Impact of Wheat–Mung Bean Intercropping on English Grain Aphid (Hemiptera: Aphididae) Populations and Its Natural Enemy

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J. Econ. Entomol. 105(3): 854–859 (2012); DOI: http://dx.doi.org/10.1603/EC11214

ABSTRACT The effects of intercropping wheat, *Triticum aestivum* L., with mung bean, *Vigna radiate* L., on the populations of English grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae), and its natural enemies were evaluated by field and laboratory experiments. The population densities of aphids and their natural enemies were evaluated in the intercropped field against different row ratio combinations of wheat–mung bean. Results showed that wheat–mung bean intercropping caused a drop in aphid densities, and the ratio 12 wheat:4 mung bean brought about the largest drop (>18%). In addition, the population densities of coccinellids (ladybirds) and parasitoids and the species diversity of all the natural enemies of aphid were higher in the intercropped field than in the field planted only with wheat. However, intercropping did not influence the community indices (evenness and index of dominance concentration) of the natural enemies. Y-tube olfactometer bioassays were carried out in the laboratory to test whether odor blends of host and nonhost plants affect the host selection of *S. avenae*. Bioassays indicated that both apterous and alate aphids significantly preferred host plant odor over odor blends of host and intercropped species. Hence, the olfactory-based host location of aphids in the field might be affected by intercropping. The intercropping experiment clearly showed that increased crop species diversity suppresses aphid population growth and preserves the population of natural enemies of aphids. Our results also provide support for the "resource concentration hypothesis" and the "enemies hypothesis."

KEY WORDS wheat, intercropping, *Sitobion avenae*, natural enemy, community index

The English grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae), is a major wheat, *Triticum aestivum* L., pest in China. It feeds on phloem sap and spreads plant viruses, consequently reducing yields. The English grain aphid is difficult to control because of its short life cycle and vigorous reproduction (Irwin et al. 1988).

The disadvantages of chemical control in combating pests such as aphids have become apparent over time. Thus, integrated pest management continues to garner much attention because one of the methods it involves for controlling pests is increasing crop diversity, and crop diversity is beneficial in terms of environmental protection and food security (Altieri and Gliessman 1983, Bach and Tabashnik 1990, Altieri 1999, Gurr et al. 2003). Intercropping wheat with other crops is an important cultivation method for increasing crop diversity in China. Many studies have examined winter wheat intercropped with other crops, especially leguminous crops. These intercropping systems form complicated and stable wheat field ecosystems that enhance the chances for survival and reproduction of beneficial insects, thereby making sustainable pest control possible (Ferguson et al. 2006, Wang et al. 2009, Zhou et al. 2009). Previous researchers have reported agronomic data on wheat–mung bean (*Vigna radiate* L.) intercropping systems (Sharma et al. 1996, Liu 1999, Quayyum et al. 2002). However, little information is available on the effects of wheat–mung bean intercropping systems on populations of aphids and their natural enemies.

Different plant species emit different odors, and host plant odor provides major chemical cues for host location of herbivorous insects; nonhost plant odor can repel insects or obscure host plant odor (Visser 1986). Numerous studies have shown the effects of odor blends of different plants on pests' host selection. For example, in studies on the host selection of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), Thiery and Visser (1986, 1987) observed that nonhost plant odor camouflage the odor of the host plant. Regarding aphids, Nottingham et al. (1991) showed that the bean aphid, *Aphis fabae* Scopoli, has a positive response to the odor of its host plant but has no response to the combined (1:1 ratio) odor of leaves from its host plant and nonhost plant. Similarly, the

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odor of soybean, *Glycine max* (L.) Merr., leaves can attract the soybean aphid, *Aphis glycine* Matsumura, but odor from a mixture of its host and nonhost plants does not influence its behavior (Du et al. 1994).

The complicated agroecosystem of intercropped fields can influence the visual and olfactory sensation of resident insects, disturbing their searching behavior, as predicted by the resource concentration hypothesis (Root 1973, Landis et al. 2000). The enemies hypothesis also states that diverse cropping systems can provide more habitats and food (e.g., pollen) compared with simple systems; therefore, diverse cropping systems harbor more enemies, thereby increasing their effectiveness (Root 1973). Aside from potentially interfering aphids' host location with volatile cues, intercropping wheat with mung bean also can offer natural enemies extra food (pollen) and habitat (Sharma et al. 1996).

