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Diel Density Patterns of Leafminer, *Liriomyza trifolii* (Diptera: Agromyzidae), and Two Parasitoids, *Opius dissitus* (Hymenoptera: Braconidae) and *Diglyphus* spp. (Hymenoptera: Eulophidae)

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Diel activity patterns of leafminer, *Liriomyza trifolii* and its parasitoids *Opius dissitus* and *Diglyphus* spp. were evaluated by using yellow sticky traps in bean (*Phaseolus vulgaris* 'Opus') fields. Densities of *L. trifolii* were found more abundant from 0800 to 1000 hours than any other time within the day during fall 2010, but did not show any clear pattern of diel abundance in spring 2011. Parasitoid *O. dissitus* was found abundant from 1001 to 1200 hours during spring 2011, however no significant diel density pattern was observed in fall 2010. *Diglyphus* spp. was more abundant during 1001 to 1200 hours and 1201 to 1400 hours than any other time in fall 2010 and spring 2011, respectively.

Agromyzid leafminers are important phytophagous flies feeding on a wide range of ornamental and vegetable plants. The American serpentine leafminer, *Liriomyza trifolii* is a serious pest of beans in south Florida (Seal et al., 2002). Both adults and larvae can cause economic damage to the plants. The female punctures the leaves by its ovipositor and inserts its mouthparts at the punctures to feed on cell contents. The larvae consume mesophyll tissues of the leaf (Fagoonee, 1984; Musgrave et al., 1975). The feeding and egg laying activities of larvae and adults can significantly impact plant physiology (Parrella and Jones, 1985) and induce proper environment for pathogens (Broadbent, 1990).

Chemical control is the main method to manage leafminers on various commercial crops (Cox et al., 1995). Frequent use of these insecticides may enhance the development of resistance in leafminers, resurgence of secondary pests and elimination of natural enemies (Ferguson, 2004; Leibee, 1981; Parrella and Keil, 1984). Thus, insecticide resistance development limits the control of leafminers (Leibee and Capinera, 1995).

To reduce sole dependence on insecticides, various research studies have been conducted to manage insect pests using biocontrol agents alone or in combination or alteration with insecticides and other control techniques. Several hymenopteran parasitoids were found to be effective for controlling agromyzid leafminers. There are at least 14 species of parasitoids of *L. trifolii* in Florida (Capinera 2001). The parasitoid wasps *O. dissitus* and *Diglyphus* spp. were found abundant on bean crops in south Florida. It was reported that *Opius* sp. and *Diglyphus* spp. were reared from *L. trifolii* from celery (*Apium graveolens*) leaves and tomato (*Solanum lycopersicum*) leaves in Florida (Schuster and Wharton, 1993; Stegmaier, 1972).

In developing an integrated pest management program, the knowledge on the biology of a pest and its natural enemies is essential. Response of natural enemies to the commonly used insecticides cannot be avoided. Considering the importance, Kaspi and Parrella (2005) and Weintraub (2001) reported on the compatibility of some insecticides with leafminer parasitoids. They found that the parasitoid *D. isaea* was more compatible with abamectin than cyromazine.

Various studies are available on the biology of *L. trifolii*. The activity of both leafminer and the parasitoid is affected by various factors. The leafminer *L. trifolii* activity is affected by temperature, light, moisture, and host plant type. Temperature is an important factor for affecting the activity of leafminer and the parasitoids in the natural environment (Abou-Fakhr-Hammad, 2000; Bordat et al., 1995). The temperature is variable during different time periods of a day; therefore, it was assumed that the activity or density levels of leafminer and parasitoids were different within various periods of the daytime. Few studies ever assessed the diel density pattern of leafminer and parasitoids. Understanding the diel density pattern of leafminer parasitoids is crucial to reduce the insecticide impact on these natural enemies in integrated pest management.

Knowledge of the diel density of leafminer and its parasitoid is important in developing an effective management program. Thus, the objectives of this study were to determine the diel density pattern of leafminer, *L. trifolii,* in bean fields. In addition, we also studied diel density pattern of leafminer parasitoids, *O. dissitus* and *Diglyphus* spp.

