to have a female-biased sex ratio. Variation in sex ratio may be host plant dependent, as the female:male ratio was 4:1 from the host *Plantago lanceolata* L. while it was 1:1 in more limited samples from *Plantago major* L. The four common local agromyzids reached peak abundance at different times of the year and together supported a stable community of both adventive and native parasitoids. Of the species reared,

several are known to attack *L. sativae* including *D. isaea*, *Hemiptarsenus varicornis* (Girault) and *Neochrysocharis formosa* (Westwood). The wasps reared include candidates for augmentative or conservation biological control that should be further considered in the event that *L. sativae* becomes a widespread pest of Australian crops.

I declare that this thesis comprises only my original work towards this Master of Philosophy, except where indicated in the preface, that due acknowledgement has been made in the text as to all the other/external material used and this thesis is fewer than the maximum word limit length, exclusive of tables, maps, bibliographies and appendices.

PREFACE

The work towards the thesis that was carried out in collaboration with others consisted of the molecular work used to support morphological identification of the specimens. All specimen preparations and barcoding were done by PhD candidate Xuefen Xu.

Dissection of the male genitalia of four agromyzid specimens was performed by Dr M. Malipatil (AgriBio, Agriculture Victoria Research, Melbourne, VIC), all photography was done by the student.

There was no third-party editorial assistance and drafts, figures and tables were all revised by the supervisors of the projects.

The student claims as original the sampling protocol, choice of sites and direction of sampling, the majority of the specimen rearing (80%) and specimen identification (95%). There was some field-work assistance for collecting the plant material under the supervision of the student.

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CHAPTER 1. General Introduction

1.1 INTRODUCTION

Strict biosecurity measures are in place in Australia to detect and stop the introduction of foreign species and limit their negative impact or proliferation within the country. The Northern Australia Quarantine Strategy (NAQS) was designed to provide an early warning system for exotic pest, weed and disease detections across northern Australia (Department of Agriculture, Water and the Environment 2020). During a NAQS survey in the Torres Strait islands in 2008, the vegetable leafminer *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) was first detected on a tomato plant on Warraber Island (IPPC 2009; Blacket *et al.* 2015). This leafminer species had been steadily making its way through Asia and South-East Asia during the 1990s. It has since crossed into Australian mainland, where it was found at Seisia, at the northern tip of Cape York, Queensland, in 2015 (IPPC 2017). Strict quarantine procedures and an early incursion management plan (Jovicich 2009) were put in place, and *L. sativae* has not yet been found outside of its point of entry. However, its spread is highly likely, and this spread could severely impact crops like tomato, beans, beetroot, pumpkins, melons and celery in Queensland as well as other crops in more southern states including Victoria.

Liriomyza sativae larvae feed on a large number of host plants from more than a dozen families which include Asteraceae, Cucurbitaceae, Fabaceaeae, and Solanaceae (CABI 2019). Integrated pest management programs that are based on biocontrol measures to supplement chemical controls are needed for a sustainable response to *L. sativae* and other invasive vegetable leafminers (Murphy & LaSalle 1999). To that end, it is important to understand the diversity and abundance of natural enemies of agromyzids already present in Australia. Such an effort can help indicate pest control that could be provided by resident natural enemies rather than relying on introduced biocontrol agents. For leafminer control, parasitoids are particularly important natural enemies (Ridland *et al.* 2020). The scope of this thesis covers field surveys of the temporal and spatial abundance of four common Victorian agromyzid leafminers and their hymenopteran parasitoids, which can potentially impact *L. sativae*.

1.1.1 Polyphagous *Liriomyza* spp.

Four highly polyphagous *Liriomyza* species are of particular concern for Australia. *Liriomyza huidobrensis* (Blanchard), *L. sativae* and *Liriomyza trifolii* (Burgess) are major agricultural pests that originated from the Americas and now have a near global distribution (Spencer 1973, 1989; Minkenberg & Van Lenteren 1986; Murphy & LaSalle 1999; Dempewolf 2004), though *L. sativae* is not established in Europe (EPPO 2020)**.** Though they started out as New World pests, these leafminers had spread to Europe and Asia by the mid-1900s (Gao *et al*. 2017; Abe 2018). In contrast, the European species closely related to *L. huidobrensis* (Spencer 1973), *L. bryoniae* (Kaltenbach), has not spread to the American continent but is widespread in Europe and Asia. Another highly polyphagous agromyzid is *Phytomyza horticola* Goureau, previously grouped with *Phytomyza syngenesiae* (Hardy) under the name *Phytomyza atricornis* Meigen. *Phytomyza horticola* is present in the Afrotropical region and widespread in Europe and Asia (CABI 2020). A number of factors may have played a role in the expansion and increased impact of these leafminers since the mid-1970s. In particular, this period coincides with the increased use of synthetic insecticides against primary pest species, which also reduce natural enemies of agromyzids. This period also coincides with the blossoming of the international trade in agricultural and horticultural crops, in particular from the Americas to Europe (Reitz *et al.* 2013; Weintraub *et al.* 2017).

A high level of polyphagy is one of the reasons that this group of leafminers pose an important threat to agriculture. *Liriomyza sativae* feeds on plants across at least 15 families (Spencer 1990; CABI 2019)., *L. trifolii* feeds on at least 25 families, *L. bryoniae* feeds on plants across 35 families, *Ph. horticola* on 36 families (Spencer

1990), and *L. huidobrensis* on more than 40 host plant families (Weintraub *et al.* 2017). All of these species are found on Fabaceae, Asteraceae, Brassicaceae, Cucurbitaceae and Solanaceae, though they vary in their host preference. This wide range of hosts increases the likelihood of leafminers being transported to new countries and establishing there.

In a typical life cycle for an agromyzid leafminer, the fertilized female fly oviposits into a leaf by piercing the epidermis with its ovipositor. Such punctures are also used by both male and female flies to feed on the exudate (Parrella 1987). This feeding behavior creates light stippling marks on the surface of the leaves. There is evidence that these wounds may facilitate the transmission of some potyviruses (Zitter & Tsai 1977), plant pathogenic bacteria (Matteoni & Broadbent 1988) and *Alternaria* fungal species (Chandler & Thomas 1991; Deadman *et al.* 2002) by creating points of entry or mechanically introducing them via their ovipositor into the plant. Most leafminer damage however comes from the larval stages. The larva develops within the leaf and feeds by tunneling through different cell layers, forming a growing mine. Photosynthesis is reduced in heavily mined leaves (Parrella *et al.* 1985). It weakens the plant and can cause entire leaves to die and fall off. Further damage in fruits and vegetables can result from UV exposure or plant weakening that severely reduces yield and value (Parrella 1987; Kwon *et al.* 2018). The late 3rd instar *Liriomyza* larva generally exits the mine and pupates in the soil; *Phytomyza* spp. generally pupate within the mine. For that reason, life stages of some agromyzid leafminers can be sheltered from chemical exposure not only in the leaf but also in the soil. The earliest methods of control consisted of removing affected plants, spraying or fumigating with nicotine-based pesticides (Smulyan 1914; Parrella & Keil 1984), and later spraying with DDT (Spencer 1973). Indiscriminate spraying resulted in the development of resistance to insecticides, and as a consequence most pyrethroids and organophosphates are no longer effective (Parrella & Keil 1984). Increased use of broad-spectrum insecticides from the mid-1900s onwards has not helped manage the problem and instead resulted in major outbreaks of *Liriomyza* agromyzids (Pohronezny *et al*. 1986; Rauf *et al.* 2000). This is due to the application of pesticides causing a reduction in parasitoid numbers and a resurgence of the agromyzids as secondary pests (Oatman & Kennedy 1976; Chavez & Raman 1987; Saito *et al.* 2008). Insecticides with translaminar properties, in particular abamectin and cyromazine, were found to be useful against *Liriomyza* spp. but have varying degrees of toxicity to parasitoids (Trumble 1985; Leibee & Capinera 1995; Weintraub 2001; Prijono *et al.* 2004; Bjorksten & Robinson 2005).

Several life-history traits complicate the control of leafminers beyond those that limit their exposure to pesticides. While the number of eggs laid by leafminers can vary among species, many species can lay several hundreds of eggs in a lifetime, up to 640 eggs in the case of *L. sativae* on kidney bean (Tokumaru & Abe 2003), resulting in rapid increases in populations. Leafminers also spend a relatively short amount of time in their larval stage mining the mesophyll layer of their host, offering a small window of time for larval parasitoids unless generations overlap. On average and under the optimal conditions of 25°C, these *Liriomyza* species spend around a week before pupation, with 4 to 5 days being the range for *L. huidobrensis* (Mujica *et al.* 2017), 5 to 6 days for *L. sativae* on cucumber (Haghani *et al.* 2007) and 3 to 5 on tomatoes (Firake *et al.* 2018), 4 days for *L. trifolii* on tomatoes (Minkenberg 1988). Rapid development as well as high reproductive output can lead to a rapid buildup of pest populations.

The ability of leafminers to fly long distances was shown to be limited in a greenhouse setting (Jones & Parrella 1986), whereas air currents and anthropogenic activities can allow for long distance movement (Iwasaki *et al*. 2008). A review of the interceptions of *Liriomyza* spp. in Europe (with a focus on *L. huidobrensis* and *L. trifolii*) found that the main pathways of entry into countries involved infested plant material (EFSA Panel on Plant Health (PLH) 2012), with interceptions of *L. huidobrensis* mostly in cut flowers and vegetables (Weintraub *et al.* 2017). In China, genetic divergence of the *L. sativae* did not mirror geographical distance, which further supports the notion that unintentional human transport leads to occasional long distance dispersal (Tang *et al.* 2016). Since these leafminers share host plants and introduction pathways, invasive *Liriomyza* species are expected to co-occur. However, ecological and environmental factors can affect their coexistence. *Liriomyza huidobrensis* has greater cold tolerance than its contemporary *L. sativae* (Chen & Kang 2002) or *L. trifolii* (Lanzoni *et al.* 2002) and in Indonesia and Guatemala, is the dominant leafminer at higher altitudes, while *L. sativae* is prevalent in the lowlands (Tantowijoyo & Hoffmann 2010; Rodriguez-Castañeda *et al.* 2017) . Species can coexist in crops but host-preferences as well as seasonal peaks of abundance vary between them (Rauf *et al.* 2000; Abe & Kawahara 2001; Tokumaru *et al.* 2007; Foba *et al.* 2015). Species displacements are common in leafminers. In Kenya, *L. trifolii* was the most abundant leafminer for several years but *L. huidobrensis* later became the dominant agromyzid, representing 90% of all leafminers collected, regardless of elevation (Foba *et al.* 2015). In California *L. sativae* was first displaced by *L. trifolii*, possibly caused by differences in insecticide resistance (Reitz & Trumble 2002). *Liriomyza langei* Frick, a cryptic species closely related to *L. huidobrensis* (Scheffer *et al.* 2014), and at one stage synonymized with it, has become the predominant species in the central coast valley of California, while *L. trifolii* remains dominant in southern California (Reitz & Trumble 2002). In Japan, displacement was reversed compared to the USA, with *L. sativae* displacing *L. trifolii* early in the 2000s (Tokumaru *et al*. 2007). In this case, *Liriomyza sativae* showed higher fecundity than *L. trifolii* on bean and tomato; moreover, while both species were parasitized by the introduced parasitoid *Dacnusa sibirica* Telenga (Braconidae), no adult wasps successfully developed on *L. sativae* (Abe *et al*. 2005, Abe & Tokumaru 2008). This suggested that local parasitoids had a role in changing the competitive advantage of the species and also in suppressing exotic leafminers.

1.1.2 Biological control – generalist parasitoids

Given the widespread insecticide resistance in leafminers but more importantly the destruction of natural enemies leading to secondary outbreaks of the pest, biological control options need to be investigated. Agromyzid flies have a number of natural enemies, including predators, entomopathogenic nematodes and entomopathogens, but the primary actors are hymenopteran parasitoids (Liu *et al*. 2009). More than 300 species associated with Agromyzidae were compiled in the

Universal Chalcidoidea Database (Noyes 2020), predominantly represented by the families Eulophidae and Pteromalidae (Chalcidoidea) In addition, parasitoid species from the Figitidae (Cynipoidea) and Braconidae (Ichneumonoidea) are common parasitoids of Agromyzidae in many parts of the world (Ridland *et al.* 2020). Diverse parasitoid communities attacking leafminers across the globe are not species specific, targeting instead the ecological niche of cryptic mining larvae. For instance, 63 parasitoid species were reared from nine agromyzid species in Peru (Mujica & Kroschel 2011), 11 species were reared from crops infested with *L. huidobrensis* and *L. sativae* in Indonesia (Rauf *et al*. 2000), 16 eulophid species were collected from *Ph. horticola* in Russia (Yefremova *et al.* 2015) and 31 species were reared from several agromyzids in Florida, USA (Stegmaier 1972). *Chrysocharis* Förster, *Diglyphus* Walker, *Hemiptarsenu*s Westwood and *Neochrysocharis* Kurdjumov are some of the common recurring genera in these parasitoid communities.

1.1.3 Parasitoid development strategies

Hymenopteran parasitoids that attack agromyzid leafminers do so by feeding and ovipositing on or in the larval or pupal stages. They cover a range of parasitism and development strategies which allows them to co-exist with other parasitoids (Harvey *et al.* 2013). Parasitoids can be categorized as being 'idiobionts' that kill or paralyze the host during the oviposition event and during feeding, or 'koinobionts' that allow the host larva to continue to develop and remain active after the oviposition event. Some of the parasitoids are highly synovigenic, such as *Diglyphus isaea* (Walker) and *Hemiptarsenus varicornis* (Girault), meaning that almost none of their eggs are present or mature at emergence and instead continue to mature throughout the females' reproductive period. This is the predominant pattern in parasitoid wasps that are idiobiont (Jervis *et al.* 2001). This strategy leads to a higher fecundity and extended longevity when the parasitoid has access to a food source, and this in turns produces additional host mortality, both from the longer ovipositing period and ongoing production of eggs as well as from the feeding-related wounds (Zhang *et al.* 2011; Cheng *et al.* 2017). Depending on the temperature, adult *H. varicornis*, *D. isaea* and *Neochrysocharis formosa* (Westwood) can kill a greater number of hosts through feeding than they do through ovipositing, all the while maintaining high levels of parasitism (Hondo *et al.* 2006).

Furthermore, the wasps of leafminers can be ecto- or endoparasitic, based on whether their egg is deposited next to their host in the mine, or directly implanted into the host. Koinobionts are more likely to be endoparasitoids as the active host larva may otherwise evade the parasitoid's larva. Attacks by these parasitoids however, do not prevent mining or damage to plants since the host larvae reaches its pupal stage. An example of an idiobiont ectoparasitoid is *D. isaea*, while *N. formosa* is an idiobiont endoparasitoid. When attacking the same host, these idiobionts nevertheless have differing fecundity peak periods, lifespans and abilities to use the nutrients from host-feeding (Zhang *et al.* 2014). Opiine, pteromalid and some eulophid parasitoids are koinobiont endoparasitoids, attacking larvae in the mines and emerging from the fly puparia. Finally, pupal endoparasitoids like *Chrysocharis pubicornis* parasitize the agromyzid pupae once formed, with some instances of them attacking 3rd instar *L. trifolii* larvae (Baeza Larios 2007). Lardner (1991) found it relatively rarely parasitizing some 2nd and 3rd instar larvae in the field. These parasitoids appear to have minor effects on *Liriomyza* spp. that pupate primarily in the soil, likely due to the difficulty of locating pupae.

1.1.4 Australian studies on parasitoids of Agromyzidae

A limited number of studies have investigated the trophic interactions of agromyzids and their parasitoids in Australia. Sampling of the adventive species *Liriomyza brassicae* (Riley), *Phytomyza plantaginis* Goureau and *Phytomyza syngenesiae* (Hardy) (Kleinschmidt 1970, Bouček 1988; Lardner 1991; Bjorksten *et al*. 2005; Lambkin *et al*. 2008) resulted in a number of parasitoid species reared from the three families Braconidae, Eulophidae and Pteromalidae (Table 1-1). Sampling of a native *Phytoliriomyza* sp. (Wood *et al.* 2010) also resulted in a number of parasitoid species being reared. The known parasitoids of agromyzids present in Australia include introduced or adventive species that have now established, such as *H.*

varicornis, *D. isaea* and *C. pubicornis* as well as parasitoids present across the Australasian region, like *Zagrammosoma latilineatum* Ubaidillah, *Opius (Opiothorax) atricornis* Fischer, and *Opius (Gerius) cinerariae* Fischer (Ridland *et al.* 2020). This diversity of generalist parasitoids already established on adventive agromyzid leafminers in Australia suggests that there is a strong ability of existing parasitoids to exploit invasive agromyzids (Ridland *et al*. 2020). It encourages the use of conservation biological control, which involves maintaining and relying on the presence of parasitoids in the environment, rather than the classical approach that consists of introducing exotic parasitoid species for pest control. Some of the existing parasitoids, both adventive and native, could be suitable for mass-rearing for use in augmentative biological control in protected cropping.

1.2 AUSTRALIAN AGROMYZIDAE SPECIES SELECTED FOR SAMPLING

Agromyzid leafminers in Australia have not been of major concern as agricultural pests. Spencer (1963b, 1977) has documented both endemic and introduced Australian Agromyzidae species. The family is poorly represented in Australia since the more than 150 species identified represent only 5% of the more than 2,900 species known globally (ITIS 2019) and belong to only 13 genera of the 31 identified (Spencer 1977; Pape & Thompson 2019). The majority of species belong to the *Ophiomyia, Melanagromyza* and *Phytoliriomyza* genera. Of those, only *Ophiomyia phaseoli* (Tryon), the bean-fly, is considered a significant agricultural pest in NSW and Queensland as it is a major pest of legumes in tropical and subtropical regions (Coquillett 1899; Queensland Government 2020). A highly polyphagous agromyzid also present in Australia is *Tropicomyia polyphyta* (Kleinschmidt), distributed in NSW and Queensland on a range of 28 host families (Spencer 1977, 1990). In the absence of economically important species like *L. sativae*, expected parasitoid interactions with pest leafminers can instead be investigated by looking at closely related species and by surveying agromyzids and parasitoids already present in the region. Four other *Liriomyza* spp. were chosen as likely candidates for parasitoids expected to transfer onto exotic *Liriomyza* flies. They are all adventive species introduced in Australia with the exception for *L. chenopodii*, a native fly found in Australia and New Zealand. They can themselves be considered to be good examples of exotic agromyzids exploited by local parasitoid populations. They are also widespread and present on commonly found host plants from urban and disturbed green spaces.

Table 1-1. Australian studies on parasitoids of Agromyzidae with number of parasitoid species included under each host agromyzid. Total = Total number of parasitoid species reared. Note that there is overlap between agromyzid species; ✓ = no parasitoid mentioned but agromyzid sampled.

Study	**State**	***Liriomyza brassicae*** (adventive)	***Liriomyza chenopodii*** (native)	***Phytomyza plantaginis*** (adventive)	***Phytomyza syngenesiae*** (adventive)	***Phytoliriomyza praecellens***[a] (native)	**Other spp.**	**Total**
Kleinschmidt (1970)	Qld	6	✓		✓		✓	**≥ 6**
Bouček (1988)	Aus	✓			✓		✓	**-**
Lardner (1991)	SA	7						**7**
Bjorksten *et al.* (2005)	Vic	4	11		6		1	**15**
Lambkin *et al.* (2008)	ACT/ NSW			3	12		✓	**14**
Wood *et al.* (2010)	SA					10		**10**

[a] Putative identification because no males have been described for this species (Spencer 1977)

1.2.1 *Liriomyza brassicae* (Riley 1885), cabbage leafminer

Oscinis brassicae Riley, 1885
Liriomyza cruciferarum Hering, 1927
Liriomyza brassicae Frick, 1952
Phytomyza mitis Curran, 1931
Liriomyza hawaiiensis Frick, 1952
Liriomyza bulnesiae Spencer, 1963
Liriomyza ornephila Garg, 1971

Liriomyza brassicae (Riley), the cabbage leafminer, is a truly cosmopolitan agromyzid with a range extending across Europe, the Nearctic, Neotropical, Oriental (including Japan), Afrotropical, and Australasian regions (Lonsdale 2011). It was first officially collected in Australia in Brisbane in 1911 on nasturtium plants (*Tropaeolum majus* L.), described by Spencer (1977) as its primary host in the country. However, there is an even earlier record in 1900 of an agromyzid leafminer, *Phytomyza affinis* Fallén*,* on cabbages and turnips in Victoria (French 1900). Based on the description, it is likely to be an amalgam of several agromyzids, including specimens subsequently described as *L. brassicae* (Kleinschmidt 1965, 1970). It has a relatively wide range of host plants which include Brassicaceae, Capparaceae and Tropaeolaceae. It has also been collected from Verbenaceae, on *Lantana camara* L. in Mexico where it forms a blotch mine instead of the usual serpentine one (Palacios Torres *et al.* 2015). There are a few records of it feeding on Fabaceae, on *Pisum* sp. and *Lathyrus* sp., including in Australia (Spencer 1973; Lardner 1991).

In addition to records from unusual host plants, the specimens reared showed some color variation, which could indicate that *L. brassicae* is a species complex (Lonsdale 2011). This is further supported by experimental evidence of host-associated selection and divergence in a population of *L. brassicae* (Tavormina 1982). In this work, a fertilized female showed a tendency to oviposit on the same host plant species they were reared from and it resulted in a shorter development time and a higher number of offspring from that plant, suggesting the possible segregation of sympatric populations onto different host plants over time. This phenomenon can lead to the association between feeding and oviposition in insects and result in host specialization or expansion (Knolhoff & Heckel 2014). Lonsdale (2011) listed *L. brassicae* from 11 families of host plants, but some records are incorrect or uncertain. A record from the plant *Foeniculum vulgare* Mill. (Umbelliferaceae) was for an adult male collected on the host but not directly reared from it. Epidermal mines on *Bulnesia arborea* Engler (Zygophyllaceae) obtained in Caracas Botanical Gardens, originally attributed to *L. brassicae* by Spencer (Spencer 1963b; Spencer & Stegmaier 1973) were since identified as *Liriomyza schmidti* (Aldrich) (Spencer 1973).

Liriomyza brassicae shares life history traits common to nearly all *Liriomyza* spp. (Parrella 1987). Females oviposit eggs under the surface of the leaf and the hatched larva forms a serpentine mine as it feeds on the mesophyll layer, reaching an average length of nearly 3 mm. The prepupal larva exits the mine by cutting a small slit in the leaf epidermis. The larva then falls to the substrate below and pupates in a concealed situation (Beri 1974). *Liriomyza brassicae* was a pest of cruciferous crops in Senegal, Hawaii, and on *Brassica campestris* in India (Spencer 1973) but has not caused substantial damage in the recent years. It was classed as a major cabbage pest in 1949 in Cleveland, Queensland, likely due to parasitoid suppression and leafminer resistance following the overuse of DDT against primary pests (Kleinschmidt 1965). After changes in chemical use, *L. brassicae* no longer was recorded as being a pest though on crops it persists throughout Australia in low numbers– with the exception of the Northern Territory (NT) where it has yet to be detected (Lardner 1991). In Victoria, it is commonly found in large numbers on brassicaceous weeds in disturbed ground in urban and peri-urban areas (Table A1-1). It is externally indistinguishable from *L. sativae* and can only be separated by characters associated with its distinct distiphallus.

1.2.2 *Liriomyza chenopodii* (Watt 1924), beet leafminer

Phytomyza betae Coquillet 1900 (primary homonym by Macquart 1855)
Haplomyza chenopodii Watt, 1924
Haplomyza imitans Malloch, 1934
Liriomyza chenopodii Spencer, 1963

A leafminer from beetroot leaves first described from Western Australia as *Phytomyza betae* by Coquillett (1900) was later deemed identical to *L. chenopodii* by Spencer (1977). In France, Macquart had already named a leafminer as *Ph. beta*e in 1855, but only under a very brief description: "*Phytomyza betae* Macq. The larva mines the leaves of the beetroot." (translated). For that reason, *Phytomyza betae* Macq. was originally considered a *nomen nudum* and not supported by enough evidence to be official. After some back and forth about which description fulfilled the requirements for a valid name (Spencer 1977, 1990: 398), Macquart's short description of the work of an insect (Art.12.2.8 in the current taxonomy code) was recognized and *Phytomyza betae* Coquillett was accepted as a homonym for *Phytomyza betae* Macq. Consequently, *Haplomyza chenopodii* Watt is the first correct name for this Australian species. Recent communications involving Neil Evenhuis (Senior Curator of Entomology at Bishop Museum), Chris Thompson (Research Associate at the Smithsonian Natural Museum of Natural History), Dan Bickel (Australian Museum, Sydney) and Mali Malipatil (Malipatil & Ridland 2008) validated *L. chenopodii* as the correct current name. *Liriomyza chenopodii* was collected in New South Wales (NSW), Victoria and New Zealand (Spencer 1973).

Though *L. chenopodii* is endemic to Australia, it has not been officially recorded on any native host plants and instead has only been reared from adventive hosts such as *Stellaria media* (L.) Vill. (Caryophyllaceae) (chickweed, a prolific annual with a large seed reserve) where it was recorded as *Haplomyza imitans* by Malloch (1934), and from *Chenopodium album* L. (Amaranthaceae) (fat hen). Both these plants are common in disturbed habitats. Other host plants include *Spinacia oleracea* L. (Amaranthaceae) (spinach) in Australia (Malloch 1934), and additional weeds like *Cerastium vulgare* L. (Caryophyllaceae) (mouse-ear chickweed) in New Zealand (Watt 1924) (Table A1-2). Arthur M. Lea, who was in contact with Coquillett, mentioned rearing *L. chenopodii* (as *Ph. betae*) from *Anthocercis* Labill. (Solanaceae) (tailflower) (Coquillett 1900), a plant genus of 10 species endemic to the southern temperate region of Australia (FloraBase 2016). This has not been confirmed as no new specimens have been reared from this genus. Based on the host plant, the mining species is more likely to have been *Phytomyza anthercidis* Spencer and there is no specimen data for the flies described by Coquillett (Spencer 1977). *Liriomyza chenopodii* is indistinguishable from *Liriomyza oleariana* Spencer without studying male genitalia. However, *L. oleariana* does not appear to share hosts with it and has only been found on *Olearia axillaris* (DC.) Benth (Asteraceae) in Western Australia (Spencer 1977). *Liriomyza caulophaga* (Kleinschmidt) is another endemic leafminer, also found on *Beta vulgaris* L. (Chenopodiaceae) (silver beet) but is less common and readily distinguished from *L. chenopodii* in both its appearance and feeding behaviours. It mines the midrib and petiole of the leaves, and pupates within a small pocket in the mine, in contrast to *L. chenopodii* which mines the mesophyll mainly and pupates externally (Kleinschmidt 1970). *Liriomyza caulophaga* has also only been recorded in Brisbane, Queensland and in the Sydney area in NSW, and there are no records of it in Victoria at this time.

1.2.3 *Phytomyza plantaginis* Goureau 1851, ribwort leafminer

Phytomyza plantaginis Goureau, 1851
Phytomyza robinaldi Goureau, 1848 *nomen nudum*
Phytomyza robinaldi Goureau, 1851
Phytomyza plantaginis Robineau-Desvoidy, 1851 [Preocc. Goureau, 1851.]
Phytomyza biseriata Hering, 1936
Phytomyza nannodes Hendel, 1936
Phytomyza plantaginicaulis Hering, 1944

There is some controversy regarding the naming authority for *Ph. plantaginis* as the original specimens were bred by Goureau and passed on to Robineau-Desvoidy to be described. Robineau-Desvoidy (1851) and Goureau (1851) each published a description of the fly in 1951 using the same name of *Ph. plantaginis.* In fact, Goureau (1851) gave it two names: *Phytomyza plantaginis* Robineau-Desvoidy and *Phytomyza robinaldi* (Goureau). Although the vast majority of current agromyzid taxonomists now use Robineau-Desvoidy as the authority for the species (Martinez 2020), Evenhuis (2016) stated that "Many publications list Robineau-Desvoidy as the author of this species; however, it is clear from the original description in Goureau (1851) that

Robineau-Desvoidy (1851) did not satisfy the criteria in Art. 50.1 of the Code to be the author in that publication". Spencer (1963a) verified this species and synonymized *Phytomyza plantaginicaulis* Hering, *Phytomyza biseriata* Hering and *Phytomyza nannodes* Hend. with *Ph. plantaginis*.

Phytomyza plantaginis is common in Australasia, and has been recorded in Tasmania, Victoria, ACT and NSW (Spencer 1963b, 1977) as well as New Zealand (Spencer 1976). In Australia it has been collected from *Plantago lanceolata* L. (Plantaginaceae) and from *Plantago major* L. (Spencer 1977) (Table A1-3). It is a genus of plants adapted to temperate climates and there are 34 species known in Australia, 10 of which were introduced, including *Ph. plantaginis* hosts (VicFlora 2019). The female fly oviposits into the leaf, very often on the abaxial surface of the oldest and most basal leaves (M. Coquilleau pers. obs.). The larva tunnels into the leaf, forming a whitish and narrow mine, sometimes in the stems and towards the petiole. The late instar larva is around 3 mm, the same length as its adult form. It pupates within the mine, forming a light or dark puparium at the widest point of the mine, its anterior spiracles anchored in the thin epidermis (Eiseman 2019; Martin 2019). No developmental data has been published for the species.

