RESEARCH ARTICLE

Influence of Garlic Intercropping or Active Emitted Volatiles in Releasers on Aphid and Related Beneficial in Wheat Fields in China

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Abstract

In order to develop biological control of aphids by a "push-pull" approach, intercropping using repellent emitting plants was developed in different crop and associated plant models. Garlic is one of the potential plant that could be inserted in crops to decrease the pest occurrence in neighboring crop plots. In this study, field works were conducted in wheat fields in Langfang Experimental Station, Hebei Province in China from October 2009 to July 2010 during wheat developmental season. The effect of wheat intercropping with garlic but also the volatiles emission on the incidence of the English grain aphid, Sitobion avenae Fabricius (Homoptera: Aphididae) was assessed. Natural beneficial occurrence and global yields in two winter wheat varieties that were susceptible or resistant to cereal aphid were also determined comparing to control plots without the use of garlic plant intercrop nor semiochemical releaser in the fields. S. avenae was found to be lower in garlic oil blend treatment (GOB), diallyl disulfide treatment (DD) and wheat-garlic intercropping treatment (WGI) when compared to the control plots for both two varieties (P<0.01). Both intercropping and application of volatile chemicals emitted by garlic could improve the population densities of natural enemies of cereal aphid, including ladybeetles and mummified aphids. Ladybeetle population density in WGI, GOB and mummified aphids densities in WGI, DD were significantly higher than those in control fields for both two varieties (P<0.05). There were significant interactions between cultivars and treatments to the population densities of S. avenae. The 1 000-grain weight and yield of wheat were also increased compared to the control. Due to their potential alternatives as a biological control agent against cereal aphid, garlic intercropping and related emitted volatiles are expected to contribute to the further improvement of integrated pest management systems and to potentially reduce the amount of traditional synthetic pesticides applied in wheat fields.

Key words: wheat, garlic, intercropping, semiochemical release, Sitobion avenae, natural enemies

INTRODUCTION

Wheat, *Triticum aestivum* L., and garlic, *Allium sativum* L., are important crops for the people in the world as

well as China. The English green aphid, *Sitobion avenae* Fabricius (Homoptera: Aphididae), is an ubiquitous pest that attacks wheat throughout its growth stages in North China (Cai *et al.* 2004; Zhao *et al.* 2009). One approach for this pest control is to develop management

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systems using diversified agroecosystems. Intercropping, the agronomic practice for the development of sustainable food production systems (Agegnehu et al. 2006; Eskandari and Ghanbari 2010), plays an important role in controlling pests and protecting beneficial insects relevant to enhancing biodiversity in an agroecosystem (Smith and McSorley 2000; Hassanali et al. 2008; Konar et al. 2010; Suresh et al. 2010; Vaiyapuri et al. 2010). For example, from 2002 to 2004, Ma et al. (2007) examined strip cropping of wheat and alfalfa, Medicago sativa, for its utility to improve the effectiveness of biological control of the cereal aphid, Macrosiphum avenae by the mite, Allothrombium ovatum. Wheat-garlic intercropping, planting row in an 8:3 ratio, can reduce the population of S. avenae by promoting natural enemies in wheat fields experiments (Wang et al. 2008). The benefits of intercropping for controlling aphids and encouraging their natural enemies have also been studied in wheat and oilseed rape, Brassica napus L. (Wang et al. 2009); cowpea, Vigna unguiculata (L.) Walp and sorghum, Sorghum bicolor (L.) Moench (Hassan 2009), wheat and pea Pisum sativum Linn (Zhou et al. 2009a, b). Intercropping has also been described potentially increasing crop yields by suppressing pest outbreaks (Sarker et al. 2007; Zhang et al. 2007; Mucheru-Muna et al. 2010; Rao et al. 2010; Vaiyapuri and Amanullah 2010). In addition, it is important to take the resistant levels to aphids of a host plant into consideration. In an intercropping system, wheat varieties that are susceptible or moderately resistant to cereal aphids may reduce cotton aphids more effectively than an aphid-resistant variety by enhancing predators to suppress cotton aphids during the cotton seedling stage (Ma et al. 2006).