Materials and Methods

STUDY SITE AND CROP PREPARATION. The study was carried out in the Tropical Research and Education Center (TREC) research

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plots, Homestead, FL. Two bean fields (site 1 and site 2), each 0.4 ha, were selected for the study. The soil type of the study sites was Krome gravelly loam soil, which consists of about 33% soil and 67% pebbles. Both fields (site 1 and site 2) were planted to bean (*P. vulgari* 'Opus', supplied from Harris Moran Seed Co., Modesta, CA) on 3 Oct. and 10 Dec. 2010, respectively. Pre-plant herbicide halosulfuron methyl (51.9 g/ha, Sandea®, Gowan Company LLC., Yuma, AZ), was applied to protect against emergence of nutsedge and broad leaf weeds 3 weeks before planting the seeds. The beans were seeded directly in rows at the rate of three seeds/ft. The bean seeds were spaced 3 ft between two adjacent rows. Granular fertilizer N–P–K (8–16–16) at the rate of 1345 kg/ha was used at planting in a band 1 m apart from the seed rows. Additionally, liquid fertilizer N–P–K (4–0–8) at the rate of 0.56 kg/ha per day was used 5 weeks after planting in each site. The plants were irrigated through drip tubes twice every day delivering 1.0 inch of water each time. Other cultural practices were as recommended for growing beans in Florida (Olson and Santos, 2011–2012). Both sites were managed for conducting the study until 15 Dec. 2010 and 22 Feb. 2011, respectively. No insecticide was used during the study period.

Plot design and diel density. Each study site was divided into 15 equal plots, each (10 m × 10 m). One yellow sticky trap 7.6 cm × 12.7 cm (3 inches × 5 inches) was set in the center of each plot from 0800 to 1800 hr within a day. For the purpose of studying diel activity pattern of leafminer and its parasitoids, sticky taps were checked at 2-h intervals (0800–1000, 1001–1200, 1201–1400, 1401–1600, and 1601–1800 hr) once in every week at the 4th, 5th, 6th, and 7th week after the bean planting in each site. The cards were checked 20 min before each interval. At the time of checking sticky cards, each insect on the card deemed to be leafminer and its parasitoids was marked with a colored pen; different color marks were selected for different intervals. At the end of the day, all traps were collected and wrapped individually with transparent polyethylene sheet to avoid any trapping of unwanted insects. Each trap was marked with plot number, date, and period. All traps were transported to the laboratory, TREC, Homestead, FL. The numbers of leafminer and various parasitoids were recorded using a bionocular microscope. The trapped leafminers and parasitoids were identified by external characteristics (Capinera, 2001; Lasalle and Parrella, 1991). Further, parasitoids were sent to the Systematic Entomology Laboratory (USDA, Beltsville, MD) for confirmation of identification to genus and species levels. Each week, a new set of sticky traps was used on the day of study.

Statistical analysis. The mean number of trapped *L. trifolii*, *O. dissitus,* and *Diglyphus* spp. from each different period was compared by analysis of variance (ANOVA, PROC GLM, SAS Institute Inc. 2003), and the means were separated by least significance difference (LSD) ($P < 0.05$).

Results and Discussion

Site 1

The population abundance of leafminer and the two species of parasitoids in each 2-h interval within the day was assessed on 9 Nov. 2010 (Fig. 1). The abundance of leafminer was significantly higher (F = 10.58; df = 4,74; $P < 0.0001$) in the first and second 2-h interval (0800–1000) (1001–1200) than other intervals. Population abundance of the leafminer decreased as daylight hours increased. Lowest activity of leafminer was observed during the fifth interval.

Population abundance of *Diglyphus* spp. was significantly higher (F = 3.33; df = 4,74; $P = 0.0148$) in the second, third, and fourth intervals than in the first and fifth intervals. *Diglyphus* spp. was almost absent during the first 2-h interval. Abundance of *O. dissitus* did not show any significant difference (F = 2.04; df = 4,74; $P = 0.0979$) among the intervals within the day (Fig. 1). Overall, *O. dissitus* capture was low during this study.