The current study evaluated 1) whether intercropping wheat and mung bean in different row-ratio combinations could reduce aphid densities and increase natural enemy species, as described by the enemies hypothesis; and 2) whether the combination of host and nonhost plants would disrupt aphids' olfactory-based host location (via Y-tube experiments), as predicted by the resource concentration hypothesis.

Materials and Methods

Field Experiment. *Varieties of Wheat and Mung Bean.* Wheat variety Beijing 837 was provided by the Institute of Plant Protection (IPP) of the Chinese Academy of Agricultural Science (CAAS). Mung bean variety Jilv 2 was provided by the Institute of Crop Sciences (ICS) of CAAS.

Field Experimental Design. The study was conducted at IPP-CAAS experimental farm in Langfang, Hebei Province, China (116° 69′ E, 39° 52′ N) in 2010. The field experiment consisted of five treatments: 10W:10MB (i.e., 10 rows of wheat:10 rows of mung bean), 12W:6MB, 12W:4MB, 16W:4MB, and W-M (wheat monoculture; 23 rows of wheat). All treatments were arranged in a randomized block design and replicated three times. Each treatment plot measured 67 m^2. One meter-wide guard rows were planted around the experimental field. Wheat was planted on 12 October 2009 in rows 30 cm apart and harvested on 25 June 2010. Mung bean was planted on 15 April 2010 in rows 40 cm apart and harvested on 5 July 2010. The space between the rows of wheat and mung bean was 40 cm. No pesticides or herbicides were used in the whole experimental area. Other field management activities were consistent with common agronomic practices in northern China.

Sampling of Insects. Sampling of apterous aphids was done following a Z-shaped" sampling pattern, in which 10 sampling sites were selected within each plot. At each sampling site, we randomly selected 10 wheat tillers and counted the number of apterous aphids on the tillers (100 tillers per plot). Yellow sticky board traps (20 by 30 cm) were used to monitor alate aphids; the number of alate aphids caught on both sides of the trap was recorded. One yellow sticky board trap was placed in the middle of each plot. Sampling of enemies was done in a 1-m^2 (1- by 1-m) zone randomly selected in each plot. Larvae of natural enemies on the wheat tillers were directly counted. Adult natural enemies were caught in a sweep net swung five times. All larvae and adults that could not be identified directly were collected and preserved in 75% ethyl alcohol, and they were taken to the laboratory for identification. Insects were sampled every 4 d from 6 May to 11 June 2010. The highest population densities of apterous aphids, alate aphids, ladybirds, and parasitoids were observed on (peak times) 30 May, 3 June, 30 May, and 7 June, respectively.

Analysis of Community Indices for Aphid Natural Enemies. Species richness index is expressed as S, indicating the number of natural enemy species.

Evenness was calculated: $J' = H'/H'_{max} = H'/\ln S$, where S is the species richness index and $H' = -\sum_{i=1}^{s} P_i \ln(P_i)$; Pi is the proportion of the total number of species i in the natural enemy community.

Index of dominance concentration was calculated: $C = \sum_{i=1}^{s} (P_i)^2$, where P_i is the proportion of the total number of species i in the natural enemy community.

Host and Nonhost Bioassays. *Aphids. S. avenae* reared on *T. aestivum* were inoculated on wheat at one- to two-leaf stage in a flowerpot at 20 ± 1°C under a photoperiod of 16:8 (L:D) h. Adult aphids were starved for 12 h before the experiment.

Odor Source. The wheat and mung bean varieties used in this experiment (same as under Field Experiment) were provided by IPP and ICS, respectively. Wheat and mung bean were grown in flowerpots at 20 ± 1°C under a photoperiod of 16:8 (L:D) h until they reached the four-leaf stage. Choice experiments were done in a Y-tube olfactometer with the following combinations: 1) five *T. aestivum* plants versus clean air, 2) five *V. radiate* plants versus clean air, 3) five *T. aestivum* plants versus five *V. radiate* plants, 4) five *T. aestivum* plants and five *V. radiate* plants versus clean air, and 5) five *T. aestivum* plants versus five *T. aestivum* plants and five *V. radiate* plants. Plants (combinations) were placed in a 1,000-ml wide-mouthed bottle fitted with a rubber stopper. The glass inlet and outlet tubes, through which clean air was introduced and replaced with odor of plants, were fitted with rubber stoppers.