1.2.4 *Phytomyza syngenesiae* Hardy 1849, cineraria leafminer

Agromyza atricornis Meigen, 1830 *nomen dubium*
Phytomyza atricornis Meigen 1838 sensu Hendel 1920
Chromatomyia syngenesiae Hardy, 1849
Phytomyza chrysanthemi Kowarz in Lintner 1891
Phytomyza syngenesiae Griffiths, 1967

Phytomyza atricornis is represented by European specimens and has been a major pest of cultivated flowers such as *Chrysanthemum* spp. L., *Cineraria stellata* L., *Calendula officinalis* L., and *Helianthus annuus* L., as well as being found on numerous other hosts. Following studies of *Ph. atricornis* specimens, it appears the species has been confused with at least 4 species, primarily *Ph. horticola* and *Ph. syngenesiae*, but also *Ph. farfarella* Hendel from Iceland collections and *Ph. aragonensis* Griffiths from

Spain (Griffiths 1967). *Phytomyza horticola* and *Ph. syngenesiae* are only identifiable based on male genitalia, as they are otherwise identical in their external morphology and pupation behavior in the mine (Griffiths 1967). For that reason, the true identity of the leafminers under the appellation of *Ph. atricornis* in past literature is dubious. Based on host plants and location, there appears to be two sister-species that do not completely overlap. *Phytomyza syngenesiae* is oligophagous, feeding primarily on a wide range of Asteraceae, with some infrequent occurrences on Apiaceae, Cucurbitaceae, Fabaceae, Labiatae and Papaveraceae (Spencer 1973; Martin 2017). While on the other hand, *Ph. horticola* is highly polyphagous, feeding on hosts from 35 families and most common on Asteraceae, Brassicaceae and Fabaceae (Spencer 1989, 1990). It has not been recorded from North America, Australia or NZ, but it is found in eastern and southern Asia as well as Africa. Where their ranges overlap in Europe, *Ph. syngenesiae* is commonly collected from Asteraceae.

Phytomyza syngenesiae is recorded throughout Australia, with the exception of NT (Kleinschmidt 1965; Spencer 1973; Osmelak 1983), from Asteraceae such as *Senecio* spp. and *Sonchus* spp. (Spencer 1977), including the widespread and invasive *Sonchus oleraceus* L. (sow thistle) (Bjorksten *et al.* 2005; Lambkin *et al.* 2008). Additional hosts from Asteraceae recorded in New Zealand and Australia include crops like *Cichorium endivia* L. (endive), *Cynara scolymus* L. (globe artichoke) and *Lactuca sativa* L. (lettuce) where it has occasionally caused severe damage in Victoria (Osmelak 1983) (Table A1-4). On rare occasions regarded as involving xenophagy ('a categorical change in diet') or more likely allotrophy ('a diversification of diet') by Griffiths (1967), *Ph. syngenesiae* was reared from *Daucus carota* (Apiaceae) (carrot) in Copenhagen, Denmark, and on *Pisum sativum* (Fabaceaeae) (pea) in California, U.S.A. In England, Spencer (1976) reared some *Ph. syngenesiae* on *Cannabis sativa* L. (Cannabaceae) (hemp) grown for experimental purposes. A few specimens were also reared from *Trifolium repens* L. (Fabaceaeeae) (white clover), *Lamium purpureum* L. (Lamiaceae) (purple deadnettle) and one male from *Nepeta cataria* L. (catnip) in New Zealand (Spencer 1976). Mining was also described on *Urtica* sp. L. (Urticaceae) (nettle), *Plantago* sp. L. (Plantaginaceae) (plantain), *Melilotus* sp. (Fabaceaeae) (sweet clover) in New Zealand. The species identity however is dubious as there are other likely leafminers of these plants, such as *L. urticae* (Watt) on nettle and *Ph. plantaginis* on plantain (Spencer 1976). It has since been reared with varying levels of confidence from one plant species from each family: Cucurbitaceae, Papaveraceae, Umbelliferae, and two species in Labiatae and Fabaceaeae (Martin 2017). The biology of *Ph. syngenesiae* was described under several names, as *Ph. chrysanthemi* by Smulyan (1914), as *Ph. atricornis* by Cohen (1936) and as *Ph. albiceps* by Watt (1924). Its lifecycle was reported by Ibrahim & Madge (1977) and Cheah (1987) detailed its temperature requirements.

It was placed for a period of time under the genus *Chromatomyia*, very closely related to *Phytomyza*. Previous traits used to differentiate *Chromatomyia* were a derived structure of the genitalia and a sclerotized puparium that remains within the mine with its spiracles extending out of the surface. However, these traits are not singular to the *Chromatomyia* leafminers and did not consistently support its generic status. Winkler *et al*. (2009) studied the phylogenetic relationships between *Phytomyza* and the closely related *Chromatomyia,* and found *Chromatomyia* to be both nested within the *Phytomyza* group and to be paraphyletic, as they were consistently separated into two groups, the *syngenesiae* and the *agromyzina*, separated by at least one *Phytomyza* species. Following these results, *Chromatomyia* is more appropriately described as a subgenus to *Phytomyza* and I will thus continue to refer to the cineraria leafminer as *Phytomyza syngenesiae*. This nomenclature still lacks consensus and *Chromatomyia syngenesiae* is used in some resources such as the Australian Faunal Directory (Elliott 2011), and by some European taxonomists (Černý & Tschirnhaus 2014).

1.1 AIMS AND OBJECTIVES

The aims of this Masters thesis were to 1) characterize the seasonal presence of target agromyzids by surveying host plants and sampling mined leaves, this took place around Melbourne and greater Victoria, over a year and a half. 2) Identify the predominant parasitoid species present. 3) Compare the parasitoid community between host leafminers by rearing flies and wasps from the plant samples.

1.3.1 Relevance of proposed research

This research will expand our knowledge about the current parasitoid population on *Liriomyza* spp. and *Phytomyza* spp. in Victoria and is the first step to identifying local agromyzids that can serve as reservoirs for biocontrol agents attacking *L. sativae.*

CHAPTER 2. Seasonality of four common leafminer flies (Diptera: Agromyzidae) and their associated parasitoid wasps in Victoria

ABSTRACT

The agricultural pest leafminer *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) was detected in far north Queensland in 2015 and measures were put in place to quarantine it while preparing for its possible spread. Integrated Pest Management (IPM) for this pest overseas uses biological controls that consist of generalist parasitoid wasps. Local parasitoid species are major candidates as they are adapted to the milieu and they do not require introduction or risk damage to the ecosystem. I investigated the presence of suitable agromyzid host species around Melbourne as well as the abundance and composition of their parasitoid community.

Four adventive agromyzids (*Liriomyza brassicae* (Riley), *Phytomyza plantaginis* Goureau, *Phytomyza syngenesiae* (Hardy)) and one endemic (*Liriomyza chenopodii* (Watt)) were surveyed to evaluate their potential as sources of parasitoids of *L. sativae*. A range of host plants was monitored at six Melbourne sites for 18 months by noting the number of plants surveyed found mined. A total of 4,678 agromyzid flies and 2,002 parasitoids were reared from collected mined leaves. Eleven wasp species were identified: seven eulophids, three *Opius* spp. Wesmael and one pteromalid species. Of the parasitoids collected, five species were reared from all leafminer fly hosts, and four species were reared from three hosts. *Chrysocharis pubicornis* (Zetterstedt) was the most abundant species but was almost entirely reared from *Phytomyza* spp.

Other common parasitoids in order of abundance were an *Asecodes* sp. Förster, *Diglyphus isaea* (Walker) and the native *Closterocerus mirabilis* Edwards & La Salle. *Opius* spp. were predominant from *Liriomyza* species, and *Zagrammosoma latilineatum* Ubaidillah was common from *L. brassicae*. There was a wide range of apparent parasitism rates based on comparisons of wasp and leafminer numbers emerging from collected material, with peaks in late spring and late autumn depending on the host. These results demonstrate a diversity of local agromyzids that persist throughout the year in southern Victoria and support a stable parasitoid

community. This community provides a local reservoir of biological control agents for controlling pest leafminers, including *L. sativae* in the advent of its spread further south.

2.1 INTRODUCTION

2.1.1 Polyphagous agromyzids

Agromyzid leafminers (Diptera: Agromyzidae) are a widespread family of small phytophagous flies, often quite morphologically similar and with larval stages feeding internally in leaves or stems. Within Agromyzidae, the genus *Liriomyza* consists of close to 400 species (ITIS 2019) out of which 23 are deemed of economic importance (Murphy & LaSalle 1999). Highly polyphagous species such as *Liriomyza sativae* Blanchard, *Liriomyza huidobrensis* (Blanchard) and *Liriomyza trifolii* (Burgess) are major agricultural pests now widespread around the world (Murphy & LaSalle 1999). Their spread and increased impact is due to a number of factors, including an increase in international trade of live plant matter such as produce and ornamental plants (Reitz *et al*. 2013), and their association with a large number of host plants predominantly from the Solanaceae, Fabaceae, Cucurbitaceae and Asteraceae families (Spencer 1973). Adult females cause indirect damage to crops by creating feeding damage and oviposition lesions in the leaf's epidermis which can also facilitate pathogen transmission (Deadman *et al.* 2002). Most of the damage comes from the larval stages however, when the larva feeds on the parenchymal layer of leaves. Extensive feeding from numerous larvae weakens the plant and can cause senescence in seedlings. Mined leaves are more likely to die and fall off and in doing affect fruit production or plant value for ornamentals (Parrella 1987). Other biological traits help explain their impact: they are multivoltine, with a quick development rate at optimal temperatures (Parrella 1987), and a high reproductive output of over 100 eggs per female (Leibee 1984) which allows source populations to build up in and around fields, maintaining the presence of the leafminers in the region.

2.1.2 Chemical and biological control

Traditional methods such as chemical controls are proving ineffective against the protected larval and pupal life stages as well as increasing insecticide resistance (Parrella & Keil 1984; Rauf *et al.* 2000). The insecticides showing the best results are the neurotoxins (abamectin and insecticides of the spinosyn class), as well as the insect growth regulator cyromazine (Prijono *et al.* 2004). These are translaminar and affect the larvae within the leaf, with few cases of resistance reported so far (Leibee & Capinera 1995; Ferguson 2004). Leafminer adults that emerge after the application of insecticide – even for other pest species present – benefit from the elimination of natural enemies. This facilitates further outbreaks of the flies once the pressure of parasitoids is lifted and they can arise as a consequential secondary pest impacting crops where their presence was not noticeable prior (Parrella & Keil 1984). This is a key factor in their spread and pest status as broad-range insecticides are now widely used in agricultural systems. For that reason, a number of studies advocate for implementing IPM programs against pest *Liriomyza* spp., using a combination of chemical and biological control methods such as parasitoid wasps (Parrella & Keil 1984; Trumble 1985; Murphy & LaSalle 1999; Mujica 2008; Gao *et al.* 2017; Weintraub *et al.* 2017).

Hymenopteran parasitoids of *Liriomyza* spp. span several families including but not limited to Braconidae, Eulophidae, and Pteromalidae (Bjorksten *et al.* 2005; Murphy & LaSalle 1999) and tend to be generalists. Females detect leafminer larvae using visual and chemical cues and oviposit near or in the larvae (Heinz & Parrella 1989; Zou *et al*. 2012). The diversity of parasitoid species is mirrored by their range of parasitic behaviours exhibited. Koinobionts parasitoids do not affect adversely their host larva's development immediately after their parasitism, it dies instead near or after pupation from the wasp's larva feeding. Idiobionts paralyze or kill the host larva before ovipositing. Idiobiont parasitoids are highly synovigenic and increase their fertility and lifespan with behaviours such as host stinging and host feeding (Jervis *et al.* 2001), which plays an additional part in leafminer mortality (Cheng *et al.* 2017). Ectoparasitoids lay eggs within the mine near the leafminer larvae and the wasp larvae feeds externally on it once hatched. Endoparasitoids on the other hand lay their egg inside the fly larva. Biological control of agromyzids relies on releases of mass-reared parasitoids, either as an augmentative and long-term addition to a field or region or as a controlled and recurring event in an enclosed area such as a greenhouse

(Liu *et al*. 2019). The cost of mass-reared biocontrol agents for leafminers is often prohibitive for growers, so they are used principally for high value crops. Relying on biocontrol agents from local populations offers several advantages since they are better suited to the environmental conditions, already part of the ecosystem and able to exploit surrounding refuges. Several factors must be taken in consideration to include local biocontrol as a component of IPM, such as temperature tolerance (Hondo *et al.* 2006), parasitism rates and intra-species competition (Harvey *et al.* 2013) and seasonality. Studies of host suitability are also critical in understanding how parasitoids make use of host reservoirs and how it will impact control in the field. Such information is limited in the Australian context and identifying host species that can serve as reservoir for pest *Liriomyza* spp. biocontrol agents would be the first step.

2.1.3 Agromyzids of interest in Australia

For this study I focused on four common agromyzids, *Liriomyza brassicae* (Riley), *Liriomyza chenopodii* (Watt), *Phytomyza plantaginis* Goureau and *Phytomyza syngenesiae* (Hardy). These are found on widespread hosts and throughout Australia excluding NT (Chapter 1). Other *Liriomyza* spp. are likely to be candidates for parasitoids that are able to transfer to exotic *Liriomyza* flies. Adventive agromyzids have had less time exposed to the existing Australian parasitoid community and can indicate the parasitoid species that are likely to first shift onto new *Liriomyza* spp. For that reason, all species chosen in this study were introduced with the exception of *Liriomyza chenopodii*. All four leafminers share the general life history traits of other agromyzids (Parrella 1987). The female flies oviposit into leaves of their host plants, and once hatched the pale-yellow larva feeds on the parenchymal layer, forming a whitish and serpentine shaped mine in the mesophyll. Females also make feeding punctures with their ovipositor to access the plant’s sap. Both *Liriomyza* species pupate externally in a rounded brown or yellow puparium, usually in the soil but occasionally on leaves or stems of their host. The adults are small, around 2 mm in length (Spencer 1977), with a shiny black mesonotum and a yellow scutellum. The two *Phytomyza* species pupate at the end of their mine; the fly exits through a small slit at the anterior end of the puparium (Cohen 1936). Their adults have a matt grey mesonotum and scutellum and are between 3-4 mm in length (Martin 2017, 2019).

Liriomyza brassicae, the cabbage leafminer, is a cosmopolitan agromyzid with a range extending into the Pacific and Oriental region. It has a relatively wide range of host plants but is primarily found on Brassicaceae, Capparaceae and Tropaeolaceae (Lonsdale 2011). Like *L. sativae* it can be found on cruciferous crops and weeds as well as ornamentals like the common *Tropaeolum majus* L. (nasturtium). Lardner (1991) reared it from a number of common invasive brassicaceous weeds including but not limited to *Raphanus raphanistrum* L. (wild radish), *Rapistrum rugosum* (L.) All. (giant mustard), *Cakile maritima* Scop. (European sea rocket), and *Sisymbrium officinale* (L.) Scop. (common hedge mustard).

Another common *Liriomyza* species, *L. chenopodii,* is also found in New Zealand and throughout Australia with the exception of the Northern Territory (Spencer 1977). Though it is considered an endemic species (Spencer 1977), it has not been found on any endemic host plant and instead has only been recorded on introduced plants from two families, Caryophyllaceae, and Amaranthaceae. This includes crops such as chard and beetroot (*Beta vulgaris* var. L.) and spinach (*Spinacia oleraceae* L.), as well as weeds like *Stellaria media* (L.) Vill., (chickweed), and *Chenopodium album* L. (fat hen), both common in disturbed habitats. A Victorian study of *L. chenopodii* in a beetroot crop showed a high parasitoid pressure, reaching 100% parasitism after only one week of leaf mines being observed (Bjorksten *et al.* 2005).

Phytomyza syngenesiae, the cineraria leafminer previously under the nomenclature *Chromatomyia syngenesiae*, is widespread around the world and an adventive species in the Oceanic region (Spencer 1963b, 1973; Kleinschmidt 1965). It feeds on a large number of host plants from Asteraceae. It can be found on valuable crops such lettuce (*Lactuca sativa* L.) and artichoke (*Cynara scolymus* L.), or ornamentals like chrysanthemums (*Chrysanthemum* L. spp.) of which it has historically been a pest (Cohen 1936; Spencer 1973). It is also found on well-established European weeds such as ragwort (*Senecio jacobaea* L.) and milk thistle (*Sonchus* spp.) (Kelsey 1937; Spencer 1977).

Phytomyza plantaginis is a leafminer found on common hosts from the *Plantago* genus (Plantaginaceae). In Australia *Ph. plantaginis* has been collected from *Plantago lanceolata* L. and *Plantago major* L. It has been recorded in New Zealand as well as in Australia (Spencer 1977). *Plantago* spp. are mostly recognized as weeds however they are being used as a pasture species in dairy fields in New Zealand (Lee *et al.* 2015) and will potentially be utilized in southern Australia as well (Dodd *et al.* 2000; Raedts *et al.* 2019).

In addition to being widespread adventive species and found on a mixture of host plants, in agricultural, garden and urban settings, they also share host plants with pest *Liriomyza* spp. *Liriomyza sativae* has been reared from a *Plantago* sp. and *Pl. major* in the USA (Oatman 1959; Stegmaier 1966). It has also been reared from *Sonchus oleraceus* and shares some brassicaceous hosts with *L. brassicae*. This further supports the argument that parasitoids reared from these four target species are likely to shift onto *L. sativae*.

2.1.4 Australian parasitoids of Agromyzidae

Hymenopteran parasitoids currently present in Australia and known to parasitize the cryptic larval stage of agromyzid leafminers, such as the ones mentioned above, are potential biocontrol agents. Studying their seasonality and host-preference can direct future IPM programs and help identify the plants and leafminers that can serve as reservoirs for the community of parasitoids of interest. Although there has only been a limited number of studies looking at parasitoids of agromyzids in Australia a number of species known to attack pest *Liriomyza* spp. have been recorded (Ridland *et al.* 2020). Three hymenopterans of particular interest are the eulophine larval parasitoids *Diglyphus isaea* (Walker), *Hemiptarsenus varicornis* (Girault), and *Zagrammosoma latilineatum* Ubaidillah found in the Indo-Australian region. *Diglyphus isaea* is already used as a biocontrol agent overseas, often released in tandem with *Dacnusa siberica* Telenga (Braconidae) in greenhouses to control *Liriomyza* pests (Chow & Heinz 2004; van der Linden 2004). *Diglyphus isaea* was possibly introduced in Australia from a New Zealand population originally brought from Pakistan for biocontrol of leafminers infesting forage brassicas in the 1970s (McGregor 1989). It was first collected in 2001 at Manly (NSW), from *Ph. syngenesiae* mines (Atlas of Living Australia 2020). *Hemiptarsenus varicornis* is a cosmopolitan parasitoid of *Liriomyza* spp. and has been known to reach high parasitism levels on *L. huidobrensis* (93%) and on *L. sativae* (60%) in Indonesia (Rauf *et al.* 2000). It has been recorded from both Melbourne and the tip of north Queensland, suggesting a wide distribution latitudinally across Australia. *Zagrammosoma latilineatum* was described from *L. huidobrensis* in Indonesia but was also caught in Qld (Ubaidillah *et al*. 2000).

Agromyzid surveys and rearing over the past few decades have provided some information on host preference and distribution of these parasitoids in Australia. Bjorksten *et al.* (2005) surveyed three agromyzid species *L. brassicae*, *L. chenopodii* and *Ph. syngenesiae* by collecting mined leaves from weeds and non-sprayed fields of Chinese cabbage and beetroot in 2002 and 2003, in Knoxfield and other sites in southern Victoria. The dominant parasitoid reared from *L. brassicae* was *H. varicornis* in both years (85% in 2002 and 48% in 2003) followed by *D. isaea*. Both parasitoids were also the dominant species found on *L. chenopodii* in beetroot crops, though their relative levels changed between seasons, with *D. isaea* low in autumn 2002 (25%) and high in spring 2002 (66%) (Bjorksten *et al*. 2005). *Hempitarsenus varicornis* was also one of the two most numerous parasitoids reared from both *Ph. plantaginis* and *Ph. syngenesiae*, in the Canberra region (ACT), and in NSW (Lambkin *et al.* 2008). *Zagrammasoma latilineatum* was one of the two most frequent parasitoids on *L. brassicae* in the Adelaide region (Lardner 1991). Eulophids such as a *Chrysocharis* sp. and *Closterocerus mirabilis* (Edward & La Salle) were also commonly found at low levels in surveys of *L. brassicae, L. chenopodii* and *Ph. syngenesiae.* Other endoparasitoids were also found in varying numbers. *Trigonogastrella parasitica* (Girault) (Pteromalidae), a species endemic to Australia, was reported to be one of the dominant species reared from *Ph. syngenesiae* mining *S. oleraceus* in Victoria and in NSW (Bjorksten *et al.* 2005; Lambkin *et al.* 2008). It was also reared from *Phytoliriomyza* sp. pupae collected from saltbushes, *Rhagodia* spp., in South Australia (SA) (Wood *et al.* 2010). When Wood *et al.* (2010) studied the parasitoid community on an endemic agromyzid, *Phytoliriomyza praecellens* Spencer, they sampled from two native host plants, the seaberry saltbush (*Rhagodia candolleana* Moq.) and the fragrant saltbush (*Rhagodia parabolica* R. Br.) and the majority of parasitoids they reared from *R. parabolica* belonged to two *Opius* species (Wood *et al.* 2010). In Australia, only three *Opius* species reared from agromyzid leafminers have been described: *O. cinerariae* Fischer, *Opius atricornis* Fischer and *Opius oleracei* Fischer (Belokobylskij *et al*. 2004). The presence of additional unknown *Opius* species requires that more DNA barcoding work be done to distinguish wasps within this morphologically similar genus.

Percent parasitism is a valuable parameter to characterize a parasitoid community, its impact on its host population numbers and its dynamics. It is commonly calculated from a host:parasitoid ratio, based on reared or dissected insect samples. Depending on the host's life-stage parasitoid species select for and based on the amount of time in the field the host larva is available for parasitism, percent parasitism will yield vastly different results. Percentage parasitism will also vary with the phenology of the host and its parasitoids (Van Driesche *et al*. 1983). Parasitism rates calculated from the number of flies versus parasitic wasps reared is instead better defined as 'apparent parasitism', from a single point in time in the field. This is facilitated by parasitoids not engaging in gregarious parasitism as it equates to one parasitoid for one host larva parasitized. In addition to the stinging of the host before oviposition, behaviours consisting of non-reproductive host feeding and host-stinging without feeding or oviposition also result in larval mortality and are not represented in the final number of adult insects present in the sample (Cheng *et al.* 2017).

Apparent parasitism is thus likely an underestimation of the impact of synovigenic wasps on the mortality rates of the leafminer population."

2.1.5 Aims

This study seeks to 1) characterize the seasonal presence of four target agromyzids and their hymenopteran parasitoids between six Melbourne sites, 2) identify the dominant parasitoids with a focus on species known to attack *L. sativae,* and 3) look at the variation in parasitoid communities between sites, host agromyzid and host plant.

2.2 MATERIALS AND METHODS

2.2.1 Study sites

The temporal abundance of flies and their parasitoids was studied between August 2018 and January 2020 at six sites around Melbourne, Victoria (Table 2-1). There was a pause in sampling between late December 2018 and mid-February 2019.

Table 2-1. Latitude and longitude coordinates for the six Melbourne-area sites.

Site names	Coordinates
Flemington Bridge	-37.7875, 144.9399
Syndal-Mt Waverley	-37.8762, 145.1472
Mt Waverley-Jordanville	-37.8754, 145.1225
Fitzroy North	-37.7778, 144.9886
Federation Trail	-37.9155, 144.6686
Diggers Road	-37.9665, 144.6856

These were chosen to ensure each of the targeted host plants was sampled at a minimum of 3 locations. Two sites are located in Werribee, a western outer suburb of Melbourne: the Federation Trail site is along a grassy corridor between a residential area and the highway, while the Diggers Rd site is close to the coast and along a field, located in the horticultural area of Werribee South. These sites are particularly abundant with *Brassica fruticulosa*, a brassicaceous weed. During the cooler months, *Plantago lanceolata* and *Sonchus oleraceus* are also present. Compared to the other sites, they receive less rainfall during the year, with an average of 36 mm per month over the last four years, compared to a range of 45 to 60 mm of rainfall per month for the four other sites (Figure A2-1). The Flemington Bridge site is situated under the Flemington Station Bridge and along the Moonee Ponds bicycle trail. This site has the most abundant and varied vegetation. All host plants are present. The fourth site is in Fitzroy North along the Merri Creek trail, in the Ottery Reserve section. All host plants are present but less abundant that at Flemington Bridge site. The last two sites are in the eastern suburb of Mount Waverley, along the railway trail. The trail is separated into two sites, between the consecutive train stops of Jordanville to Mt Waverley Station, then from Mt Waverley to the intersection of the trail with Blackburn Road, just before Syndal Station. These sites abound with *Pl. lanceolata* and *S. oleraceus* while the other hosts are less present. Access to the vegetation lining the sides of the pathway was limited between Blackburn Rd and Lawrence Rd (approximately half of the trail) by metal fencing built in November 2019. Sampling of accessible host plants was continued along the fence and sampled as previously undertaken along the unfenced areas. Sampling along the Merri Creek trail ceased in October 2019 due to construction happening on a bridge over the trail and creek, cutting access to the main sampling portion for this site. Sampling further north on the same trail was unfavorable due to the presence of grass instead of perennial plants on the banks of the creek.

2.2.2 Sampling protocols

The targeted leafminers consisted of two *Liriomyza* species, *L. brassicae* and *L. chenopodii,* and two *Phytomyza* species, *Ph. plantaginis* and *Ph. syngenesiae*. A targeted host plant was specified for each agromyzid, based on its abundance and presence at sites (Table 2-2).

Table 2-2. Details of host plants targeted for quantitative sampling in this field study.

Agromyzids	**Target host plants***
Liriomyza brassicae	*Brassica fruticulosa* Cirillo, twiggy turnip
L. chenopodii	*Chenopodium album* L., fat hen
	Stellaria media L., Cirillo, chickweed
Phytomyza plantaginis	*Plantago lanceolata* L., ribwort plantain
	Plantago major L., greater plantain[a]
Ph. syngenesiae	*Sonchus oleraceus* L., sow thistle

*Botanical nomenclature is based on VicFlora list of common names.

[a]Not a main host plant but targeted at Flemington Bridge site.

A pilot sampling study (“target sampling”) was undertaken at each site in August 2018 to assess the presence and ease of sampling for all targeted host plants. Four consecutive stops, distant of at least 20 m were randomly made at each site, and all target host plants present were surveyed in 15 min. All mined material was collected and then pooled for each host plant species at the end of the total hour of sampling. This method was abandoned since it did not permit a comparison of presence of leafminers between sampling events and did not result in equal sampling efforts between host leafminers, sampling events and sites.

The quantitative protocol ("COUNT sampling") involved surveying host plants and noting the ratio of mined to un-mined plants, and where present, removing a single mined leaf from each plant found to be mined. The percentage of surveyed plants found 'mined' is defined as the infestation level for that host, at that sampling event. All the leaves on the plant were visually assessed for mining. Mined leaves were inspected and whenever possible, leaves with empty mines were discarded in favour of mines with clear signs of larvae present. Fifty plants for each host plant species were surveyed at each site, unless there were not enough present in which case the subsequent maximum number of plants surveyed was noted and used instead. This protocol was applicable for the host plants of *L. brassicae*, *Ph. plantaginis* and *Ph. syngenesiae* but not for *L. chenopodii* since its targeted host plant *Stellaria media* grows as a ground cover so individual plants cannot readily be differentiated and *Chenopodium album* was not commonly found. Plants other than the six targeted species were collected whenever suspicious mining was observed, to identify other potentially common agromyzid species and to note target leafminer species shifts of host plants or other preferred host-associations. All *Plantago* species were surveyed when available as they are the only known hosts for *Ph. plantaginis*. Besides *Pl. lanceolata*, two other species: *Pl. major* and *Plantago coronopus* L. (buck's horn plantain), were sampled when found mined.

Sampling bias of later instars with more conspicuous mining was avoided by selecting a leaf with a high number of mines for the quantitative count instead of the first mined leaf noticed on the plant. The leaves from the same host plant species were pooled and placed in a sampling bag labelled as quantitative sampling. Whenever possible, all additional mined leaves found on the plant were placed in another sampling bag and labelled as qualitative sampling. This sampling was not performed for every sampling event and was limited by time and the volume of vegetation sampled. The "COUNT sampling" protocol will now be referred to as the quantitative sampling and the qualitative sampling will be used to describe both the "target sampling" and "EXTRA sampling".

Apparent parasitism percentage, which does not take in count the larval mortality numbers caused by behaviours other than ovipositing, was calculated using the following equation:

$$\Sigma \text{Parasitism}_{estimated} = \text{Parasitoids}_{emerged} / (\text{Parasitoids}_{emerged} + \text{Leafminer}_{emerged})$$

2.2.3 Rearing methods

Collected plant material was brought into the laboratory at the end of each sampling trip and leaves from the same site, host plant and sampling protocol were grouped and placed into labeled Ziploc bags lined with paper towel. The number of leaves per bag was dependent on their size and Ziploc bags were only filled halfway. All bags were manually inflated before being closed. Bags were then placed upright in boxes for ease of storage and to facilitate the verification of wasp emergence. Ziploc bags were kept in a Constant Temperature (CT) cabinet at 20°C, with a 16:8 h photoperiod of light:dark.

