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Seasonal Abundance of Various Hymenopteran Parasitoids of Leafminers in Beans and Comparative Abundance in Bean, Tomato, and Squash

Dakshina R. Seal [1,*], Oscar Liburd [2] and Jian Li [3]

1 Tropical Research and Education Center, University of Florida-IFAS, Homestead, FL 33033, USA
2 Entomology and Nematology Department, University of Florida-IFAS, Gainesville, FL 32603, USA; oeliburd@ufl.edu
3 Independent Researcher, 1828 SW 63RD AVE, Gainesville, FL 32608, USA; lijian59@gmail.com
* Correspondence: dseal3@ufl.edu

Simple Summary: Vegetable leafminer, *Liriomyza trifolii* (Burgess) is a common pest of almost all vegetable crops grown in Florida, USA. Leafminer infestation begins as soon as vegetable seedlings are above the ground. Growers start using insecticides of various chemical classes to control leafminer. They continue insecticide sprays until 2–3 weeks before harvesting crops. This practice of using insecticides deleteriously affects our biotic and abiotic environments. In our four-years research study, we recorded thirteen species of hymenopteran parasitoids of *L. trifolii* belonging to three families: Braconidae, Eulophidae, and Pteromalidae. Among all parasitoids, *Opius*, *Euopius*, *Diglyphus*, and *Diaulinopsis* were the predominant ones on beans and other vegetables. Their peak abundance occurred in November–January while most vegetable crops are in their peak growing stage. This study also investigated the preference of parasitoids for the three major vegetable crops: bean, squash, and tomato planted in randomized complete block design in field plots. This information could be of great benefit in developing an IPM of vegetable leafminers balancing biocontrol agents and insecticide application.

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Citation: Seal, D.R.; Liburd, O.; Li, J. Seasonal Abundance of Various Hymenopteran Parasitoids of Leafminers in Beans and Comparative Abundance in Bean, Tomato, and Squash. *Agriculture* **2023**, *13*, 1460. https://doi.org/10.3390/agriculture13071460

Academic Editor: David João Horta Lopes

Received: 26 May 2023
Revised: 15 July 2023
Accepted: 18 July 2023
Published: 24 July 2023

Abstract: The composition and seasonal abundance of hymenopteran parasitoids of *Liriomyza trifolii* (Burgess) was investigated on snap bean (*Phaseolus vulgaris* L.), tomato (*Solanum lycopersicum* L.), and squash (*Cucurbita pepo* L. '*Enterprise*') from 2010 to 2016 in South Florida in two studies. In the first study (2010–2016), 13 species of parasitoids were collected from the snap bean crop. *Opius dissitus* Muesebeck (Braconidae) was the most abundant parasitoid throughout the study period from September 2010 to February 2016. *Diaulinopsis callichroma* Crawford (Eulophidae) was the second most abundant parasitoid on bean in 2010, 2012, 2014, and 2016. Other parasitoids included *Euopius* sp. (Braconidae)., *Diglyphus begini* (Ashmead), *D. intermedius* (Girault), *D. isaea* (Walker), *Neochrysocharis* sp., *Closterocerus* sp., *Chrysocharis* sp., *Zagrammosoma lineaticeps* (Girault), *Z. muitilineatum* (Ashmead), *Pnigalio* sp. (all Eulophidae), and *Halticoptera* sp. (Pteromalidae). In the second study on the comparative abundance of parasitoids in three crops conducted in 2014 and 2016 using bean (*Phaseolus vulgaris* L., tomato (*Solanum lycopersicum* L.) and squash (*Cucurbita pepo* L.) arranged in a randomized complete block design, bean attracted more parasitoids than tomato and squash irrespective of parasitoid species and years. This information will help in devising a biocontrol-based integrated program for managing leafminers in beans and other vegetable crops.

Keywords: *L. trifolii*; hymenopteran parasitoids; seasonal abundance; biological control; vegetable crops

1. Introduction

South Florida is a major vegetable production area in the USA, ranking first in the value of fresh market snap bean and cucumber production and second in squash and tomato production [1]. Various insect pests including sweet potato whitefly (*Bemisia tabaci*

Biotype B or MEAM1) (Hemiptera: Aleyrodidae), thrips of various species (Thysanoptera: Thripidae), aphids (Homoptera: Aphididae), cucumber beetle (*Diabrotica* spp., Coleoptera: Chrysomelidae), armyworms (*Spodoptera frugiperda* (J.E. Smith, Lepidoptera: Noctuidae), and leafminers (Diptera: Agromyzidae) infest these vegetable crops and cause economic damage. Among the insect pests, the leafminers (*Liriomyza* spp.), comprising of more than 300 spp., have a significant economic impact on vegetable production worldwide [2,3]. Yield losses may account for >70% in snap bean and potato [4]. Leafminers, *Liriomyza trifolii* (Burgess), *L. sativae* (Riley), and *L. huidobrensis* (Blanchard) are important in threatening US agriculture [5–8]. Among leafminers, *L. trifolii* has a broad host range consisting of about 400 species of plants [6,9] and poses a serious threat to global agriculture [10]. Commercial growers use broad spectrum insecticides as a primary means for controlling *Liriomyza* leafminers [11,12]. As the development of leafminers takes place inside leaf-mines, most of these insecticides only target the adult fly and fail to provide effective control against other life stages. The ultimate result of widespread and intensive use of insecticides has a high potential for developing leafminer resistance against these insecticides [13,14]. Most importantly, the use of insecticides decimates potential natural enemies of leafminers and causes an outbreak of leafminer abundance [13,15].