Y-Tube Olfactometer Bioassays. The glass Y-tube olfactometer used to test the behavioral responses of the aphids to odor source was similar to the olfactometer described by Du et al. (1996), with 2-cm-internal diameter and 60° inside angle. The length of its stem was 10 cm. A pump provided a stream of air that successively passed through an activated charcoal filter, distilled water, odor source, and a flow meter, before entering into each arm. Airflow was maintained at 100 ml/min. All connections between the parts were Teflon (PTFE) tubing. The olfactometer was placed in a chamber with a temperature of 20 ± 1°C. Aphids were released 5 cm from the opening of the stem of the

Y-tube. Choosing occurred 5 cm into the olfactometer arm. Aphids that did not make a choice or moved <5 cm into the olfactometer arm 5 min after release were considered nonresponsive. The positions of the two arms were switched after testing five aphids. For each combination (plants or versus air), 50 aphids were tested individually. Before each bioassay, the system was washed and rinsed with acetone and placed in an oven at 160°C for 2 h.

Statistical Analysis. All data on population densities were analyzed using analysis of variance (ANOVA), and means were compared using the Bonferroni correction (SAS institute 2001). The significant level was set at $P = 0.05$. The number of apterae was transformed using $\sqrt{x}$, and the number of alatae, ladybirds, and parasitoids was transformed using $\sqrt{(x + 1)}$, to meet the assumption of normality. The frequencies of aphid choices observed in the Y-tube experiment were compared using a chi-square test.

Results

Population Densities of Aphids and Their Natural Enemies. *Apterous S. avenae.* During the sampling period, the average densities of the apterous aphids in the wheat monoculture field were not different from the average densities in the 10W:10MB, 12W:6MB, and 16W:4MB fields, but they were significantly higher than the average densities in the 12W:4MB field ($F = 5.83$; df = 4, 10; $P = 0.02$; Fig. 1A). The day (30 May) the highest densities of apterous aphids were observed, there were more apterous aphids in the monoculture field than in the 12W:4MB and 16W:4MB fields, but no significant difference was detected between the densities of apterous aphids in these two fields ($F = 23.10$; df = 4, 10; $P < 0.001$; Fig. 1A).

Alate S. avenae. During the sampling period, the populations of alate aphids observed in the wheat monoculture field were significantly higher than the populations of alate aphids in the 12W:4MB field. The number of alate aphids was smallest in the 12W:4MB field compared with the other intercropped fields ($F = 10.92$; df = 4, 10; $P < 0.01$; Fig. 1B). The densities of alate aphids in both 12W:6MB and 12W:4MB fields were also lower than the densities in the monoculture field on the day (3 June) that the highest densities of alate aphids were observed ($F = 8.49$; df = 4, 10; $P < 0.01$; Fig. 1B).

Ladybirds. Being indicators of the abundance of the natural enemies of aphids, the total population sizes of the following species were estimated: *Coccinella septempunctata* L., *Propylaea japonica* Thunberg, and *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). The densities of these ladybirds were significantly lower in the monoculture field than in all the intercropped fields. The populations of the indicators were highest in the 12W:4MB field, but this population was not significantly different from the populations in the 10W:10MB and 16W:4MB fields ($F = 24.20$; df = 4, 10; $P < 0.001$; Fig. 1C). The densities of the ladybirds in all the treatments were not signif-