When handling particularly fragile plant matter or when there was limited material collected, leaves and sections of plants mined and with live larva were isolated – cut out with scissors when from a large leaf – and placed into a Petri dish (60 mm x 15 mm) partially filled with 1% agar. Agar was poured into the Petri dish kept at an angle to allow it to cool covering a portion of the bottom of the dish. The edge of the cut leaf or the stem of the cut leaf was inserted into the agar, and a circle of blotting paper was placed on the other half of the Petri dish to control condensation. All Petri dishes were labeled and taped shut. In some instances, pupae were found in large numbers at the bottom of Ziploc bags (i.e. species pupating outside of the leaf). These pupae were removed and placed into 3 cm diameter transparent glass and plastic tubes, partially filled with pressed-down sand moistened with water as per Lambkin *et al.* 2008. Squares of fine mesh were placed on top of the tube and held secure with a rubber band. No more than 30 pupae were placed in a single pupal tube. When large numbers of pupae were recovered, Petri dishes containing moistened sand and with the lid taped shut were used to rear up to 50 pupae. The dryness of the sand was also checked when looking for emerged insects and water was added if it appeared dry. This method of rearing permitted me to confirm any parasitoids reared as endoparasitoids, pupating inside the fly pupa. Petri dishes and pupal tubes were kept in the same conditions as the Ziploc bags. Rearing containers were checked twice a week and examined for the presence of flying insects at the top of the bag. If present, the Ziplocs were partially opened and using a moistened paint brush the flies and wasps were caught and deposited in a dish filled with 100% EtOH. Once all visible specimens were removed, the Ziploc bag was fully opened, and the paper towels were removed and changed. Special care was taken to monitor for any fly or parasitoid hidden under plant material or between paper towel sheets.

2.2.4 Identification of specimens

Once gathered, all flies and wasps that emerged were separated into morphologically similar groups and placed with a label into 2 mL vials of 100% ethanol and kept at -20°C. A subsample of the specimens was kept in 80% ethanol at 4°C for future dissection. Identifications were carried out under a light microscope (Optik microscope series SZM) and the vials were numbered and registered into a database. Agromyzids were identified using the LucID key to polyphagous agromyzid leafminers (Malipatil & Ridland 2008) and the dichotomous key from Spencer (1977) for *Ph. plantaginis*. Parasitoids were identified using two LucID keys (Fisher *et al.* 2005; Reina & La Salle 2003) and the key to Australasian Chalcidoidea (Bouček 1988). Additional keys were used for specific genera, such as for *Diglyphus* (Zhu *et al.* 2000; Hansson & Navone 2017), *Chrysocharis* (Hansson 1985), *Opius* (Belokobylskij *et al*. 2004) or for specific species such as *Closterocerus mirabilis* (Edwards & LaSalle 2004, Berry 2007), or *Zagrammosoma latilineatum* (Ubaidillah *et al.* 2000). The illustrated key to the hymenopteran parasitoids of *L. trifolii* in Japan, though in Japanese, offered a useful series of diagrams to differentiate the main expected genera, including *Opius* spp., *Dacnusa* and *Gronotoma* (Konishi 1998). Samantha Ward (The University of Melbourne) looked over unknown braconids to confirm and exclude any aphid parasitoids mistakenly present in samples. Whenever possible, morphological identification was corroborated with molecular work (DNA barcoding) by Xuefen Xu (The University of Melbourne). A subsample of these parasitoids has been dried and pinned and will be deposited in the Victorian Agricultural Insect Collection at AgriBio, La Trobe University. Some Entodoninae specimens have been sent to Ryan Perry (University of California, Riverside) for study and the two *Opius* morphospecies are being sent to Cornelis van Achterberg (Department of Terrestrial Zoology, Naturalis Biodiversity Center, Leiden) for possible identification (Figure A2-3, A2-5).

2.3 RESULTS

2.3.1 Rearing of agromyzids

A total of 4,678 agromyzids were reared from the six sites, 203 of them collected during the pilot sampling in August 2018. The quantitative sampling of the main host plants yielded 2,311 agromyzids in total, including 154 *L. brassicae*, 1,861 *Ph. plantaginis* and 296 *Ph. syngenesiae*. The additional qualitative sampling at the sites on all host plants available yielded 62 *L. brassicae* and 1,118 *L. chenopodii*: the latter included 196 from *Chenopodium album* and 922 from *S. media*. *Chenopodium album* was rarely present and only found mined at two sites, Diggers Rd in Werribee South and at the Fitzroy North site. A mix of host plants yielded another 871 *Ph. plantaginis* and 227 *Ph. syngenesiae* flies (Table 2-1). In addition to these agromyzid leafminers, two species of *Scaptomyza* Hardy (Diptera: Drosophilidae) were reared from *B. fruticulosa* and *S. media*, respectively. Identification to genus was performed using a key to Drosophilidae genera (Okada 1989), and DNA sequencing confirmed the identity of *Scaptomyza flava* (Fallén), an introduced leafminer (Martin 2004), on *B. fruticulosa*. The drosophilid leafminers reared from *S. media* were identified as *Scaptomyza australis* Malloch using the key in Bock (1977), who also noted that one specimen had been reared from *S. media* in NSW. DNA barcoding confirmed the genus and matched the *Scaptomyza* species reared from *S. media* to specimens found and barcoded in Sydney.

Table 2-3. Four target agromyzid species reared from all host plants found at the six Melbourne sites. Quantitative and quantitative sampling data are separated.

Agromyzidae	**Host plant**	**Numbers from sites**	
		Quantitative	**Qualitative**
Liriomyza brassicae			
	Brassica fruticulosa[1]	154	62
L. chenopodii			
	Stellaria media		922
	Chenopodium album		196
Phytomyza plantaginis			
	Plantago lanceolata[1]	1,861	159
	Pl. major	72	601
Ph. syngenesiae			
	Sonchus oleraceus[1]	296	145
	S. asper	8	11
	Taraxacum sp.	9	32
	Arctotheca calendula		3

[1] Main host plants targeted for quantitative sampling

Phytomyza plantaginis was mostly reared from *Pl. lanceolata* (n=2,120; ♀=1,452; ♂=413) but *Pl. major* was another important host plant (n= 673; ♀= 256; ♂=276) (Chapter 3). *Plantago major* was not present at all sites but was abundant at the Flemington Bridge site, where it was clustered near to the creek, and reached impressive sizes (up to 30 cm in length and 20 cm in width). It was mined for a short period of time, but no flies were reared.

Raphanus raphanistrum L. (wild radish) was present at the Fitzroy North and at the Mount Waverley sites. Its hairy stems and leaves made it difficult to handle while surveying and sampling, so most surveying was done solely visually. It was readily recognized by its inflorescence colour, leaf surface and shape as well the larger size and appearance of its seed pods. Two *S. flava flies* were reared from *R. raphanistrum* at the Flemington Bridge site (25 October 2019). Another known ornamental host plant of *L. brassicae*, *Tropaeolum majus* L. (nasturtium) was only present in a single patch at the Syndal-Mt Waverley site. Other brassicaceous weeds were also present but in low abundance and for that reason not observed for mines.

Phytomyza syngenesiae was reared in low numbers from other Asteraceae host plants (Table 2-3). *Sonchus asper* is another thistle host plant it was sporadically found during sampling, while the dandelion *Taraxacum* sp. F. H. Wigg. was present at every site and particularly abundant at the Flemington Bridge site. Numerous mines from a *Taraxacum* sp. were observed in June 2019 at the Flemington Bridge site, just as the first mines on *S. oleraceus* were appearing. No other *Ph. syngenesiae* samples were collected at other sites at that time of the year. Occasionally, mined leaves were noticed on other flowering hosts such as *Arctotheca calendula* (L.) (capeweed), *Bidens pilosa* L. (cobbler's pegs) and *Senecio* spp. L. (ragworts) (outside of the six sites) and were collected.

2.3.2 Temporal presence of agromyzids

2.3.2.1 *Liriomyza brassicae*

Following quantitative sampling over the period of two years, 82% of the *L. brassicae* were collected during autumn (March to May), and less than 10% were reared in any other season. At all sites, *L. brassicae* infestations started to increase in late September 2018, with the highest infestation levels between March and May 2019 (Figure 2-1). The highest infestation levels in *B. fruticulosa* by *L. brassicae* were found at the Flemington Bridge site where they reached 90 to 94% over the three sampling events in March and April. In Werribee, both Federation Trail and Diggers Rd sites had abundant host plant cover, but infestation levels were lower. *Brassica fruticulosa* was present at the Fitzroy North site throughout the entire year and mined between October 2018 and May 2019 with a peak of 77% mined in mid-March. In 2019, the first appearances of *L. brassicae* mines were delayed to late October, compared to 2018 (Figure 2-1).

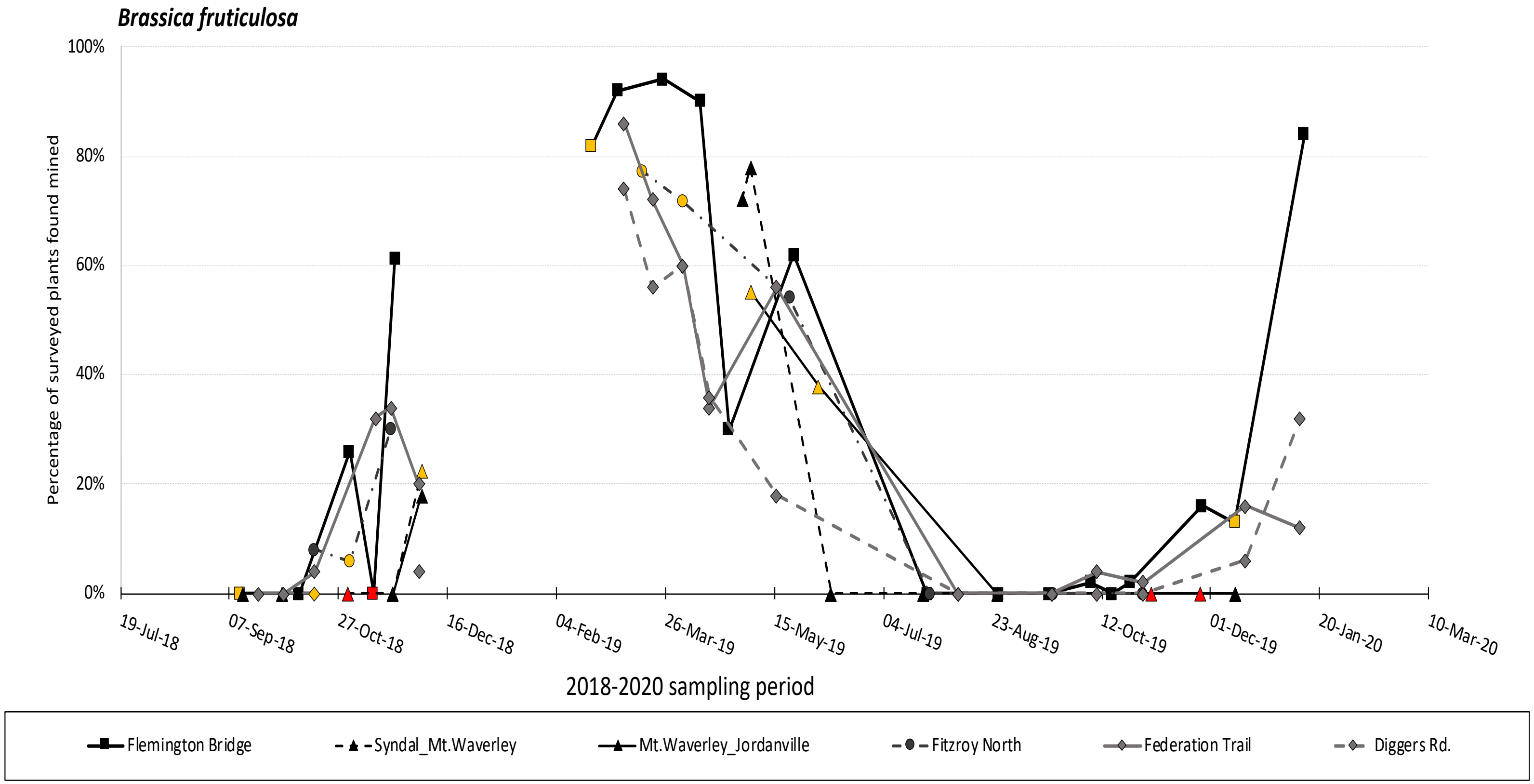


Figure 2-1. Peak activity of *Liriomyza brassicae* based on the percentage of *Brassica fruticulosa* found with at least one leaf mined based on 50 plants surveyed or fewer plants if less than 50 occurring. Host plants with foliage were not evident at all sites throughout the sampling months. All markers as depicted in the legends represent 50 host plants surveyed; yellow data points (yellow box) <50 plants surveyed; red data points (red box) <10 plants surveyed. No sampling between December 2018 and January 2019.

2.3.2.2 *Phytomyza plantaginis*

Phytomyza plantaginis abundance was less clearly associated with seasons; 65% of the leafminers were collected in spring, 20% in summer (from 1st of December to the end of February) and 10% in autumn. Infestations (Figure 2-2) of its main host, *Plantago lanceolata*, varied between sites and across the year; Flemington Bridge had the highest overall infestation levels (average of 59%) but there were periods of low infestation in July and October. At another location, Diggers Rd, host plants were not abundant but were heavily infested at the start of the sampling period and then only at a low level by late summer 2019 (Figure 2-2). *Plantago lanceolata* was abundant at the Fitzroy North site, where 50 host plants were surveyed on all sampling occasions with the exception of March 2019 and late-October 2019. However, there were only two infestation periods in the spring of each year. This leafminer appears to show weak seasonality but strong site differences.

2.3.2.3 *Phytomyza syngenesiae*

Sonchus oleraceus was infrequently infested at all sites and in contrast to *Ph. plantaginis*, *Ph. syngenesiae* had clear activity peaks (Figure 2-3); 81% of the flies were collected in spring, none in autumn and only 3% in winter and 16% in summer. The highest number of mined plants were seen in October, and mines were observed as early as April in 2019, while buildup was slower in 2018 (Figure 2-3). There was a brief increase in the number of mines at the Werribee sites in spring, with 100% infestation levels of the 50 host plants surveyed at the Federation Trail site in October 2019. The Flemington Bridge site recorded high levels of infestation, though it was followed by a reduction in mined leaves found in mid-October, before reaching close to 80% infestation the next sampling event. The Mount Waverley sites showed a delay in the increase of infestation levels compared to the Flemington Bridge and Werribee sites. Overall, this leafminer shows very strong seasonality across all sites.

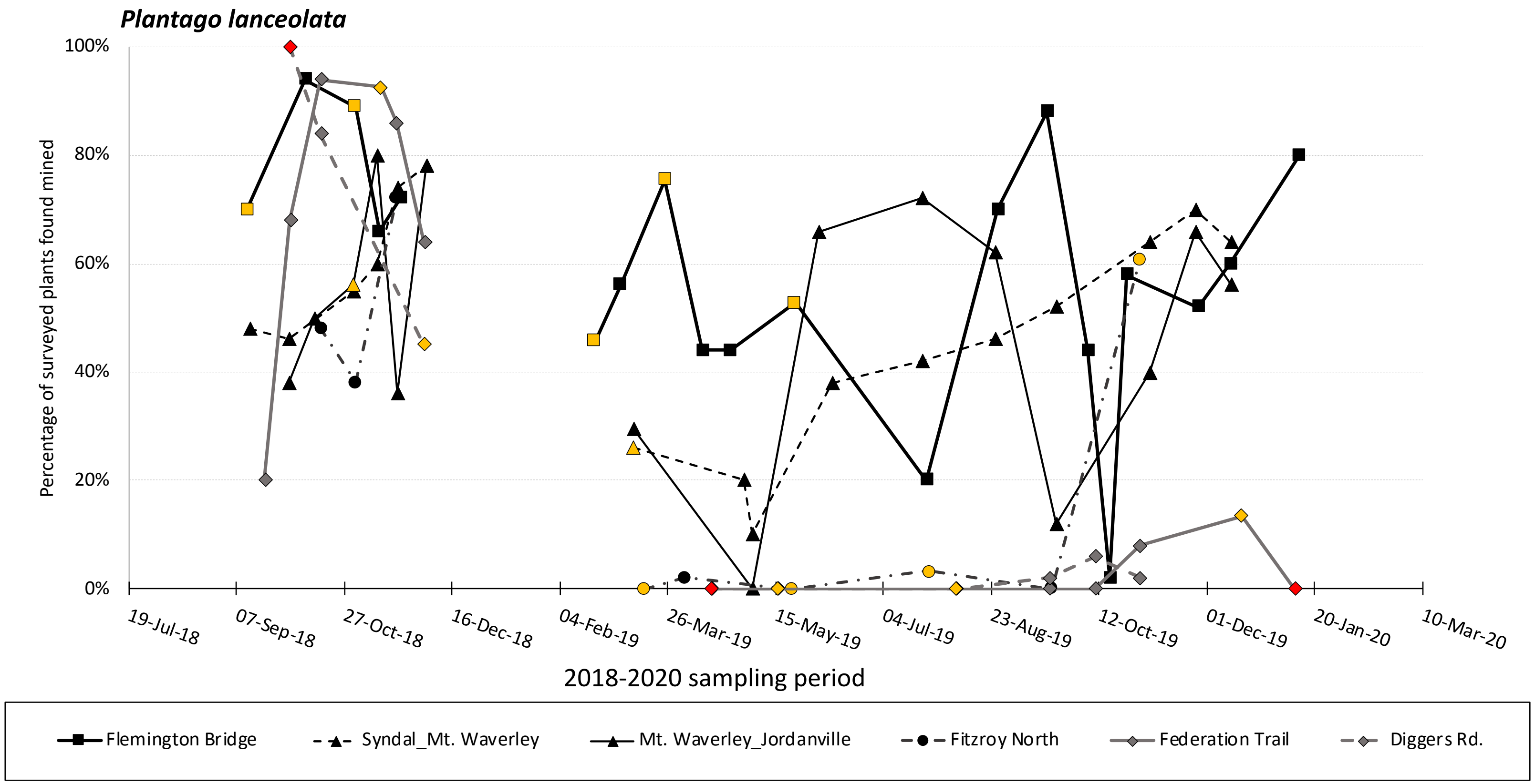


Figure 2-2. Peak activity of *Phytomyza plantaginis* based on the percentage of *Plantago lanceolata* found with at least one leaf mined based on 50 plants surveyed or fewer if less than 50 occurring. Host plants were not present at all sites throughout the sampling months. All markers as depicted in the legends represent 50 host plants surveyed; yellow data points (▭) <50 plants surveyed; red data points (▭) <10 plants surveyed. No sampling between December 2018 and January 2019.

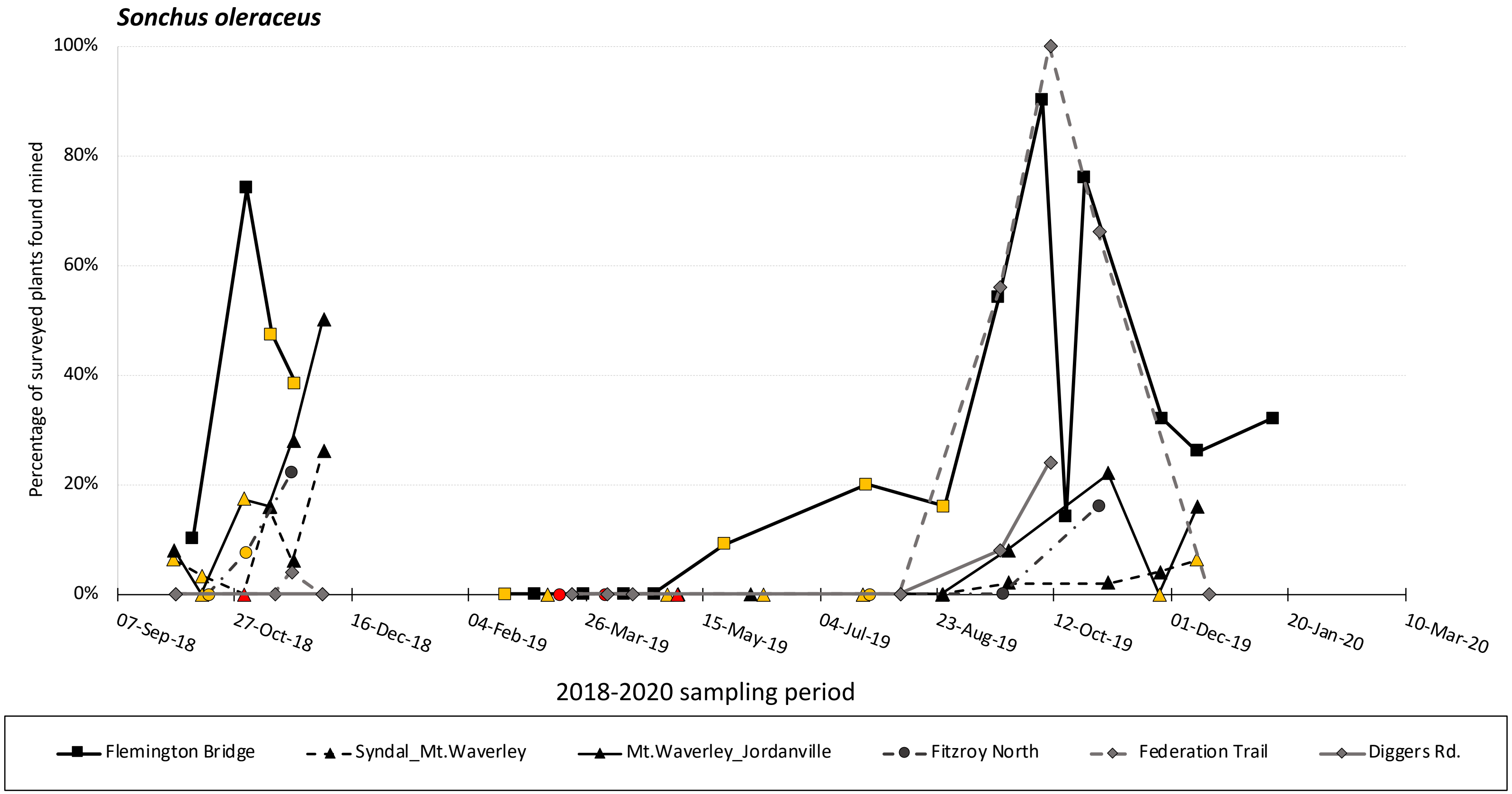


Figure 2-3. Peak activity of *Phytomyza syngenesiae* based on the percentage of *Sonchus oleraceus* found with at least one leaf mined based on 50 plants surveyed or fewer if less than 50 occurring. Host plants were not present at all sites throughout the sampling months. All markers as depicted in the legends represent 50 host plants surveyed; yellow data points (▭) <50 plants surveyed; red data points (▭) <10 plants surveyed. No sampling between December 2018 and January 2019.

2.3.2.4 *Liriomyza chenopodii*

Overall, 90% of *L. chenopodii* were collected in spring, as part of the qualitative sampling. Their host plants were harder to find at the sites; *S. media* in particular produces very small leaves and often grows as ground cover among other plants (M. Coquilleau pers. obs.), making it hard to locate and sample. Mines were observed on *S. media* between August and early December on both years, but plants were observed as early as May (Figure 2-4). *Chenopodium album* was rarely observed at three sites: Diggers Rd in Werribee South, Fitzroy North site, and Flemington Bridge where it was detected only twice (Figure 2-4).

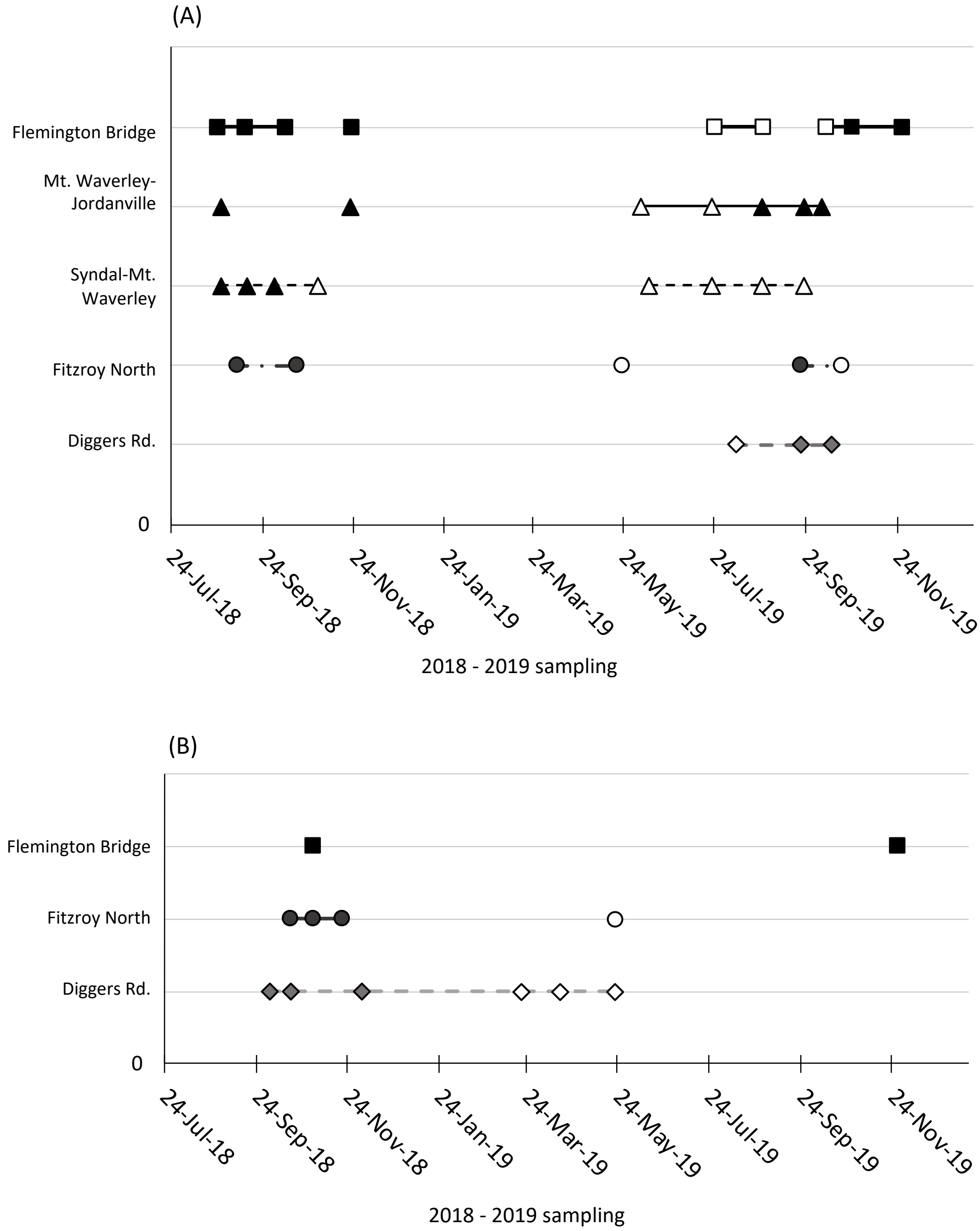


Figure 2-4. Temporal presence of *Liriomyza chenopodii* on the host plant (A) *Stellaria media* and (B) *Chenopodium album* at sites based on qualitative sampling (no sampling during December and January 2018). White markers represent the presence of host plants but absence of observed mining; black markers represent successful sampling of mined plants. Lines and dotted lines (site-dependent) connect consecutive sampling events at a site. The absence of lines between sampling events indicate the absence of host plants during surveys.

2.3.3 Parasitoid observations

The presence and composition of the parasitoid communities were compared for each agromyzid, between sites and sampling events, using only quantitative data (Tables 2-4 to 2-11). The temporal data for the site/agromyzid combinations not discussed in detail in the Results are included in the Appendix (5.2.2 Appendix).

2.3.3.1 Parasitoids from *Liriomyza brassicae*

Liriomyza brassicae was the host with the highest number of species of parasitoids: 10 identified species from the Eulophidae and Braconidae. The highest diversity of parasitoids was observed at the Flemington Bridge site from November 2018 to March 2019 and again in January 2020. No agromyzids were reared from this site without wasps also being present at the same time (Table 2-4). Very few parasitoids were reared from the Mount Waverley sites, all during peak agromyzid presence from March to June 2019, and no insects were reared afterwards (Tables A2-1, A2-2). In Werribee, *L. brassicae* was present at both the Federation Trail (Table 2-5) and Diggers Rd sites, but only one fly was reared from quantitative sampling at Diggers Rd. Another 18 were reared in the qualitative sampling undertaken there on the same day. At both Werribee sites, parasitoids were reared between December and May, with all parasitoid species collected at Diggers Rd on a single day in March 2019, the day with the highest infestation, but no flies emerged (Table A2-3). Parasitoids consisted of *Neochrysocharis formosa* (Westwood), *O. cinerariae* and *Z. latilineatum.* In Fitzroy North, only five *L. brassicae* emerged and no wasps were reared from the quantitative sampling (Table A2-4). Only two *Opius* sp. 1 were reared from additional qualitative sampling in October 2018. At least one *Opius* species was present at every site. *Hemiptarsenus varicornis* was only reared from the Flemington Bridge site, while *D. isaea* was present at the Werribee sites but only collected in the quantitative sampling at Flemington Bridge.

Table 2-4. Number of *Liriomyza brassicae* and parasitoids reared from *Brassica fruticulosa* collected at the Flemington Bridge site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *L. b = Liriomyza brassicae;* Σ Par. = Sum of parasitoids; *O.* sp.1 = *Opius* morphospecies 1; *D. isa = Diglyphus isaea; A.* sp. = *Asecodes* sp.; *N. for = Neochrysocharis formosa; H. var = Hemiptarsenus varicornis; C. mir = Closterocerus mirabilis*; Un = Unidentified.