Hymenopteran parasitoids possess potentials in suppressing initial populations of leafminers [6]. Noyes [16] reported about 300 species of parasitoids that attack agromyzid leafminers, and about 80 of them were listed to parasitize larvae of *Liriomyza* species. Stegmaier [17] recorded 31 species of hymenopteran parasitoids in 19 genera and five families that parasitize agromyzid leafminers in South Florida. Many of these species in Braconidae and Eulophidae are potential parasitoids of agromyzid leafminers [3]. Several regional reviews on leafminer parasitoids in Asia include Petcharat et al. [18], Xu et al. [19], Zhu et al. [20]. *Neochrysocharis formosa* (Westwooe) (Hymenoptera: Eulophidae) is a larval endoparasitoid and has been used to control leafminers in tomato, bean, and eggplant in Japan [21,22]. Over 40 species of parasitoids have been reported from *Liriomyza* leafminer worldwide [23,24]. Johnson et al. [15] reared 40 parasitoid species in four families from the major leafminer *L. sativae* Blanchard in Hawaii. Oatman and Johnson [25] recorded as many as 20 parasitoid spp. from *Liriomyza* sativae. Among all parasitoids, *Diglyphus begini*, *Halticoptera circulus* (Walder), and *Chrysomotomyia punctiventris* (Crawford) were the most abundant.

Insecticide-based management for leafminers can also negatively impact natural enemies and enhance the resurgence of leafminer populations. Johnson et al. [15] found two leafminer parasitoids, *Diglyphus begini* (Ashmead) and *C. punctiventris* (Crawford), that were negatively impacted by treatments of methomyl and chlordimeform. Leafminer population density increased and hymenopterous parasitoids decreased after application of broad-spectrum insecticides [5,26]. Saito [27] observed a high level of resistance in leafminer with reduction in parasitism after application of insecticides in commercial garden pea fields.

Several studies have been conducted to identify leafminer parasitoids in various regions and on different plant hosts in North America [8,17,28,29]. Schuster and Wharton [8] reported four families and 15 species of leafminer parasitoids on tomato in Florida. The leafminers' hymenopteran parasitoids were found in the bean crop during various growing seasons. Understanding parasitoids composition and status are primary steps for investigating new potential biological agents for leafminer control. In addition, leafminers' parasitoid interactions and community structure are important factors to develop an IPM program for *Liriomyza* species.

Snap bean is an important agricultural product in Florida, valued at $131,280 million [1]. The leafminer *L. trifolii* is the most significant pest on bean crops.

The objective of this study was to investigate the composition and seasonal abundance of *L. trifolii* parasitoids on snap beans in South Florida. We also studied the comparative abundance of four parasitoid species in three commonly grown major vegetable crops.

2. Materials and Methods

2.1. Snap Bean Sites

The leafminer parasitoids' composition and abundance in snap beans were investigated in 2010, 2012, 2014, and 2016. Each year, we had three plantings of snap beans: 1st planting in the second week of September 2nd planting in the second week of October, and 3rd planting in the second week of December. Bean growing season in South Florida starts in September and continues through March. As bean is a short time crop (58–65 days), we planted bean three times to cover the growing season. Each year, all three plantings were performed on the same site spacing 61 m between the plantings, where each planting site was 2 acres. In 2010 and 2012, study sites were established in the Tropical Research and Education Center (TREC), University of Florida-IFAS, Homestead, FL, USA (25°30′33.7″ N 80°30′17.1″ W). In 2014 and 2016, the sites were in growers' fields. Raised, open soil beds (91 cm wide, 20 cm height) were prepared with a Kennco superbedder (Kenco Manufacturing Company Inc., Atoka, OK, USA) to plant beans in each site. Beds were 60 cm wide with one row of bean plants in the center of each bed. Plants within the row were spaced 5 cm. Snap beans were seeded directly on the raised beds of Rockdale soil in all sites by placing Caprice snap bean seeds individually into a 2.5-cm-deep hole performed by a seed planter (3-Point Crop-Seeder, Zoro #: G204039667, China). Planting space within the bed was 7.6 cm and in between the bed 91.4 cm. Snap bean seeds were supplied by Harris Moran Seed Company, Modesta, CA, USA. Pre-plant herbicide, halosulfuron methyl (Sandea®, Gowan Company LLC., Yuma, Arizona), was applied at 51.9 g/ha to control emergence of nutsedge (*Cyperus esculentus*) and broad-leaf weeds. At the time of preparing beds, granular fertilizer 6:12:12 (N:P:K) was applied in the furrow on both sides of the seed row on each bed at 1345 kg/ha. Two drip tubes were placed parallel with 30.5 cm spacing on the center of the bed for irrigating plants. Plants were irrigated once a day, delivering approximately 2.5 cm to maintain soil moisture. Liquid fertilizer (4:0:8) was applied through the drip irrigation system at the rate of 0.56 kg N/ha/day 3, 4, and 5 weeks after planting. The fungicide Chlorothalonil (Bravo®, Syngenta Crop Protection, Inc., Greensboro, NC, USA) was used at 2.81 L/ha 4 weeks after planting to prevent fungal diseases. No insecticide was used at these study sites.