On 16 Nov. 2010, the density of *L. trifolii* was significantly higher (F = 4.24; df = 4,74; $P = 0.004$) in the first 2-h interval than in the second and fifth intervals (Fig. 2). However, population abundance of leafminers in the first 2 h did not differ from the third and fourth intervals within the day. The highest peak of

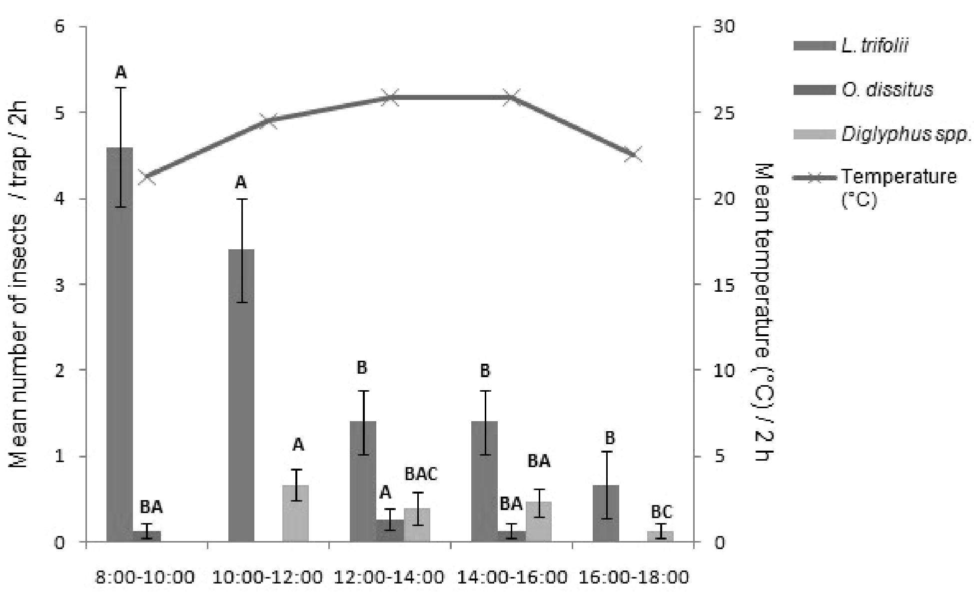

Fig. 1. Bean site 1, 9 Nov. 2010. Mean (±SE) number of the *L. trifolii*, *O. dissitus,* and *Diglyphus* spp./yellow sticky trap during each 2-h interval. Means with the same letter are not significantly different ($P < 0.05$, LSD test).

leafminers populations was observed at 0800–1000 HR followed by 1401–1600, 1201–1400, 1601–1800, and 1001–1200 HR.

Diglyphus spp. was significantly more abundant (F = 5.06; *df* = 4,74; *P* = 0.0012) in the second 2-h interval than in the other intervals (Fig. 2). *Diglyphus* spp. was almost absent in the first 2-h interval. Like *Diglyphus* spp., *O. dissitus* was absent in the first interval (0800–1000); and increased insignificantly in the subsequent intervals (F = 0.88; *df* = 4,74; *P* = 0.4778).

On 23 Nov. 2010, the *L. trifolii* density was significantly higher (F = 17.8; *df* = 4,74; *P* < 0.0001) in the first 2-h interval (0800–1000) than the subsequent intervals within a day (Fig. 3). Diel density of leafminer decreased as the day progressed. The lowest activity of leafminer was observed at the end of the day (1601–1800).