☐ = Agromyzids and parasitoids; ☐ = Parasitoids only

W#	**Date** dd/mm/yyyy	**#P**	**#L**	**%M**	***L. b***	**Σ Par.**	***O.* sp.1**	***D. isa***	***A.* sp.**	***N. for***	***H. var***	***C. mir***	**Un**
37	12/09/2018	40	0	0%									
41	09/10/2018	**50**	0	0%									
44	01/11/2018	**50**	13	26%	2	1					1		
46	12/11/2018	3	0	0%									
47	22/11/2018	36	22	61%	10	10	2		3		3	2	
Pause in sampling													
8	20/02/2019	11	9	82%	3	5	5						
10	04/03/2019	**50**	46	92%	55	10	9	1					
13	25/03/2019	**50**	47	94%	2	18		13		5			
15	11/04/2019	**50**	45	90%		4			2	1			1
17	24/04/2019	**50**	15	30%	11	4	3					1	
21	24/05/2019	**50**	31	62%	8	1			1				
26	24/06/2019	**50**	0	0%									
35	26/08/2019	**50**	0	0%									
38	18/09/2019	**50**	0	0%									
41	07/10/2019	**50**	1	2%									
43	25/10/2019	**50**	1	2%									
48	27/11/2019	**50**	8	16%									
50	12/12/2019	31	4	13%									
3	13/01/2020	**50**	42	84%	12	1			1				
Grand Total					**103**	**54**	**19**	**14**	**7**	**6**	**4**	**3**	**1**

Table 2-5. Number of *Liriomyza brassicae* and parasitoids reared from *Brassica fruticulosa* collected at the Federation Trail site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *L. b = Liriomyza brassicae;* Σ Par. = Sum of parasitoids; *N. for = Neochrysocharis formosa*; *O.* sp. 1 = *Opius morphospecies* 1*; O. cin = Opius cinerariae; C. pub = Chrysocharis pubicornis;* Un = Unidentified.

[yellow] = Agromyzids only; [green] = Agromyzids and parasitoids; [blue] = Parasitoids only

W#	Date dd/mm/yyyy	#P	#L	%M	*L. b*	Σ Par.	*N. for*	*O.* sp.1	*O. cin*	*C. pub*	Un
38	20/09/2018	**50**	0	0%							
40	02/10/2018	**50**	0	0%							
42	16/10/2018	**50**	2	4%	1						
46	13/11/2018	**50**	16	32%	2						
47	20/11/2018	**50**	17	34%	1						
49	03/12/2018	**50**	10	20%	2	1					1
Pause in sampling											
10	07/03/2019	**50**	43	86%		2	1			1	
12	20/03/2019	**50**	36	72%	10						
14	03/04/2019	**50**	30	60%		3	2		1		
16	15/04/2019	**50**	17	34%							
20	16/05/2019	**50**	28	56%		2		2			
26	24/06/2019	**50**	1	2%							
32	07/08/2019	**50**	0	0%							
38	19/09/2019	**50**	0	0%							
41	10/10/2019	**50**	2	4%							
44	31/10/2019	**50**	1	2%							
51	17/12/2019	**50**	8	16%	7						
2	11/01/2020	**50**	6	12%							
Grand Total					**23**	**8**	**3**	**2**	**1**	**1**	**1**

2.3.3.2 Parasitoids from *Phytomyza plantaginis*

Parasitoids reared over time were consistent and across sites comprised mainly of *D. isaea*, *H. varicornis*, *N. formosa* and *Asecodes* sp. This was not the case for the Flemington Bridge site however (Table 2-12), where 84% of the wasps reared were *C. pubicornis* which were found throughout the year (Table 2-6). In contrast, this species was nearly absent from Mount Waverley samples (Tables 2-7, 2-8, 2-12). In comparison there were only two infestation periods in the spring of 2018 and 2019 in Fitzroy and only one parasitoid, *T. parasitica* (Pteromalidae), was reared in November 2019 (Table A2-7). At the Werribee sites, parasitoids were reared throughout the sampling events of 2018, during the months of October until December, and consisted predominantly *D. isaea* (n=42). At Diggers Rd, wasps were only reared from the sampling event on the 16/10/2018, with one *T. parasitica* reared on 31/10/2019 (Tables A2-5, A2-6). There was consistent overlap of parasitoids and agromyzids with the exception of the two Glen Waverley sites from April to November where parasitoids were uncommon and not present at every sampling event (Tables 2-7, 2-8).

Table 2-6. Number of *Phytomza plantaginis* and parasitoids reared from *Plantago lanceolata* collected at the Flemington Bridge site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. p = Phytomyza plantaginis;* Σ Par. = Sum of parasitoids; *C. pub = Chrysocharis pubicornis; D. isa = Diglyphus isaea; T. par = Trigonogastrella parasitica; A*. sp. = *Asecodes* sp.; *H. var = Hemiptarsenus varicornis;* Un = Unidentified.

☐ = Agromyzids only; ☐ = Agromyzids and parasitoids

W #	**Date** dd/mm/yyyy	**#P**	**#L**	**%M**	***P. p***	**Σ Par.**	***C. pub***	***D. isa***	***T. par***	***A. sp***	***H. var***	**Un**
37	12/09/2018	10	7	70%	12	1	1					
40	09/10/2018	**50**	47	94%	194	32	28	1		2	1	
41	01/11/2018	45	40	89%	20	35	29	6				
44	12/11/2018	**50**	33	66%	1	4	2					2
46	22/11/2018	**50**	36	72%	10	1	1					
Pause in sampling												
8	20/02/2019	35	16	46%	13	4			2	1		
10	04/03/2019	**50**	28	56%	9	2	1		1			
13	15/03/2019	45	34	76%	4	10	6	1	3			
15	11/04/2019	**50**	22	44%	9	4	4					
17	24/04/2019	**50**	22	44%	21	19	19					
21	24/05/2019	36	19	53%	40	4	4					
26	24/06/2019	**50**	10	20%	26	7	7					
35	26/08/2019	**50**	35	70%	4							
38	18/09/2019	**50**	44	88%	36	15	13	1			1	
41	07/10/2019	**50**	22	44%	4	5	4					1
43	25/10/2019	**50**	29	58%	20	1	1					
48	27/11/2019	**50**	26	52%	21	9	5					4
50	12/12/2019	**50**	30	60%	15	30	29	1				
3	13/01/2020	**50**	40	80%	29	18	16					2
Grand Total					**477**	**201**	**170**	**10**	**6**	**3**	**2**	**9**

Table 2-7. Number of *Phytomyza plantaginis* and parasitoids reared from *Plantago lanceolata collected* at the Syndal-Mt Waverley site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. p = Phytomyza plantaginis;* Σ Par. = Sum of parasitoids; *A.* sp. = *Asecodes* sp.; *H. var = Hemiptarsenus varicornis; D. isa = Diglyphus isaea; N. for = Neochrysocharis formosa*; *T. par = Trigonogastrella parasitica*; *C. mir = Closterocerus mirabilis;* Un = Unidentified.

[yellow box] = Agromyzids only; [green box] = Agromyzids and parasitoids

W #	Date dd/mm/yyyy	#P	#L	%M	*P. p*	Σ Par.	*A.* sp.	*H. var*	*D. isa*	*N. for*	*T. par*	*C. mir*	Un
37	13/09/2018	**50**	24	48%	13	2	2						
40	01/10/2018	**50**	23	46%	41	3			1		1		1
41	13/10/2018	**50**	24	48%	52	12	6	1	4		1		
44	31/10/2018	40	22	55%	25	4	3			1			
46	11/11/2018	**50**	30	60%	22	4			1		1		2
47	21/11/2018	**50**	37	74%	59	6		2	3	1			
49	04/12/2018	**50**	39	78%	10	4							3
Pause in sampling													
11	10/03/2019	**50**	13	26%	4	4				3			1
18	30/04/2019	**50**	10	20%	6								
18	04/05/2019	**50**	5	10%	2								
24	10/06/2019	**50**	19	38%	13								
30	22/07/2019	**50**	21	42%	1								
35	25/08/2019	**50**	23	46%	47	8	5		2		1		
39	22/09/2019	**50**	26	52%	19								
45	04/11/2019	**50**	32	64%	12	1		1					
48	26/11/2019	**50**	35	70%	12	6	1			1		1	3
50	12/12/2019	**50**	32	64%	24	11		9			2		
Grand Total					**362**	**65**	**17**	**13**	**11**	**6**	**6**	**1**	**10**

Table 2-8. Number of *Phytomyza plantaginis* and parasitoids reared from *Plantago lanceolata* collected at the Mt Waverley-Jordanville site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. p = Phytomyza plantaginis;* Σ Par. = Sum of parasitoids; *H. var = Hemiptarsenus varicornis; D. isa = Diglyphus isaea; A.* sp. = *Asecodes* sp.; *T. par = Trigonogastrella parasitica; C. mir = Closterocerus mirabilis; N. for = Neochrysocharis formosa; C. pub = Chrysocharis pubicornis;* Un = Unidentified.

☐ = Agromyzids only; ☐ = Agromyzids and parasitoids

W #	Date dd/mm/yyyy	#P	#L	%M	*P. p*	Σ Par.	*H. var*	*D. isa*	*A.* sp.	*T. par*	*C. mir*	*N. for*	*C. pub*	Un
40	01/10/2018	**50**	19	38%	15	3	1				2			
41	13/10/2018	**50**	25	50%	37	1				1				
44	31/10/2018	**50**	28	56%	23	14	5	1	5	1		1		1
46	11/11/2018	**50**	40	80%	28	5	2		2					1
47	21/11/2018	**50**	18	36%	33	1						1		
49	04/12/2018	**50**	39	78%	111	16	6	10						
Pause in sampling														
11	10/03/2019	17	5	29%										
18	30/04/2019	**50**	10	20%										
18	04/05/2019	**50**	16	32%	5									
24	04/06/2019	**50**	33	66%	50									
30	22/07/2019	**50**	36	72%	30	1		1						
35	25/08/2019	**50**	31	62%	29									
39	22/09/2019	**50**	6	12%	8									
45	04/11/2019	**50**	20	40%	14									
48	26/11/2019	**50**	33	66%	18	5	2	2		1				
50	12/12/2019	**50**	28	56%	22	5	3			1			1	
Grand Total					**423**	**51**	**19**	**14**	**7**	**4**	**2**	**2**	**1**	**2**

2.3.3.3 Parasitoids from *Phytomyza syngenesiae*

Phytomyza syngenesiae was recorded infrequently at the sites and so were parasitoids reared from this host. However, after substantial growth of *S. oleraceus* at the Federation Trail site in early October 2019, the next sampling event in late October yielded a large number of parasitoids (n=128) and no flies. The parasitoids consisted mostly of *Asecodes* sp. (70%), as well as some *D. isaea, H. varicornis* and *C. mirabilis*. The next sampling event in December found no mines and no parasitoids were reared. At the Diggers Rd site, there was an increase in agromyzid activity between September and December and the majority of the parasitoids at this site were reared over the last two sampling events in this period (Tables A2-8, A2-9). Low infestation levels were noted for the Fitzroy North site and very few parasitoids were reared, only in November 2018 and October 2019 (Table A2-10). As in the case of *Ph. plantaginis*, *Ph. syngenesiae* was mostly parasitized by *C. pubicornis* at the Flemington Bridge site, though *Closterocerus mirabilis* was the major parasitoid over the same time period overall (Table 2-9). *Closterocerus mirabilis*, *C. pubicornis* and *Asecodes* sp. were the most common parasitoids throughout the year though abundance was site-specific (Tables 2-9, 2-10, 2-11).

Overall, there was substantial overlap of flies and parasitoids for all three leafminers observed in the quantitative sampling. The highest numbers of agromyzids and parasitoids were collected from the Flemington Bridge site (Table 2-12). With the exception of July for *Asecodes* and *C. pubicornis* and June for *D. isaea*, these three parasitoids were reared throughout the sampling period. *Diglyphus isaea* was reared from *L. brassicae* between March and May, and then only from *Phytomyza* spp. until the end of the year, and this was also the case for *N. formosa*. Parasitoids present for part of the year included *H. varicornis* (5 months) and *C. mirabilis* (8 months), and more than 50% of them were reared between the months of September and December. *Zagrammosomaa latilineatum* was only reared in the month of December and March. Limited sampling in January and February could be the reason for its lack of detection during that period. *Opius* species were noticeably absent from June to October.

Table 2-9. Number of *Phytomyza syngenesiae* and parasitoids reared from *Sonchus oleraceus* collected at the Flemington Bridge site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. s = Phytomyza syngenesiae;* Σ Par. = Sum of parasitoids; *C. mir = Closterocerus mirabilis; C. pub = Chrysocharis pubicornis; A*. sp. = *Asecodes* sp.; *H. var = Hemiptarsenus varicornis; D. isa = Diglyphus isaea; O. cin = Opius cinerariae*; *T. par = Trigonogastrella parasitica*; Un = Unidentified.

☐ = Agromyzids only; ☐ = Agromyzids and parasitoids; ☐ = Parasitoids only

W #	Date dd/mm/yyyy	#P	#L	%M	*P. s*	Σ Par.	*C. mir*	*C. pub*	*A.* sp.	*H. var*	*D. isa*	*O. cin*	*T. par*	Un
37	12/09/2018	40	0	0%										
41	09/10/2018	**50**	5	10%		7	5	1	1					
44	01/11/2018	**50**	37	74%	11	98	33	51	9		2	2	1	
46	12/11/2018	19	9	47%		6	5		1					
47	22/11/2018	26	10	38%	10	14	9		4	1				
Pause in sampling														
8	20/02/2019	37	0	0%										
10	04/03/2019	**50**	0	0%										
13	25/03/2019	**50**	0	0%										
15	11/04/2019	**50**	0	0%										
17	24/04/2019	35	0	0%										
21	24/05/2019	22	2	9%	3									
26	24/07/2019	45	9	20%	23	8		3	5					
35	26/08/2019	**50**	8	16%	21	5			4				1	
38	18/09/2019	**50**	27	54%	4	3	1	1						1
41	07/10/2019	**50**	45	90%		2		1	1					
43	25/10/2019	**50**	38	76%	18	44	11	7	14	5				7
48	27/11/2019	**50**	16	32%		3	3							
50	12/12/2019	**50**	13	26%	1	12	8	1	1	1		1		
3	13/01/2020	**50**	16	32%	3	2	1				1			
Grand Total					**94**	**204**	**76**	**65**	**40**	**7**	**3**	**3**	**2**	**8**

Table 2-10. Number of *Phytomyza syngenesiae* and parasitoids reared from *Sonchus oleraceus* collected at the Syndal-Mt Waverley site during all quantitative surveys.

W# = week number*;* #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. s = Phytomyza syngenesiae;* Σ Par. = Sum of parasitoids; *C. pub = Chrysocharis pubicornis; C. mir = Closterocerus mirabilis; A*. sp. = *Asecodes* sp.; *H. var = Hemiptarsenus varicornis; T. par = Trigonogastrella parasitica*.

(yellow box) = Agromyzids only; (green box) = Agromyzids and parasitoids; (cyan box) = Parasitoids only

W#	**Date** dd/mm/yyyy	**#P**	**#L**	**%M**	***P. s***	**Σ Par.**	***C. pub***	***C. mir***	***A.* sp.**	***H. var***	***T. par***
37	13/09/2018	**50**	10	20%	15						
40	01/10/2018	47	3	6%	11						
41	13/10/2018	30	1	3%	1						
44	31/10/2018	9	0	0%	9	1		1			
46	11/11/2018	19	3	16%	2	8	6	1		1	
47	21/11/2018	**50**	3	6%	5	6	3	3			
49	04/12/2018	**50**	13	26%	3	5		1	2	1	1
Pause in sampling											
11	10/03/2019	**50**	0	0%							
18	30/04/2019	26	0	0%							
18	04/05/2019	24	0	0%							
24	10/06/2019	31	0	0%							
30	22/07/2019	6	0	0%							
35	25/08/2019	17	0	0%							
39	22/09/2019	**50**	1	2%		1			1		
45	04/11/2019	**50**	1	2%	1						
48	26/11/2019	**50**	2	4%							
50	12/12/2019	32	2	6%							
Grand Total					**47**	**21**	**9**	**6**	**3**	**2**	**1**

Table 2-11. Number of *Phytomyza syngenesiae* and parasitoids reared from *Sonchus oleraceus* collected at the Mt Waverley-Jordanville site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. s = Phytomyza syngenesiae;* Σ Par. = Sum of parasitoids; *C. mir = Closterocerus mirabilis; T. par = Trigonogastrella parasitica; A*. sp. = *Asecodes* sp.; *H. var = Hemiptarsenus varicornis.*

☐ = Agromyzids and parasitoids; ☐ = Parasitoids only

W#	Date dd/mm/yyyy	#P	#L	%M	*P. s*	Σ Par.	*C. mir*	*T. par*	*A.* sp.	*H. var*
40	01/10/2018	**50**	4	8%		4	1			3
41	13/10/2018	39	0	0%						
44	31/10/2018	23	4	17%	5	1			1	
46	11/11/2018	**50**	8	16%	5	2			2	
47	12/11/2018	**50**	14	28%	13	3	3			
49	04/12/2018	**50**	25	50%	15	6		4	1	1
Pause in sampling										
11	10/03/2019	15	0	0%						
18	30/04/2019	9	0	0%						
18	04/05/2019	32	0	0%						
24	04/06/2019	**50**	0	0%						
30	22/07/2019	**50**	0	0%						
35	25/08/2019	**50**	0	0%						
39	22/09/2019	**50**	4	8%						
45	04/11/2019	**50**	11	22%	12	3	1	2		
48	26/11/2019	37	0	0%						
50	12/12/2019	**50**	8	16%	2	1	1			
Grand Total					**68**	**20**	**6**	**6**	**4**	**4**

Table 2-12. Summary table for agromyzids and parasitoids reared during the quantitative sampling of the target host plants at each site.

∑ par = Sum of parasitoids; *O.* sp. 1 = *Opius* morphospecies 1; *O.* sp. 2 = *Opius* morphospecies 2; *O. cin = Opius cinerariae*; *D. isa = Diglyphus isaea*; *A*. sp. = *Asecodes* sp.; *N. for = Neochrysocharis formosa*; *C. mir = Closterocerus mirabilis*; *C. pub = Chrysocharis pubicornis; Z. lat = Zagrammosoma latilineatum; T. par = Trigonogastrella parasitica;* Unid. = Unidentified; Unkn. = Unknown species; U/ble = Unidentifiable

Liriomyza brassicae (host plant: *Brassica fruticulosa*)															
Site	**Agromyzid**	**∑ par**	***O.* sp. 1**	***O.* sp. 2**	***O.* cin**	***D. isa***	***A.* sp.**	***N. for***	***C. mir***	***C. pub***	***Z. lat***	***T. par***	**Unid.**	**Unkn**	**U/ble**
Diggers Rd	1	10	-	-	1	-	-	8	-	-	1	-	-	-	-
Federation Trail	23	9	1	-	2	-	-	3	-	1	-	-	-	-	1
Fitzroy North	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Flemington Bridge	91	54	19	-	-	14	7	6	3	-	-	-	-	2	1
Mt Waverley-Jordanville	5	4	-	1	-	-	-	2	-	-	-	-	-	-	1
Syndal-Mt Waverley	23	9	6	-	-	-	2	-	-	-	-	-	-	-	-
Total	**148**	**86**	**26**	**1**	**3**	**14**	**9**	**19**	**3**	**1**	**1**	**0**	**0**	**2**	**3**
Phytomyza plantaginis (host plant: *Plantago lanceolata*)															
Site	**Agromyzid**	**∑ par**	***O.* sp. 1**	***O.* sp. 2**	***O.* cin**	***D. isa***	***A.* sp.**	***N. for***	***C. mir***	***C. pub***	***Z. lat***	***T. par***	**Unid.**	**Unkn**	**U/ble**
Diggers Rd	122	16	-	-	-	13	1	-	-	-		1	-	-	-
Federation Trail	381	67	-	-	-	29	1	22	-	9		-	-	5	-
Fitzroy North	84	1	-	-	-	-	-	-	-	-		1	-	-	-
Flemington Bridge	477	203	-	-	-	10	3	-	-	170		6	-	1	10
Mt Waverley-Jordanville	423	55	-	-	-	14	7	2	2	1		4	4	1	1
Syndal-Mt Waverley	374	69	-	-	-	11	17	6	1	-		6	3	3	9
Total	**1,861**	**411**	**0**	**0**	**0**	**77**	**29**	**30**	**3**	**180**	**0**	**18**	**7**	**10**	**20**
Phytomyza syngenesiae (host plant: *Sonchus oleraceus*)															
Site	**Agromyzid**	**∑ par**	***O.* sp. 1**	***O.* sp. 2**	***O.* cin**	***D. isa***	***A.* sp.**	***N. for***	***C. mir***	***C. pub***	***Z. lat***	***T. par***	**Unid.**	**Unkn**	**U/ble**
Diggers Rd	22	62	-	-	-	31	13	-	3	-	3	-	1	-	5
Federation Trail	62	166	-	-	-	12	116	-	4	-	-	-	-	12	12
Fitzroy North	40	16	-	-	-	-	8	1	7	-	-	-	-	-	-
Flemington Bridge	85	193	-	-	3	3	34	-	74	65	-	2	-	-	8
Mt Waverley-Jordanville	47	15	-	-	-	-	3	-	5	-	-	6	-	-	-
Syndal-Mt Waverley	12	21	-	-	-	-	3	-	6	9	-	1	-	-	-
Total	**301**	**473**	**0**	**0**	**3**	**46**	**215**	**1**	**105**	**74**	**3**	**9**	**1**	**12**	**25**

2.3.4 Key parasitoids across sites and hosts

Across the entire sampling period, a total of 2,002 parasitoids were reared and identified to 11 species in 3 families (Table 2-13). An additional 40 specimens were deemed too damaged for morphological identification and 48 were not conclusively identified, primarily belonging to Pteromalidae. They possess five-segmented tarsi but lack the wing cells specific to Braconidae and all other traits defining other five-segmented tarsi chalcids (Bouček 1988). For all host leafminers of interest, with the exception of *L. chenopodii*, eulophid parasitoids were predominant in both the quantitative and qualitative sampling. For *L. brassicae* the overall rate of 43% parasitism consisted of 80% eulophids; with the exception of one unknown pteromalid, the rest were braconids. Less than 10% of the insects reared from *L. chenopodii* pupae were parasitoids, and more than 50% of those were braconids (Figure 2-5). The highest proportion of parasitism was obtained for *Ph. syngenesiae*, with 60% of the insects reared being parasitoids, and 95% of these wasps consisted of eulophids. Apparent parasitism was 25% for *Ph. plantaginis*, with 95% of them also being eulophids (Figure 2-6).

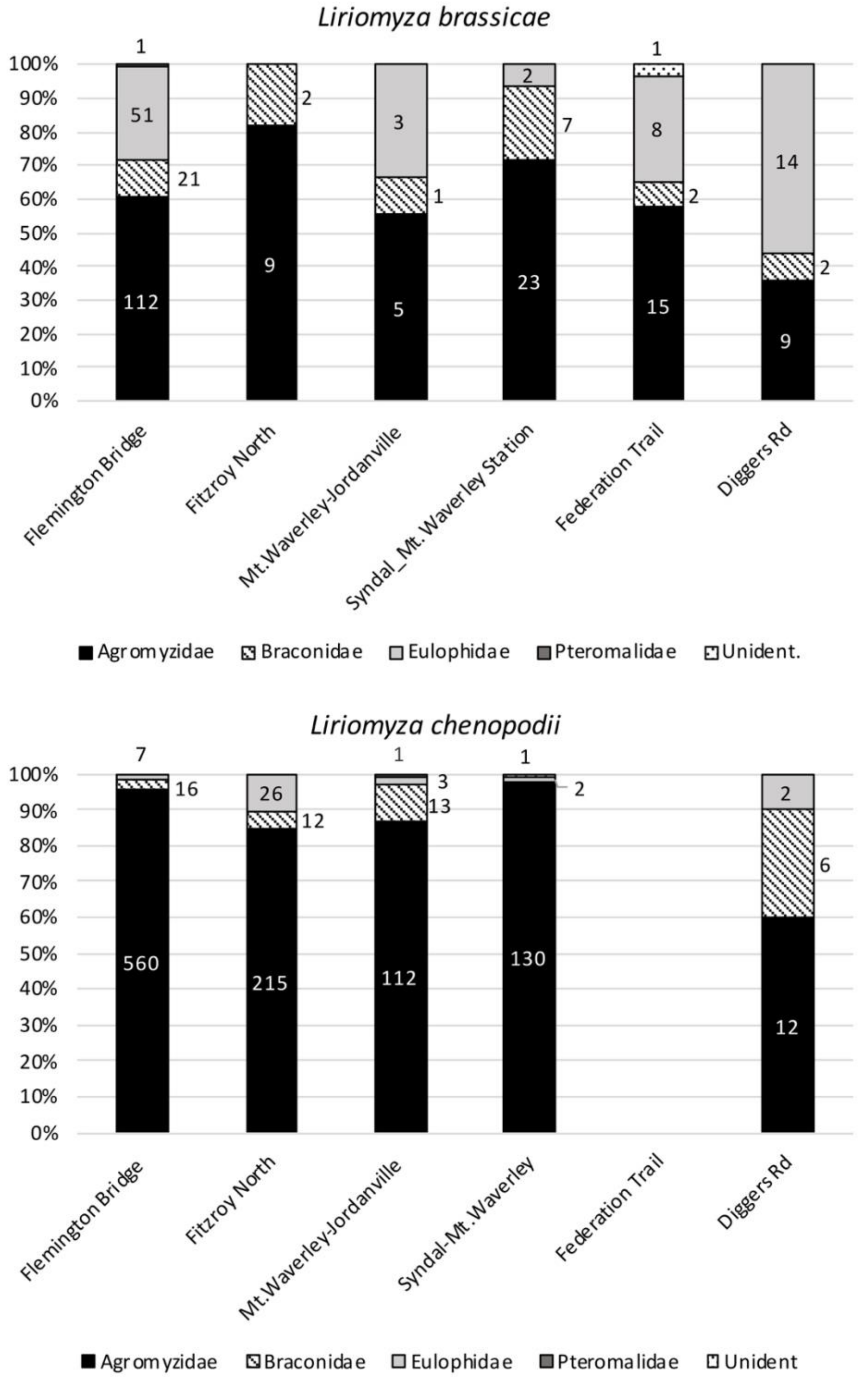
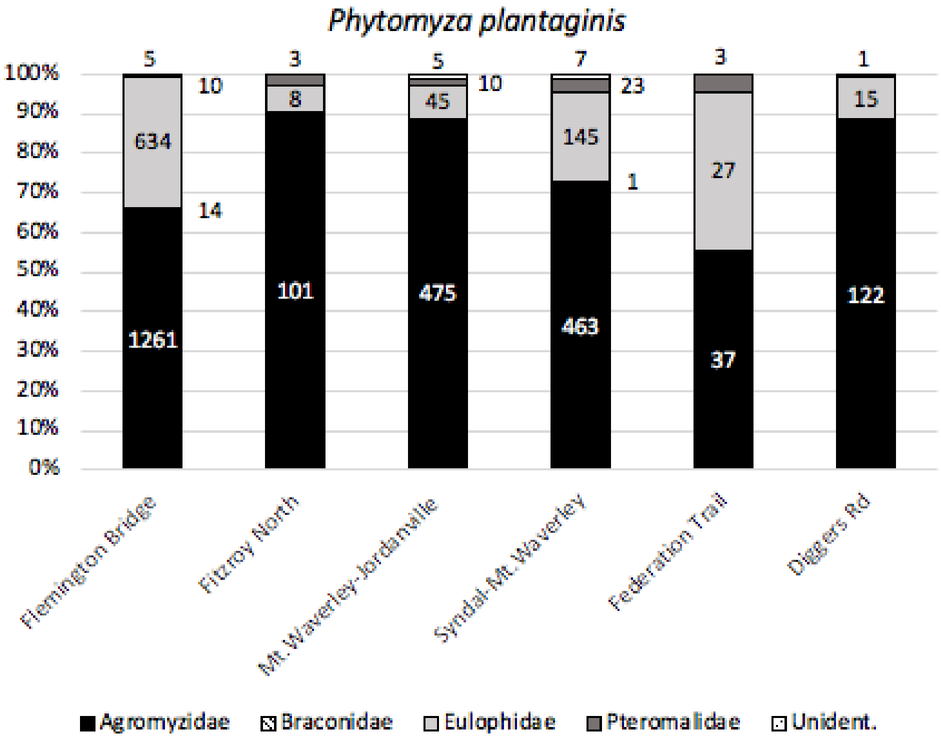


Figure 2-5. Observed parasitism proportions for the two *Liriomyza* spp. at each sampling site. Data includes specimens reared from all known host plants of the agromyzid, collected from quantitative and qualitative sampling protocols. Unident = includes all unknown and unidentifiable wasps.