In the second study, we recorded abundance and composition of various parasitoids in three commonly grown major vegetable crops including tomato, bean, and squash planted in TREC research plots (25°30′33.7″ N 80°30′17.1″ W) in 2014 and 2016. Florida ranks first or second in growing these crops. The experimental field for this study consisted of 16 beds, each 67 m long, 1.8 m wide, and 15.2 cm high. Four such adjacent beds constituted one block. Each block was divided into 4 equal plots of 15.2 m long, 7.32 m wide which constituted a plot. The abovementioned three crops were planted using a randomized complete block design, each replicated four times. All crops were planted on raised beds of Rockdale soil covered with white on black plastic mulch. In 2014, bean and squash were seeded on 12 September, and tomato was transplanted two weeks after bean and squash. In 2016, the same planting dates as 2014 were used. Plant spacing for tomato was 45.7 cm within the bed and 183 cm in between the beds. The spacing for squash and bean was 30.5 cm and 15.2 cm within the bed, respectively. All other methods for maintaining crops were as discussed above or otherwise following standard cultural practices mentioned in the Vegetable Production Handbook of Florida [30]. Chlorothalonil (Bravo Weather Stik, Syngenta Crop Protection LLC, St. Louis, MO, USA. 1.7 L/ha), mancozeb (Manzate® Pro-Stick, United Phosphorus Inc., Cary, NC 27513, USA. 1.7 L/ha), pyrimethanil (Scala®, Bayer CropScience, St. Louis, MO, USA. 0.5 L/ha), and penthiopyrad (Fontelis®, 1.0 L/ha) were used in weekly rotation to prevent fungal and bacterial diseases (target spot, Rhizoctonia root rot, leaf spot, late blight). No other pesticides were used to control foliage pests except *Bacillus thuringiensis* subsp. *Aizawai* strain ABTS 1857, (Valent BioSciences Corporation, Libertyville, IL, USA, 1.7 kg/ha) two times to control beet armyworm on tomato.

2.2. Leaf Sampling and Insect Rearing

In the first study, parasitoids were collected from foliage of bean. Sampling began when bean plants had two primary leaves fully unfolded. To address all variability in sampling, four sections (=plots), each consisting of four rows × 15.2 m long, were randomly selected from each experimental field. Ten full grown and widely open leaves, one leaf per plant, were randomly collected from each plot-row weekly (40 leaves per week). When the bean plants became mature and their primary leaves dropped off, the older bottom leaves were always collected from the plants because of *L. trifolii* feeding preference for the older and mature leaves [31,32]. The sampled leaves from each plot-row were placed into 15 cm diameter Petri dishes (10 leaves per Petri dish) and marked with the date and plot number. The samples were then transported to the vegetable IPM laboratory at the Tropical Research and Education Center and placed into a growth chamber at 25 °C, 70% RH, 14:10 (L:D) h for the development of leafminer and its parasitoids. The bean leaves were checked daily for collecting leafminer pupae. The emerging pupae in each Petri dish were separated and transferred to a new Petri dish and labeled with the same sampling date. The separated pupae were also checked daily for recording the larval endoparasitoids' emergence. Bean leaves were checked daily for collecting the larval ectoparasitoids from mines on the leaves.

In the second study, from each plot of four rows each 15.2 m long, the sample consisted of randomly selected 10 full grown infested leaves/row, 10 × 4 = 40 leaves/plot. For the convenience of parasitoid collection, leaves from each row (10 leaves) were placed separately in a Petri dish (10 cm diam). All other procedures were accomplished following the above study.

2.3. Insects Collection and Identification

All the emerged parasitoids were collected and preserved in 75% alcohol for identification. The leafminers were identified following morphological characters described by Capinera [7] (2001), and further verified by the Division of Plant Industry (DPI), Gainesville, FL, USA. The parasitoids were identified based on the external characters used in previous studies [3,28,33]. The leafminers parasitoids were sent for verification to the Division of Plant Industry (DPI), Gainesville, FL, USA, and further confirmed by Systematic Entomology Laboratory, USDA, MD. Voucher specimens were stored in the Entomology, Tropical Research and Education Center, Homestead, FL, USA.

2.4. Statistical Analysis

All data in the first study (abundance and composition of parasitoids in beans) and second study (comparative abundance in three crops) were square root transformed before analyses. Non-transformed means are reported in the tables. Transformed data of both studies were analyzed using a mixed model ANOVA. In the first study, fixed effect consisted of parasitoid species, year of study, and months, and random effect consisted of replications, whereas in the second study (comparative abundance of parasitoid species in three crops), fixed effect consisted of parasitoid species, crops, years, and months, and random effects consisted of plots. Both fixed and random effects in both studies were used to account for experimental design (PROC GLIMMIX model, SAS Version 9.3, SAS Institute Inc., Cary, NC, USA [34]). In the PROC GLIMMIX model, the Kenward–Roger method was used to estimate the degree of freedom. Means were separated using Tukey's Honestly Significant Difference (HSD) test in SAS. All data were analyzed at a 5% level of significance.