The peak increase in diel activity of *Diglyphus* population was observed during the second interval (10:01–12:00) (F = 4.28; *df* = 4,74; *P* = 0.0037) (Fig. 3). Decrease in activity of *Diglyphus* sp. was observed before and after the second interval. Population

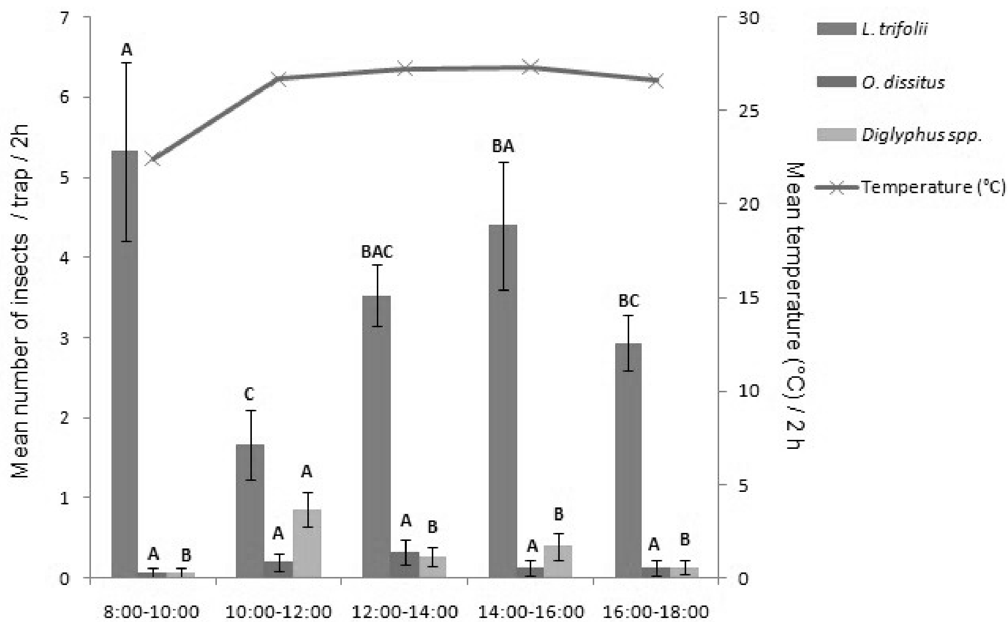

Fig. 2. Bean site 1, 16 Nov. 2010. Mean (±SE) number of the *L. trifolii*, *O. dissitus,* and *Diglyphus* spp./yellow sticky trap during each 2 h interval. Means with the same letter are not significantly different (*P* < 0.05, LSD test).

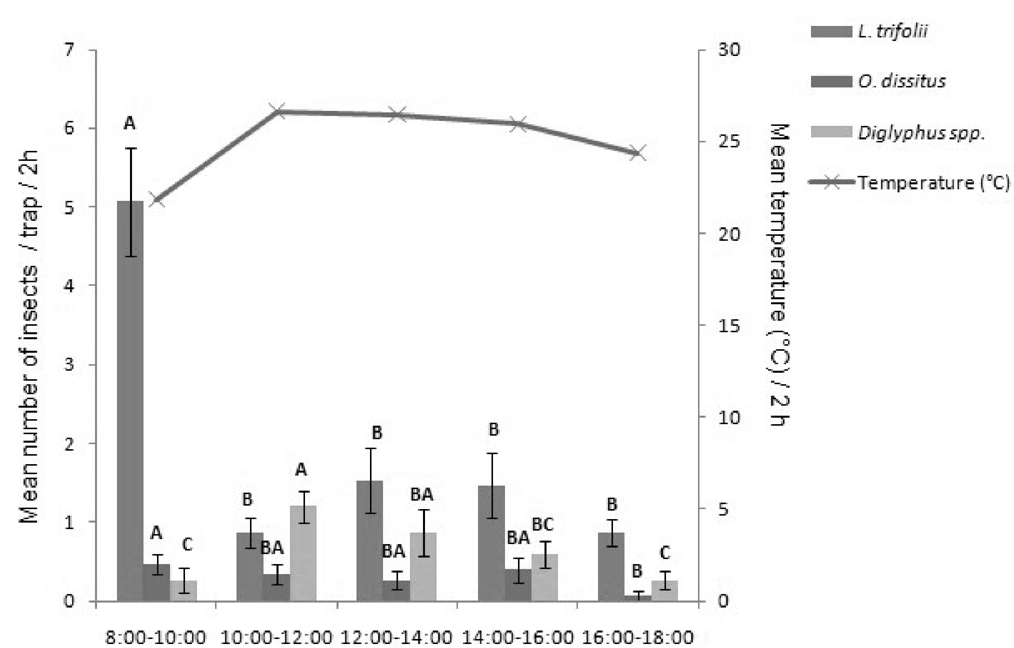

Fig. 3. Bean site 1, 23 Nov. 2010. Mean (± SE) number of the *L. trifolii*, *O. dissitus* and *Diglyphus* spp. / yellow sticky trap within each 2 h interval. Means with the same letter are not significantly different (*P* < 0.05, LSD test).