However, volatiles produced by non-host plants often affect the behavior of pests and their natural enemies, these may vary genetically among plants (Bai *et al.* 2011). Intercropping with the non-host molasses grass, *Melinis minutiflora*, significantly decreased levels of infestation by stem-borers in the main crop and also increased larval parasitism of stemborers by *Cotesia sesamiae*. Volatile agents produced by *M. minutiflora* repelled female stem-borers and attracted foraging female *C. sesamiae* (Khan *et al.* 1997). Due to the inherent variability, an important modification of this method is the external application of volatile semiochemicals in the field, which have a stabilizing effect and may reduce populations of the aphids *Diuraphis noxia* (Prinsloo *et al.* 2007), and *Rhopalosiphum padi* (Ninkovic *et al.* 2003). Essential oils, obtained by steam distillation of plant foliage, and even the foliage itself of certain aromatic plants have traditionally been used to protect stored grain and legumes, and to repel flying insects (Isman 2000). Diallyl disulfide, an essential component of garlic volatiles (Edris and Fadel 2002), and in a fumigation bioassay, had insecticidal activity on the larvae of Japanese termite, *Reticulitermes speratus* (Park and Shin 2005) and mushroom fly, *Lycoriella ingenua* (Park *et al.* 2006). However, few studies have investigated the effects of garlic oil blend and its components on *S. avenae* control in wheat fields.

The objectives of this study were thus to compare the effects of wheat monoculture, wheat-garlic intercropping (wheat cultivars with different resistant levels to English grain aphid), treatments with a garlic oil blend, and diallyl disulfide emissions in wheat fields on *S. avenae*, their natural enemies, and overall crop yield. It could provide a potential strategy that can contribute to the biological control to reduce the aphid infestations.

RESULTS

Aphid population density

S. avenae population densities differed significantly among the four treatments at several sampling dates in Beijing 837 and Zhengzhou 831 (Fig. 1). *S. avenae* populations decreased dramatically from late May to early June, and peak numbers were found in late season sampling in both cultivars examined.

Aphid population reached its peak in the two cultivars on May 26 and 30, respectively. However, during this peak period, aphid population density with the control treatment was significantly higher than that seen in any other treatments in both cultivars (Beijing 837: $F_{3, 12}$ =111.62, *P*<0.01; Zhengzhou 831: $F_{3, 12}$ =215.41, *P*<0.01). The highest abundance of aphids was observed in the CK treatment, and the lowest in diallyl disulfide treatment (DD) and garlic oil blend treatment (GOB) with both Beijing 837 and Zhengzhou 831.



Sampling dates (d-mon)

Fig. 1 Population dynamics of *S. avenae* (mean±SE) in different treatments. A, Beijing 837. B, Zhengzhou 831.

Ladybeetle population density

There were three species of ladybeetles, *Coccinella* septempunctata L., *Harmonia axyridis* Pallas and *Propylaea japonica* Thunber, found in wheat fields during the sampling period. The ladybeetle populations (all species) of each plot for two cultivars (Beijing 837 and Zhengzhou 831) are shown in Fig. 2. The ladybeetle populations in wheat-garlic intercropping treatment (WGI), DD and GOB plots were significantly higher compared to the CK plots for both cultivars at the peak dates with the exception of DD in Beijing 837 (Beijing 837: $F_{3,12}$ =52.34, *P*<0.01; Zhengzhou 831: $F_{3,12}$ =131.46, *P*<0.01). And WGI had the highest number of ladybeetles according to the whole investigation period, followed by GOB and DD.

Mummified aphids density

The peak Mummified aphid densities was occurred in all treatments on May 30 (Fig. 3). On this date, mum-



Fig. 2 Population dynamics of ladybeetles (mean±SE) in different treatments. A, Beijing 837. B, Zhengzhou 831.

mified aphids densities were lower in CK plots than in DD and WGI plots (Beijing 837: $F_{3, 12}$ =20.41, P<0.05; Zhengzhou 831: $F_{3, 12}$ =21.32, P<0.01). Although GOB also increased parasitism of *S. avenae*, there was no significant difference between GOB and CK treatments in the two cultivars.

Two-factor effects

A summary of the statistical analyses on the effects of treatments on the mean number of *S. avenae*, ladybeetles, and mummified aphids are given in Table 1. There was a significant difference in *S. avenae* (P<0.01) between different treatments. Wheat cultivars also influenced observed numbers of *S. avenae* (P<0.01) and ladybeetles (P<0.01). There were sig-



Fig. 3 Population dynamics (mean±SE) of mummified aphids treatments. A, Beijing 837. B, Zhengzhou 831.

nificant interactions between cultivars and treatments for *S. avenae* (P=0.048). However there was no significant difference detected in mummified aphids in the wheat cultivars and treatments and their interactions.