3. Results and Discussion

3.1. Seasonal Abundance in Beans

We recorded 13 species of hymenopteran parasitoids of *L. trifolium* (Figure 1) on beans in south Florida during the four years (2010, 2012, 2014, and 2016) of the present study. They belonged to three families including Braconidae (*Opius dissitus* (Muesebeck) and *Euopius* sp.), Eulophidae (*Diaulinopsis callichroma* (Crawford), *Diglyphus begini* Ashmead), *D. intermedius* (Girault), *D. isaea* (Walker), *Neochrysocharis* sp. Kurdjumov, *Closterocercus* sp. Westwood, *Zagrammosoma lineaticeps* (Girault), *Z. multilineatum* (Ashmead), *Pnigalio* sp. Schrank, *Chrysocharis* sp. Hahn), and Pteromalidae (*Halticoptera* sp.). Among these, *Opius dissitus* and *Diaulinopsis callichroma* were the most abundant species in all four years of study followed by *Euopius* sp., *Diglypohus begini*, and *Neochrysocharis* sp. (Table 1). Other species were present in a low density, 0.23–0.56 in 2010 and 0.17–0.51 in 2012, 0.45–0.97 in 2014 and 0.17–0.64 in 2016. The highest number of parasitoids, irrespective of species, was recorded in 2014 ($\overline{x}$ = 13.29) followed by 2010 ($\overline{x}$= 11.93), 2012 ($\overline{x}$ = 9.51) and 2016 ($\overline{x}$ = 8.69). Total parasitoid abundance, irrespective of species, showed a strong positive relationship (r = 0.94, $p < 0.0001$) with leafminers' abundance. Rauf et al. [35] also recorded 10 Eulophid parasitoids out of 11 spp. from infested vegetable leaves in Indonesia. Variation in the density among parasitoid species in different years could be due to the variation in abundance of the host *Liriomyza*, quality of crop, and temperature. During the present study over four years, mean monthly temperature (°C) varied from 19 °C in December to 24.4 °C in October. Johnson and Hara [36] emphasized the matching of parasitoid species, with favorable *Liriomyza* species and crop for the development of effective biological control.

Figure 1. *Liriomyza trifolii* (Agromyzidae). Photo credit: J. Li.

Table 1. Mean number of leaf miner parasitoids per bean leaf sample for four years study.

Family & Species	**2010**	**2012**	**2014**	**2016**
Braconidae				
Opius dissitus	12.05 ± 0.90 aA	10.08 ± 0.81 aA	7.63 ± 0.84 aA	6.17 ± 0.93 aA
Euopius sp.	1.12 ± 0.14 cdA	1.10 ± 0.15 cA	2.00 ± 0.20 cA	1.50 ± 0.16 cA
Eulophidae				
Diaulinopsis callichroma	6.83 ± 0.64 bA	5.38 ± 0.52 bA	4.60 ± 0.73 bA	3.23 ± 0.37 bA
Diglyphus begini	1.98 ± 0.30 cA	0.77 ± 0.12 cdA	1.77 ± 0.25 cdA	0.70 ± 0.12 dA
D. intermedius	0.92 ± 0.14 deA	0.63 ± 0.12 ceA	0.93 ± 0.18 egA	0.47 ± 0.10 deA
D. isaea	0.64 dib	1.12 ± 0.14 dfB	7.63 ± 0.84 cfA	7.63 ± 0.84 deB
Neochrysocharis sp.	1.40 ± 0.19 cdA	0.82 ± 0.14 cdA	1.23 ± 0.16 ceA	0.47 ± 0.09 deA
Closterocercus	0.58 ± 0.10 efA	0.370.08 dfaA	0.97 ± 0.17 dgA	0.40 ± 0.08 deA
Zagrammosoma lineaticedps	0.37 ± 0.08 gfA	0.25 ± 0.06 efA	0.53 ± 0.12 fgA	0.27 ± 0.08 deA
Z. multilineatum	0.85 ± 0.15 dfA	1.03 ± 0.17 fA	1.03 ± 0.17 egA	0.23 ± 0.08 deA
Pnigalio sp.	0.33 ± 0.08 gA	0.67 ± 0.14 efA	0.67 ± 0.14 egA	0.33 ± 0.09 deA
Chrysocharis sp.	0.28 ± 0.07 gA	0.67 ± 0.14 fA	0.67 ± 0.14 egA	0.17 ± 0.07 eA
Pteromalidae				
Halticoptera sp.	0.27 ± 0.07 gA	0.47 ± 0.10 efA	0.47 ± 0.10 gA	0.23 ± 0.08 deA
	df = 122,003, F = 191.33 $p < 0.0001$	df = 122,003, F = 170.39 $p < 0.0001$	df = 122,003, F = 46.82 $p < 0.0001$	df = 122,003, F = 49.20 $p < 0.0001$

According to the Tukey HSD test, means within a column followed by same lowercase letter and within a row followed by same uppercase letter do not differ statistically at $p \geq 0.05$.