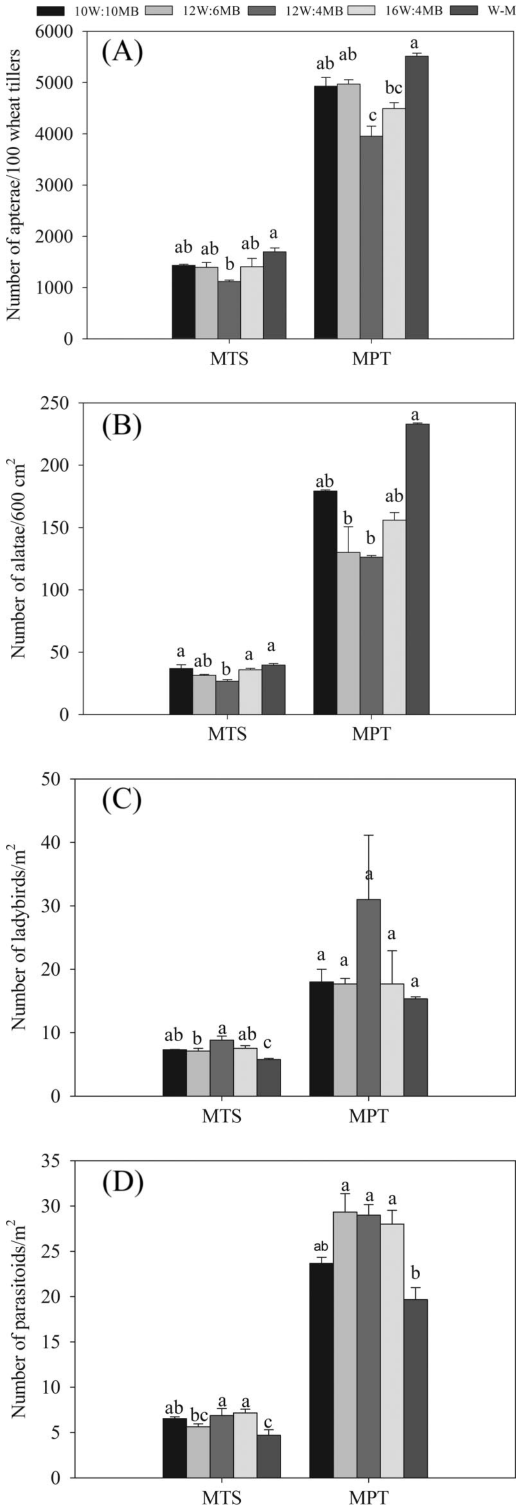

Fig. 1. Numbers (mean ± SE) of *S. avenae* and its natural enemies. (A) Apterous aphids. (B) Alate aphids. (C) Ladybirds. (D) Parasitoids. MTS, mean of 10 samples from 6 May to 11 June 2010; MPT, mean at peak time. Bars topped by different letters are significantly difference at 0.05 level.

Table 1. Species richness index, evenness, and index of dominant of aphid natural enemies in different treatments

Treatment	Accumulative total species (*S*)	Mean of evenness (*J*′)	Mean dominant concn index (*C*)
10W:10MB	12.00 ± 0bc	0.87 ± 0.02a	0.25 ± 0.02a
12W:6MB	13.67 ± 1.20ab	0.83 ± 0.03a	0.26 ± 0.02a
12W:4MB	15.67 ± 0.33a	0.79 ± 0.02a	0.25 ± 0.03a
16W:4MB	15.67 ± 0.33a	0.83 ± 0.02a	0.25 ± 0.03a
W-M	10.00 ± 0.33c	0.86 ± 0.02a	0.30 ± 0.03a

Means within columns followed by the same letter are not significantly different between treatments ($P < 0.05$).

icantly different on the peak time (30 May) of ladybird densities (Fig. 1C).

Parasitoids. Being indicators of the abundance of aphid parasitoids, the total population sizes of the following species were estimated: *Aphidius gifuensis* Ashmead and *Aphidius avenae* Haliday (Hymenoptera: Aphidiidae). The densities of these parasitoids in the wheat monoculture field were significantly lower than their densities in the intercropped fields except in the 12W:6MB field; the densities of the parasitoids in the 10W:10MB, 12W:4MB, and 16W:4MB fields were not different from one another ($F = 20.80$; df = 4, 10; $P < 0.001$; Fig. 1D). On the peak time (7 June) of parasitoid densities, the densities were higher in the 12W:6MB, 12W:4MB, and 16W:4MB fields than in the wheat monoculture field ($F = 8.80$; df = 4, 10; $P < 0.01$; Fig. 1D).

