Effect of intercropping pepper with sugarcane on populations of *Liriomyza huidobrensis* (Diptera: Agromyzidae) and its parasitoids

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**ABSTRACT**

The effect of intercropping pepper with sugarcane on populations of the leafminer *Liriomyza huidobrensis* Becker was investigated over two seasons (2007, 2008) in Yunnan, China. Populations of adults, the female:male sex ratios and the densities of larval mines were significantly higher in pepper monoculture than in intercropping in a 2:2 pattern (2 rows of pepper to 2 rows of sugarcane), which in turn were significantly higher than intercropping in a 1:2 pattern (1 row of pepper to 2 rows of sugarcane). The densities of the parasitoids of the leafminer were significantly higher in intercropping than in the pepper monoculture. The total parasitism rates were 71.6%, 22.5% and 5.9% in 2007, and 69.5%, 20.6% and 5.9% in 2008, in the 1:2, 2:2 and pepper monoculture, respectively. The lower densities of leaffminer adults, the lower female:male sex ratio, and smaller numbers of larval mines in the intercropping systems were attributed to the intercropping pattern and the growth of pepper. These results indicate that intercropping is suitable for the control of *L. huidobrensis* on pepper plants. These results demonstrate the utility of intercropping of pepper with sugarcane in the management of *L. huidobrensis*.

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1. Introduction

*Liriomyza huidobrensis* Becker (Diptera: Agromyzidae) is one of the most damaging pests of vegetables and ornamentals (Zhou et al., 2000). Larvae feed within the lower mesophyll layer of leaves resulting in reduced photosynthesis, which directly impacts growth and yield (Heinz and Chaney, 1995). The pest was first reported in Yunnan Province of China in 1993 (Jiang, 1997). Since then the insect has spread over a larger area attacking an ever increasing number of plant species. Up to 1990, it was reported from elsewhere to feed on plants belonging to 14 families (Spencer, 1990), while in China, so far, it has been reported to attack 196 plant species belonging to 39 families (He et al., 2001). Certain plant species are preferred over others (Carolina and Marshall, 1992; He et al., 2001; Li et al., 2004). In some areas, *L. huidobrensis* is more serious than the long-established *Liriomyza sativae* (Yang et al., 1999; Zhou and Luo, 2000).

Several species of natural enemies play an important role in the regulation of leafminer populations (Waterhouse and Norris, 1987; Murphy and LaSalle, 1999) and at least 23 species of parasitoids have been applied in biological control programs against *Liriomyza* spp. (Waterhouse and Norris, 1987; Greenhead and Greenhead, 1992; Johnson, 1993) resulting in as much as 90% parasitism. Even then, the leafminer populations can be still high enough to cause serious damage to the crop. Although frequent use of insecticides results in pests becoming resistant to insecticides (Murphy and LaSalle, 1999; Civelek and Weintraub, 2003), by reducing natural enemy populations (Heinz and Chaney, 1995; Weintraub and Horowitz, 1996), thus leading to a resurgence of pest population (Parrilla et al., 1983; Keil et al., 1985; Smith, 1986), insecticides are widely used in China to combat leafminers. This has resulted in *L. huidobrensis* becoming a primary pest from its earlier status of a secondary one (Spencer, 1973; Minkenberg and van Lenteren, 1986).

Intercropping has been adopted by many Chinese farmers owing to its economic and agronomic advantages over monoculture (Jeffers and Triplett, 1979; Chan et al., 1980; Jeffers, 1987, 1990). Sugarcane (*Saccharum sinensis* Roxb) is an important commercial crop in Mile County of Yunnan Province of China. Intercropping of sugarcane with maize (*Zea mays* L.), potato (*Solanum tuberosum* L.),
and peanut (*Arachis hypogea* L.) is common practice there. The objective of this study was to compare infestation of *L. huidobrensis* and activities of its parasitoids on pepper (*Capsicum annuum* L.), an important vegetable in Yunnan, which has potential for being suitable for intercropping with sugarcane.