Two different Braconid parasitoids, *O. dissitus* (Figure 2) and *Euopius* sp. (Figure 3), were observed on beans. *Opius dissitus* is a solitary larval endo parasitoid of *L. trifolii* [37–39]. The adults are black in color with long antennae and are 1.5 mm in length. The parasitoid females deposit their eggs inside the leafminer larvae. The parasitized host larvae continue to develop throughout the larval stage and emerge from the puparium. This observation is supported by Bordat et al. [38], who reported that parasitoid females lay their eggs directly inside their host larvae. At the end of the development, the parasitoid adults emerged from the host puparium. One *O. dissitus* adult was found to emerge from a single leafminer pupa in our study.

Figure 2. *Opius dissitus* (Braconidae). Photo credit: J. Li.

Figure 3. *Euopius* sp. (Braconidae). Photo credit: J. Li.

Opius dissitus was the most abundant leafminer parasitoid on snap beans in all four years of this study in the Miami-Dade County (Table 1). A significantly higher number of *O. dissitus* was recorded in each of this study than the other parasitoid species. *Opius* sp. occurred on bean during all months of the study (Figure 4a). *Opius dissitus* abundance was high in November, December, and January during four years of the study. Population density decreased after January. The density of *O. dissitus* was positively correlated (r = 0.80, $p < 0.0001$) with leafminer density within the whole growth season. The density of *O. dissitus* was high when the temperature was cool (15~19 °C) in December 2010 and January 2011. The optimum temperature for both *O. dissitus* male and female was 20 °C, and the female had a higher reproduction at 25 °C [39]. *Opius dissitus* was reported as the most abundant larval parasitoid from leafminer infested tomato foliage in Florida [8].

Euopius sp. (Figure 3) is a larval endoparasitoid. One *Euopius* sp. adult was found to emerge from a single host puparium. *Euopius* sp. has almost the same body size and antennae as *O. dissitus*. However, *Euopius* sp.'s body has a yellow color, which differentiates it from *O. dissitus* (being black in color). The abundance of *Euopius* sp. (Table 1) was low in 2010 ($\bar{x}$) = 0.83) and 2012 ($\bar{x}$) = 0.78) and did not differ statistically from 2016 ($\bar{x}$ = 1.12). The highest density of *Euopius* was recorded in 2014 ($\bar{x}$) = 1.33). Peak density of *Euopius* was recorded in September of 2010, December of 2012 and 2016, and January of 2014, which differed significantly from the rest of the years (Figure 4b). The *Euopius* sp. was reared from *Liriomyza*-infested weeds on *Bidens alba* (L.) (Asterales: Asteraceae) [40].

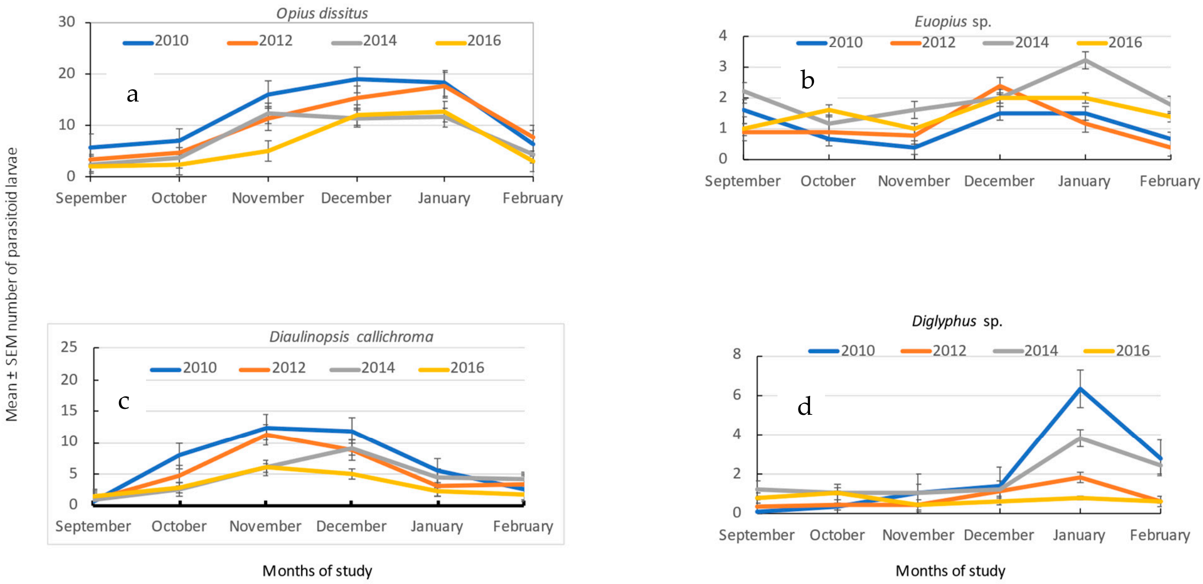

Figure 4. Seasonal pattern of abundance of four species of leafminers' parasitoids in bean in bean in 2010, 2012, 2014 and 2016.