Yields

Wheat 1000-grain weights and yield were both increased when compared with CK, and significant differences were detected in all pairwise comparisons between WGI, DD, GOI, and CK except with DD in 1000-grain weight of Zhengzhou 831. The highest 1000-grain weight and yield were observed with the DD treatment, but there was no significant difference among WGI, DD and GOB except in yield of Zhengzhou 831, data was shown in Table 2.

DISCUSSION

Increasing agrobiodiversity by crop intercropping and application of plant essential oils in fields may provide potential alternatives to those currently used to control S. avenae. Intercropping garlic or related volatiles releasing in wheat fields has potentially direct effects on the aphids, such as repellent, toxic, masking host plant odours, and masking visual orientation, or indirect effect, such as stimulating natural enamies and inducing resistance in host plant. The push-pull strategy is an useful tool for integrating pest management programs, reducing pesticide input (Cook et al. 2007), and maximizing the efficacy of behavior manipulating stimuli through the additive and synergistic effects of a non-host crop. Our results indicated that the abundance of S. avenae was lower, with both varieties, in the wheatgarlic intercropping system than in wheat monoculture. This may be due to two factors: (1) garlic is a stimulus for push components to make wheat resources hard to locate, unattractive, or unsuitable to aphids; and/or (2) intercropping systems that increase crop diversity in the agroecosystem significantly preserved and augment more ladybeetles and mummified aphids than monoculture wheat fields. Similar phenomenon was also observed in wheat-garlic (Wang et al. 2008), wheat-alfalfa (Ma et al. 2007) and maize-sorghum (Khan et al. 1997) intercropping system. The results of this study further demonstrated the effects of intercropping on aphids and their natural enemies. Wheat varieties (Beijing 837) that are susceptible to wheat aphid might reduce wheat aphids more effectively than an aphid-resistant variety (Zhengzhou 831) in the intercropping system. This may occur by an attracting more ladybeetles to suppress cereal aphids. There was no significant difference on mummified aphids densities between the varieties.

Aphid behavior is affected by volatile compounds which can increase the sensitivity of aphids to disturbance, and promote mobility of non-settled individuals (Pettersson *et al.* 1995), such as methyl salicylate (Ninkovic *et al.* 2003). Some volatile compounds (e.g., *cis*-jasmone) also may induce plant defenses and reduce *S. avenae* population in the field test (Bruce *et al.* 2003). Lower densities of aphids and increase in their natural enemies were found in

Source of variation	df	S. avenae		Ladybeetles		Mummified aphids	
		F	Р	F	Р	F	Р
Wheat variety	1	155.52	< 0.0001	24.58	< 0.0001	1.24	0.2786
Treatment	3	370.47	< 0.0001	0.18	0.9071	1.04	0.3943
Wheat variety×Treatment	3	3.13	0.0475	0.66	0.5857	0.72	0.5521

Table 1 F-test on effects of wheat cultivars and treatments on the abundance of S. avenae and natural enemies in wheat field

Table 2 1000-grain weight and yield in different treatments

Treatment	1 000-grain v	weight (g)	Yield (kg ha-1)		
	Beijing 837	Zhengzhou 831	Beijing 837	Zhengzhou 831	
Intercropping	35.29±2.17 a	36.62±1.39 a	4771.2±228.5 a	5726.4±204.0 b	
Garlic oil blend	35.60±1.44 a	36.40±2.24 ab	4737.6±153.9 a	5736.0±94.5 b	
Diallyl disulfide	37.38±0.98 a	39.18±2.79 a	5020.8±152.4 a	6182.4±228.8 a	
Control	32.53±1.03 b	33.38±1.49 b	4075.2±269.4 b	4814.4±115.6 c	

Mean values±SE in the same column followed by different letters are significantly different (ANOVA, LSD test, differences considered significant at P<0.05).

fields with applications of semiochemicals from garlic. Although no significant difference in mummified aphids densities were seen between fields with garlic oil blend and control was measured, ladybeetle population density in fields with garlic oil blend and mummified aphids densities in fields with diallyl disulfide were significantly higher than those in control fields. A significant effect of semiochemicals treatment on aphids and their natural enemies was observed between susceptible (Beijing 837) and resistant (Zhengzhou 831) varieties, possibly due to complex interactions between the chemical, plant variety and growing environment (Prinsloo et al. 2007). 1000-grain weight and yield of wheat were also increased in treatment fields, except for the 1000-grain weight in the GOB field for Zhengzhou 831, and there were no differences among WGI, GOB and DD except in yield of DD treatments for Zhengzhou 831.

