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ORIGINAL ARTICLE

Use of ryegrass strips to enhance biological control of aphids by ladybirds in wheat fields

Zhao-Ke Dong[1,2], Feng-Juan Gao[1,2] and Run-Zhi Zhang[1,3]

[1]*Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences,* [2]*Graduate University of Chinese Academy of Sciences,* [3]*State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*

Abstract Non-crop habitats may play a vital role in conservation biological control. This study tested the effect of ryegrass (*Lolium multiflorum* L.) strips on aphid and ladybird populations in adjacent winter wheat fields. The field experiment was conducted in three ryegrass-margin wheat plots and three control plots in 2010 in North China. In spring, the same aphid species, *Sitobion miscanthi* (Takahashi), was found in both the ryegrass strips and wheat plots. The population density of ladybirds in the ryegrass strips ($3.5 \pm 0.9/m^2$) was significantly higher than in the wheat plots ($1.5 \pm 0.5/m^2$). We cut the ryegrass, forcing the ladybirds to migrate to the wheat fields. Three and eight days after cutting the ryegrass, the aphid numbers in the ryegrass-margin wheat plots decreased significantly: they were 19.9% and 53.6%, respectively, lower than in control plots. In the early period of ladybird population development, the percentage of larvae was greater in the ryegrass-margin wheat plots than in controls, and the peak number of pupae in the ryegrass-margin wheat plots occurred 5 days earlier than in the control plots. The results suggest that ryegrass strips may promote the development of ladybird populations. Cutting ryegrass can manipulate ladybirds to enhance biological aphid control in wheat fields. The efficiency of this management approach is discussed.

Key words coccinellids, habitat management, natural enemies, non-crop habitat

Introduction

In an agro-ecosystem, creating a suitable habitat within the agricultural landscape to provide resources such as food (pollen, nectar, alternative prey or hosts) or shelter for natural enemies could enhance biological control (Landis *et al.*, 2000). As part of conservation biological control, habitat management has received a good deal of attention (reviews in Gurr & Wratten, 1999; Fiedler & Landis, 2007; Jonsson *et al.*, 2008).

Many studies have been conducted on habitat management for the biological control of wheat aphids. Thomas *et al.* (1991) proposed the creation of mid-field grassy ridges (known as beetle banks) to provide an overwintering habitat for predators, which would facilitate early colonization of the crop by the predators in the spring. Collins *et al.* (2002) documented that the presence of a beetle bank in the middle of a cereal field had a significant impact in reducing aphid population. Langer and Hance (2004) found that non-crop habitats with alternative host aphids within wheat fields may increase the parasitism of wheat aphids.

Ladybirds have been identified as important biological control agents of aphids (Hagen, 1962; Kring *et al.*, 1985; Hodek & Michaud, 2008). The potential of ladybirds in suppressing pests in agricultural ecosystems is evident in the many attempts to use them in importation, conservation and augmentation as part of biological control (Obrycki & Kring, 1998). Some theoretical and exploratory studies suggested employing non-crop

Correspondence: Run-Zhi Zhang, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Beijing 100101, China. Tel: +86 10 64807270; fax: +86 10 64807099; email: zhangrz@ioz.ac.cn

habitats to enhance wheat aphid control by ladybirds (Bianchi & van der Werf, 2003; Alhmedi *et al.*, 2009), though few reports have detailed the effect of ladybirds following the establishment of non-crop habitats to promote aphid suppression in wheat fields.

We explored the potential of intercropping ryegrass, *Lolium multiflorum*, with wheat to enhance aphid predation by attracting and conserving aphid predators. The aphids present on ryegrass are mostly *Sitobion miscanthi* (Takahashi). In northern China, the population growth of the three wheat aphid species, *S. miscanthi* (Takahashi), *Schizaphis graminum* (Rondani) and *Rhopalosiphum padi* (L.), starts in late April and abruptly ceases a month later as the wheat crop ripens and as a result of natural enemy pressure. The damage caused by aphids has become an increasing problem over the past decade in China. Even though there is routine insecticide use in aphid control, the average damage is up to 0.45 million tonnes per year (Cao *et al.*, 2006). Ladybirds, mostly *Harmonia axyridis* Pallas and *Propylaea japonica* (Thunberg), are already present in early spring. Greater densities of ladybirds have been recorded in ryegrass than in wheat fields (Huang *et al.*, 2008). It was thought that the aphids in the ryegrass strips could act as a food resource to encourage early establishment of ladybird populations. When the aphid population in the wheat field needs to be reduced, the ryegrass is cut to force the ladybirds to migrate to the wheat field and control the aphids. We proposed the hypothesis that ladybirds oviposited earlier in ryegrass and could be manipulated to suppress aphids in nearby wheat fields.