There were ten different species of parasitoids found in the family of Eulophidae. *Diaulinopsis callichroma* (Figure 5) was found to be the second largest group of *L. trifolii* parasitoids in this study (Table 1). The highest population density was recorded in 2010, followed by 2012 and 2014. The lowest density was recorded in 2016, which did not differ statistically from 2014. *D. callichroma* occurred across the season during the four years of this study. However, population abundance peaked in November followed by December. The population abundance of *D. callichroma* a showed positive correlation (r = 0.65, $p < 0.0001$) with leafminers abundance when its populations across the four years are considered. The hind femora of adult *D. callichroma* is dusky basally, while the fore and middle femora are pale [33]. The male adult's basal antennal segments are black and enlarged. The body size of adults is about 1.1–1.3 mm in length. This parasitoid is a larval ectoparasitoid of leafminer. *D. callichroma* larvae feed directly on the host larva and eventually kill the larva. The parasitoid larvae pupate inside the mine, eventually emerging from the mine. The abundance of *D. callichroma* might be affected by both host density and temperature condition.

Figure 5. *Diaulinopsis callichroma* (Eulophidae). Photo credit: J. Li.

D. callichroma population density was the highest in 2010 ($\bar{x}$) = 2.35), which did not differ statistically from 2012 ($\bar{x}$) = 2.09). The lowest population density of *D. callichroma* was observed in 2016 ($\bar{x}$) = 1.690). Its abundance was high in November and December of each year (Figure 4c). Population density decreased before and after this duration (November–December). The abundance of *D. callichroma* might be affected by both host density and temperature.

Three different species of *Diglyphus* parasitoids (Figures 6–8: *D. begini*, *D. intermedius* and *D. isaea*) were reared from bean foliage. The abundance of *D. begini* was significantly higher than that of *D. intermedius* and *D. isaea* in 2010 and did not differ in other years. The *D. begini* population density was low until December during this study (Figure 4d). The population abundance peaked in January.

Figure 6. *Diglyphus begini* (Eulophidae). Photo credit: J. Li.

Figure 7. *Diglyphus intermedius* (Eulophidae). Photo credit: J. Li.

Figure 8. *Diglyphus isaea* (Eulophidae). Photo credit: J. Li.

Diglyphus spp. parasitoids were the third largest group in the study. These are larval ectoparasitoids, and adult females generally lay more than one egg by the side of the host larva [41]. *Diglyphus* spp. are characterized by their antennae with two funicular segments. The forewings of these three species of *Diglyphus* have dense setose, whereas the basal cell has uniformly dense setose. These three abovementioned species of *Diglyphus* can be distinguished from each other based on the dark area on their hind tibia. *D. begini* basal hind tibia has a short and less than 25% metallic dark area (Figure 6).

D. intermedius basal tibia has a relatively larger area of 25–35% dark color and with extended dusky color (Figure 7).

Alternatively, *D. isaea* basal hind tibia has over 75% metallic dark color proportion (Figure 8) [28,33].

Schuster and Wharton [8] reported that *Diglyphus* parasitoids were the most abundant larval ectoparasitoids on the tomato crop in Florida and that both species (*D. intermedius* and *D. begini*) were recorded in the current study. In our study, the most abundant larval ectoparasitoid on bean crop was *D. callichroma*. It should be noted that *D. isaea* has been used as an effective biological control agent in greenhouse for controlling *Liriomyza* spp. leafminer in the Netherlands [41,42].

Neochrysocharis sp. (Figure 9) was recorded as the fifth largest group of leafminer parasitoids during our four years of study. The population abundance was higher in 2010 ($\bar{x}$ = 0.89) and 2014 ($\bar{x}$ = 0.97) than that of 2012 ($\bar{x}$ = 0.61) and 2016 ($\bar{x}$ = 0.47). Adults emerge from mines on the leaves. The adult body is metallic green, and eyes are red. The fore, middle, and hind tibia are pale. *Neochrysocharis* sp. is reported as an endoparasitoid of the leafminer larva [8]. Tran [43] reported a high abundance of *Neochrysocharis* in the north central coast region of Vietnam. This species was also reported from Indonesia and is considered as a potential biocontrol agent for invasive agromyzid pests [44].

Figure 9. *Neochrysocharis* sp. (Eulophidae). Photo credit: J. Li.

The population of *Closterocerus* sp. (Figure 10) was low on bean foliage during this study. Based on our four years of study, it is the eighth largest group of leafminer parasitods in bean. Density of *Closterocerus* ranged from 3.58% in 2012 to 5.79% in 2014. *Closterocerus* is an endoparasitoid of the leafminer's young instars [3].

Figure 10. *Closterocerus* sp. (Eulophidae). Photo credit: J. Li.

The adult body length is 1.0–1.3 mm with characteristic dark bands on the forewings. Stegmaier [29] reported that *C. cinctipennis* is a parasitoid of the leafminer *L. trifolii* in Florida. Rauf et al. [35] reported *Closterocerus* sp. from snow peas (*Pisum* sp.) infested with this Asian leafminer species *Chromatomyia horticola* (Goureau). *C. mirabilis* has been reported as a potential parasitod of agromyzid leafminers in Australia [45].