2. Materials and methods

2.1. Study location

The experiment was conducted 2007 and 2008 in Zhuyuan District, Mile County, in Yunnan Province of China. Zhuyuan is at an altitude of 1170 m, with annual average temperature of 19.4 °C. A randomized complete block design was used with three treatments and three replicates. Sugarcane (variety Yuetang 93–159) was a ratoon crop after the first harvest. Pepper (variety Qiangfeng No.1) seedlings were planted in mid-February between two 1.4 m wide and 30 m long rows of sugarcane, in a pattern of 1:2 (1 row of pepper to 2 rows of sugarcane), 2:2 (2 rows of pepper to 2 rows of sugarcane) and 2:0 (no sugarcane, pepper monoculture). Three blocks, each of 630 m², were set up. Cultural practices included fertilizer application, irrigation and mechanical weeding; no insecticide was applied. Experiments in both years were conducted from March to June. Observations on pest infestation and natural enemies were recorded from 15/March, the third week after pepper planting and ended on 7/June, with harvest of the crop in 2007 and 2008, when the sugarcane was still standing.

2.2. Sampling and collection of adult *L. huidobrensis*

Adult leafminers as well as parasitoids, on and near pepper plants, were sampled once a week using a RYOBI Leaf Blower W/VAC suction sampler. The collection cone was 13.0 cm in diameter (Model 310BVR-EZ) (Dietrick, 1961; Timothy and Ronald, 2001). The collection cone of the suction sampler was lowered as near to the plant as possible making sure there was no damage to the plants, for 2 s at 10 randomly selected locations within a plot. For each subsample, collection locations were regularly distributed over the pepper rows. Within each pepper row, sampling was done randomly. All insect specimens removed from the sampler were frozen at −10 °C. No samples were collected on windy days.

2.3. Observations on leaf mines and punctures

Once a week, 15 plants were selected from 5 rows of pepper in each plot, with 3 plants taken from each row. The number of mines in the leaves from the lower and upper parts of the pepper plants was carefully examined and recorded.

2.4. Parasitism study

A total of 30 leaves was randomly plucked from the lower canopy of 10 pepper plants in each plot, 5 plants being sampled in each row. Sampled leaves were stored at room temperature (25–28 °C). Emerging leafminer and parasitoid adults were counted and viewed under stereoscopic microscope, sorted, and identified based on difference in the external morphology of the adeagus (Spencer, 1973; Hansson, 1990; Wang et al., 1998; Xu et al., 1999). Cumulative parasitism and the contribution of each species were estimated using a method developed by Horváth and Benedk (2007).

2.5. Data analysis

All the data were subject to the one-way analysis of variance for repeated measures with post hoc LSD multiple comparisons. Densities of the parasitoids were transformed using log_{10}(x + 1) before statistical analyses. All procedures were carried out by using SPSS 11.0 software program for Windows.

3. Results

3.1. Populations of adult *L. huidobrensis*

*L. huidobrensis* was the dominant species of leafminer on pepper and which contributed 97.7%, even though the species L. sativae (Blanchard) was found on the pepper plants, which contributed only 2.3% in number. In the initial stages, no significant difference was found between the population of adults or immatures on monocropped and intercropped pepper. The population densities of the leafminer were 1.13, 0.90 and 0.67 leafminers per suction sampler in the monocrop field, intercropping of 2:2 and 1:2 field in 2007, respectively. They were 0.60, 0.70 and 0.57 leafminers per suction sampler in the monocrop field, intercropping of 2:2 and 1:2 field in 2008, respectively. However, as the season progressed, the trend of the average population density of the leafminer from different treatments was similar, and a significant level of suppression of the leafminer population density occurred in pepper-sugarcane intercropping compared with pepper monoculture (Fig. 1). Insect numbers increased with the growth of the pepper, with a rapid increase from the 4th week after transplanting of the pepper. Pest populations first peaked at 13.2, 7.1, and 2.9 leafminers per suction sampler in 2007 for monoculture, 2:2 and 1:2 intercropping, respectively. Insect numbers differed significantly. In 2008, pest populations first peaked at 13.2, 5.0, and 0.9 *L. huidobrensis* adults/suction sample on pepper in the pepper monoculture, 2:2 and 1:2 cropping patterns, respectively. By the
6th week after the transplanting, the population of *L. huidobrensis* increased more quickly in the pepper monoculture plots than in 2:2 and 1:2 intercropping patterns. The second peak was in the 13th week after the transplanting of pepper in both years, with populations of 17.8, 11.9, and 7.4 per suction sample, respectively, for the three cropping patterns in 2007 and 18.3, 11.1, 5.5 *L. huidobrensis* per suction sampler in 2008. Pest populations increased concurrently with the growth of the pepper, and the maximum insect numbers were observed in the final sampling date, when the populations in the pepper monoculture, 2:2 and 1:2 intercropping were 28.5, 14.9, 10.5 per suction sampler in 2007, and 34.3, 15.7, 8.4 per suction sample in 2008. They were significantly different (2007: $F_{2,320} = 351.2, P < 0.01$; 2008: $F_{2,320} = 312.4, P < 0.01$). The tests of within-subject effects showed there was a statistical significance of total variation of the density of leafminer from the third week to the 13th week after pepper transplanting (2007: $F_{2,260} = 23.4, P < 0.05$; 2008: $F_{2,260} = 29.8, P < 0.05$). Compared with the monocrop group at the first peak of population density, it was lower ($P < 0.05$ in 2:2 and $P < 0.01$ in 1:2).