abundance of *O. dissitus* was low in all intervals (F = 1.5; *df* = 4,74; *P* = 0.2128) (Fig. 3). No clear peak in the diel pattern of *O. dissitus* was observed during this study (F = 1.5; *df* = 4,74; *P* = 0.2128).

On 1 Dec. 2010, the *L. trifolii* population abundance was still significantly higher in the first 2-h interval (F = 12.77; *df* = 4,74; P = 0.0002) (Fig. 4) followed by the fourth, third, second and fifth intervals. Population activity of leafminers was minimum at the end of the day.

Population of *Diglyphus* spp. peaked at the second 2-h interval (F = 6.46; *df* = 4,74; *P* < 0.0001) (Fig. 4). Activity of *Diglyphus* decreased thereafter with the increase or decrease of daylight hours. However, *Diglyphus* populations were present all across the day. Population of *O. dissitus* was significantly lower during this study (Fig. 4). No significant difference (F = 0.2; *df* = 4,74; *P* = 0.9394) of the *O. dissitus* density was found among all the intervals.

When data across all intervals of a day were combined, a distinct pattern in the diel activity of leafminers was observed (Fig. 5). Peak activity of leafminers was observed during the first 2-h interval, which was significantly different (F = 7.22; *df* = 4,19; *P* = 0.0019) from all other intervals. The abundance of leafminers did not differ among the subsequent intervals.

Unlike leafminer, the density of *Diglyphus* spp. was highest (F

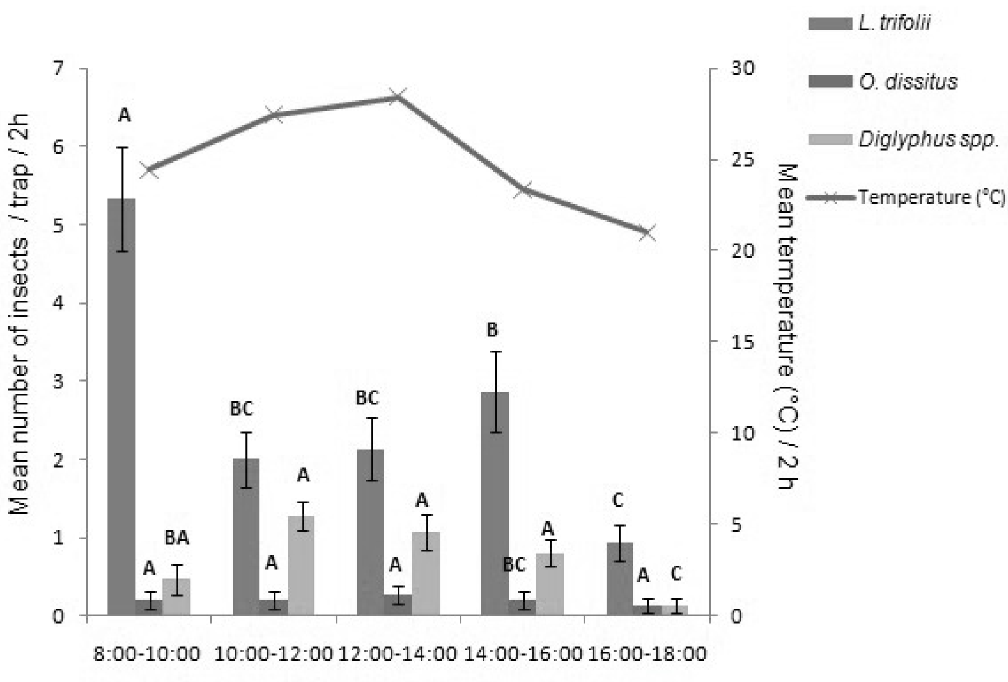

Fig. 4. Bean site 1, 1 Dec. 2010. Mean (± SE) number of the *L. trifolii*, *O. dissitus* and *Diglyphus* spp. / yellow sticky trap during each 2 h interval. Means with the same letter are not significantly different (*P* < 0.05, LSD test).

