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Nakahira, Kengo

Laboratory of Insect Natural Enemies, Institute of Biological Control, Faculty of Agriculture,  
Kyushu University

Iwase, Shun-ichiro

Laboratory of Insect Natural Enemies, Division of Biological Control, Department of Applied  
Genetics and Pest Management, Graduate School of Bioresource and Bioenvironmental Sciences,  
Kyushu University

Takagi, Masami

Laboratory of Insect Natural Enemies, Division of Biological Control, Department of Applied  
Genetics and Pest Management, Graduate School of Bioresource and Bioenvironmental Sciences,  
Kyushu University

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## Bottom-up Effects of Crop Seeding Methods on Densities of the Alfalfa Weevil *Hypera postica* and Its Introduced Parasitoid *Bathyplectes anurus*

Kengo NAKAHIRA<sup>1\*</sup>, Shun-ichiro IWASE<sup>2</sup> and Masami TAKAGI<sup>2</sup>

Laboratory of Insect Natural Enemies, Institute of Biological Control, Faculty of Agriculture,  
Kyushu University, Fukuoka 812–8581, Japan

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The alfalfa weevil *Hypera postica* (Gyllenhal) is the most destructive pest of leguminous crops such as alfalfa, Chinese milk vetch, and hairy vetch throughout the world, including Japan. To control *H. postica*, the solitary endoparasitoid *Bathyplectes anurus* (Thomson) was released in Japan as a classical biological control agent. In our study, we investigated the bottom-up effects of hairy vetch seeding methods (i.e., control of seeding timing and density) on the *B. anurus* density to develop a field production method for *B. anurus*. The average densities of *H. postica* and *B. anurus* were significantly higher with early timing and a higher seeding density compared with early timing and a lower seeding density, late timing and a higher seeding density and late timing, and a lower seeding density. This confirmed that the densities of *H. postica* and *B. anurus* could be adjusted by the bottom-up effect of hairy vetch seeding methods. During the tri-trophic interaction among hairy vetch, *H. postica*, and *B. anurus*, the interaction between the hairy vetch weight and *H. postica* densities was high, whereas the interaction between the *H. postica* and *B. anurus* densities was very high. These results suggested that early timing and high density seeding indirectly enhanced the density of *B. anurus* by strong direct effects of the *H. postica* density on the *B. anurus* during the tri-trophic interaction. Overall, we concluded that a combination of early timing and high density hairy vetch seeding may facilitate efficient field productions of *B. anurus*.

**Key words:** classical biological control, green manure, honey source, tri-trophic interaction, weed control

### INTRODUCTION

The alfalfa weevil *Hypera postica* (Gyllenhal) is the most destructive pest of alfalfa *Medicago sativa* (L.) in USA etc. (Radcliffe and Flanders, 1998; Moradi-Vajargah *et al.*, 2011), and it also infects on the leguminous weeds *Vicia angustifolia* L., *Trifolium repens* (L.), and *Melilotus officinalis* (L.) (Byrne and Blickenstaff, 1968; Shobu *et al.*, 2005). In Japan, *H. postica* is the most serious invasive pest of Chinese milk vetch *Astragalus sinicus* L., which is an important secondary crop in rice paddy fields (Shobu *et al.*, 2005; Kuwata *et al.*, 2005). Heavy infection with *H. postica* larvae dramatically damages the growth and honey production in Chinese milk vetch (Shobu *et al.*, 2005; Kuwata *et al.*, 2005). Pesticide applications are effective for controlling *H. postica* (Cummings *et al.*, 2004), but their side-effects on honeybees and contamination of honey products are problematic (Shobu *et al.*, 2005). Therefore, the solitary endoparasitoid *Bathyplectes anurus* (Thomson) (Hymenoptera: Ichneumonidae), an effective classical biological control agent against *H. postica* in the USA and Canada (Radcliffe and Flanders, 1998), has been released since 1991 and it is established in the Fukuoka Prefecture of Japan (Shobu *et al.*, 2005). However, *B.*

*anurus* was collected only around the release area in Japan, more than a decade after its initial release in 2003 (Shobu *et al.*, 2005) and later.

The slow spread of *B. anurus* has also been reported in the USA (Radcliffe and Flanders, 1998) and Canada (Harcourt, 1990). Therefore, large numbers of *B. anurus* cocoons or parasitized hosts were produced and collected from alfalfa fields to expand its distribution area, which were released into non-colonized areas (Radcliffe and Flanders, 1998). In Japan, the alfalfa is not a common crop. *B. anurus* occurs on Chinese milk vetch and leguminous weeds during spring in Japan (Shobu *et al.*, 2005), but it is killed immediately by continuous cultivation and weed control in summer.