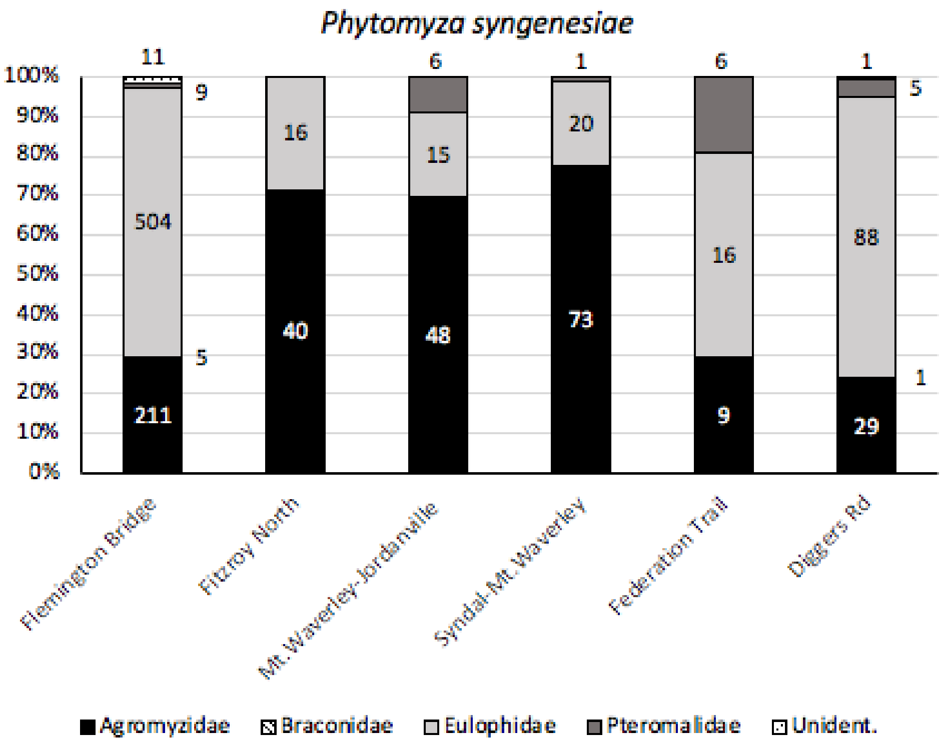


Figure 2-6. Observed parasitism proportions for the two *Phytomyza* spp. at each sampling site. Data includes specimens reared from all known host plants of the agromyzid, collected from quantitative and qualitative sampling protocols. Unident = includes all unknown and unidentifiable wasps.

Looking at the quantitative and qualitative data for parasitoids reared from all six sites shows host-specific differences. For *L. brassicae*, *N. formosa* was the most abundant parasitoid overall (n=40), followed by *Opius* Msp1 (n=30) and *D. isaea* (n=23). The three species represented close to 66% of the parasitoid community on that host, across those sites. The dominant parasitoid across both quantitative and qualitative sampling of *Ph. plantaginis* was *C. pubicornis* (n=574), representing 60% of the total number of wasps reared. Of these *C. pubicornis* wasps, 98% (n=564) were reared from *Plantago* spp. collected at the Flemington Bridge site. *Diglyphus isaea* (n=187) and *H. varicornis* (n=63) are the next two most abundant species parasitizing this agromyzid. The main parasitoid on *Ph. syngenesiae* across the six sites was *C. pubicornis* (n=280), with *Asecode*s sp. (n=249) and *C. mirabilis* (n=137) the second and third most sampled wasps, respectively.

All host leafminers were found to be attacked by seven of the 11 hymenopteran species identified, and ten of the parasitoid species were represented in the samples reared from *L. brassicae* (Table 2-13). *Asecodes* sp., *C. mirabilis*, *H. varicornis* and *O. cinerariae* were found across all host leafminers. *Diglyphus isaea* was not reared from *L. chenopodii*. *Opius* morphospecies had distinct host preferences; low numbers of *Opius* Msp2 were reared from *Liriomyza* species, and *Opius* Msp1 was reared almost solely from *L. brassicae*, while *O. cinerariae* was mostly reared from *L. chenopodii* samples. *Liriomyza brassicae* was a main host for several parasitoids like *Opius* Msp 1 but also for *Z. latilineatum*, as only three were reared out of *Phytomyza* species and none from *L. chenopodii*. *Phytomyza* species were also the main source of *C. pubicornis* and *T. parasitica*, with only one reared out of from each *Liriomyza* sp. *Closterocerus mirabilis* was almost entirely reared from *Ph. syngenesiae* with very few reared from the other leafminers. Among all parasitoids, only *N. formosa* had no males identified, with a total of 76 wasps reared, of which 74 were female and 2 could not conclusively be sexed. This parasitoid was found in similar numbers on both *L. brassicae* and *Ph. plantaginis* (Table 2-13).

Table 2-13. Flies and wasps reared from all plants collected during sampling, from both qualitative and quantitative protocols (see Materials and Methods), at all six sampling sites. Unsexed specimens are included in the totals.

Plant host	**Agromyzidae**		**Parasitoids**				
Species	**Species**	**n**	**Family**	**Species**	**n**	**♀**	**♂**
Brassica fruticulosa	*Liriomyza brassicae*	**216**	Eulophidae		**152**	74	45
				Asecodes sp.	**13**	7	5
				*Chrysocharis pubicornis**	**1**		1
				Closterocerus mirabilis	**4**	1	
				Diglyphus isaea	**55**	9	30
				Hemiptarsenus varicornis	**12**	8	4
				Neochrysocharis formosa	**44**	43	
				Zagrammosoma latilineatum	**19**	4	5
				unidentified	**2**		
				unidentifiable	**2**		
			Pteromalidae		**1**		
				unknown	**1**		
			Braconidae		**39**		
				Opius cinerariae	**5**		
				Opius sp. 1	**30**		
				Opius sp. 2	**3**		
				unknown	**1**		
			Unidentifiable		**1**		
	Scaptomyza flava	**49**	*No parasitoids*	/			
Raphanus raphanistrum	*S. flava*	**2**	*No parasitoids*	/			
Stellaria media	*Liriomyza chenopodii*	**922**	Eulophidae		**15**	5	10
				Asecodes sp.	**2**	1	1
				Diglyphus isaea	**1**		1
				Hemiptarsenus varicornis	**12**	4	8
			Pteromalidae		**1**	1	
				Trigonogastrella parasitica	**1**	1	
			Braconidae		**30**	2	
				Opius cinerariae	**28**	2	
				Opius sp. 2	**2**		
			Unidentifiable		**4**		
	Scaptomyza australis	**203**	*No parasitoids*	/			

**Chrysocharis pubicornis* barcoding indicated the presence of two closely related clades, though morphologically undistinguishable

Plant host	Agromyzidae		Parasitoids				
Species	Species	n	Family	Species	n	♀	♂
Chenopodium album	*L. chenopodii*	**196**	Eulophidae		**27**	6	12
				Asecodes sp.	**24**	6	9
				Closterocerus mirabilis	**3**		3
			Braconidae		**16**		
				Opius cinerariae	**11**		
				Opius sp. 1	**3**		
				Opius sp. 2	**1**		
				unidentifiable	**1**		
Plantago lanceolata	*Phytomyza plantaginis*	**2,120**	Eulophidae		**448**	211	152
				Asecodes sp.	**33**	20	11
				*Chrysocharis pubicornis**	**226**	99	65
				Closterocerus mirabilis	**3**	3	
				Diglyphus isaea	**89**	36	42
				Hemiptarsenus varicornis	**59**	23	34
				Neochrysocharis formosa	**31**	30	
				Zagrammosoma latilineatum	**1**		
				unidentifiable	**6**		
			Pteromalidae		**39**	15	5
				Trigonogastrella parasitica	**22**	11	2
				unidentifiable	**3**		
				unknown	**14**	4	3
			Braconidae		**1**		
				unidentifiable	**1**		
Pl. major	*Ph. plantaginis*	**673**	Eulophidae		**374**	193	168
				Asecodes sp.	**9**	6	3
				*Chrysocharis pubicornis**	**337**	170	157
				Closterocerus mirabilis	**3**		
				Diglyphus isaea	**20**	13	7
				Hemiptarsenus varicornis	**4**	3	1
				unknown	**1**	1	
			Braconidae		**2**		
				Opius cinerariae	**2**		
			Pteromalidae		**3**	1	
				Trigonogastrella parasitica	**3**	1	
			unidentifiable		**6**		
Pl. coronopus	*Ph. plantaginis*		Pteromalidae		**2**	1	
				Trigonogastrella parasitica	**1**	1	
				unknown	**1**		

**Chrysocharis pubicornis* barcoding indicated the presence of two closely related clades, though morphologically undistinguishable

Plant host	Agromyzidae		Parasitoids				
Species	**Species**	**n**	**Family**	**Species**	**n**	**♀**	**♂**
Sonchus oleraceus	*Phytomyza syngenesiae*	**413**	Eulophidae		**734**	224	155
				Asecodes sp.	**241**	92	63
				*Chrysocharis pubicornis**	**242**	56	27
				Closterocerus mirabilis	**133**	47	26
				Diglyphus isaea	**59**	15	14
				Hemiptarsenus varicornis	**38**	13	25
				Neochrysocharis formosa	**2**		
				Zagrammosoma latilineatum	**2**		
				unidentifiable	**17**	1	
			Pteromalidae		**34**	8	10
				Trigonogastrella parasitica Girault	**11**	5	5
				unidentifiable	**10**		2
				unknown	**13**	3	3
			Braconidae		**3**		
				Opius cinerariae	**3**		
			unidentifiable		**9**		
Sonchus sp.[b]	*Ph. syngenesiae*	**64**	Eulophidae				
				Asecodes sp.	**1**		1
				Closterocerus mirabilis	**2**		1
				Hemiptarsenus varicornis	**3**	2	1
Sonchus asper	*P. syngenesiae*	**19**	Eulophidae		**2**	2	
				Asecodes sp.	**1**	1	
				Closterocerus mirabilis	**1**	1	
Taraxacum sp.	*Ph. syngenesiae*	**41**	Eulophidae		**48**	26	22
				Asecodes sp.	**5**	3	2
				*Chrysocharis pubicornis**	**38**	21	17
				Closterocerus mirabilis	**1**	1	
				Diglyphus isaea	**2**		2
				Hemiptarsenus varicornis	**2**	1	1
			Braconidae		**2**		
				Opius cinerariae	**2**		
Arctotheca calendula (Capeweed)	*Ph. syngenesiae*	**3**	Eulophidae		**1**		1
				Hemiptarsenus varicornis	**1**		1

**Chrysocharis pubicornis* barcoding indicated the presence of two closely related clades, though morphologically undistinguishable

[b] *Sonchus* plants likely *S. oleraceus* but ID was uncertain

2.4 DISCUSSION

All four agromyzids supported a diverse parasitoid community, with overlap between species and four parasitoids attacking all hosts. The peak abundance of the host leafminers varied between species and was staggered temporally, resulting in a year-long presence of agromyzids at most sites. Delayed density dependence was also observed as parasitoid presence and apparent parasitism peaked accordingly, with recurring peaks at the end of spring and autumn. The parasitoids reared included likely candidates for biological control such as *H*. *varicornis*, also reared from *L. sativae* in far North Queensland, and *D. isaea*, a known agent in biocontrol of pest *Liriomyza* overseas. The parasitoid community appeared stable during the sampling period, both due to host availability across species and across seasons. This supports the notion that local introduced and native agromyzid species can serve as reservoirs for pest natural enemies (Ridland *et al.* 2020).

2.4.1 Temporal and spatial variation

One of the limitations of sourcing beneficials from surroundings instead of from commercial enterprises is the difficulty of ensuring their presence in high enough numbers before the pest threat crosses a threshold at which it will negatively affect crops and yield (Macfayden & Muller 2013). For this reason, knowing the periods of buildup and abundance of parasitoids and their reservoir host species is of value. The seasonality and overlap of the leafminers across the 6 sites showed that peak abundance was not synchronized across the four species, with *L. brassicae* most active in summer and autumn, while *Ph. plantaginis* was found throughout the year at certain sites. *Phytomyza syngenesiae* was active in December 2018 but sampling was stopped for the next two months and no mines were observed in February 2019, though host plants was available. Sampling of *L. chenopodii* was also stopped in November 2018 and 2019 and it was not detected afterwards though *C. album* was still present. This could be due to climate variation and should be further investigated as mined *S. oleraceus* were observed after December 2020. In the case of *S. media*, the main host plant surveyed for *L. chenopodii*, is a spring annual and was very rarely found at sites once the temperatures increased in summer. Survival of *Liriomyza* species across the summer and winter months could be facilitated by their pupation within the substrate however since *Ph. syngenesiae* pupates within its mine and its host plants are annuals, the process by which it survives summer and winter is unclear. When *Sonchus* host plants remained present after summer, they were not observed to be mined and were often damaged or in poor condition, likely due to aphids or plant pathogens (M. Coquilleau pers. obs.). *Phytomyza plantaginis* was found over winter months as pupae, likely protected from the elements by remaining on the ventral side of the lowest leaves of the *Plantago* host, often by having mined towards the base of the midrib. *Chrysocharis pubicornis*, as a pupal endoparasitoid, likely benefits from the lingering of pupae in plant matter. For *Ph. plantaginis* at the Flemington Bridge site, it was the main parasitoid reared from every sample collected including during the winter months. These observations suggest that the leafminers and parasitoids exhibit a range of strategies to counter seasonally unfavourable conditions depending on the nature of their host plant.

While only a few sites were considered in this study, site conditions varied. The Werribee sites were exposed to the sun and had fewer host plants present year-round. Other sites like Flemington Bridge and Fitzroy North are along a waterway and had most host plants present throughout the year. The vegetation present is likely to reflect differences in a number of variables, including the micro-climate based on the urban nature of the site (Figure A2-1).

Brassicaceous weeds, including *B. fruticulosa*, and monocot weeds were the predominant plants at the Werribee sites. These differences in site characteristics will have contributed to insect differences across the sites. A key difference between sites was the low number of *L. brassicae* reared from Fitzroy North and the two Werribee sites compared to the Flemington Bridge site, even when high plant infestation levels were recorded. *Diglyphus isaea* and *H. varicornis* were present at every site, while 98% of all *C. pubicornis* reared from *Ph. plantaginis,* and 96% of those reared from *Ph. syngenesiae,* were from the Flemington Bridge site. Similarly, 72% of all *C. mirabilis* were reared from *Ph. syngenesiae*, at Flemington Bridge. *Zagrammosoma latilineatum* was only detected in Werribee South, Diggers Rd site, with the exception of a single wasp reared from *Ph. plantaginis* at the Syndal-Mt Waverley site.

2.4.2 Non-agromyzid leafminers

In this survey, the selected host plants were mined by a single agromyzid species with the exception of *B. fruticulosa* and *S. media* that were also found to be mined by two species of *Scaptomyza* flies. Whenever possible, sampling of *S. flava* on *B. fruticulosa* was avoided by selecting mined leaves not presenting their characteristic mining pattern or by segregating their distinct pupae once they had left the mines. *Scaptomyza flava* mines started in a serpentine pattern with frass spotting along it much like for *L. brassicae* mines, however, they then converge towards the leaf midrib where they fuse into a large discolored blotch mine (Martin 2017). *Scaptomyza australis* mines could not be differentiated from *L. chenopodii* on *S. media*. *Scaptomyza* spp. were easily differentiated and isolated from the agromyzid leafminers in their pupal stage as their pupae are recognizably larger, more elongated in shape and with longer spiracles. In Europe, *S. flava* was found on *S. asper* (Godfray 1985), but none were reared from the *Sonchus* species collected in this project. In New Zealand, it is parasitized by an unknown *Opius* species and by *Asobara persimilis* (Papp) (Braconidae), and this parasitoid was also reared from *S. australis* in Australia (Osmelak 1983; Martin 2004, 2012). *Scaptomyza flava* was found to be parasitized by *H. varicornis* on *R. raphanistrum* in Victoria (Bjorksten *et al.* 2005), however no parasitoids were reared from *Scaptomyza* in my study.

2.4.3 Comparison with past Australian surveys

There are a limited number of faunal surveys looking at the parasitoid community on *Liriomyza* and *Phytomyza* species in Australia (Lardner 1991; Bjorksten *et al.* 2005; Lambkin *et al.* 2008; Wood *et al.* 2010). Bjorksten *et al.* (2005) investigated parasitism of *L. brassicae* and *S. flava* from crops of Chinese cabbage and *L. chenopodii* from beetroot in Knoxfield, Victoria. These three leafminers as well as *Ph. syngenesiae* were also reared from weedy host plants at various locations around Victoria. Only *H.*

varicornis was confirmed to parasitize *S. flava* collected from *Raphanus raphanistrum* and the same has been true for the *S. flava* samples in the current study, collected from *Brassica fruticulosa*. Bjorksten *et al.* (2005) found *L. chenopodii* to be the leafminer with the highest diversity of parasitoids, with 8 distinct species reared, while in our sampling *L. chenopodii* had the lowest number of hymenopteran parasitoids, possibly due to differences in sample sizes and host plants or other site attributes. No *D. isaea* was reared from *L. chenopodii* in my study but they were present in the previous study (Bjorksten *et al*. 2005) study. Another parasitoid not detected in this study is *Dacnusa areolaris* (Nees ab Esenbeck) (Braconidae), an endoparasitoid. Bjorksten *et al*. (2005) found them in reasonable numbers (n=25; out of a total n=146) parasitizing *Ph. syngenesiae* mining *S. oleraceus* around Melbourne. No *Opius atricornis* were reared in Victoria but Lardner (1991) found it in small numbers between January and February in SA on *L. brassicae*. He suggested a host plant preference for larvae attacking *Tropaeolum majus* and *R. rugosum.* The absence of *O. atricornis* from my samples could be due to the very limited sampling of *T. majus*, sporadically present at only one of the six main sites but only found mined in other external sampling locations. The only opiine wasp reared in the past Victorian survey was *O. cinerariae*, on *L. chenopodii* (Bjorksten *et al.* 2005). In comparison, *Opius* was a well-represented genus in our samples, with three morphospecies present, each on multiple agromyzid host species. This was similar to the ACT collections, where another 3 *Opius* species were obtained from *Ph. syngenesiae.* but they did not include *Opius cinerariae.* Only their first species seemed to correspond to this study's Msp.1 but it was not conclusive and the other two were distinct (Lambkin *et al.* 2008) (5.3.4 Appendix).

Chrysocharis pubicornis was not reared from either *Phytomyza* species in the ACT samples (Lambkin *et al.* 2008). It was also not reared from *Ph. syngenesiae* collected in the previous Victorian samplings, and there is no point of comparison with *Ph. plantaginis* as it was not one of the agromyzids collected (Bjorksten *et al.* 2005). Unknown *Neochrysocharis* specimens were reared in the ACT samples from *Ph. plantaginis* and *Ph. syngenesiae* (Lambkin *et al.* 2008). The late chalcid expert John La

Salle identified the parasitoids reared from this study and could not identify these specimens. I assume that they are not *N. formosa* with which he was familiar. There is no indication that the unknown *Neochrysocharis* species reared from Victoria and identified by La Salle is the same species as in the ACT samples (Bjorksten *et al.* 2005) (Table A2-11). La Salle also examined *Asecodes* sp. reared from *Ph. syngenesiae* in the ACT but he did not identify them as *A. erxias*, another species he was familiar with (Lambkin *et al.* 2008). These could be the same species as the unknown *Asecodes* specimens reared in this project, and further morphological and molecular studies from the pinned specimens kept at the Australian National Insect Collection (ANIC) could determine if that is the case. From the SA samples of *L. brassicae* collected by Lardner (1991), it is likely that the *Zagrammosoma* species reared was *Z. latilineatum* and that the *Closterocerus* species was *C. mirabilis*. *Zagrammosoma latilineatum* was found by Wood *et al.* (2010) in SA. Additionally, Lardner (1991) recorded a parasitoid of *L. brassicae* in SA identified only as *Chrysonotomyia* sp. This could be a *Neochrysocharis* specimen, as the two genera were previously synonymous. Further morphological and molecular studies from the voucher specimens deposited at the Waite Institute are needed to clarify the situation. Finally, in the Bjorksten *et al*. (2005) collections, *D. isaea* and *H. varicornis* were the most abundant parasitoids. They were also the only two species found at least once on all host agromyzids across all three surveys (Table A2-11). My collections expand on the parasitoid-host interactions and the parasitoid community reared is comparable to the one from the previous sampling in Victoria. This suggests some stability in parasitoid presence over the past decade.

2.4.4 Parasitoid community composition and host preference

The four common agromyzids sampled across the six Vic sites were found to support a range of generalist parasitoids, of which eulophids were most abundant. Among all samples, *Asecodes* sp., *C. pubicornis* and *D. isaea* were the predominant parasitoids, closely followed by *C. mirabilis* and *H. varicornis*. *C. pubicornis*, *D. isaea, H. varicornis*, and *N. formosa*. *Closterocerus mirabilis*, and *T. parasitica* are known only from Australia, and *O. cinerariae* from Australia and New Zealand (Chapter 1).

Sampling methods deployed in this study resulted in the plant material containing a range of life-stages for the agromyzids, including eggs and first instar larvae too small for the majority of parasitoids to attack, influencing the total emergences towards a higher number of agromyzids. This can be further exacerbated by the availability of all agromyzid life-stages in the plant material collected. *Liriomyza* flies pupate into the soil which prevented the collection of pupae at the time of the sampling and limits the number of flies to that of the live larvae present in the sample. Pupal trays could not be placed under the mined plants due to their public location. On the other hand, *Phytomyza* species pupate within the leaf mine and allows for the sampling of already pupated flies within fresh leaf matter. *Phytomyza* samples are expected to have a higher number of endoparasitoids than *Liriomyza* where a portion of them escape collection the moment the fly has pupated. Sentinel mined plants have been used to compare parasitoid abundance between instars and species of agromyzids (Amano *et al.* 2008) and reared more parasitoids than collecting mined leaves (Bjorksten *et al.* 2005). Combined with pupal trays they would allow for a more complete picture of the parasitoid community.

The most abundant parasitoid was identified as an *Asecodes* Förster sp., recognizable predominantly by two rows of setae radiating from the stigmal vein and a distinct occipital groove (5.3.3 Appendix). This entedonine genus has a complex taxonomical history, it has been synonymized and separated multiple times with the other genera *Omphale*, *Ionympha* and *Teleopterus* (Hansson 1996). *Asecodes* species have been collected on invasive *Liriomyza* flies in South-East Asia. *Asecodes delucchii* (Bouček) was the predominant parasitoid on *L. sativae* in Vietnam (Tran *et al.* 2007), and an unknown *Asecodes* was reared from plants mined by *L. sativae*, *L. trifolii* and *Phytomyza horticola* (Goureau) in Indonesia (Rauf *et al.* 2000). In Australia there are reports of *Asecodes* collected from Qld as well as from Vic (Atlas of Living Australia 2020b), and Bouček (1988) noted that 4 to 5 species were present in the Australasian region. In addition, with the other entedonine *C. mirabilis*, further study of its impact on *Liriomyza* species is needed as in my samples it was predominantly reared from the

Phytomyza leafminers. The next most abundant parasitoids were *D. isaea* and *C. pubicornis*, both exotic. *Diglyphus isaea* was a species of particular interest due to its status as a mass-reared biocontrol agent (Chow & Heinz 2006; Maharjan *et al.* 2017). As it is known to parasitize all four major leafminer species including *L. chenopodii* (Bjorksten *et al.* 2005), local populations can potentially be used to rear parasitoids for augmentative control. The limits of its range in Australia are not yet confirmed. More than 50 wasps were reared from *Ph. syngenesiae* as far north as Manly (Sydney, NSW) (P. Ridland pers. comm.) (Atlas of Living Australia 2020b) but it could be found further north.

Chrysocharis pubicornis specimens were identified using the key to Palearctic *Chrysocharis* (Hansson 1985). Molecular testing revealed that two distinct genetic clades existed within the sample pool (X. Xu unpublished data). No morphological differences were observed between specimens in either clade, so they were all referred as *C. pubicornis*. *Chrysocharis pubicornis* is a koinobiont endoparasitoid that is well established in Australia, found in NSW, SA, Vic and Tas (Bouček 1988). Since it primarily targets the pupae of their host (Baeza Larios 2007; Hansson 1985), it is less suited as a parasitoid for *L. brassicae*, *L. chenopodii* or *L. sativae*, as all three leave the mine as a late third instar larva and pupate in the soil, complicating the locating and parasitizing of the pupae (Baeza Larios 2007). This is supported by our results, as almost all *C. pubicornis* specimens reared were from the *Phytomyza* leafminers, which pupate in the leaf.

In comparison, *Opius* are better able to exploit leafminers that pupate in the soil, since they oviposit into larvae but do not develop until pupation. The potential role of opiine parasitoids in biocontrol in Australian horticulture was suggested by Wood *et al.* (2010) in their study of parasitoids on native *Phytoliriomyza* spp. on native saltbush as two unspecified *Opius* species were the most abundant wasps from one host plant and reared in smaller numbers from the other. Among the different braconid morphospecies found in NSW and ACT in 2005 and reared from *L. brassicae*, *Ph. plantaginis* and *Ph. syngenesiae,* six were originally identified as *Opius* (Lambkin *et* *al.* 2008). Three of those were confirmed in 2020 as *Opius* sp. (morphospecies 1, 2 and 3, pers. obs.) and the other three were *Aphidius* (Braconidae) (morphospecies 4, 5 and 6, S. Ward pers. comm.), and are aphid parasitoids likely introduced with the plant matter collected. In samples in Lambkin *et al.* (2008), the *Opius* morphospecies 1, 2 and 3 were reared from *Ph. syngenesiae* and no *O. cinerariae* were collected. In my Vic samples, three *Opius* sp. were collected; the predominant one found primarily on *L. brassicae* is *O. cinerariae,* however the other two have not been conclusively identified (5.2.4 Appendix). *Opius* Msp1 resembles most closely *Opius* sp. 1 collected in ACT. *Opius* Msp2 was very similar to *Opius (Opiothorax) chromatomyiae* Belokobylskij & Wharton, however some traits, including the distinctive "bottle opener" mandibular shape, were absent in my specimens.

Another wasp of particular interest is the eulophid *Z. latilineatum*, described by Ubaidillah *et al.* (2000) from *L. huidobrensis* on potato, in West Java, Indonesia and from specimens from Qld. It is the first species from this genus described from the Indo-Australian region. *Zagrammosoma latilineatum* is of particular interest due to its extended distribution across Australia's east-coast, given its presence in far north Qld and in Vic, as well as the fact that it has been reared from *L. sativae* in Australia. Observations in the field (E. Pirtle pers. comm.) also seem to suggest that it remains active during periods of high temperatures (40°C). Further studies on its biology and physiology should be undertaken, as heat-resistance is an appealing trait in local biocontrol agents, and one of the possible barriers to successful use of exotic parasitoids. Though not one of the major parasitoids in this study, the third parasitoid of interest, *H. varicornis,* was found to exploit all agromyzids reared, including those from host plants other than the target species such as *Ph. plantaginis* on *Pl. major* and *Ph. syngenesiae* on *A. calendula*. It is not only well established, but also found on *L. sativae* in Qld as well as in East-Asia on other *Liriomyza* spp. (Rauf *et al.* 2000) and on *Ph. horticola* (Matsuda *et al.* 2009). Its appeal as a biocontrol agent is increased by its significant host-feeding and stinging behavior, increasing agromyzid larval mortality (Cheng *et al.* 2017).

The rearing of *N. formosa* in this study marks the first confirmation of this species in Vic, reared from both *L. brassicae* and *Ph. plantaginis*. While in Japanese field collections there was a sex ratio strongly biased towards females (Arakaki & Kinjo 1998), Vic sampling has only yielded females, with two individuals unsexed. For ACT, nearly half of the specimens were not available for sexing, but all sexed wasps were female. This suggests a thelytokous population of *N. formosa* may be present in Australia. In Japan, a strong female bias in field samples of *N. formosa* suggested both sexual and asexual populations co-existed (Arakaki & Kinjo 1998). A laboratory thelytokous strain was sequenced for the endosymbionts *Wolbachia* and *Cardinium* and RNA sequences were screened for the bacteria *Rickettsia* (Adachi-Hagimori *et al*. 2006). All three are known to manipulate the reproduction of arthropods and in the case of *Wolbachia,* induce parthenogenesis in parasitoids (Arakaki *et al.* 2001). Analyses showed that the wasps were infected with a strain of *Rickettsia* and that it was the cause for the parthenogenesis (Adachi-Hagimori *et al*. 2011). Multiplex PCR assays developed by Adachi-Hagimori *et al.* (2008) led to differentiation of thelytokous and arrhenotokous strains in Japan. The thelytokous strain was also found in China (Yang *et al.* 2017). Testing the Victorian *N. formosa* for the strain-specific sequence and for the presence of *Rickettsia* is warranted. *Neochrysocharis formosa* is recognizable from other *Neochrysocharis* spp. in the South-East Asian region, such as *N. okazakii* and *N. beasleyii,* from its wing infumation near the stigmal vein and its nearly complete notauli (Fisher & LaSalle 2005). Collections of a *Neochrysocharis* species in ACT and Vic have yet to be identified (Bjorksten *et al.* 2005; Lambkin *et al.* 2008). There have been records of *N. okazakii* in Vic (Bjorksten *et al.* 2005).