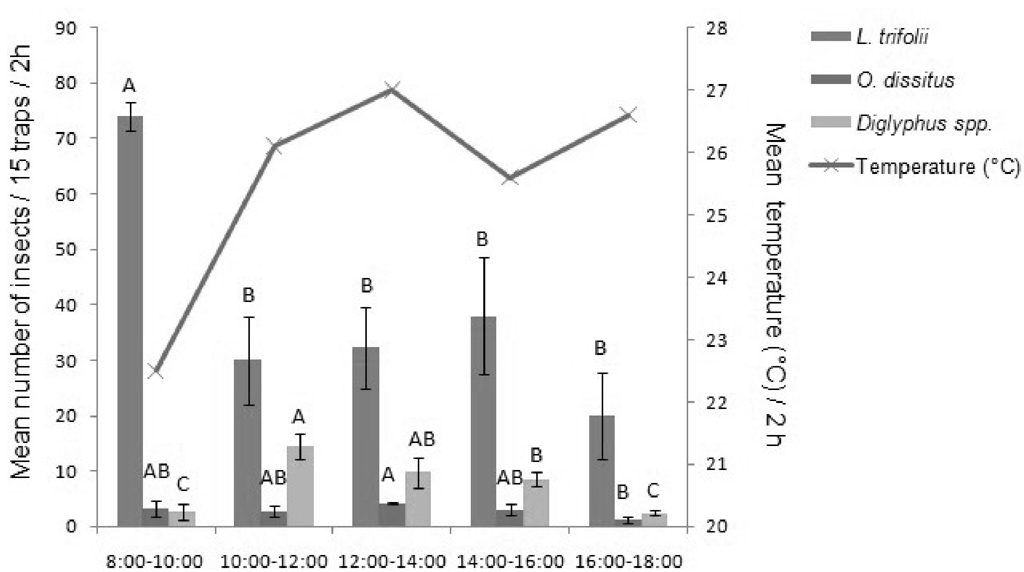

Fig. 5. Bean site 1. Mean (± SE) number of the *L. trifolii*, *O. dissitus* and *Diglyphus* spp. / 15 yellow sticky traps during each 2 h interval (based on combined data of 9, 16, 23 Nov. and 1 Dec. 2010). Means with the same letter are not significantly different (*P* < 0.05, LSD test).

= 7.6; *df* = 4,19; *P* = 0.0015) during the second 2-h interval than other intervals (Fig. 5). The lowest density level of *Diglyphus* was observed during the first and last 2-h intervals. No significant difference (F = 1.44; *df* = 4,19; *P* = 0.2696) was found in *O. dissitus* density level among all the intervals within the day.

Site 2

The dial activity pattern was studied based on the combined 4 d of data from 15 yellow sticky traps, because of the low abundance of insects in spring season 2011. The leafminer density did not show any significant difference (F = 0.7; *df* = 4,19; *P* = 0.6049) (Fig. 6) within the daytime.

Unlike site 1, the *Diglyphus* spp. had a significantly higher density level (F = 6.43; *df* = 4,19; *P* = 0.0032) (Fig. 6) in the third 2-h interval (12:01 – 14:00). The *O. dissitus* density presented a significant difference (F = 3.8; *df* = 4,19; *P* = 0.0251) during the daytime, and its density was highest in the second 2 h (1001–1200) among all the intervals.