Materials and methods

Field experiment design

The field experiment was conducted in 2010 at Yucheng Experimental Station of the Chinese Academy of Sciences, Shandong Province, China (116°36′E, 36°57′N). The wheat field was divided into three blocks; within each block, a strip plot and a control plot were positioned at random. Each strip plot (20 m × 12 m) had two parallel ryegrass strips (2.5 m × 12 m) running along both of its long edges. The length of the ryegrass strips was 12 m. Ryegrass (cv Dongmu 70) was sown by hand along the 12-m margins of the strip plots on October 7, 2009. The margins of the control plots were left bare and weeded during the season. All plots were 2 m apart and separated by bare ground. Wheat with cultivar Kenong 199 was sown by machinery on October 15, 2009 and harvested on June 15, 2010. No insecticide was applied during the study.

Ryegrass strip manipulation

Granular fertilizer (NPK 40-30-20 kg/ha) was applied to ryegrass strips on April 20 for rapid growth. The ryegrass was cut at a height of 1.5 m by sickle on June 1, while most ladybirds in the ryegrass strips were at the larval stage. After cutting, the ryegrass was left in the field margin for 24 h to allow the ladybirds to migrate from the ryegrass strip to wheat field.

Survey of aphids and natural enemies

Aphids, aphid predators, and parasitoids were sampled at 5-day intervals in the winter wheat fields and ryegrass strips from May 12 to June 14. Aphid sampling was carried out as follows. In each plot, 100 tillers were cut at 10 random locations (10 tillers per location). Each group of 10 tillers was placed in bags and transported to the laboratory. Samples were treated in the laboratory with 75% alcohol, and all aphids were counted and identified. All data were expressed as the density of aphids per 100 tillers. We used visual counts to quantify the abundance of aphid natural enemies in 10 randomly placed 0.5 m × 0.5 m subplots per wheat plot. Wheat and ryegrass samplings were synchronous. The sampling started in early May and finished in mid-June, when the wheat was harvested.

Data analyses

The total mean number of aphids per 100 tillers was $\log_{10}(x + 1)$ transformed. Aphids in wheat fields were analyzed using repeated-measures analysis of variance (MANOVA), with treatment (strip *vs*. controls) as factors and sample date as the split-plot factor. On each sampling date, the difference between treatment and control was analyzed using one-way ANOVA. The growth rate of aphids between ryegrass strips and wheat plots were also compared. The rate of increase was calculated as:

$$\lambda = (N_{t+\Delta t}/N_t)^{1/\Delta t},$$

where N_t is the density of aphids at time t, and $N_{t+\Delta t}$ is the time between samples (Lotka, 1925). The data of total ladybird numbers that did not fit the assumptions of ANOVA after transformation were analyzed using a non-parametric statistical test (Mann–Whitney test). The percentage of ladybirds at different stages of development was compared between plots using a χ^2-test. The data of ladybird eggs were not analyzed because of their low number. All statistical analyses were performed using the SPSS statistical package (version 18.0, SPSS Inc., Chicago, IL, USA).

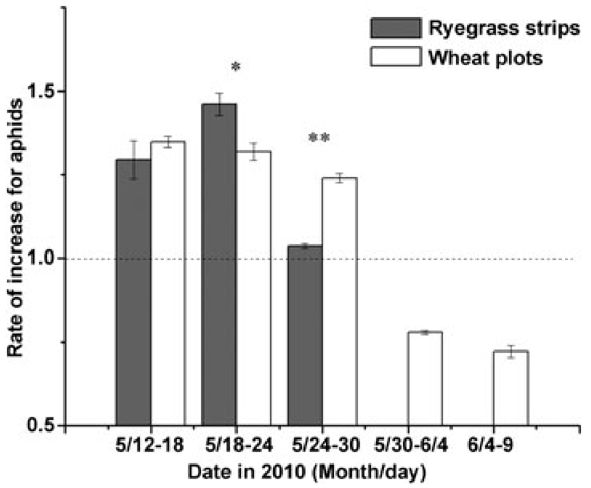

Fig. 1 The rate of increase of aphids in ryegrass strips and wheat plots ($^*P < 0.05$, $^{**}P < 0.01$).