We recorded two species of *Zagrammosoma* (Figures 11 and 12), *Z. lineaticeps* (Girault) and *Z. multilineatum* (Ashmead). The density of *Z. lineaticeps* ranged from 2.63% in 2012 to 3.54% in 2014 and that of Z. *multilineatum* ranged from 0.17% in 2012 to 0.80% in 2014. Both species are larval ectoparasitoids of *Liriomyza* leafminer. Both *Z. lineaticeps* and

Z. multilineatum were found in Florida [8,17]. *Zagrammosoma* spp. were also collected from leafminer infested vegetable leaves in Indonesia [38]. *Zagrammosoma lineaticeps* is black or dark-colored and the forewing has a dark line along the apical margin (Figure 10), while *Z. mulitilineatum* is predominately yellow and mesoscutum has dark stripes (Figure 11). The abundance of both species was high in October and November 2010. In the study, *Z. multilineatum* was relatively more abundant (20 adults) than *Z. lineaticeps* (10 adults) (Table 1). *Zagrammosoma* spp. were also collected from *Liriomyza*-infested faba bean (*Vicia faba*) in Egypt [46].

Figure 11. *Zagrammosoma lineaticeps* (Eulophidae). Photo credit: J. Li.

Figure 12. *Zagrammosoma multilineatum* (Eulophidae). Photo credit: J. Li.

Pnigalio sp. (Figure 13) is the 10th largest group of parasitoids. It occurred in a low density on bean ranging from 2.43% in 2010 to 4.29% in 2016. *Pnigalio* sp. is a larval ectoparasitoid of the leafminer *Liriomyza*. The genus was also reported to parasitize Lepidoptera, Hymenoptera, and Coleoptera [3]. The body length of adult females is 1.7~1.9 mm and male is 1.5~1.7 mm. The antennae have four funicular segments, and for the male, it is a laterally branched structure antenna [3]. Lasalle and Parrella [28] indicated that only one Nearctic species, *P. flavipes* (Ashmead), attacks *Liriomyza*. However, Schuster and Wharton [8] reported species of *P. maculipes* (Crawford) reared from tomato foliage in Florida. *Pnigalio* spp. abundance was 12.3% among the hymenopteran parasitoid complex of citrus leafminer in Texas [47].

Chrysocharis sp. (Figure 14) is a larval-pupal endoparasitoid of leafminer and was the second most abundant larval-pupal endoparasitoid (Table 1). *Chrysocharis* sp. showed a consistent population density during the four years of this study. In our study, *Chrysocharis* density ranged from 1.79% in 2012 to 4.29% in 2014. *Chrysocharis* sp. Post-marginal vein is always longer than the stigma vein, and the petiole is present and distinct. *Chrysocharis* sp. Is a primary parasitoid of leaf-mining larvae of Diptera, Lepidoptera, and Hymenoptera [48]. The authors [48] found a low percentage of *Chrysocharis* sp (0.04–0.17) and highest percentage of *Halticoptera* sp. (32–52%) on vegetable crops in winter and autumn in the Middle

Volga Basin in Russia. *Chrysocharis* sp. was reported from *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae)-infested vegetable leaves in Indonesia [35].

Figure 13. *Pnigalio* sp. (Eulophidae). Photo credit: J. Li.

Figure 14. *Chrysocharis* sp. (Eulophidae). Photo credit: J. Li.

Halticoptera sp. (Figure 15) belongs to the family of Pteromalidae. This species is another larval-pupal endoparasitoid of leafminers in beans. The abundance of this species was the lowest in 2010 (1.93%) and highest in 2014 (3.39%). The antenna has six funicular segments. Schuster and Wharton [8] reported a high population of *Halticoptera* sp. on tomato. Shahein and El-Maghraby [49] recorded a very low percentage of *Halticoptera* sp. on broad beans (*Vicia faba* L.) in Egypt. They found *Diglyphus* sp. and *Opius* as the most abundant species on bean. In addition, *H. circulus* was reported to be the only one Nearctic species of *Halticoptera* known to parasitize *Liriomyza* leafminer [28]. *Halticoptera arduine* (Walker) successfully parasitized *L. trifolii*, *L. sativae*, and *L. huidobrensis* leafminers and developed in them as endoparasitoids [50].

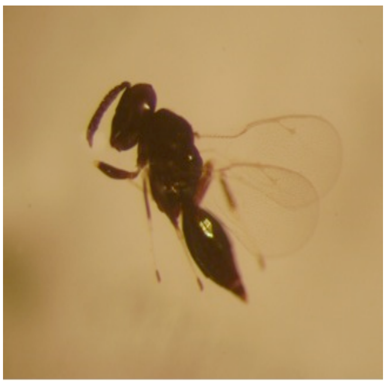

Figure 15. *Halticoptera* sp. (Pteromalidae). Photo credit: J. Li.