2.4.5 Biological control candidates for *L. sativae*

Previous samples made in Seisia and the Torres Strait found multiple species of parasitoids attacking the invasive *L. sativae* population currently behind a quarantine zone at the tip of Cape York in Qld. These include *H. varicornis*, *Cirrospilus brevicorpus* Shafee & Risvi, *C. mirabilis* and *Z. latilineatum* (Ridland *et al.* 2020). With the exception of *C. brevicorpus*, these parasitoids were collected in my study. Based on previous records of parasitoid communities on Agromyzidae in Australia, Ridland *et al.*

(2020) identified 27 genera of hymenopteran parasitoids from eight families as possible candidates in the control of exotic pest agromyzids such as *L. sativae*. Among those, nine genera were deemed to be most promising as biological control candidates, namely six eulophids: *Diglyphus*, *Hemiptarsenus*, *Zagrammosoma*, *Chrysocharis*, *Closterocerus*, *Neochrysocharis*, two pteromalids: *Callitula*, *Trigonogastrella* and three braconid morphospecies from *Opius*. Of these nine genera, eight were represented in this study, and it is interesting to note that the *Asecodes* sp. was a predominant parasitoid and should be added to this list of potential biocontrol agents. It is likely that based on the location of the agromyzid community, additional genera should be considered as a major source of *Liriomyza* parasitoids. For example, there is little known about endoparasitoids from the *Asecodes* genus in Australia. Another unknown *Asecodes* was reared in ACT (Lambkin *et al.* 2008) and was not identified despite examination by La Salle. Specimens from that study were not available for comparison with the current ones.

2.4.6 Reservoir agromyzids for biocontrol agents

An important question is whether the local parasitoids will transfer onto pest species, both in crops and non-crop host plants, to reduce infestation pressure at the landscape level. Insecticide use has facilitated agromyzid proliferation as a secondary pest (Reitz *et al.* 2013) in the absence of biological enemies, and parasitoids risk being negatively affected by present-day chemical control used in Australia as in other countries (Reitz *et al.* 2013). In Japan, organophosphates and pyrethroids applied on field populations of *Ph. horticola* and *L. sativae* led to a reduction in almost all parasitoids, including *D. isaea* and *N. formosa*, and a resurgence of leafminers afterwards (Saito *et al.* 2008). Toxicity to parasitoids is insecticide specific, as demonstrated by Tran & Ueno (2012) for *N. okazakii,* a parasitoid of *Liriomyza* species in Vietnam, and by Hernández *et al.* (2011) for *N. formosa* (abamectin). Thus, insecticide use should be done in tandem with carefully choosing which chemical control to use against leafminers in crops and when to spray them to preserve existing biocontrol agents. The first step in making use of agromyzid parasitoids in Australian horticultural areas is by providing a space for healthy reservoirs and parasitoid communities to accumulate over time. This study showed that green spaces in urban and peri-urban areas, as well as near horticultural fields as was the case of the Werribee sites, support a number of non-pest agromyzids and their parasitoids. Green corridors in agriculture have been advocated for the maintenance of beneficials.

2.4.7 Future research

Identifying candidate agromyzids and parasitoids to serve as a source of biocontrol agents is only the first part of future IPM programs against *L. sativae* and other pest *Liriomyza* spp. in Australia. The biology of some introduced species is well characterized, as is the case for *D. isaea* since they are mass-reared overseas, however that is not in the context of the Australian region. Long-established populations have the potential of being better suited to the local climate and their development rates should be re-assessed based on local stocks. Other parasitoids with high abundance on local agromyzids are also of interest for further studies. Experiments could be undertaken to look at the actual percentage parasitism of the agromyzids (not just observed), which can be achieved using sentinel pots each with a specific host larval development stage (Amano *et al.* 2008). Future sampling of agromyzids should focus further on their temporal presence and stability. Sampling over a period of two years showed fluctuations between seasons as well as substantial site variation. Changing climate is likely to have an impact on the availability of agromyzids to act as reservoirs for biocontrol agents, especially if temperatures increase, as it could facilitate the spread of species from tropical climates and extend the period of activity of temperate agromyzids. Native and naturalized agromyzids in Australia remain a potentially untapped source of parasitoids. In other cases, like for *Asecodes*, *Zagrammosoma* and *Neochrysocharis* spp., cryptic larvae from lepidopteran leafminers are also possible hosts and could serve as reservoirs (Ubaidillah *et al.* 2000; Luna *et al.* 2011; Noyes 2019).

Besides observing the presence, seasonality, and diversity of parasitoids on a local community of agromyzid leafminers, sampling from a range of host plants brings in an additional layer of information. Studying host plant preference of both flies and their parasitoids is needed to identify plants best suited as reservoirs for source populations of parasitoids. Host plant species have an effect on the development of the leafminer larvae as well as on its offspring's host preference for oviposition as shown for *L. brassicae* in the US (Tavormina 1982). A divergence in behavior and phenology was demonstrated in individuals fed on different host plant species, originating from a genetic divergence present in a wild population (Tavormina 1982; Okoth *et al.* 2014). Interestingly, Wood *et al.* (2010) showed that there was a significant difference in agromyzid abundance and parasitoids reared from two saltbushes (Chenopodiaceae: *Rhagodia* spp.) growing in the same conditions in SA. They sampled 10 times the number of *Phytoliriomyza* flies on *R. candolleana* compared to the *R. parabolica*, and the abundance and relative frequency of parasitoids present was also host plant specific. *Trigonogastrella* sp., *Z. latilineatum* and *H. varicornis* were found primarily on *R. candolleana*, representing 73% of all of the parasitoids reared, while the *Opius* spp. were more abundant on *R. parabolica*, representing 60% of its parasitoids reared. Lardner (1991) also noted that *O. atricornis* in SA was only reared from *L. brassicae* on *T. majus* and *R. rugosum*, while *O. cinerariae* was only reared from *L. brassicae* on *R. rugosum* and *Sisymbrium officinale*. Wood *et al.* (2010) suggested adding *R. candolleana* to green belts around agricultural zones to improve parasitoid diversity and offer a refuge from insecticide spraying. Intentional planting of certain species to attract non-pest agromyzid hosts and their parasitoids is yet another control method to be considered in future Australian IPM plans.

In conclusion, this study has helped identify a diverse parasitoid fauna attacking common leafminers found in host plants common in urban and agricultural areas. The four target agromyzids had separate abundance peaks, *L. brassicae* active in summer and autumn while *L. chenopodii* and *Ph. syngenesiae* were abundant in spring. *Phytomyza plantaginis* was active throughout the year. Presence of parasitoids overlapped with that of the agromyzids, and each agromyzid supported at least five different species. Parasitoid represented in this sampling include known parasitoids of

L. sativae overseas (*D. isaea* and *N. formosa*) and in far north Qld (*H. varicornis* and the native *C. mirabilis* and *Z. latilineatum*). The dominant parasitoids were an *Asecodes* sp., *C. pubicornis* and *D. isaea*. This study supports the value of multiple agromyzid species in green spaces, including the overlooked *Ph. plantaginis*, as hosts to a stable community of parasitoids.

<u>CHAPTER 3.</u> First record of males and population variation in sex ratio in the leafminer *Phytomyza plantaginis* Goureau (Diptera: Agromyzidae) from Victoria, Australia

ABSTRACT:

Parthenogenetic reproduction has only been found in two species of agromyzid leafminers (Diptera: Agromyzidae), both from the genus *Phytomyza* Fallén. *Phytomyza plantaginis* Goureau, originally from Europe, has been introduced into North America, Australia and New Zealand, where only females have been collected. It is also found in Japan where both sexes have been collected. I have now reared both sexes from *Plantago* spp. collected in Victoria, the first record of male *Ph. plantaginis* in Australia. In sites around Melbourne, the sex ratio was female-biased, with females comprising 75% of sampled individuals overall. Males were absent from collections in western and northern Victoria, Australian Capital Territory, New South Wales and Western Australia, suggesting that males are only present in populations within a restricted area. I reared 2,549 female and 746 male adult flies from three *Plantago* species. *Plantago lanceolata* was the most abundant and widespread host but some flies were also reared from *Pl. major* and *Pl. coronopus*. A female sex bias was observed in samples from *Pl. lanceolata* but not in those from the other two host plants, however, sampling from *Pl. coronopus* was particularly limited. The mechanism responsible for apparent parthenogenesis in this leafminer and causes for the localized presence of males remain unknown although endosymbionts and their interactions with environmental factors may play a role.

3.1 INTRODUCTION

The adventive agromyzid species, *Phytomyza plantaginis* Goureau, is thought to have been introduced into Australia from Europe by early settlers (Spencer 1963b). Its host plants from the *Plantago* genus (Plantaginaceae), are associated with human settlement, and seeds were likely introduced as contaminants (Smith *et al*. 2020) even before 1850 (Atlas of Living Australia 2020c). A common species in Europe, *Ph. plantaginis* is mostly reared from the ribwort or narrow-leaved plantain *Plantago lanceolata* L. and the greater plantain *Plantago major* L. (Spencer 1972). Its European range spans latitudinally from Norway to Italy and longitudinally from Spain to Belarus (Martinez 2020). *Phytomyza plantaginis'* range was expanded by anthropogenic movements and it has now been recorded in Canada and the USA (Spencer 1969, 1981; Eiseman *et al.* 2019), as well as in East Siberia and Japan where it had been described as *Phytomyza nannodes* Hendel (Sasakawa 1961; Spencer 1963b). It is also found in New Zealand and Australia (Spencer 1963a; 1976b, 1977) (Table 3-1).

Although the species is widespread in southern Australia, very little is known about its ecology here. In the Australian Capital Territory (ACT) *Ph. plantaginis* was parasitized by *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), by a *Neochrysocharis* sp. and an unidentified pteromalid (Lambkin *et al.* 2008). These wasps are likely to attack a range of leaf mining agromyzids (Chapter 1). In New Zealand, five wasps have been recorded from *Ph. plantaginis*. They consist of the adventive eulophids *Chrysocharis pubicornis* (Zetterstedt), *D. isaea*, *Hemiptarsenus varicornis* (Girault) and braconid *Opius cinerariae* Fisher, and an endemic *Proacrias* n. sp. (Martin 2019). For that reason, *Ph. plantaginis* may act as a potential source of parasitoids for other economically damaging agromyzids (Chapter 2). As with other agromyzids, the female fly uses her ovipositor to pierce the epidermis of the leaves and feed on the exudate. Males feed from the same wound, and the presence of leafminers can sometimes be noticed by the numerous circular puncture wounds before mines are visible. Eggs are laid under the plant's epidermis, and the larva tunnels into the mesophyll after it has hatched. The resulting mine appears silvery or whitish when fresh and turns to a darker coloration once the plant has aged; the frass is large and widely spaced. The larva can feed on either side of the leaf and sometimes tunnels into the stems. The larva pupates within the leaf, forming an oval-shaped, slightly flattened puparium with some variation in color, ranging from off-white to shiny black (M. Coquilleau pers. obs.). Similar feeding patterns have been observed and illustrated in Europe (Ellis 2020), in the USA (Eiseman 2019) and New Zealand (Martin 2019). The anterior spiracles of the puparium protrude from the epidermis and anchor it to the top layer of the mine (Eiseman 2019).

Frick (1951) confirmed parthenogenesis in an American population of *Ph. plantaginis* by rearing three generations from isolated females. A sample of *Ph. plantaginis* from the host *Plantago lanceolata* from central California yielded only females (n=125). Samples of this species from New York and Pennsylvania were noted as having both sexes, but little information was provided (Frost 1924). An early case of parthenogenetic reproduction in the genus *Phytomyza* was described by Hering (1951), in a species closely related to *Ph. plantaginis* (Winkler *et al.* 2009), when he isolated unmated females of *Phytomyza crassiseta* Zetterstedt from a German population in an enclosure containing the host plant *Veronica* sp. L. (Plantaginaceae), and obtained mines and later an all-female generation of offspring. Spencer (1981) however, noted that both male and female *Ph. plantaginis* are found in their original range, in Europe. Flies from both sexes have been reared from *Plantago lanceolata* and native *Plantago* spp. in Japan (Sasakawa 1961), while collections from New Zealand and from Australia have yielded only females (Spencer 1963a, 1976, 1977) (Table 3-1).

3.1.1 Aims

In this Chapter, I investigate the sex ratio of *Ph. plantaginis* based on sampling undertaken in 2018 and 2019 with a focus on the Melbourne area, Victoria (VIC). *Phytomyza plantaginis* were common in these collections (Chapter 2). This provided me with an opportunity to 1) verify the identity of *Ph. plantaginis* using morphological and molecular techniques, 2) assess the relative abundance of *Ph. plantaginis* on

Plantago spp. in urban settings in Melbourne, 3) collate information for other sites in Australia. and 4) compare the sex-ratio of this species in different Australian populations.

Table 3-1. Published records of *Phytomyza plantaginis* specimens reared and sexed from different locations outside of its endemic range.

Country	**State/location**	**♀**	**♂**	**Author**
Australia	New South Wales	3		(Spencer, 1977)
	Australian Capital Territory	3		(Spencer, 1977)
	Victoria	3		(Spencer, 1977)
	Tasmania	16		(Spencer, 1977)
	Western Australia	4		(Spencer, 1977)
New Zealand	North Island	45		(Spencer, 1976)
	South Island	10		(Spencer, 1976)
Canada	Alberta	4		(Sehgal, 1971)
	Ontario	4		(Spencer, 1969)
	Quebec	1		(Spencer, 1969)
USA	Central California	171		(Frick, 1951)
	Hawaii	16		(Sasakawa, 1964)
Japan	Ehime (Matsuyama)		1	(Sasakawa, 1961)
	Nishinakama	24	13	(Sasakawa, 1956)
	Takarajima		2	(Sasakawa, 1956)

3.2 MATERIALS AND METHODS

3.2.1 Victorian sampling – Temporal and spatial

Sampling was done at six sites in the Melbourne area (Table 3-2), as part of a temporal survey (Chapter 2). Quantitative sampling was done for flies reared from *Pl. lanceolata* over a period of 10 to 18 months depending on the sites. Fifty *Pl. lanceolata* host plants were surveyed during each sampling event and the total number found mined was recorded. A single mined leaf from each plant was removed and kept as part of the quantitative sample. Additional leaves from each *Pl. lanceolata* plant found mined were collected, especially when the plants were heavily infested, as part of the qualitative sampling. *Plantago major* was also sampled following this protocol at the Flemington Bridge site. All sampling of *Plantago* spp. at the 6 Melbourne sites was completed in a 20 to 30 min period at each site.

Table 3-2. Melbourne area sites. GPS coordinates of the Melbourne area sites where temporal quantitative sampling took place.

Site	Latitude	Longitude
Werribee area		
Federation Trail	-37.91558	144.66863
Diggers Rd	-37.96652	144.68568
Mount Waverley area		
Syndal – Mt Waverley	-37.87625	145.14724
Mt Waverley - Jordanville	-37.87549	145.12258
Flemington Bridge	-37.78756	144.93993
Fitzroy North	-37.77787	144.98869

Australian sampling

Supplementary opportunistic sampling was done by M. Coquilleau, P. Ridland and X. Xu outside of the Melbourne sites, in locations north of Melbourne as well as in Canberra (ACT), Lismore, Ballina and Bangalow in New South Wales (NSW) and Bunbury in Western Australia (WA) (Table 3-3). Plants were collected from urban and peri-urban areas, such as road verges and along parks and green spaces. *Plantago lanceolata* was the sole host plant at these sites with the exception of Forrest (-38.5501, 143.7454) (VIC) where *Pl. major* was the only host plant, and Sunbury (VIC) where *Plantago coronopus* was found mined along *Pl. lanceolata*. Sampling duration was variable and dependent on the level of infestation.

Table 3-3. Australian sites. GPS coordinates of the sites where qualitative sampling took place.

State - Locations	Latitude	Longitude
ACT (collector: X. Xu)		
Canberra	-35.2380	149.0850-
NSW (collector: P. Ridland)		
Lismore	-28.8209	153.3445
	-28.8108	153.2803
	-28.8760	153.5797
Ballina	-28.8694	153.5755
	-28.8691	153.5574
Bangalow	-28.6892	153.5195
WA (collector: P. Ridland)		
Bunbury	-33.3704	115.6387

3.2.2 Rearing of agromzids

Mined *Plantago* leaves were placed into a labelled Ziploc bag, with the host plant, location, date, collector and sampling type indicated on it, and brought into the lab. Sheets of paper towel were laid between each layer of leaves to prevent condensation. Leaves were folded as needed to allow for space near the top of the bag, where insects gathered once emerged. The paper towel was replaced every week with new sheets. *Plantago major* leaves were often large and released a lot of moisture. For that reason, whenever possible, the area of these leaves mined was separated from the rest of the leaf to limit plant matter in the Ziploc bag and additional sheets of paper towel were added to these as required. All bags were inflated before being sealed to avoid crushing the leaves and kept upright in boxes for ease of storage. All Ziplocs were kept in a CT cabinet at 20°C, with a 16L:8D photoperiod.

3.2.3 Identification and handling of specimens

Ziploc bags were inspected for the presence of flies or wasps at the top of the bag twice a week and any insect found was removed using a paint brush moistened with 100% EtOH and placed into a dish containing 100% EtOH. Flies and parasitoids were separated under a dissecting microscope and sexed based on external genitalia before being transferred to 2 mL vials with 100% EtOH and kept at -20°C. Some of the samples were placed in vials of 80% EtOH and kept at 4°C for future dissection. All vials were labelled and assigned a numbered tag associated with the database. Morphological identification of the flies was done by M. Coquilleau, using the key in Agromyzidae of Australia (Spencer 1977). The dissection of the male genitalia to confirm the identity of male specimens was done by Dr M. Malipatil, (AgriBio, Agriculture Victoria Research, Melbourne, VIC). DNA sequencing and molecular analyses were undertaken by X Xu, PEARG (University of Melbourne).

3.2.4 Statistical analyses

Contingency tests to compare male and female numbers were performed using R (R Core Team 2020) with the Rcmdr package and RStudio.

3.3 RESULTS

3.3.1 Distribution sampling

Samples from NSW, ACT or WA yielded no males, but in Vic 25% of the sexed flies were males. The lowest ratio of females (54%) was from the Flemington Bridge site (Figure 3-1). In a contingency test comparing male to female numbers across the four states (sites pooled, excluding unsexed flies), a highly significant difference among states was obtained ($X^2 = 115.71$, df = 3, $P < 0.001$) (Table 3-4).

Table 3-4. Total number of females, males and unsexed *Phytomyza plantaginis* collected across 4 states, all host plants included (*Plantago coronopus*, *Pl. lanceolata* and *Pl. major).*

States	**Σ ♀**	**Σ ♂**	**Sum unsexed[1]**
ACT	140	0	6
NSW	153	0	0
VIC	2,196	746	398
WA	60	0	8
Total	**2,549**	**746**	**412**

Unsexed[1]: Condition of specimens was too poor for accurate sexing

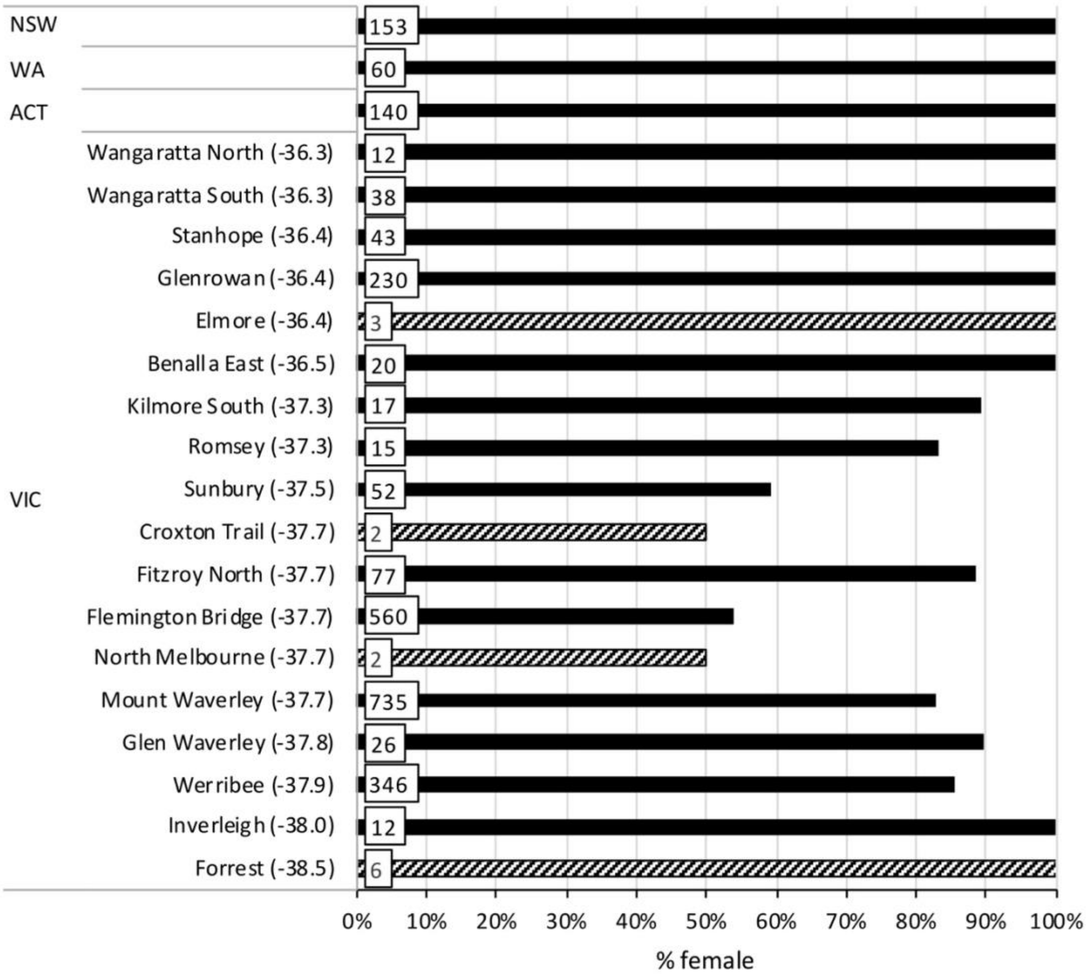


Figure 3-1. Proportion of female *Phytomyza plantaginis* from all collection sites (north to south distribution, Latitude is indicated next to the site) and all host plants, excluding unsexed individuals due to poor condition. Crosshatch bars indicate samples of less than 10 flies in total. The number on each bar is the total number of flies sexed at each site.

In Table 3-5, underlined locations represent the quantitative sampling sites in the Melbourne area that were sampled over time. Inverleigh was sampled on 17/05/2019, Sunbury on 27/09/2019, and the rest of the sampling was undertaken between 14-19/06/2019, for a 40 to 60 minutes sampling effort at each site. *Plantago lanceolata* was found sporadically mined. No males were reared from locations above Kilmore South (37° South), nor from the southernmost location, Inverleigh (Figure 3-2). The six female flies collected from Forrest were not included in this as they were reared from *Pl. major*. However, it should be noted that Forrest is further south than Inverleigh and further sampling might extend the southern limit of this male presence.

Three historical Vic samples each of a single female fly were included from Spencer (1977), Apollo Bay, Tasmania (collected 1/1/1967), Melton (27/11/1948) and Wyperfeld National Park (51 km N.-W. of Hopetoun) (4/11/1974). They are part of the "Females only <10 flies" data points (Figure 3-2). The samples clustered around the Melbourne area include both sexes, with the exception of a single female fly sample reared from Werribee South beach (-37.9741, 144.6938) from *Pl. coronopus*. However, this location is in the Werribee region, where both sexes were reared from *Pl. lanceolata* (Table 3-5). The distribution of the fly collections is patchy, as host plants in some parts of Victoria were not found mined. This was the case for Bolinda (-37.4524; 144.7355), a location surveyed between Sunbury and Romsey where plants were infested.

Table 3-5. Numbers of female, male and unsexed *Phytomyza plantaginis* from *Plantago lanceolata* across all Victorian sites. Locations are listed in a south-to-north gradient, shaded locations are all within the Melbourne area. Main sites are underlined and were regularly sampled (quantitative protocol). All other locations were only occasionally sampled.

VIC	Sample date	Latitude	Longitude	Σ ♀	Σ ♂	Unsexed[1]
Inverleigh	17/04/2019	-38.0943	144.0092	12	0	0
Werribee	(multiple)	-37.9155 -37.9665	144.6686 144.6856	345	58	104
Glen Waverley	(multiple)	-37.8812	145.1507	26	3	0
Mt Waverley	(multiple)	-37.8762 -37.8754	145.1472 145.1225	735	152	62
North Melbourne	(multiple)	-37.7974	144.9478	2	2	0
Flemington Bridge	(multiple)	-37.7875	144.9399	301	196	76
Fitzroy North	(multiple)	-37.7778	144.9886	75	9	14
Croxton Trail	(multiple)	-37.7653	144.9979	2	2	0
Sunbury	27/09/2019	-37.5717	144.7292	6	4	0
Romsey	14/06/2019	-37.3544	144.7473	15	3	0
Kilmore South	17/06/2019	-37.308	144.9517	17	2	0
Benalla East	16/06/2019	-36.5584	145.9990	20	0	0
Elmore	14/06/2019	-36.4931	144.6082	3	0	0
Glenrowan	15/06/2019	-36.4619	146.2243	230	0	0
Stanhope	15/06/2019	-36.4616	144.7911	43	0	0
Wangaratta South	16/06/2019	-36.3738	146.3163	38	0	0
Wangaratta North	16/06/2019	-36.3417	146.3009	12	0	0
Total				**1,882**	**431**	**257**

Unsexed[1]: Condition of specimens was too poor for accurate sexing

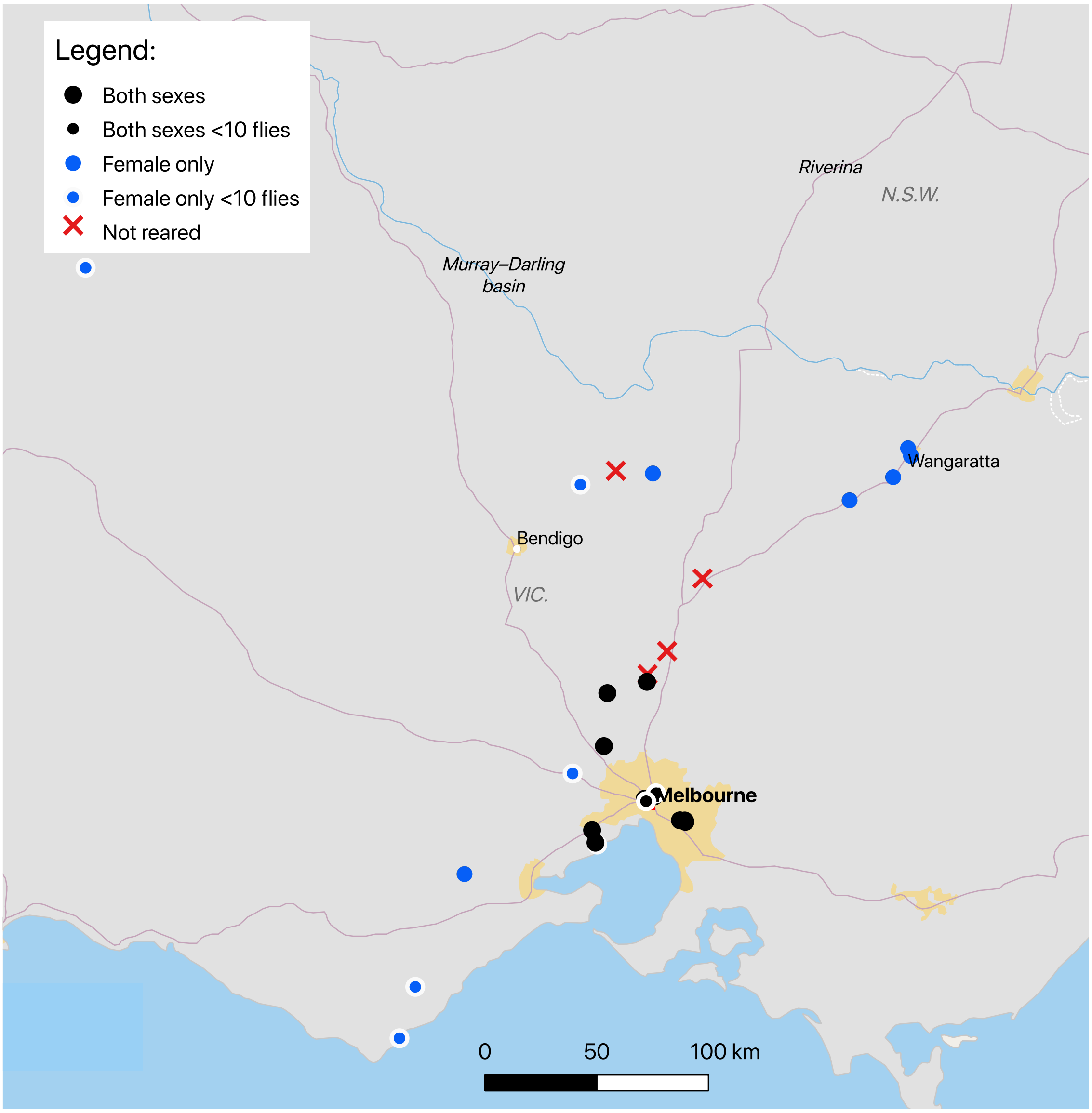


Figure 3-2. Map of sampling sites in greater Victoria for *Phytomyza plantaginis,* including the category "Not reared", where plants were found mined (n<10 leaves mined per site), but no flies were successfully reared.

3.3.2 Sex-ratio biases associated with host species

Plantago lanceolata was the only host plant sampled in states outside of Victoria. However, in Victoria, three *Plantago* spp. were surveyed (Table 3-6), so the influence of host plant on sex ratio could be examined. A contingency test showed a different distribution of males to females across host species (excluding unsexed flies) (X^2 = 245.5, df = 2, p-value < 0.001). This reflected a higher incidence of females collected from *Pl. lanceolata*, though both sexes were collected from all three host plants and host plant comparisons are to some extent confounded by collection location and the time of collection.