Aphid perception of volatile cues is adapted for avoidance of non-host plants, and they can detect a wide range of chemical compounds (Pickett and Glinwood 2007; Prinsloo *et al.* 2007). Garlic oil blend and diallyl disulfide are components of the essential oils of garlic, which represent non-hosts to cereal aphids, and are likely to be unsuitable for their orientation. Intercropping with garlic can also be used as an approach to control wheat aphids by conserving and enhancing populations of their natural enemies, and consequently also reducing the chemical dependency in wheat agroecosystems.

CONCLUSION

Crop intercropping and application of plant essential oils in fields could obtain better effects in conserving and enhancing abundance of natural enemies, and simultaneously reducing the cereal aphids damage in agroecosystems. Alternative strategies can also avoid environmental pollution and health problems caused by the extensive use of traditional synthetic pesticides. They are expected to contribute to the further improvement of integrated pest management systems. Further research needs to be done to evaluate the mechanisms of how garlic and its volatiles affect natural enemies of cereal aphids in a complex agroecosystem.

MATERIALS AND METHODS

Wheat and garlic varieties

Two wheat varieties, *T. aestivum*, *cv*. Beijing 837 (susceptible) and *cv*. Zhengzhou 831 (resistant), with different levels of resistance to *S. avenae* were provided by the Institute of Plant Protection at the Chinese Academy of Agricultural Sciences in Beijing. The garlic variety, *Allium sativum* L. *cv*. Zhongnong 4 was also used in this study. This variety is currently used commercially in Huang-Huai-Hai Plain, China.

Chemicals

Diallyl disulfide (purity 80%, remainder mainly allyl sulfides) and garlic oil blend (30-50 wt. % diallyl disulfide, 10-13 wt. % diallyl trisulfide, 5-13 wt. % allyl disulfide) were purchased from Sigma-Aldrich, Inc., Missouri, US.

Field experimental design

Field experiments were conducted at the Langfang Experimental Station of the Institute of Plant Protection, CAAS, Hebei Province of China (39°30'N, 116°37'E) in 2010. Wheat and garlic were planted with 20 and 40 cm distance between rows in wheat and garlic, respectively.

A conventional randomized plot design was used, with treatment plots (10 m×8 m) randomly repeated in each of four plots. The following treatments were compared: (i) WGI, wheat-garlic intercropping by planting row in 8:3 ratio (eight rows of wheat with three rows of garlic); (ii) GOB, the release of garlic oil blend in wheat field; (iii) DD, the release of diallyl disulfide in wheat field; (iv) CK, control, wheat monoculture without garlic plant intercrop nor semiochemical releaser use in the fields. A 2-m wide area was set around the each plot to decrease potential border effects on insect dispersion. No pesticides were applied on the fields during the entire growth stage of wheat and garlic.

Release of chemicals in fields

A rubber tube (10 cm×0.05 cm diameter, Pherobio Technology Co. Ltd., Beijing, China) as the releaser loaded with 10 μ L candidate volatile substances was hung in wheat fields at a height of 10 cm above the top of wheat plant, and five releasers were used completely randomized design in each single plot. The first introduction of releasers was made on 22 April (at the setting stage of wheat), and chemicals were subsequently supplied every 7 d until aphid counting ended.

Sampling of insects

Due to aphid parasitoids being difficult to count in the field, the number of mummified aphids found was examined. Mummified aphids and aphid densities on plants were counted and recorded in each plot in five 1 m^2 plots. Within each sampling plot, thirty randomly selected wheat tillers were used as one sampling unit. Lady beetles on all wheat plants within the 1 m^2 plots, and covering three rows of wheat were counted in the center of each plot. Aphids was sampled in wheat every 4-d from April 24th to June 7th. Ladybeetles and mummified aphids were sampled every 4-d from May 16th to June 5th.

Crop yields

Yields and 1 000-grain weights of wheat were assessed by harvesting and weighing crop products from each plot. This resulted in the calculation of yields in kg ha⁻¹. 1 000-grain weight was evaluated by weighing two samples of 500 kernels for each plot.

Statistical analysis

All data of insect population densities, yield and 1 000grain weight related to the different treatments in field were analyzed using one-way analysis of variance (ANOVA) (SAS 2001) followed by least-significant difference test (LSD). The effects of varieties and treatments on aphids and their natural enemies were analyzed using the general linear model (GLM) procedure. The data used in ANOVA and GLM were transformed by square root, when necessary, to meet assumptions of normality before variance analysis. A probability level of <0.05 was considered statistically significant.

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