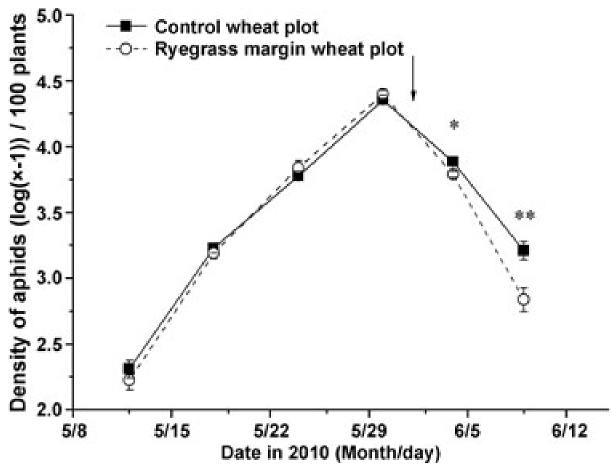

Fig. 2 Population dynamics of aphids in control and ryegrass margin wheat plots. Arrow refers to the date of cutting ryegrass ($^*P < 0.05$, $^{**}P < 0.01$).

Results

Aphid and natural enemy populations in ryegrass strips

The aphid population in ryegrass strips increased rapidly until late May, a period during which the ryegrass was at the flowering stage. Aphid density increased 41.8-fold over an 18-day period, from a low of 57.8 ± 17.1 per 100 tillers on May 12 to a peak of 2 474.7 ± 198.4 per 100 tillers on May 30. Rate of increase of aphid population in ryegrass strips is significantly higher than in wheat plots during May 18 to 24 ($t = 3.324$; $P = 0.029$). Afterward, the growth rate in ryegrass strips became low during May 24 to 30, whereas the growth rate in wheat plots was high. The aphids in ryegrass strips disappeared after cutting ryegrass (on June 1), and aphids in wheat plots began to decline (Fig. 1). Aphids peaked earlier in ryegrass strips than in wheat plots. The major predators in the ryegrass strips were *H. axyridis* and *P. japonica* (Coccinellidae). The percentages of these two species of ladybirds were 51.7% and 48.3%, respectively. Small numbers of Syrphidae were also observed. The major parasitoid was *Aphidius avenae* Haliday. The parasitism rate was lower than 1% during the study. At the beginning of the experiment, ladybird densities in the ryegrass strips were low. They then increased with aphid population growth. On May 30, the density of ladybirds was significantly higher in the ryegrass strips than in the wheat plots (total ladybirds: ryegrass, 3.5 ± 0.9 / m^2; wheat, 1.5 ± 0.5 / m^2; $t = 2.004$; $P = 0.051$). The density of larvae in the ryegrass strips (2.9 ± 0.8 / m^2) was 2.4 times that in the wheat plots (1.2 ± 0.4 / m^2). The ryegrass strips were cut on June 1, and the ladybirds were forced to migrate from the ryegrass strip to the nearby wheat plots.

Influence of ryegrass strips

The ryegrass strips had a significant effect on aphid densities in the ryegrass-margin wheat plots (repeated-MANOVA, $P = 0.019$). The differences occurred in June after the ryegrass was cut, when the aphid population declined. The aphid density in the ryegrass-margin wheat plots was significantly (19.9%) lower than controls (June 4, $F_{1,4} = 7.956$, $P = 0.048$). Five days later, the aphid numbers were still significantly (53.6%) lower in the ryegrass-margin wheat plots (June 9, $F_{1,4} = 11.102$, $P = 0.029$) (Fig. 2). No difference was found for ladybird densities between the ryegrass-margin wheat plots and control wheat plots (Table 1).