Overall, for entire season from the third week of the transplanting to the harvest of the pepper, there were statistically significant differences between the insect population in 1:2: 2:2 intercropping and pepper monoculture fields. Monoculture harbored higher *L. huidobrensis* numbers than the 2:2 or 1:2 intercropping systems (2007: $F = 54.6, P < 0.01$; 2008: $F = 35.7, P < 0.01$); the highest density of leafminer was in the monoculture cropping. Pest populations on pepper plants in 1:2 intercropping were always lower than that in the 2:2 intercrop system, and the density of leafminer from the different cropping system followed the order of monoculture cropping > 2:2 intercropping > 1:2 intercropping. Moreover, the density of leafminer in the 2:2 intercropping was significantly higher than that in the 1:2 intercropping (2007: $F = 79.3, P < 0.01$; 2008: $F = 84.3, P < 0.01$). The seasonal abundance of leafminers in intercropping systems was usually delayed, relative to pepper monoculture.

Finally, the multivariate test of repetitive measures ANOVA (2007: $F = 156.5, P < 0.01$; 2008: $F = 149.8, P < 0.01$) indicated that the interaction between the intercropping system and time showed a statistical significant effect on the density of leafminer.

### 3.2. Female: male sex ratio of *L. huidobrensis*

The populations of *L. huidobrensis* female adults were far higher than that of the males throughout the season in the pepper monoculture than pepper intercropping with sugarcane and it changed with the growth of the pepper (Fig. 2). The female: male sex ratio increased in favor of females with the growth of the pepper plants starting from the third week after transplanting of the pepper in all planting patterns. The maximum female: male sex ratio was 3.95 in favor of females in the 7th week after the transplanting in the pepper monoculture, and 2.6 and 1.7 in the 8th week after the transplanting in the 2:2 and 1:2 intercropping, respectively. The female: male sex ratio decreased with the growth and maturity of the pepper, especially nine weeks after transplanting. The tests of within-subjects effects showed that the total variation of sex ratio at survey dates was statistically significant (2007: $F = 24.4, P < 0.05$; 2008: $F = 27.6, P < 0.05$). Overall, the female: male sex ratio of *L. huidobrensis* was significantly higher in the pepper monoculture than in the intercropping systems at all time points throughout the survey season (2007: $F = 28.5, P < 0.01$; 2008: $F = 22.4, P < 0.01$). The highest female: male sex ratio was in the pepper monoculture and the lowest in the 1:2 intercropping. Moreover, the female: male sex ratio of leafminer in the intercropping of 2:2 was significantly higher than that in the 1:2 (2007: $F = 32.64, P < 0.01$; 2008: $F = 45.51, P < 0.01$). But the density of females remained higher in the pepper monoculture than in the intercropping systems even though the female: male sex ratio decreased with the growth of the pepper and sugarcane plants. By the end of the season, at the time of the harvest, the female: male sex ratio decreased. It coincided with the reduced oviposition and survivorship. Even then, female adults were still significantly greater in numbers in pepper monocropping than in the pepper intercropping plots.

There was a significant interaction between treatment and time by the multivariate test of repetitive measures ANOVA (2007: $F = 125.23, P < 0.01$; 2008: $F = 117.34, P < 0.01$). Compared with the result of within-subjects, the effect of the time point on the female: male sex ratio changed with the cropping system.