To investigate plant host effects more directly, the male to female ratios in flies sourced from *Pl. major* (n=1,140; ♀=466; ♂=455) and *Pl. lanceolata* (n=431; ♀=268; ♂=163) collected on the same dates at Flemington Bridge were pooled and compared. A contingency test showed a significant difference between the hosts (X^2 =15.9, df = 1, P <0.001), suggesting that sex ratio may depend on the host plant, with relatively higher numbers of males from *Pl. major* (Table 3-7).

Table 3-6. Total number of females, males and unsexed *Phytomyza plantaginis* across all sampling sites in Victoria reared from the three *Plantago* species.

Phytomyza plantaginis	*Plantago lanceolata*	*Pl. major*	*Pl. coronopus*	**Total**
Σ ♀	2,193	476	48	**2,717**
Σ ♂	630	456	33	**1,119**
Unsexed[1]	453	193	1	**647**
Grand Total	**3,276**	**1,125**	**82**	

Unsexed[1]: Condition of specimens was too poor for accurate sexing

3.3.3 Temporal sampling of *Plantago major*

At the Flemington Bridge site, the seasonal abundance of *Ph. plantaginis* mining *Pl. lanceolata* and *Pl. major* fluctuated, with sporadic peaks in spring (September 2018 and October 2019) and in summer. The agromyzid was present on *Pl. lanceolata* throughout the year, and both sexes were found across seasons (Table 3-7.) (Chapter 2). *Plantago major* was a common secondary host plant present at the site and was occasionally found mined. It was also found in small numbers at the Fitzroy North site but not surveyed. It was not a target species of the current study and not sampled following a quantitative protocol. It was also not surveyed at every sampling event, for that reason, the seasonality of infestation of *Pl. major* is unclear. Nevertheless, *Pl. major* was abundantly mined in October to December 2018 (n=889; ♀=363; ♂=347) and from January to May 2019 (n=176; ♀=67; ♂=70). It was also collected later in 2019 but it was less noticeably mined in spring of 2019 (M. Coquilleau pers. obs.). At other Vic sites, two females and one male were reared from *Pl. major* at the Fitzroy North site and six females were reared from Forrest, Victoria.

3.3.4 Morphological and molecular identification

Samples of male *Phytomyza* flies reared from *Plantago* spp. were brought to Dr M. Malipatil from AgriBio, Agriculture Victoria Research (La Trobe University, Melbourne) for genitalia dissection. He confirmed that male genitalia from the Victorian samples matched the male genitalia description by Spencer (1977) (Figure A3-1). Voucher specimens have been lodged in the Victorian Agricultural Insect Collection (VIAC) at AgriBio. Specimens of male and female *Ph. plantaginis* were also passed to Ms. Xu for barcoding using mitochondrial primer pair leafminer-CO1-F/R for the 3’ end (Blacket *et al.* 2015). Specimens from Victoria and ACT were sequenced. They returned with 99% similarity to *Ph. plantaginis* specimens from California.

Table 3-7. Total *Phytomyza plantaginis* flies reared from the main host plants at the Flemington Bridge site (VIC) over time. Each host plant was surveyed during a window of 20 to 30 min for each sampling event (both quantitative and qualitative sampling).

	Plantago lanceolata			*Pl. major*		
Site	Σ ♀	Σ ♂	Unsexed[1]	Σ ♀	Σ ♂	Unsexed[1]
2018	**203**	**124**	**78**	**363**	**347**	**179**
25/06/2018	0	0	1			
20/08/2018	1	0	0			
24/08/2018	13	20	19			
12/09/2018	8	4	4	67	77	2
09/10/2018	144	79	8	257	234	129
01/11/2018	12	7	36	21	24	24
12/11/2018	2	1	4			
22/11/2018	2	3	6			
12/12/2018	1	0	1			
29/12/2018	5	1	6	8	5	24
31/12/2018	15	9	0	10	7	0
2019	**200**	**133**	**77**	**103**	**108**	**40**
20/02/2019	7	7	3	15	10	1
04/03/2019	8	4	2	19	27	4
25/03/2019	11	3	0	18	15	31
11/04/2019	5	5	6	13	13	3
24/04/2019	16	22	2	2	4	0
24/05/2019	20	18	2	0	1	0
24/06/2019	16	8	9			
26/08/2019	5	1	0	34	37	0
18/09/2019	48	39	14			
07/10/2019	12	8	1			
12/10/2019	4	3	0			
25/10/2019	7	6	24			
27/11/2019	12	3	5	2	1	1
12/12/2019	29	6	9			
2020	**32**	**16**	**9**			
13/01/2020	32	16	9			
Grand Total	**435**	**273**	**164**	**466**	**455**	**219**

Unsexed[1]: Condition of specimens was too poor for accurate sexing

3.4 DISCUSSION

3.4.1 Distribution of parthenogenetic versus sexual populations

Male *Ph. plantaginis* were confirmed for the first time in Victoria and reared primarily from *Pl. lanceolata* at six urban and suburban sites in Melbourne surveyed across 17 months. Additional sampling outside of Melbourne and in three other states showed males present in populations near Melbourne, and a reduction in their numbers as distance increased from the city, such that there were only females reared from all other states and at Victorian locations distant to Melbourne. A reduction in males in a northerly direction has been noted in populations from Sweden and Germany (Block 1969). Limited samples south of Melbourne and historical samples from Tasmania suggest that males may also be absent there, and all samples from New Zealand have also yielded only females (Spencer 1976). The reasons for this urban and peri-urban pattern of male presence are unclear.

Among the two species of *Phytomyza* with parthenogenesis, female-biased populations are not distributed consistently. The European range of *Phytomyza crassiseta* overlaps with *Ph. plantaginis*, extending slightly further south to northern Turkey (Martinez 2020). The German population of *Ph. crassiseta* tested by Hering was highly female-biased, however, he noted that more southern populations in Europe had an equal male-to-female ratio. Block (1969) documented apparently female-biased populations in several localities in Sweden, as well as in Helsinki in Finland and in other Scandinavian locations. For *Ph. plantaginis*, both sexes are present in Europe but only females have been confirmed from North America and New Zealand, suggesting female-only, parthenogenetic settlers. Invasions that included males have also occurred however, since both sexes are present in Japan. Frick (1951) investigated the likelihood of parthenogenetic reproduction in the Californian *Ph. plantaginis* by reproducing Hering's method, isolating unmated females with a *Plantago* sp. host plant and rearing the following female-only generation. This process was followed for a single additional generation and showed that new larvae were present in the plant. Interestingly, he reported that the females had a functional spermatheca and suggested that they could possibly reproduce sexually in the presence of male flies. It would also be worthwhile carrying out experimental validation of parthenogenesis in the Australian flies, including experimental crosses and observations on the spermatheca.

3.4.2 Host plants and other *Plantago* spp. agromyzids

The sex-ratio of flies reared from *Pl. lanceolata* was biased towards females in the Vic populations. The same bias was not observed in samples from the other two host species, where male and female flies were equally represented. While the sample size for flies reared from *Pl. coronopus* was small, this is not the case for the *Pl. major* samples. *Plantago major* favors moist disturbed habitat (VicFlora 2019) and was found only at the two sites bordering a creek, Fitzroy North and Flemington Bridge. Both *Pl. lanceolata* and *Pl. major* were abundant there in spring and throughout the year at the Flemington Bridge site. Since those samples share geography and season, the host plants are an obvious variable that might have an effect on the sex ratio of *Ph. plantaginis*. As the mechanism behind the presence of males and female bias in this agromyzid is unknown, it is uncertain whether fertilized females may have a preference for *Pl. major*, resulting in the significantly higher number of male offspring reared from it or if it is endosymbiont related. *Phytomyza plantaginis* feeds on other host plants that could be studied to compare their flies' sex-ratios. For example, in Japan they were reared from *Phytomyza kamtschatica* Link, and *Phytomyza mohnikei* Miq. (Sasakawa 1961) while in the USA, *Plantago rugelii* Decne and *Plantago wrightiana* Decne are also recorded as hosts (Eiseman *et al.* 2019). In Australia, there are 34 *Plantago* spp. recorded, 10 of which are introduced which potentially might be hosts for *Ph. plantaginis* (VicFlora 2019). Fecundity could vary between all-female and sexual populations and might be relevant to IPM plans. Higher numbers of viable larvae would improve their potential as a parasitoid reservoir, however whether there are differences is still unknown.

3.4.3 Future directions

Phytomyza plantaginis is of increasing interest in horticulture as a source of generalist hymenopteran parasitoids attacking pest *Liriomyza* spp. (Lambkin *et al.* 2008; Ridland *et al.* 2020) (Chapter 2). Its range in Australia is extensive but not very well studied and limited by the distribution of its host plant. Though originating from a temperate climate, *Plantago lanceolata* has been recorded not only throughout Tas, Vic and the east coast of NSW, but also in Alice Springs, and as far north as Cairns (Atlas of Living Australia 2020c) (Figure A3-2). Additional sampling will help delimit the distribution of *Ph. plantaginis* in Australia as well as the extent of the male and female populations. Narrow-leaved plantain cultivars are already used in New Zealand for dairy pastures (Lee *et al.* 2015) and are being considered as a drought-tolerant component to south-eastern Australian dairy pastures (Langworthy *et al.* 2018; Raedts & Langworthy 2019). Plants are already common on field margins and unless sprayed, an increased abundance in pastures will augment its potential as a source of parasitoids.

There is no literature on its temperature requirements or if its biology differs when reared on weedy *Plantago lanceolata* or cultivars used in pastures, both relevant in the event plantain pastures become more common and are sprayed. Furthermore, the agromyzid's parthenogenesis is of interest to study the appearance of asexuality as a reproduction mode. It needs to be confirmed in a systematic manner and the mechanism behind it should be investigated. It may involve endosymbionts such as *Wolbachia*, known to manipulate their host reproduction in a variety of ways, including in some cases by inducing parthenogenesis (Werren *et al.* 2008). The first steps would be to screen for their presence and compare life-histories and longevity between colonies of sexual and of female-only lines. Crossing experiments can then address the ability for parthenogenetic females to mate with males and if so, how their daily fecundity, fertility rate, egg and larval viability compares to unfertilized females and females from the sexual line. Additional rearing techniques will have to be developed to ensure flies can be easily collected and transferred for such experimental work.

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5 APPENDICES

5.1 APPENDIX 1.

Table A1-1. Australian and New Zealand literature records of *Liriomyza brassicae* host plants.

Common Name	Scientific Name	Family	State	Reference
Twiggy turnip	*Brassica fruticulosa*	(Brassicaceae)	Vic	**in this study**
Cabbage	*Brassica oleracea*	(Brassicaceae)	Qld	Kleinschmidt (1965)
Cauliflower	*Brassica rapa*	(Brassicaceae)	SA	Spencer (1977)
Turnip	*Brassica rapa* subsp. *rapa*	(Brassicaceae)	Vic	Spencer (1977)
Chinese cabbage	*Brassica rapa* subsp. *pekinensis*	(Brassicaceae)	Vic	Bjorksten (2005)
Sea Rocket	*Cakile maritima*	(Brassicaceae)	SA	Lardner (1991)
Spider flower	*Cleome graveolens*	(Capparaceae)	NZ	Spencer (1976)
Spider flower (bladderpod)	*Cleome isomeris (=Isomeris arborea)*	(Capparaceae)	SA	Spencer (1977)
Lincoln Weed	*Diplotaxis tennifolia*	(Brassicaceae)	SA	Lardner (1991)
Peppercress	*Lepidium africanum*	(Brassicaceae)	SA	Lardner (1991)
Wild Radish	*Raphanus raphanistrum*	(Brassicaceae)	SA	Lardner (1991)
			Vic	Bjorksten (2005)
Giant Mustard	*Rapistrum rugosum*	(Brassicaceae)	SA	Lardner (1991)
			Vic	Bjorksten (2005)
Hedge Mustard	*Sisymbrium officinale*	(Brassicaceae)	SA	Lardner (1991)
Indian Hedge	*Sisymbrium orientale*	(Brassicaceae)	SA	Lardner (1991)
Nasturtium	*Tropaeolum majus*	(Tropaeolaceae)	SA	Lardner (1991); Spencer (1977)
			NZ	Spencer (1976)
			Vic	Bjorksten (2005)

Table A1-2. Australian and New Zealand literature records of *Liriomyza chenopodii* host plants.

Common name	**Latin name**	**Family**	**State**	**Reference**
Beet/ White beet	*Beta vulgaris* subsp. *vulgaris*	(Amaranthaceae)	SA	Coquillett (1900)
			NZ	Watt (1924)
			VIC	Bjorksten (2005)
Chard	*Beta vulgaris* var *cicla*	(Amaranthaceae)	VIC	Bjorksten (2005)
Fodder beet	*Beta vulgaris* var *crassa*	(Amaranthaceae)	VIC	Bjorksten (2005)
			Tas.	Lea (from Coquillett, 1900)
Common chickweed	*Stellaria media*	(Caryophyllaceae)	NZ	Watt (1924)
			VIC	Bjorksten (2005)
			NSW	Malloch (1934)
Common mouse-ear chickweed	*Cerastium vulgare*	(Caryophyllaceae)	NZ	Watt (1924)
Fat hen	*Chenopodium album*	(Amaranthaceae)	Qld	Kleinschmidt (1965)
Spinach	*Spinacia oleracea*	(Amaranthaceae)	VIC	Bjorksten (2005)
			NSW	Malloch (1934)

Table A1-3. Australian and New Zealand literature records of *Phytomyza plantaginis* host plants.

Common Name	**Scientific Name**	**Family**	**State**	**Reference**
Buck's horn plantain	*Pl. coronopus*	(Plantaginaceae)	VIC	**in this study**
Ribwort plantain	*Pl. lanceolata*	(Plantaginaceae)	VIC	Bjorksten (2005)
			NZ	Spencer (1976)
Greater plantain	*Pl. major*	(Plantaginaceae)	VIC	Bjorksten (2005)
			NZ	Spencer (1976)

Table A1-4. Australian and New Zealand literature records of *Phytomyza syngenesiae* host plants.

Common Name	Scientific Name	Family	State	Reference
New Zealand celery	*Apium prostratum*	(Umbelliferae)	NZ	Martin (2017)*
Cape weed	*Arctotheca calendula*	(Asteraceae)	VIC	*in this study*
Common burdock	*Arctium minus*	(Asteraceae)	NZ	Spencer (1976)
Hedge artemisia	*Artemesia arborescens.*	(Asteraceae)	NZ	Martin (2017)*
Tarragon	*Artemisia dacunculus*	(Asteraceae)	NZ	Martin (2017)*
Marguerite daisy	*Argyranthemum frutescens* subsp. *foeniculum*	(Asteraceae)	VIC	Lambkin (2008)
Common daisy	*Bellis* sp.	(Asteraceae)	NZ	Harrison (1959)
Cobbler's pegs	*Bidens pilosa*	(Asteraceae)	VIC	*in this study*
Chrysanthemum	*Chrysanthemum sp.*	(Asteraceae)	NZ	Watt (1924)
Endive	*Cichorium endivia*	(Asteraceae)	VIC	Osmelak (1983)
Cineraria	*Cineraria sp.*	(Asteraceae)	NZ	Watt (1924)
Scotch thistle	*Cirisium sp.*	(Asteraceae)	NZ	Harrison (1959)
	Cryptostemma calendulacea	(Asteraceae)	NZ	Watt (1924)
Artichoke	*Cynara scolymus*	(Asteraceae)	VIC	Osmelak (1983)
Dahlia	*Dahlia* sp.	(Asteraceae)	NZ	Watt (1924)
German ivy	*Delairea odorata*	(Asteraceae)	NZ	Martin (2017)*
Fireweed	*Erechtites* sp.	(Asteraceae)	NZ	Watt (1924)
Toothed fireweed	*Erechtites minima*	(Asteraceae)	NZ	Spencer (1976)
Oxtongue	*Helminthotheca echioides*	(Asteraceae)	NZ	Martin (2017)*
Flatweed	*Hypochaeris radicata*	(Asteraceae)	NZ	Martin (2017)*
Candle plant	*Kleinia articulata*	(Asteraceae)	NZ	Martin (2017)*
Lettuce	*Lactuca sativa*	(Asteraceae)	VIC	Osmelak (1983)
			NZ	Martin (2017)*
Nipplewort	*Lapsana communis*	(Asteraceae)	NZ	Martin (2017)*
Catnip	*Nepeta cataria*	(Labiatae)	NZ	Spencer (1976)
Field-poppy	*Papaver rhoeas*	(Papaveraceae)	NZ	Martin (2017)*
Garden pea	*Pisum sativum*	(Fabaceaeae)	NZ	Martin (2017)*
Ragworts,	*Senecio* spp.	(Asteraceae)	VIC	*in this study*
			NZ	Spencer (1976)

Martin (2017)* Data retrieved by author from Plant-SyNZ database (15 April 2017), only plants with a reliability score of 10/10 for the evidence of host association were included.

Common Name	Scientific Name	Family	State	Reference
Prickly sow thistle	*Sonchus asper*	(Asteraceae)	VIC	*in this study*
			NZ	Spencer (1976)
Common sow thistle	*Sonchus oleraceus*	(Asteraceae)	VIC	Bjorksten (2005)
			NZ	Spencer (1976)
Dandelion	*Taraxacum* sp.	(Asteraceae)	VIC	*in this study*
			NZ	Spencer (1976)
White clover	*Trifolium repens*	(Fabaceaeeeae)	NZ	Spencer (1976)
Cocklebur	*Xanthium*	(Asteraceae)	NZ	Harrison (1959)

5.2 APPENDIX 2.

5.2.1 Temporal sampling tables

Table A2-1. Number of *Liriomyza brassicae* and parasitoids reared from *Brassica fruticulosa* collected at the Mt Waverley-Jordanville site during all quantitative surveys.

W# = week number; *L. b* = *Liriomyza brassicae*; Σ Par. = Sum of parasitoids; *O.* sp.2 = *Opius* morphospecies 2; *N. for* = *Neochrysocharis formosa*.

[green box] = Agromyzids and parasitoids; [cyan box] = Parasitoids only

W#	Date dd/mm/yyyy	#Plants	#Leaves	%Mined	*L. b*	Σ Par.	*O.* sp.2	*N. for*
40	01/10/2018	2	0	0%				
41	13/10/2018	0						
44	31/10/2018	0						
46	11/11/2018	0						
47	21/11/2018	11	0	0%				
49	04/12/2018	**50**	9	18%				
Pause in sampling								
11	10/03/2019	0						
18	30/04/2019	21	13	61%				
18	04/05/2019	38	21	55%	5	1	1	
24	04/06/2019	37	14	38%		1		1
30	22/07/2019	0						
35	25/08/2019	8	0	0%				
39	22/09/2019	0						
45	04/11/2019	2	0	0%				
48	26/11/2019	4	0	0%				
50	12/12/2019	2	0	0%				
Grand Total					**5**	**2**	**1**	**1**

Table A2-2. Number of *Liriomyza brassicae* and parasitoids reared from *Brassica fruticulosa* collected at the Syndal – Mt Waverley site during all quantitative surveys.

W# = week number; *L. b* = *Liriomyza brassicae*; Σ Par. = Sum of parasitoids; *O.* sp.1 = *Opius* morphospecies 1; *A.* sp. = *Asecodes* sp.; *O. cin* = *Opius cinerariae*.

= Agromyzids only; = Agromyzids and parasitoids

W#	**Date** **dd/mm/yyyy**	**#Plants**	**#Leaves**	**%Mined**	***L. b***	**Σ Par.**	***O.* sp.1**	***A.* sp.**	***O. cin***
37	13/09/2018	**50**	0	0%					
40	01/10/2018	11	0	0%					
41	13/10/2018	0							
44	31/10/2018	8	0	0%					
46	211/11/018	0							
47	21/11/2018	22	0	0%					
49	04/12/2018	27	6	22%	1				
Pause in sampling									
11	10/30/2019	10	2	20%	2	2	1		1
18	30/04/2019	**50**	36	72%	20	3	2	1	
18	04/05/2019	**50**	39	78%	9	4	3	1	
24	10/06/2019	40	0	0%					
30	22/07/2019	**50**	0	0%					
35	25/08/2019	**50**	0	0%					
39	222/09/019	0	0						
45	04/11/2019	0	0						
48	26/11/2019	4	0	0%					
50	12/12/2019	0	0						
Grand Total					**32**	**9**	**6**	**2**	**1**

Table A2-3. Number of *Liriomyza brassicae* and parasitoids reared from *Brassica fruticulosa* collected at the Diggers Rd site during all quantitative surveys.

W# = week number; *L. b* = *Liriomyza brassicae*; Σ Par. = Sum of parasitoids; *N. for* = *Neochrysocharis formosa*; *O. cin*= *Opius cinerariae*; *Z. lat* = *Zagrammosoma latilineatum*.

☐ = Agromyzids only; ☐ = Parasitoids only

W#	Date dd/mm/yyyy	#Plants	#Leaves	%Mined	*L. b*	Σ Par.	*N. for*	*O. cin*	*Z. lat*
40	02/10/2018	**50**	0	0%					
42	16/10/2018	47	0	0%					
46	13/11/2018	0							
47	20/11/2018	0							
49	03/12/2018	**50**	2	4%					
Pause in sampling									
10	07/03/2019	**50**	37	74%		9	7	1	1
12	20/03/2019	**50**	28	56%					
14	03/04/2019	**50**	30	60%	1				
16	15/04/2019	**50**	18	36%			1		
20	16/05/2019	**50**	9	18%					
26	24/06/2019	**50**	0	0%					
32	07/08/2019	**50**	0	0%					
38	19/09/2019	**50**	0	0%					
41	10/10/2019	**50**	0	0%					
44	31/10/2019	**50**	3	6%					
51	17/12/2019	**50**	16	32%					
2	11/01/2020	**50**	0	0%					
Grand Total					**1**	**9**	**8**	**1**	**1**

Table A2-4. Number of *Liriomyza brassicae* and parasitoids reared from *Brassica fruticulosa* collected at the Fitzroy North site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *L. b* = *Liriomyza brassicae*; Σ Par. = Sum of parasitoids.

☐ = Agromyzids only

W#	Date dd/mm/yyyy	#P	#L	%M	*L. b*	Σ Par.
42	16/10/2018	**50**	4	8%	2	
40	01/11/2018	34	2	6%		
47	20/11/2018	**50**	15	30%		
Pause in sampling						
11	15/03/2019	22	17	77%		
14	03/04/2019	46	33	72%	3	
21	22/05/2019	**50**	27	54%		
30	25/07/2019	**50**	0	0%		
38	20/09/2019	**50**	0	0%		
42	17/10/2019	35	0	0%		
44	31/10/2019	**50**	0	0%		
Grand Total					**5**	

Table A2-5. Number of *Phytomyza plantaginis* and parasitoids reared from *Plantago lanceolata* collected at the Federation Trail site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. p* = *Phytomyza plantaginis*; Σ Par. = Sum of parasitoids; *D. isa* = *Diglyphus isaea*; *N. for* = *Neochrysocharis formosa*; *C. pub* = *Chrysocharis pubicornis*; *A.* sp. = *Asecodes* sp.; *H. var* = *Hemiptarsenus varicornis*; Un = Unidentified.

☐ = Agromyzids only; ☐ = Agromyzids and parasitoids

W#	Date dd/mm/yyyy	#P	#L	%M	*P. p*	Σ Par.	*D. isa*	*N. for*	*C. pub*	*H. var*	*A.* sp.	Un
38	20/09/2018	**50**	10	20%	5							
40	02/10/2018	**50**	34	68%	109	5			5			
42	16/10/2018	**50**	47	94%	168	42	23	6	4	6	1	2
46	13/11/2018	40	37	93%	74	12	5	6				1
47	20/11/2018	**50**	43	86%	19	7	1	6				
49	03/12/2018	**50**	32	64%	4	4		4				
Pause in sampling												
10	07/03/2019	0										
12	20/03/2019	0										
14	03/04/2019	0										
16	15/04/2019	5	0	0%								
20	16/05/2019	30	0	0%								
26	24/06/2019	36	0									
32	07/08/2019	25	0	0%								
38	19/09/2019	**50**	0	0%								
41	10/10/2019	**50**	0	0%								
44	31/10/2019	**50**	4	8%	2							
51	17/12/2019	37	5	14%								
2	11/01/2020	4	0	0%								
Grand Total					**381**	**70**	**29**	**22**	**9**	**6**	**1**	**3**

Table A2-6. Number of *Phytomyza plantaginis* and parasitoids reared from *Plantago lanceolata* collected at the Diggers Rd site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. p* = *Phytomyza plantaginis*; Σ Par. = Sum of parasitoids; *D. isa* = *Diglyphus isaea*; *A.* sp. = *Asecodes* sp.; *H. var* = *Hemiptarsenus varicornis*; *T. par* = *Trigonogastrella parasitica*.

= Agromyzids only; = Agromyzids and parasitoids; = Parasitoids only

W#	**Date** dd/mm/yyyy	**#P**	**#L**	**%M**	***P. p***	**Σ Par.**	***D. isa***	***A.* sp.**	***H. var***	***T. par***
40	02/10/2018	6	6	100%	39					
42	16/10/2018	**50**	42	84%	72	15	13	1	1	
46	13/11/2018	0								
47	20/11/2018	0								
49	03/12/2018	31	14	45%	9					
Pause in sampling										
10	07/03/2019	0								
12	20/03/2019	0								
14	03/04/2019	0								
16	15/04/2019	7	0	0%						
20	16/05/2019	3	0	0%						
26	24/06/2019	0								
32	07/08/2019	1	0	0%						
38	19/09/2019	**50**	1	2%	1					
41	10/10/2019	**50**	3	6%	1					
44	31/10/2019	**50**	1	2%		1				1
51	17/12/2019	0								
2	11/01/2020	8	2	25%						
Grand Total					**122**	**16**	**13**	**1**	**1**	**1**

Table A2-7. Number of *Phytomyza plantaginis* and parasitoids reared from *Plantago lanceolata* collected at the Fitzroy North site during all quantitative surveys.

W# = week number; *P. p* = *Phytomyza plantaginis*; Σ Par. = Sum of parasitoids; *T. par* = *Trigonogastrella parasitica*.

[yellow] = Agromyzids only; [green] = Agromyzids and parasitoids

W#	Date dd/mm/yyyy	#plants	#leaves	%mined	*P. p*	Σ Par.	*T. par*
42	16/10/2018	**50**	24	48%	47		
40	09/11/2018	**50**	19	38%	22	1	
47	20/11/2018	**50**	36	72%	7		1
Pause in sampling							
11	15/03/2019	46	0	0%			
14	03/04/2019	**50**	1	2%			
21	22/05/2019	30	0	0%			
30	25/07/2019	30	1	3%			
38	20/09/2019	**50**	0	0%			
42	17/10/2019	**50**	1	2%	1		
44	31/10/2019	23	14	61%	7		
Grand Total					**84**	**1**	**1**

Table A2-8. Number of *Phytomyza syngenesiae* and parasitoids reared from *Sonchus* oleraceus collected at the Federation Trail site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. s* = *Phytomyza syngenesiae*; Σ Par. = Sum of parasitoids; *A.* sp. = *Asecodes* sp.; *D. isa* = *Diglyphus isaea; H. var* = *Hemiptarsenus varicornis*; *C. mir* = *Closterocerus mirabilis*; Un = Unidentified.

(green) = Agromyzids and parasitoids; (blue) = Parasitoids only

W#	**Date** dd/mm/yyyy	**#P**	**#L**	**%M**	***P. s***	**Σ Par.**	***A.* sp.**	***D. isa***	***H. var***	***C. mir***	**Un**
38	20/09/2018*	**50**	0	0%							
40	02/10/2018	**50**	0	0%							
42	16/10/2018	0									
46	13/11/2018	**50**	0	0%							
47	20/11/2018	**50**	2	4%							
49	03/12/2018	24	0	0%							
Pause in sampling											
10	07/03/2019	24	0	0%							
12	20/03/2019	6	0	0%							
14	03/04/2019	0									
16	15/04/2019	0									
20	16/05/2019	0									
26	24/06/2019	0									
32	07/08/2019	29	0	0%							
38	19/09/2019	**50**	28	56%							
41	10/10/2019	**50**	50	100 %	62	37	26		5		8
44	31/10/2019	**50**	33	66%		128	90	12	5	3	18
51	17/12/2019	3	0	0%							
2	11/01/2020	0									
Grand Total					**62**	**165**	**116**	**12**	**10**	**3**	**24**

*then only identified as "*Sonchus* sp."

Table A2-9. Number of *Phytomyza syngenesiae* and parasitoids reared from *Sonchus oleraceus* collected at the Diggers Rd site during all quantitative surveys.

W# = week number; #P = # plants; #L = # leaves mined; %M = % of plants found mined; *P. s* = *Phytomyza syngenesiae*; Σ Par. = Sum of parasitoids; *D. isa* = *Diglyphus isaea*; *A*. sp. = *Asecodes* sp.; *H. var* = *Hemiptarsenus varicornis; C. mir* = *Closterocerus mirabilis*; *Z. lat* = *Zagrammosoma latilineatum*; Un = Unidentified.