Community Indices of Natural Enemies. Except in the 10W:10MB field, the species richness of the natural enemies was significantly higher in the intercropped fields than in the wheat monoculture field; but there was no difference among the species richness of the natural enemies in these intercropping treatments ($F = 19.92$; df = 4, 10; $P < 0.001$; Table 1).

The mean evenness of the natural enemies in the wheat monoculture field was not significantly different from their mean evenness in all the intercropped fields ($F = 4.66$; df = 4, 10; $P = 0.03$; Table 1).

There was also no significant difference between the mean index of dominance concentration of the natural enemies in the wheat monoculture and in all intercropping treatments (Table 1).

Bioassays of Aphid Preference for Host Plant and Nonhost Plant Odors. *Apterous S. avenae.* Apterous aphids preferred the odor of *T. aestivum* over clean air ($\chi^2 = 18.75$, $P < 0.001$; Fig. 2A). Relative to clean air, the odor of *V. radiate* did not significantly attract the apterous aphids ($\chi^2 = 0.53$, $P = 0.47$). Apterous aphids showed significant preference to the host plant odor over the odor of *V. radiate* ($\chi^2 = 10.26$, $P < 0.01$), whereas they had no response to the odor blend of *T. aestivum* and *V. radiate* ($\chi^2 = 1.04$, $P = 0.31$). Host plant odor significantly attracted the apterous aphids compared with the odor blend of *T. aestivum* and *V. radiate* ($\chi^2 = 7.71$, $P = 0.01$).

Alate S. avenae. Compared with the control, the odor of *T. aestivum* significantly attracted alate aphids

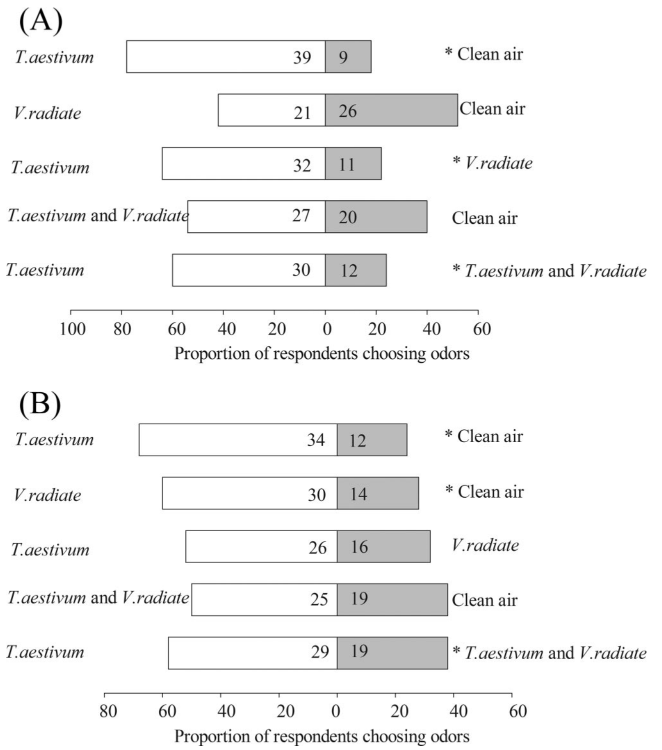

Fig. 2. Selective preference of *S. avenae* to different treatments. (A) Apterous aphids. (B) Alate aphids. Values in the bars represent number of reactive *S. avenae*. *, $P < 0.05$.

($\chi^2 = 10.52$, $P < 0.01$; Fig. 2B). The odor of *V. radiate* also attracted alate aphids ($\chi^2 = 5.82$, $P = 0.02$). Alate aphids showed no preference to host plant odor over nonhost plant odor ($\chi^2 = 2.38$, $P = 0.12$), and they showed no preference to the odor blends of host and nonhost plants over clean air ($\chi^2 = 0.82$, $P = 0.37$). However, like the apterous aphids, alate aphids were more attracted by host plant odor than by odor blends of host and nonhost plants ($\chi^2 = 6.10$, $P = 0.01$).