### 3.3. Mines and punctures on leaves of pepper plants

Adult *L. huidobrensis* usually puncture leaves for both feeding and oviposition, with the latter type of punctures leading to leaf mines after egg hatch. Mines on leaves of pepper plants made by leafminer larvae, significantly increased in number as the season progressed ($P < 0.05$ in 2007 and 2008), rapidly from six weeks after transplanting in both 2007 and 2008 (Fig. 3). The mine densities were 0.35, 0.30, 0.20 from the monocrop, 2:2 and 1:2 intercrop pattern, respectively in 2007, and 0.50, 0.80, 0.45 in 2008, on the initial survey date. On the 6th survey date, they were 9.8, 6.3, 3.5 in the monocrop, 2:2 and 1:2 intercrop pattern, respectively in 2007, and 8.7, 7.4, 6.1 in 2008. The variance of mines with time was tested by one-way ANOVA. The result showed that the mines differed significantly between the different survey dates in monoculture and intercrop treatments in 2007 and 2008 ($P < 0.05$ for the monocrop, 2:2 and 1:2 intercropping pattern). The cumulative number of mines per plant
increased to a maximum of 29.7, 21.7, and 15.0 at final sampling in 2007 and 31.3, 23.3, and 15.7 in 2008, respectively, in pepper monoculture, 2:2, and 1:2 intercropping patterns, respectively. Tests of between-subject effects were conducted. The results showed that the mine numbers were significantly greater in the pepper monocrop than in the 2:2 and 1:2 intercropping pattern (F = 33.1, P < 0.01 in 2007; F = 15.1, P < 0.01 in 2008), the mine numbers in the 1:2 intercropping were significantly lower than that in the 2:2 (2007: F = 23.5, P < 0.01; 2008: F = 21.43, P < 0.01).

Moreover, the interaction between the intercropping treatment and time was statistically significant for the numbers of mines by multivariate tests of repetitive measure ANOVA (2007: F = 39.8, P < 0.01; 2008: F = 41.5, P < 0.01). Moreover, the density of the parasitoids increased quickly in the intercropping of 1:2 than that of in the 2:2 and monoculture from the third week to the 9th week after the transplanting pf pepper, and the density of parasitoids was inversely related to the density of leafminer from the intercropping of 2:1 (P < 0.01) and 2:2 (P < 0.05), but there were no significant interactions between the density of parasitoids and the density of leafminer in the monocropping systems.

Three species of parasitoids; Diglyphus isaea Walker, Opius sp. and Hemiptarsenns varicornis (Girault) were the main natural enemy species in all three cropping patterns. During March to June, a total of 399 and 474 parasitoid specimens was collected from the sampled pepper leaves in 2007 and 2008, respectively. The number of specimens of D. isaea, Opius sp., and H. varicornis amounted to 76, 169, 154 in 2007, and 299, 132, 43 in 2008, respectively. D. isaea and Opius sp. contributed 62.4% and 27.3% of parasitoids in 2007, and 63.1% and 27.9% in 2008.

### 3.4. Parasitoid species and parasitism of *L. huidobrensis* 3.4.1. Suction trapping of adults

In the first survey, in the 3rd week after pepper transplanting, very few parasitoid adults were captured in suction samplers in both 2007 and 2008. From the 4th week onwards, however, the number of parasitoid increased rapidly and peaked in the 9th week in the 1:2 intercropping; the density was 0.60 and 0.30 per suction sampler in 2007 and 2008, respectively. From the 10th week after the transplanting of pepper, the density of parasitoids in the 1:2 intercropping decreased with the growth of the pepper and sugarcane. The maximum parasitoid density in the 1:2 intercropping and pepper monoculture in 2007 was in the 11th week and 14th week, with density of 0.27 and 0.10 parasitoids per suction sampler, respectively. In 2008, the maximum density of the parasitoids in the 1:2 intercropping and pepper monoculture was in the 10th week and 13th week, with densities of 0.11 and 0.05 parasitoids per suction sampler, respectively. The cumulative density of the parasitoids was significantly higher in 1:2 intercropping than in 2:2 intercropping and the pepper monoculture planting (2007: F = 39.8, P < 0.01; 2008: F = 41.5, P < 0.01). Moreover, the density of the parasitoids increased quickly in the intercropping of 1:2 than that of in the 2:2 and monoculture from the 3rd week to the 9th week after the transplanting pf pepper, and the density of parasitoids was inversely related to the density of leafminer from the intercropping of 2:1 (P < 0.01) and 2:2 (P < 0.05), but there were no significant interactions between the density of parasitoids and the density of leafminer in the monocropping systems.