= Agromyzids only; = Agromyzids and parasitoids; = Parasitoids only

W#	**Date** dd/mm/yyyy	**#P**	**#L**	**%M**	***P. s***	**Σ Par.**	***D. isa***	***A.* sp.**	***H. var***	***C. mir***	***Z. lat***	**Un**
40	02/10/2018	40	0	0%								
42	16/10/2018	0										
46	13/11/2018	0										
47	20/11/2018	0										
49	03/12/2018	4	0	0%								
Pause in sampling												
10	07/03/2019	0										
12	20/03/2019	6	0	0%								
14	03/04/2019	12	0	0%								
16	15/04/2019	13	0	0%								
20	16/05/2019	0										
26	24/06/2019	3	0									
32	07/08/2019	28	0	0%								
38	19/09/2019	**50**	4	8%	1	2				1		1
41	10/10/2019	**50**	12	24%	15							
44	31/10/2019	**50**	11	22%		15		3	4	4		4
51	17/12/2019	**50**	24	48%	6	46	31	10	3		2	
2	11/01/2020	8	2	25%		1					1	
Grand Total					**22**	**61**	**31**	**13**	**7**	**5**	**3**	**5**

Table A2-10. Number of *Phytomyza syngenesiae* and parasitoids reared from *Sonchus oleraceus* collected at the Fitzroy North site during all quantitative surveys.

W# = week number,*P. s* = *Phytomyza syngenesiae*; Σ Par. = Sum of parasitoids; *A.* sp. = *Asecodes* sp.; *C. mir* = *Closterocerus mirabilis*; *N. for* = *Neochrysocharis formosa*.

☐ = Agromyzids only; ☐ = Agromyzids and parasitoids

W#	Date dd/mm/yyyy	#plants	#leaves	%mined	*P. s*	Σ Par.	*A.* sp.	*C. mir*	*N. for*
42	16/10/2018	20	0	0%					
40	01/11/2018	26	2	8%	1				
47	20/11/2018	**50**	11	22%	3	2	1		1
Pause in sampling									
11	15/03/2019	3	0	0%					
14	03/04/2019	4	0	0%					
21	22/05/2019	5	0	0%					
30	25/07/2019	14	0	0%					
38	20/09/2019	**50**	0	0%					
42	17/10/2019	**50**	1	2%	2	1	1		
44	31/10/2019	**50**	8	16%	2	2	1	1	
Grand Total					**8**	**5**	**3**	**1**	**1**

5.2.2 Weather data

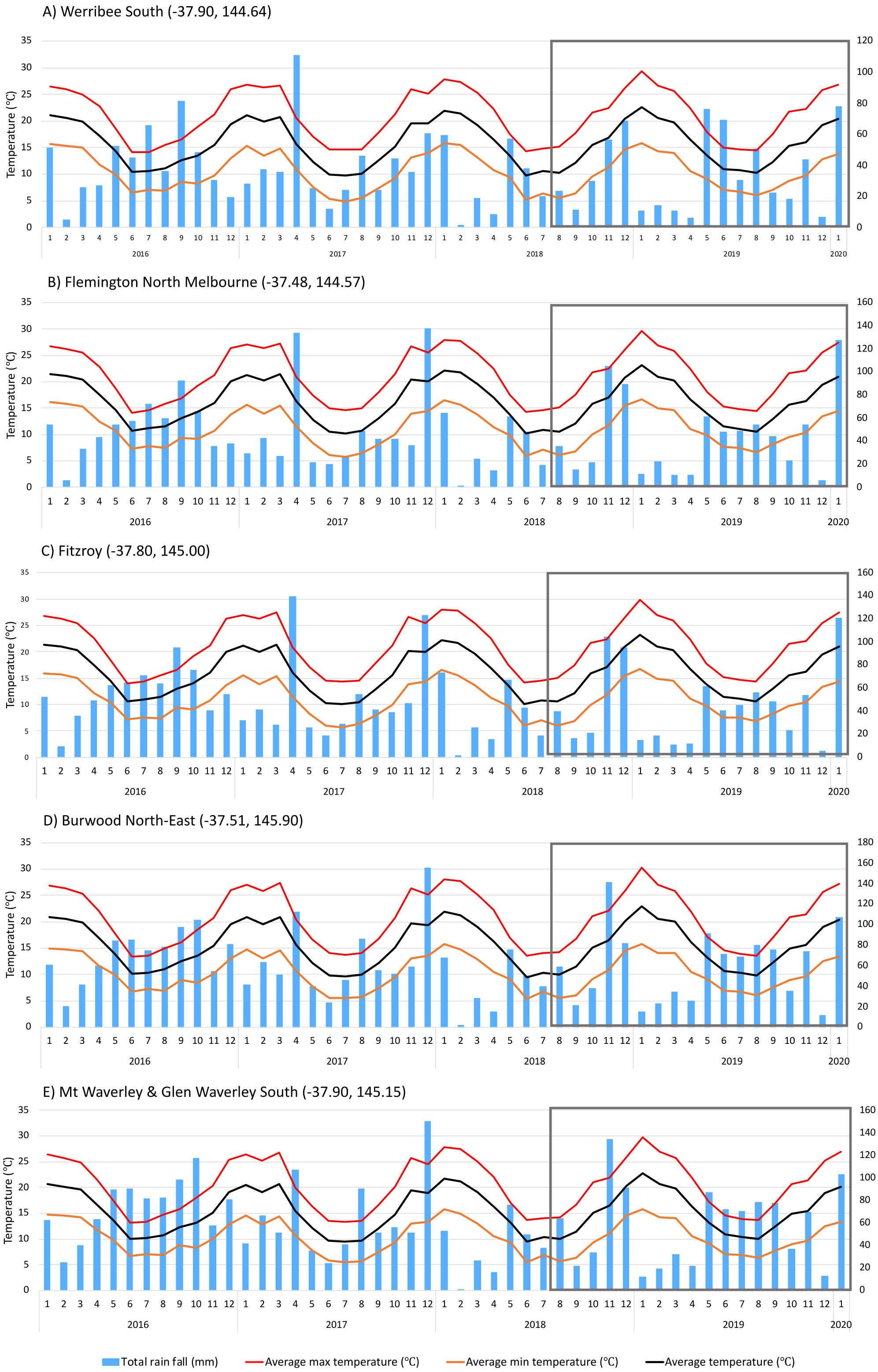


Figure A2-1. Total rainfall, and average temperatures for localities around Melbourne. A) for Federation Trail and Diggers Rd sites, B) for Flemington Bridge, C) North Fitzroy, D) & E) are north and south from the Mount Waverley sites. Graphs based on SILO, Australian climate database (Jeffrey *et al.* 2001).

5.2.3 *Asecodes* sp.

The species was confirmed to belong to the subfamily Entedoninae based on the following traits: two setae present dorsally on the submarginal vein and only one pair of setae on the scutellum (Figure A2-2:1), A2-2:2)). The presence of sensory pores on the ventral edge of the male scape could not be confirmed. It possesses two characteristics unique to the genus *Asecodes* as described by Hansson (1996). Its head has a distinct and elongated occipital groove (Figure A2-2: 1)), and it also has two rows of setae radiating from the stigmal vein of the forewing towards the apical end (Figure A2-2: 3)). Other traits corresponding to the genus and observed in the specimens include the flagellum, with two funicular segment and a three-segmented club, a postmarginal vein shorter than the stigmal vein and a very short pronotum without transverse carina. It is lightly sculptured, with a smooth propodeum and with a dark blueish metallic coloration for the head and body.

Hansson 1996 synonymized *Teleopterus* Silvestri with *Asecodes*. *Teleopterus* was originally diagnosed by the presence of two to three lines of setae from the stigmal vein, and all *Asecodes* parasitoids reared from leafminers in this study possess two lines of setae.

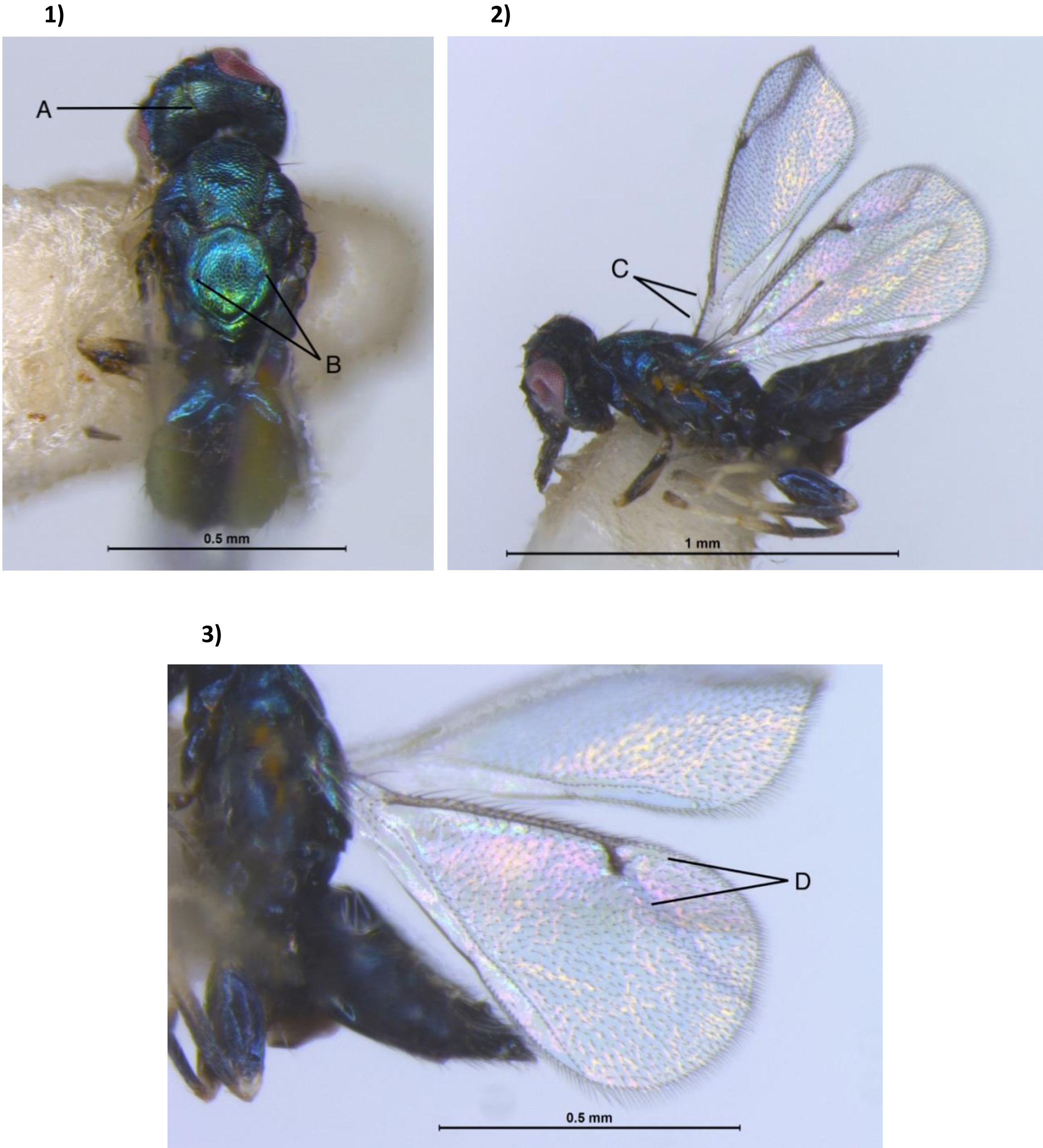


Figure A2-2. Morphological details for *Asecodes* sp. (♀); **1)** head and mesosoma, dorsal view; **2)** body, lateral view; **3)** wing close-up. A = occipital groove; B = one pair of scutellar setae; C = two setae on submarginal vein; D = two rows of setae stemming from the stigma vein.

5.2.4 *Opius* morphospecies

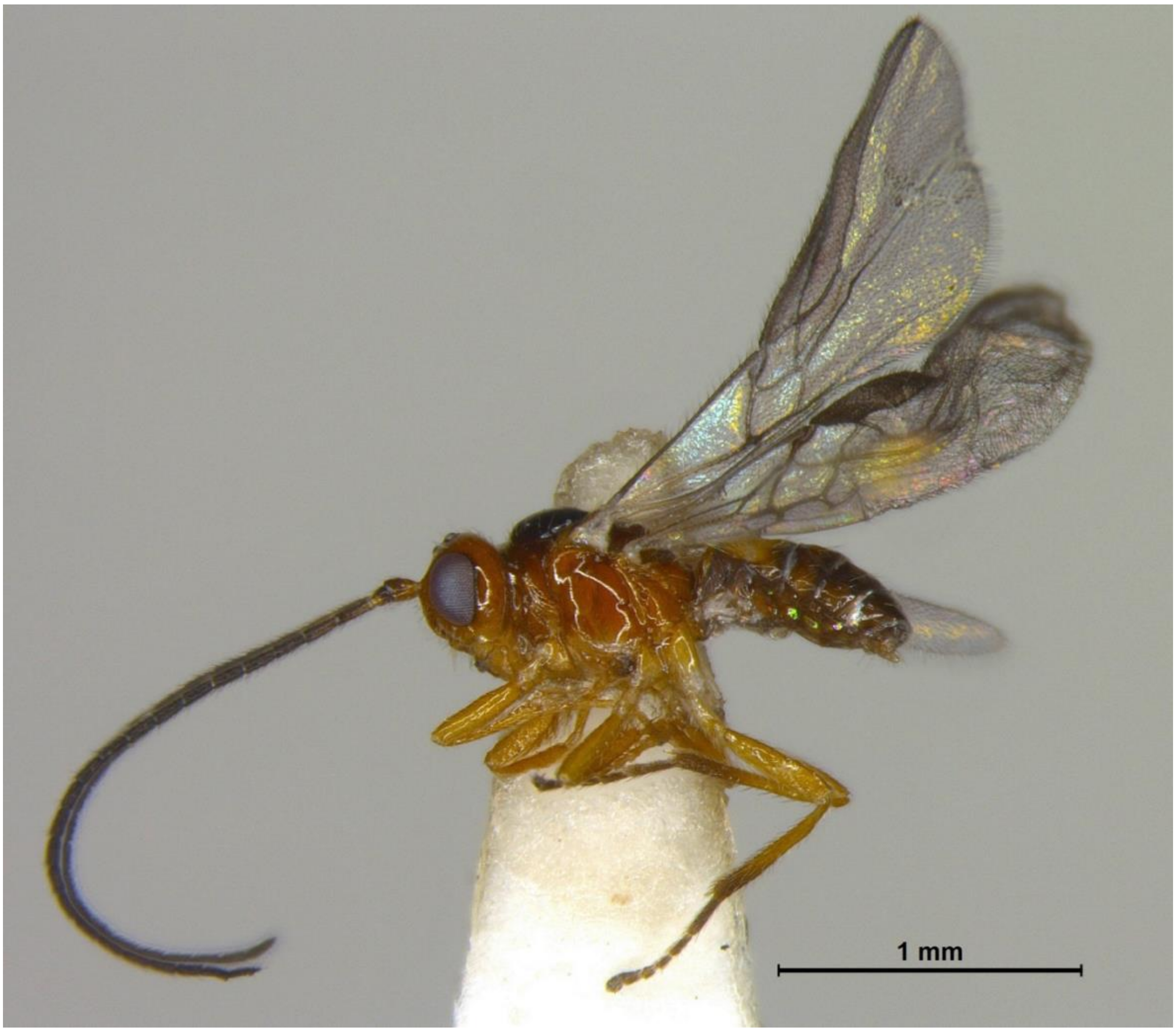
Opius Msp1. (Figure A2-3).

Head. distinctly transverse*,* rounded behind eyes, ocelli positioned in equilateral triangle. Malar suture absent, clypeus narrow and crescent shaped, face with a depression between the antennal sockets. *Antennae.* slender and filiform, 22 segmented (based on single specimen). *Body.* Absence of any pits on the mesosoma. Notauli present, minimally curved, reaching the posterior edge or the mesoscutum. Postmesoscutum border faintly rounded towards the posterior. Scutellar sulcus with depression narrow and long, across the width of the scutellum, crenulate. Meso-posterior scutellum depression present. Subalar depression shallow, sternauli absent, mesopleura slightly concave. Episternauli not distinctly present, visible as an indentation across the mesopleura. Minute indentation just below the halfway point along the posterior edge of the mesopleura. *Wing*. Pterostigma not wedge shaped, instead slightly oblong.

Sculpture. Head smooth. Mesonotum entirely smooth. Propleura smooth, with some faint narrow oblique grooves on the anterior end. Metapleura smooth, some ripples closest to the hind wing. Propodeum largely smooth, second metasomal tergite finely coriaceous. Remaining tergites smooth.

Color. Body and head burnt orange, majority of the mesoscutum shiny black, with some variation to a lighter brown color. Some darkening between the ocelli. Below eyes, clypeus and mandibles lighter orange, yellowish. Palpi pale yellow. Antennae brown, proximal half of the scape burnt orange. Ventral aspect of the body, up to $1/5^{th}$ of the mesopleuron, from head to metapleuron, pale orange, yellowish. Coxae and legs, first and second metasomal tergites are pale orange, yellowish. Remaining tergites brown. Pterostigma and major veins brown. Fore wings appear mostly hyaline. In appearance closest to the *Opius* sp. 1 reared by Lambkin *et al.* (2008) (Figure A2-4).

4)



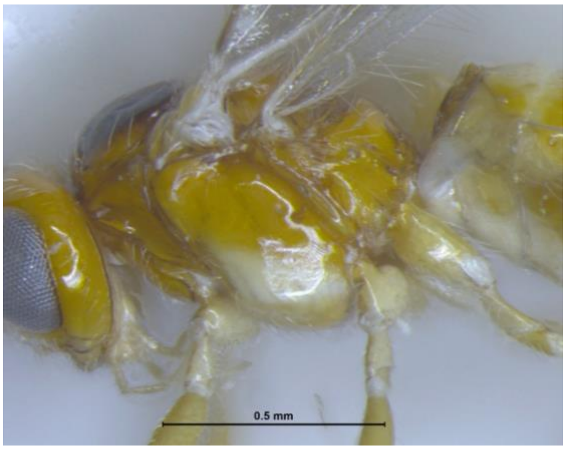
5)

6)

7)

Figure A2-3. Morphological details for *Opius* Msp 1; **4)** lateral view; **5)** mesosoma, lateral view; **6)** head, frontal view; **7)** head and mesosoma, dorsal view.

8)



9)

Figure A2-4. Morphological details for *Opius* species 1 reared from *Phytomyza syngenesiae* in the Canberra area (ACT) by Lambkin *et al.* (2008); **8)** mesosoma, lateral view (only photo not white-corrected, coloration closer to image 9)); **9)** head and mesosoma, dorsal view.

Opius Msp2. (Figure A2-5).

Head. distinctly transverse, rounded behind the eyes, and ocelli positioned in equilateral triangle. Malar suture absent. Clypeus narrow and crescent shaped, face with a depression between the antennal sockets. *Antennae.* slender and filiform, 19 segmented. *Body.* Absence of any pits on mesosoma. Notauli present, minimally curved, does not reach the posterior edge of the mesoscutum, instead one third its length. Postmesoscutum border rounded towards anterior end. Scutellar sulcus with depression narrow and long, across the width of the scutellum, crenulate. Meso-posterior scutellar depression present. Sternauli absent, mesopleura slightly concave. Episternauli indistinct, visible as an indentation across the mesopleura. Minute indentation just below the halfway point along the posterior edge of the mesopleura. Propodeum smooth. Metanotum narrow and rugose. *Wing.* Pterostigma narrow and elongated, wedge shaped.

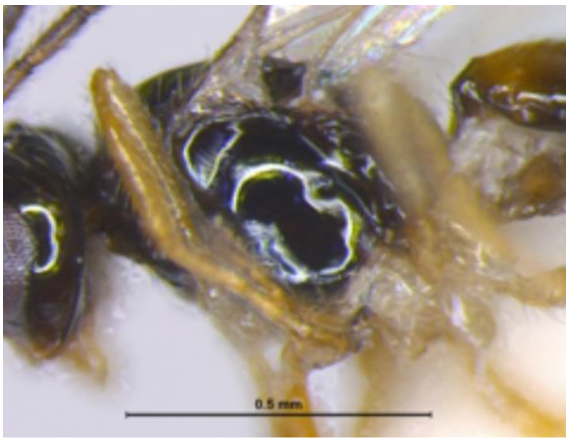
Sculpture. Head smooth. Mesonotum entirely smooth. Propleura smooth, with some faint oblique grooves on the anterior end. Mesopleura smooth, some ripples closest to the dorsal half. Propodeum largely smooth, first metasomal tergite coriaceous (striae not visible on photos). Remaining tergites smooth.

Color. Body shiny black. Below eyes, clypeus and mandibles yellowish. Palpi pale yellow. Antennae and scape brown. Coxae and legs yellowish. First mesosomal tergite with light brown tinge, remaining tergites brown nearly black. Pterostigma and major veins brown. Fore wings appear mostly hyaline.

Opius cinerariae (Figure A2-6).

10)

11)

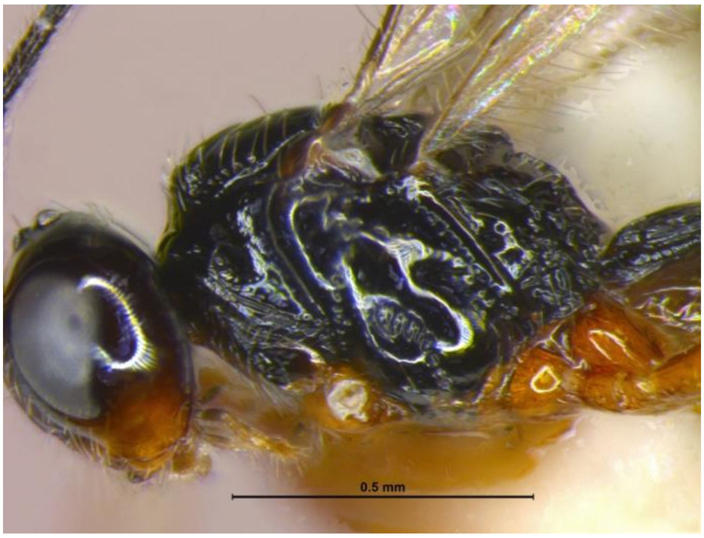


12)

13)

Figure A2-5. Morphological details for *Opius* Msp2; **10)** lateral view; **11)** mesosoma, lateral view; **12)** head, frontal view; **13)** head and mesosoma, dorsal view.

14)



15)

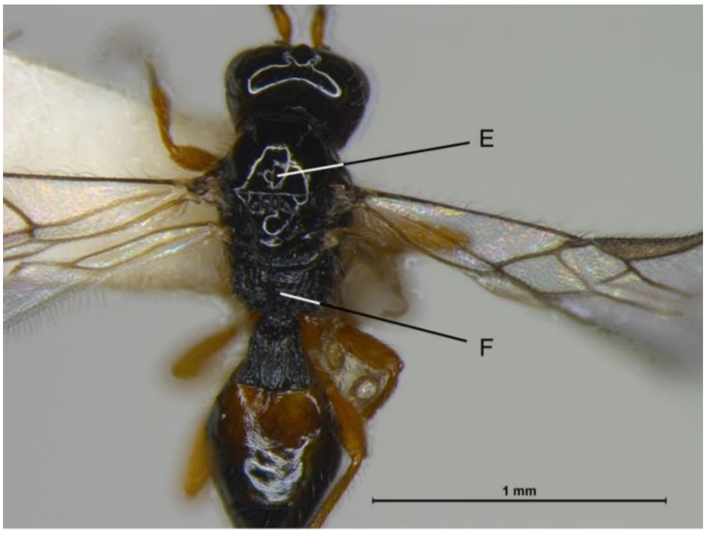


Figure A2-6. Morphological details for *Opius cinerariae*; **14)** mesosoma, lateral view; **15)** head and mesosoma, dorsal view. E = single round median pit on the mesoscutum; F = propodeum textured or rugose.

Comparison of *Opius* morphospecies

The main combination of features distinguishing *Opius* Msp1 and 2 are their body and head coloration, as well as the shape of their postmesoscutum border (rounded anteriorly in *Opius* Msp1 and straight for *Opius* Msp2). This project's *Opius* Msp1 most closely resembles *Opius* species 1 collected by Lambkin *et al.* (2008) in Canberra (ACT) from *Phytomyza syngenesiae* on a range of hosts, due to its body coloration, mesoscutum and mesopleura appearance.

Opius Msp2 from alternative hosts

Four wasps were reared from *L. chenopodii*, three from *L. brassicae*, while the rest of the *Opius* Msp2 were reared from mined saltbush *Rhagodia parabolica*, similar to the Wood *et al.* (2010) study that reared parasitoids out of two saltbushes, *R. candolleana* and *R. parabolica*. *Opius* Msp2 is similar to *O. chromatomyiae,* with *O. chromatomyiae* different based on the following traits: a wider pterostigma vein, a wider clypeus (twice as wide as it is high, semicircular), narrower hypoclypeus, mandibles with a characteristic shape (apical two-thirds narrow before a widening of the basal border). Both photo angle and magnification did not allow for the clypeus and mandible shapes to be compared between the two *Opius.* Folding on the forewing of *Opius* Msp2 makes it hard to determine with certainty the pterostigma shape, and for that reason it cannot be conclusively said that *Opius* Msp2 is not *O. chromatomyiae*. No molecular work done to date has confirmed its identity.

Table A2-11. Parasitoid wasp species reared from past surveys of four agromyzid leafminers in Australia.

		Liriomyza brassicae		*L. chenopodii*	*Phytomyza plantaginis*	*Phytomyza syngenesiae*	
Family	Species	Lardner (1991) SA	Bjorksten *et al.* (2005) VIC	Bjorksten *et al.* (2005) VIC	Lambkin *et al.* (2008) ACT	Bjorksten *et al.* (2005) VIC	Lambkin *et al.* (2008) ACT
Braconidae	Braconidae sp.	-	-	-	-	-	X
	Dacnusa areolaris	-	-	X	-	-	-
	Opius atricornis	X	-	-	-	-	-
	O. cinerariae	X	-	X	-	-	-
	Opius sp. 1	-	-	-	-	-	X
	Opius sp. 2	-	-	-	-	-	X
	Opius sp. 3	-	-	-	-	-	X
Eulophidae	*Asecodes* sp.	-	-	-	-	-	X
	Closterocerus sp.*	X	-	-	-	-	-
	C. mirabilis	-	X	X	-	X	X
	Chrysocharis pubicornis	X	-	-	-	-	-
	Chrysonotomyia sp.***	X	-	-	-	-	-
	Diglyphus isaea	-	X	X	X	X	-
	Entedoninae genus A	-	-	X	-	-	-
	Entedoninae genus B	-	-	X	-	-	-
	Entedoninae genus C	-	-	-	-	X	-
	Hemiptarsenus varicornis	X	X	X	X	-	X
	Neochrysocharis sp.	-	-	-	X	X	-
	N. formosa	-	-	-	-	-	-
	N. okazakii	-	X	-	-	-	-
	Stenomesius sp. **	-	X	-	-	-	-
	Zagrammosoma sp.*	X	-	-	-	-	-
	Z. latilineatum	-	X	-	-	-	-
Pteromalidae	*Pachyneuron* sp.	-	-	-	-	X	-
	Pteromalidae sp.	-	-	-	X	-	-
	Trigonogastrella sp.	-	-	-	-	-	-
	T. parasitica	-	-	-	-	-	X
Diapriidae	Unknown sp.***	-	-	X	-	-	-

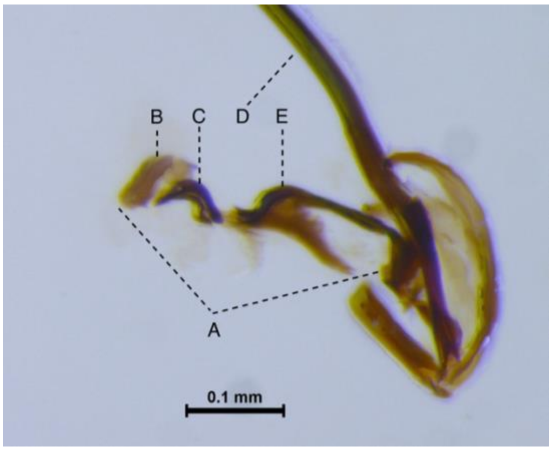
**Closterocerus* sp. likely *Closterocerus mirabilis*; **Zagrammosoma* sp. likely *Zagrammosoma latilineatum;* ***Stenomius* sp. is a genus known as lepidopteran parasitoids.

5.3 APPENDIX 3.

Male genitalia dissection

The genitalia preparation was performed by Dr M. Malipatil, following the methods described by Malipatil & Ridland (2008) for polyphagous agromyzid leafminers. More detailed instructions can be found in EPPO (2005) and Spencer (1981). The only differences in methodology was the use of 10% potassium hydroxide KOH at room temperature and a longer period of soaking for the specimens of 15 min instead of boiling KOH and soaking for 2 to 3 min. Specimens had previously been stored in an ethanol concentration of 80%.

(a)



(b)

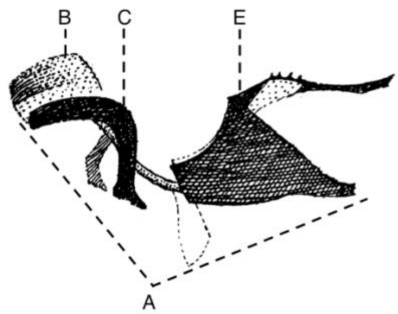


Figure A3-1. (a) *Phytomyza plantaginis* male genitalia dissection, aedeagus, side view, photographed with compound microscope, magnification (x400); (b) Spencer (1987) European specimen *Ph. plantaginis* male genitalia, aedeagus, side view, illustrated.

A = aedeagus; B = distiphallus; C = mesophallus; D = aedeagal apodeme; E = basiphallus.

Figure A3-2. Map of the distribution of records for the host plant *Plantago lanceolata* L. (Plantaginaceae) ribwort plantain (Atlas of Living Australia 2020c).