Discussion

This research demonstrated that wheat aphid densities are significantly lower in most intercropped fields than in a wheat monoculture field, consistent with previous studies on intercropping of wheat with other crops (Ma et al. 2006; Li et al. 2007; Zhou et al. 2009; Wang et al. 2009, 2010). The complex planting structure in a field intercropped with wheat and mung bean might disturb the migration of aphids between wheat plants (He 2009). Based on the resource concentration hypothesis, a complex planting structure also disturbs herbivores' olfactory-based host location (Root 1973, Hou and Sheng 1999, Landis et al. 2000). The choice bioassays in this research provide support for this hypothesis. The experiments demonstrated that apterous and alate aphids significantly preferred host plant odor over clean air, but they did not respond to odor blends of host and nonhost plants. Consequently, the blended odors of wheat and mung bean in the field increased the difficulty of aphids in searching for hosts, which indicates that the selection of appropriate plant species is crucial to pest colonization in an intercropped wheat field. Ventilation also was enhanced in the field intercropped with wheat and mung bean, and this change in microclimate also might ultimately influence aphid survival (Bertrand and Wilson 1996, Ebwongu et al. 2001, Rao et al. 2004).

The population densities of *S. avenae* in the 12W:4MB field were significantly lower than those in the wheat monoculture during both the sampling period and the peak times. The densities of apterous aphids in the 12W:4MB and 16W:4MB fields were not significantly different. The appropriate/inappropriate theory suggests that insects prefer landing on green plants to landing on brown soil (Finch and Collier 2000). There was substantial bare soil in the mung bean fields during the early stages of plant growth, but more wheat rows and less bare soil existed in the 12W:4MB and 16W:4MB fields than in the other intercropped fields. As a result, the aphids and their natural enemies were more easily attracted in the two treatments mentioned. A large number of natural enemies could suppress the population growth of aphids in the 12W:4MB and 16W:4MB fields.

Higher densities of primary enemies (ladybirds and parasitoids) were observed in the intercropped fields than in the monoculture field. Although the number of ladybirds in the intercropped fields was not significantly different from their number in the wheat monoculture field during the peak time of ladybird population densities, the average densities of ladybirds throughout the sampling period were significantly higher in the intercropped fields. Mung bean bloomed at later period than wheat, so it could have provided the ladybirds with pollen during wheat grain filling, which enhanced the survival of the ladybirds. The densities of parasitoids in the 12W:4MB and 16W:4MB fields were significantly higher than in the monoculture field during both the sampling period and the peak time of population densities. Tylianakis et al. (2004) indicated that proximity to floral resources could increase the rates of parasitism. The natural enemies were more abundant in the intercropping treatments, except in the 10W:10MB treatment, than in the monoculture. These results also provide support to the enemies hypothesis, stating that diverse habitats increase the number of species of natural enemies, sustain their life cycles, and encourage immigration (Root 1973). However, the diverse population of the enemies did not have an influence on the evenness and index of dominance concentration of the aphids, which promotes stability of enemy communities resulting in sustained pest control (Li et al. 2007).

Our results suggest that diversity of crop species could suppress aphid population growth and enhance the biological control of aphids by diverse enemies. These findings also provide support for the resource concentration hypothesis and the enemies hypothesis.

Different intercropping treatments are related to biological control effectiveness. The lower densities of aphids and higher diversity of enemy species were found in the 12W:4MB and 16W:4MB fields. Moreover, a larger proportion of the main crop (wheat) also existed in these two treatments. Hence, these treatments will be implemented more easily. More work is required to evaluate the predatory efficiency and yield in intercropped systems.

Acknowledgments

We thank Professor John A. Gatehouse (Durham University, United Kingdom) for review of the manuscript. This research was supported by Dr. Gezhi Weng (SunRise Realty and Financing, Inc., Piedmont, CA); grants from the National S & T Major Project (2009ZX08012-007B), the Modern Agroindustry Technology Research System (2060302), and China-Belgium Cooperation Project (2010DFA32810). The Cooperation Universitaire au Development (CUD) funded an interuniversity targeted project between Belgium and China (PICShandong).

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Received 2 July 2011; accepted 10 April 2012.