Discussion

Plant cutting is an effective way to manipulate the movement of natural enemies. Lin *et al.* (2003) showed that cutting alfalfa in an alfalfa/cotton intercropping system increased predator numbers in the cotton field and delayed the development of the cotton aphid population. In our study, we analyzed the functional mechanism of the ryegrass strips in terms of the dynamics of the different developmental stages of the ladybirds. The ladybird larvae were first found on May 30. Both ladybird density and the larval density were significantly higher in

Table 1 Mean number of ladybirds (± SE) per square meter and statistical comparison between control and ryegrass margin wheat plots.

Date (2010)	Control wheat plots	Ryegrass-margin wheat plots	Statistical comparison between control and ryegrass-margin wheat plots	
			Z	*P*
May 12	0.3 ± 0.2	0	–	–
May 19	0	0	–	–
May 24	0.3 ± 0.2	0	–	–
May 30	1.2 ± 0.6	1.9 ± 0.8	−0.680	0.502
June 4	3.0 ± 1.6	3.2 ± 0.8	−0.115	0.909
June 9	8.1 ± 2.0	11.3 ± 2.2	−1.080	0.290
June 14	18.5 ± 2.7	14.9 ± 2.6	0.958	0.346

the ryegrass strips than in the wheat plots. It suggested that ladybirds oviposited earlier in ryegrass strips than in wheat plots. The larvae of ladybirds might disperse back and forth between ryegrass strips and nearby wheat fields. On May 30, the percentage of larvae was significantly higher in the ryegrass-margin wheat plots (92.9%) than in controls (55.6%; $\chi^2 = 4.515$; $P = 0.034$). After we cut the ryegrass, ladybirds in the ryegrass strips might migrate to the nearby wheat fields in search of prey. The percentage of pupae in the ryegrass-margin wheat plots (54.5%) was significantly higher than in controls (25.5%) on June 9 ($\chi^2 = 3.878$; $P = 0.049$). The number of pupae in the ryegrass-margin wheat plots reached a peak 5 days earlier than in controls (Fig. 3). The ladybird larvae in ryegrass strips were older than those in wheat plots, therefore, the recruitment of older ladybird larvae in ryegrass-margin wheat plots changed the composition of ladybird stages. Both searching and handling rates of larvae increase with the ladybird developmental stage which increases aphid consumption rates (Xia *et al.*, 2003). Colonization of the ryegrass-margin wheat plots by these older larvae may have contributed to the decline in the aphid population in these plots and explain the difference between aphid populations in the control and ryegrass-margin wheat plots after the ryegrass was cut.

Food is important for supporting ladybird reproductive capacity and a female's offspring. The quantity or quality of food directly influences a female's decision to oviposit (Seagraves, 2009). The ryegrass and wheat areas had the same aphid species (*S. miscanthi*). But the aphid population in ryegrass strips peaked earlier than those in wheat plots. The advanced phenology of aphids in ryegrass strips possibly caused earlier oviposition in ryegrass strips.

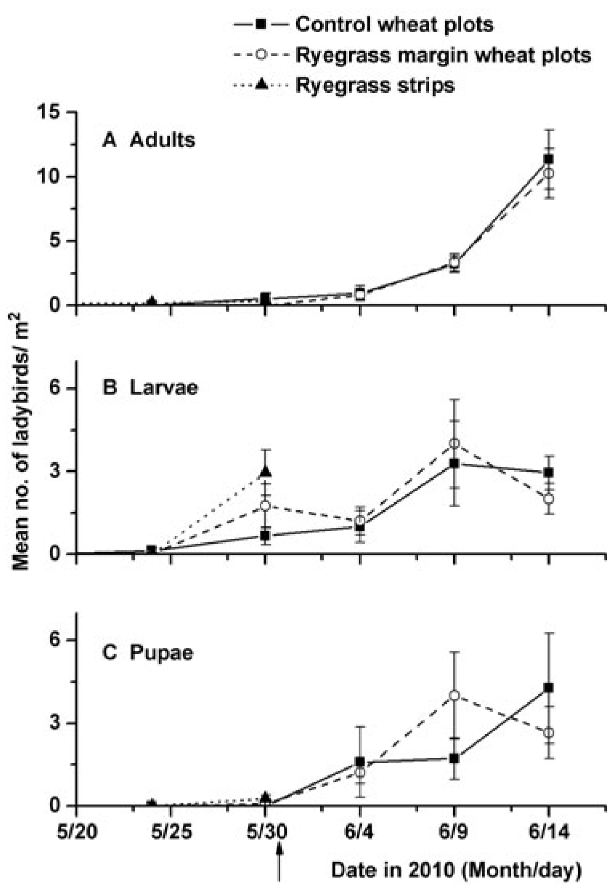

Fig. 3 Mean number of adults (A), larvae (B), pupae (C) of ladybirds per square meter in control wheat plots, ryegrass margin wheat plots and ryegrass strips during the sampling period. Arrow refers to the date of cutting ryegrass.