3.2. Comparative Abundance of Parasitoids in Three Crops

Opius sp. occurred in all three vegetable crops with the highest number in bean, followed by tomato and squash in both years' studies (F = 218.34, df = 2, 53.4, p = 0.001) (Figure 16A,B). The population abundance of *Opius* sp. was negatively correlated with the temperature increase (r = −0.80) in all crops during this study. In the instance of *Euopius* sp., the highest abundance was recorded in bean, followed by squash and tomato (F = 6.32; df = 2, 32.1; p = 0.004). Month did not influence population abundance of *Euopius*, irrespective of crops (F = 0.16; df = 5, 44.48; p = 0.16). Year (F = 4.59, df = 1, 53.24; p = 0.036), crop x year (F = 4.78; df = 2, 32.1; p = 0.015), and crop x month (F = 3.43; df = 10, 16.95; p = 0.012) significantly impacted the *Euopius* population in tomato in 2014 (Figure 16C,D). However, in 2016, the highest abundance was recorded in bean, followed by tomato and squash. On all crops, *Euopius* population abundance was negatively correlated with temperature increase in both years' studies (r = −0.25 to −0.52). The abundance of *Diaulinopsis* was significantly higher in bean followed by tomato and squash in both years (F = 54.92; df = 2, 43.19; p = 0.001) (Figure 16E,F). Year of study did not impact the population abundance of *Diaulinopsis* sp. Month of study alone (F = 10.14; df = 5, 29.11; p = 0.0001) and crop x month (F = 8.44; df = 10, 41.35; p = 0.001) significantly increased population abundance of *Diaulinopsis*. The population increase in *Diaulinopsis* was positively correlated with temperature in squash (r = 0.36 in 2014); otherwise, it followed the same trend of population increase like the above species. The population of *Diglyphus* sp. was higher in bean followed by squash and tomato in both years (F = 7.99; df = 2, 25.06; p = 0.0025) (Figure 16G,H). The year of study positively impacted the population of *Diglyphus* sp. (F = 24.49; df = 11, 081; p = 0.0005). The month of study did not cause a statistical difference in the population increase in *Diglyphus*. Crop × year and crop–month interactions had a positive effect on the increase in the *Diglyphus* population. The *Diglyphus* population abundance was positively correlated with temperature in squash (r = 0.51) and tomato (r = 0.14), and negatively correlated in bean in both years' studies (2014: r = −0.47; 2016: r = −0.2015). Overall, population abundance of parasitoids were high when density of leafminer was high and temperature was relatively low (Figure 17).

Figure 16. Mean number of parasitoids/leaf sample on bean, tomato and squash in different months of 2014 and 2016; *Opius* sp. (**A**,**B**), *Euopius* sp. (**C**,**D**), *Diaulinopsis* sp. (**E**,**F**) and *Diglyphus* sp. (**G**,**H**).

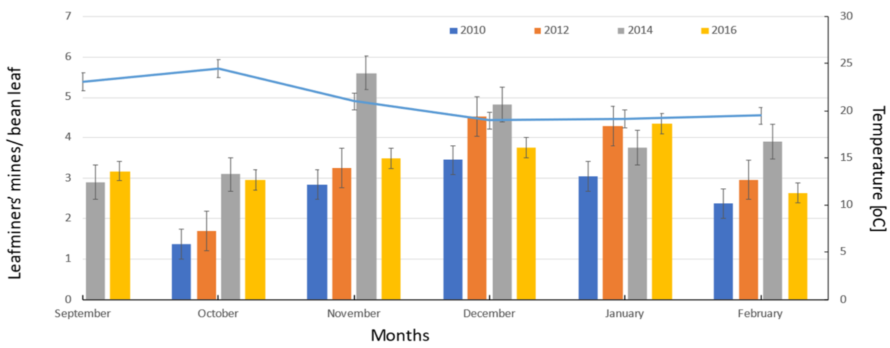

Figure 17. Mean temperature (mean ± SD) represented by line and mean number of leafminers' mines/bean leaf (mean ± SD) represented by bars in different months.

Very little information is available on the comparative abundance of parasitoids using replicated vegetable crop plots. Tran et al. [51] surveyed various vegetable crop fields for the abundance of parasitoids associated with agromyzid leafminer and reported *Neochrysocharis formosa* as the most abundant parasitoid on French bean, tomato, and casava in Vietnam.

4. Conclusions

Vegetable crops are mostly infested by leafminers. The present study provided information about thirteen species of hymenopteran parasitoids that attack leafminers. Among all parasitoids, four species were recorded to be more abundant on beans, squash, and tomato than the rest of the parasitoid species recorded in this study. Leafminer abundance was high between 7.2° and 23.8 °F. Parasitoid species presence followed the pattern of leafminers abundance. Insecticide applications severely impact the population abundance of parasitoids, although this was not studied in the current study. Biocontrol of leafminers will be effective if insecticides are applied selectively.

Author Contributions: D.R.S. conceptualized the project, collected resources to set up the field experiment, analyzed data, and prepared final version of the manuscript. O.L. helped in writing the manuscript. J.L. collected data and prepared images of parasitoids. All authors have read and agreed to the published version of the manuscript.

Funding: The research was funded by obtaining in kind support from vegetable growers.

Institutional Review Board Statement: The manuscript was checked by TREC faculty, and their comments were addressed to finalize the manuscript.

Data Availability Statement: Dakshina Seal maintains excel file of all data used in this study.

Acknowledgments: Cathie Sabines assisted in collecting samples. Tomato, bean, and squash growers supported this study by providing land and growing crops. USDA and DPI helped in confirming parasitoid species.

Conflicts of Interest: There is no conflict of interest with any coauthors, or any researchers in conducting and publishing this work.

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