Temperature was a major factor for leafminer and the parasitoid abundance as reported by the following author. The high daily temperature (≥30 °C) could reduce the adult's density (Abou-Fakhr-Hammad, 2000). At the bean site 1 (Nov. to Dec. 2010), the average temperature within the daytime changed greatly (22 °C to 27 °C), and it was relatively low (<25 °C) in the morning and high (>25 °C) during the rest of the day. The dial density of leafminer, *L. trifolii* was significantly higher in the first 2-h interval when the temperature was relatively lower (<25 °C) than other periods of the day. The leafminer population started to decrease when the temperature increased above 25 °C after the second 2-h interval. Therefore, the low temperature in the first

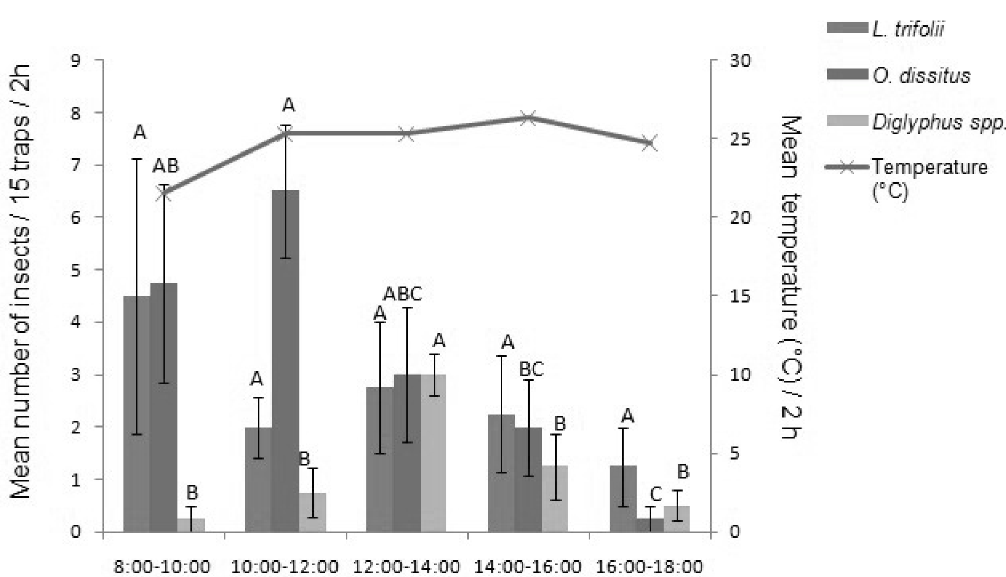

Fig. 6. Bean site 2. Mean (± SE) number of the *L. trifolii*, *O. dissitus* and *Diglyphus* spp. / 15 yellow sticky traps during each 2 h interval (based on combined of 31 Jan. 17 Feb. and 1 Mar. 2011). Means with the same letter are not significantly different (*P* < 0.05, LSD test).