Frere *et al.* (2007) showed that the presence of rose bush, *Rosa rugosa* (Thunb.), strips did not influence the aphid population in a wheat field and that the aphids' natural enemies might have stayed among the rose bushes, where prey were abundant, and did not migrate to the wheat field. In our study, there were significantly greater numbers of ladybirds in the ryegrass strips than in the wheat field. The parasitism level in ryegrass strips and wheat fields were both quite low. Ladybirds were the main natural enemies to control aphids. Our results indicate that ryegrass strips can reserve a certain amount of ladybirds and that cutting ryegrass strips on the border of a wheat field has a significant impact in reducing aphid populations. The moment of ryegrass cutting could also affect the efficacy of this habitat manipulation. A shortcoming of our study is that the cutting moment is not considered well enough. For example, if the ryegrass had more treatments like no cut, early cut and late cut, we would have a better understanding of the potential use of ryegrass manipulation. The early infestation and rapid build-up of the aphid population in adjacent wheat was partly due to a lack of natural enemies. Edwards *et al.* (1979) showed that predation in spring and early summer was important for reducing the number of aphids at the population peak. At the beginning of our experiment, ladybirds were largely absent from the field before May 30, so they were not active early enough to inhibit aphid population establishment. Climate factors may explain the heavy aphid infestation and low ladybird predation in the present study. Vickerman (1977) pointed out that severe winter and spring temperatures produce higher aphid populations. In the study area, low temperatures in the spring of 2010 persisted for a long time, and wheat phenology was delayed by about 10 days. The aphid peak density in this study was approximately 200 aphids per tiller, which may have exerted an important influence on wheat production. Larsson (2005) presented a crop-loss model for the grain aphid *Sitobion avenae* (F.) in winter wheat that showed rapidly increasing damage occurs when aphid numbers increase 4–10 aphids/tiller. Elliott *et al.* (2000) studied the effect of temperature on the searching activity of adult ladybirds and suggested that extended periods of cold weather during the growing season could inhibit biological control of cereal aphids by coccinellids. Our results indicate that ryegrass has a significant effect in reducing the aphid population during the later period of aphid population development. The reduction amounted to about 1 500 per 100 tillers; however, it might be quite limited in saving production since the economic loss had already been caused. Future research should focus on the early-season control of aphids to reduce their numbers below the economic threshold.

Prey availability in non-crop habitats may play a significant part in the conservation of ladybirds and the related biological control in agro-ecosystems (Bianchi & van der Werf, 2004). Some characteristics of ladybirds, such as mobility and the ability to aggregate rapidly on aphid populations, make them effective in suppressing aphids. Muller and Godfray (1997) demonstrated an earlier population decline in nettle aphid colonies adjacent to grass plots as a result of migration. Accompanying weed strips with olfactorily attractive plants can also contribute to the promotion of predators (e.g., ladybirds) in biological pest control (Sengonca *et al.*, 2002). Ryegrass is a type of forage grass that has some agronomic characters, such as abundant tillers, strong resistibility, and earlier maturation than wheat. It may be that some factors produced by ryegrass, such as pollen, that affect the microenvironment might promote the development of ladybirds. Ryegrass is grown as stock feed in many parts of the world. Strip-planting ryegrass in wheat fields appears to improve biological control of aphids and may replace at least one insecticide use. Although intercropping ryegrass would occupy some areas of wheat, this habitat manipulation may have many advantages, such as economic and environmental benefits.

Generalist aphid predators could provide more effective control if early-season densities are increased through augmentative releases or encouragement of crop colonization (Latham & Mills, 2010). Future research could combine the augmentative release of ladybirds and ryegrass-strip management to control wheat aphids.

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Disclosure

This manuscript and the authors of the manuscript are not involved in any potential conflicts of interest, including financial interests and relationships and affiliations.

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