### Table 1

Rate of the parasitism of *Liriomyza huidobrensis* infesting pepper planted as monoculture and intercropped with sugarcane in a 2 rows pepper:2 rows of sugarcane(2:2), and 1 row of pepper:2 rows of sugarcane(1:1) pattern in Yunnan, China, in 2007 and 2008(df = 8).

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<th>2008 Monoculture</th>
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SED: standard error of difference. ***: P < 0.01. **:** P < 0.001.
parasitoid adult emerged an individual leafminer larva/pupa. In the initial stages, the parasitism was lower and no significant difference was found between the parasitism on leafminer in pepper in the monocrop and intercrop treatments. However, as the season progressed and the population of leafminer increased, a significant difference was found in pepper-sugarcane intercropping compared to pepper monoculture (Table 1). The total parasitism increased quickly from the 4th week and peaked at the 5th week after the transplanting of the pepper, with parasitism of 3.3%, 6.3%, 9.6% on pepper in pepper monoculture, 2:2 and 1:2 cropping patterns in 2007, and 5.1%, 11.3%, 20.6% in 2008, respectively. By the 6th week after the transplanting, the parasitism decreased, and it increased again from the 7th week, and the second peak was in the 11th week, with parasitism of 2.7, 7.6 and 14.5% in the pepper monoculture, 1:2 and 2:2 intercropping patterns, respectively, and that in the 1:2 pattern was significantly higher than that of in the 2:2 and monoculture pattern ($F = 56.3, P < 0.01$). In 2008, the second peak was in the 14th week after the transplanting of pepper, with parasitism of 3.6%, 9.4% and 13.0% in the pepper monoculture plots than in 2:2 and 1:2 intercropping patterns, respectively. The maximum parasitism was in the 1:2 intercropping pattern and it was significantly higher than that of in the 2:2 intercropping pattern and the monoculture ($F = 338.6, P < 0.01$). Throughout the season, there were statistically significant differences between the parasitism in 1:2, 2:2 intercropping and pepper monoculture fields. The parasitism in the pepper intercropping was significantly higher than in the pepper monoculture (2007: $F = 43.9, P < 0.01$; 2008: $F = 49.8, P < 0.01$).

The tests of within-subjects effects showed that the total variation of parasitism did not vary significantly in 2007 ($F = 5.76, P > 0.05$) and 2008 ($F = 7.86, P > 0.05$), but there was a significant effect of interaction between the treatment and time by multivariate test of repetitive measures ANOVA (2007: $F = 115.4, P < 0.01$; 2008: $F = 126.2, P < 0.01$), so that the intercropping pattern significantly affected the parasitism.

4. Discussion

Pepper is an important cash crop in sub-tropical areas in China, but leafminers are an important constraint to its production. The system of intercropping short-season crops with sugarcane, an all-year-round crop, has been adopted by Chinese farmers quickly because it increases farm income per unit area (Chen et al., 1989; Li, 1989; Lai, 1998; Feng, 1996; Su et al., 1998; Li et al., 2009). Soon after planting new or rotting of recently harvested sugarcane crop, large portion of land between sugarcane rows remains underutilized for 3–4 months. Quick-growing crops, therefore, are planted between rows of sugarcane, which improve productivity of land. Popularly grown intercrop species are pepper, corn, cabbage, potato, and tomato. Our study revealed that intercropping pepper with sugarcane suppressed leafminer damage to pepper significantly more monochop of pepper. We also found the female:male sex ratio of pepper than in the intercropping system. These findings are consistent with similar studies with other intercropping systems (Rabb et al., 1976), which are not available in monoculture. Higher abundance of natural enemies in polyculture has also been found in other field studies (Root, 1973; Risch et al., 1983).

Multiple cropping often results in a significant reduction in pest problems (Altieri and Letourneau, 1982). Intercropping short-duration crops with sugarcane is a common practice in Yunnan, a major sugarcane and vegetable growing province of China. Such intercropping system increases farm income by US$15,000 per ha (personal communiqué from local agricultural technicians and interviews with farmers). Our study shows that this practice also reduces pest damage and contributes to increased savings in pest control practices as well as resulting in safer food and a cleaner environment.

Acknowledgments

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References