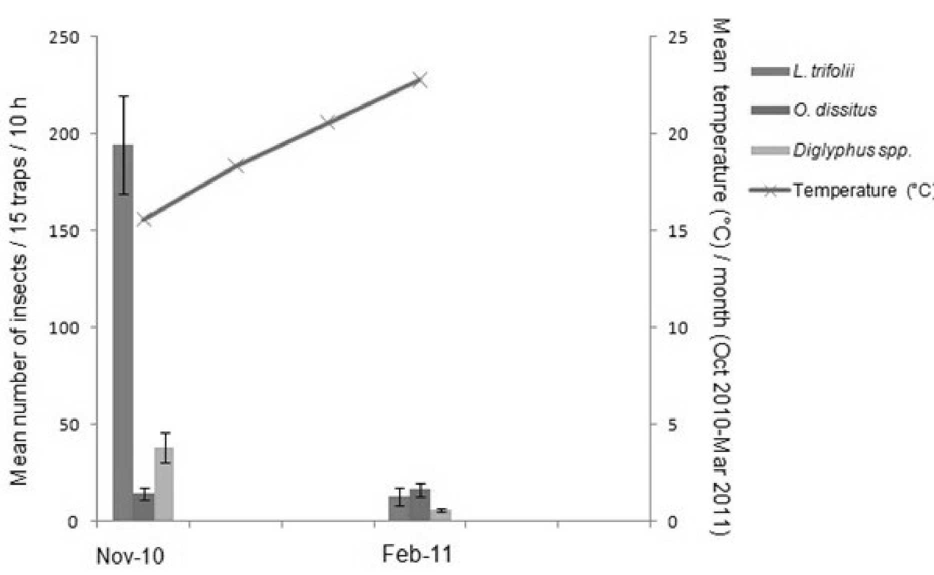

Fig. 7. Seasonal density: mean (± SE) number of the *L. trifolii*, *O. dissitus* and *Diglyphus* spp. per 15 yellow sticky traps / 10 h at bean site 1, 2010 and site 2, 2011. The mean temperature was from Nov. 2010 to Feb. 2011.

2 h (8:00–10:00) might be the main reason for higher density of leafminer in the morning than other periods of a day. Parasitoid of *O. dissitus* did not show any significant density difference among the periods within the day. The *O. dissitus* was reported that optimum temperature for both of male and female adults was 20 °C, and female had a higher reproduction at 25 °C (Bordat et al. 1995). The *Diglyphus* spp. density was always higher in the second 2-h interval than any other periods when the temperature was above 25 °C within the day.

There were three different species in *Diglyphus* spp. found in our study, and they are *D. begini* (Ashmead), *D. intermedius* (Girault), and *D. iseae* (Walker). The *Diglyphus* spp., as a larval ecto-parasitoid, has been used as leafminer biological control agents in greenhouse (Minkenberg, 1987), and the optimum temperature for rearing *D. isaea* from *L. trifolii* ranged between 32.3 °C and 32.6 °C (Bazzocchi et al., 2003). The relatively low temperature (<25 °C) in the first 2-h interval in the daytime might reduce the *Diglyphus* spp. density level. The density of *Diglyphus* increased to a higher level at the second 2-h interval when the temperature increased (>25 °C).

The 4 d of combined data in site 2 in spring 2011, shown in Fig. 6, did not provide a similar diel density pattern as in bean site 1. The average daily temperature was relatively high during late February and early March. The temperature in the morning was low but not significantly different from the temperature at other periods within the day, and it may not affect the diel density level of *L. trifolii*. *O. dissitus* density is relatively higher in the second 2-h interval. The highest density level of *Diglyphus* spp. was in the third 2-h (1201–1400) interval. These results agreed with the study of temperature influence on *O. dissitus* and *D.* sp. (Bazzocchi et al., 2003; Bordat et al., 1995).

The abundance of insects might be another factor for affecting the diel density pattern. The abundance of leafminer was significantly high in the bean site 1 but low in bean site 2 (Fig. 7). This might be another reason that the leafminer showed a significant density level in the first 2-h interval (0800–1200) within a day in the site 1 during the cool season. The high density of leafminer in fall season contributed a diel density pattern. However, there was not a significant diel density level of leafminer during daytime at the site 2 where the leafminer density was very low.

Yellow sticky traps were used for monitoring leafminer and its parasitoids diel density pattern in the study. The numbers of leafminers caught on yellow sticky traps were more at high population density than that of at low population density. Parrella and Jones (1985) revealed yellow sticky trap was a useful and rapid tool to estimate *L. trifolii* density on *Chrysanthemum* in greenhouse. In our study, the results indicated that the yellow sticky trap was an effective tool for monitoring the seasonal abundance of *Diglyphus* parasitoids density. Therefore, yellow sticky traps can be used as an effective tool in the research on leafminer and some parasitoid ecology.

The parasitoids of *O. dissitus* and *Diglyphus* spp. were found to be the most abundant parasitoid wasps of leafminer *L. trifolii* on bean crops. *O. dissitus* is a larval-pupa endoparasitoid of *Liriomyza* leafminer and adults emerge from the pupae of leafminer. *Diglyphus* parasitoids were ectoparasitoid of leafminer larvae and adults emerge from the mines on foliage. Understanding the diel biological behavior of leafminer and parasitoid could improve pest management strategies in open field crops. Releasing the leafminer parasitoids during the appropriate time and season could enhance the biological control effectiveness in the open field environment.

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