The hairy vetch *Vicia villosa* Roch, a leguminous host plant of alfalfa weevil (Byrne and Blickenstaff, 1968), is an agricultural agent that is used as a weed control (Campiglia *et al.*, 2012), green manure in rice paddy fields (Sato *et al.*, 2011), and a honey source for honeybees in weed lands and rice paddy fields (Morse and Calderone, 2000). These agricultural characteristics of hairy vetch make it suitable for rice farmers and honeybee keepers, although *B. anurus* production using hairy vetch and the cultivation methods of hairy vetch have not been studied. In our study, we selected hairy vetch as a candidate plant species for producing *B. anurus*, and we investigated the bottom-up effect of seeding methods (i.e., the control of timing and density) in hairy vetch on the *B. anurus* density to develop a field production method for *B. anurus*. We also evaluated the tri-trophic interaction among hairy vetch, *H. postica*, and *B. anurus* to elucidate the mechanism underlying the increase in *B. anurus* and to establish the method of collection

<sup>1</sup> Laboratory of Insect Natural Enemies, Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan

<sup>2</sup> Laboratory of Insect Natural Enemies, Division of Biological Control, Department of Applied Genetics and Pest Management, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

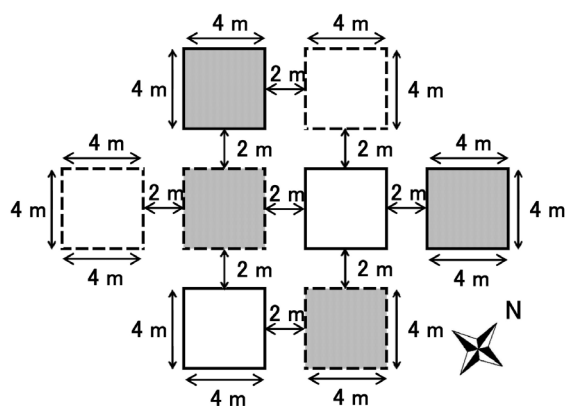
\* Corresponding author (E-mail: nakahira@agr.kyushu-u.ac.jp)

and release of *B. anurus*.

## MATERIALS AND METHODS

This study was conducted between July 2011 and May 2012 in an agricultural field located in Hisayama Town, Fukuoka Prefecture, Japan. The agricultural land was a weed land for five years before the experiment, and the field was plowed before the experiment was commenced in July 2011. The liquid herbicide N-(phosphonomethyl)-glycine potassium salt (192 ppm) was applied on September 5, 2011 to remove the weeds. Eight blocks were established in the farmland with 2 m intervals between each block on September 22, 2011 (Fig. 1). Hair vetch seeds were sown on two dates (early timing: September 23, 2011; late timing: November 22, 2011) and at two seeding densities (low density: 3.5 g/m<sup>2</sup>; high density: 7.0 g/m<sup>2</sup>). Late April is the most suitable period for estimating the damage to hairy vetch and estimating densities of *H. postica* and *B. anurus* in the Fukuoka Prefecture of Japan, because *H. postica* is in its mature larval or prepupal stages (Shobu *et al.* 2005). On April 23, 2012, a quadrat (30 cm×30 cm) was set five times randomly within each block and the plants with insects in each quadrat were cut and taken to the laboratory with paper bag. In the laboratory, the hairy vetch *V. villosa*, narrow-leaved vetch *V. sativa* subsp. *nigra*, and other plants from each quadrat were divided and weighed, and the damaged hairy vetch leaves per 100 leaves were counted in each quadrat. These plants were maintained in the laboratory to obtain *H. postica* adults and *B. anurus* cocoons until mid June. The number of emerged adult weevils and parasitoid cocoons were counted during mid June. No alfalfa weevils and introduced parasitoids were released onto the experimental site. Furthermore, no pesticides or water were applied to the plants on the farmland between September 6, 2011 and at the end of our study.

We defined the parasitism of *B. anurus* as the number of parasitoid cocoons / the number of host alfalfa weevil (i.e., emergence of adult alfalfa weevils plus parasitoid cocoons) based on laboratory rearing results using



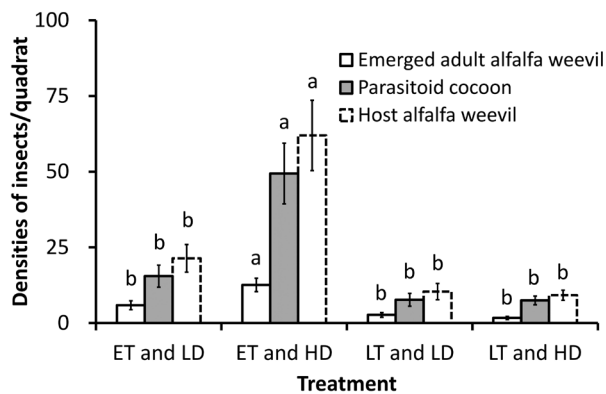
**Fig. 1.** Experimental design of hairy vetch seeding. Blocks with solid and broken lines indicate early and late seeding, respectively. Blocks with gray and white colors indicate low and high seeding density, respectively.

the same method as Shobu *et al.* 2005. We used general linear models to investigate the effects of the timing and density of hairy vetch seeding on the following discrete response variables: the numbers of *H. postica* and *B. anurus*, the arcsine-transformed parasitism rate of *B. anurus* on *H. postica*, the arcsine-transformed leaf damage rates for hairy vetch, and the weights of hairy vetch, *V. sativa* subsp. *nigra*, and other plants in each quadrat. In these analyses, the categories of the seeding timing treatment (early or late), the categories of the seed density treatment (low or high) in each block, and the interactions of the two treatments were treated as explanatory variables. If the interaction of seeding timing and density treatments in the general linear model was significant at the 5% level, a Tukey–Kramer multiple comparison test was used to analyze the average differences in the discrete response variables among treatment combinations. We used a simple linear regression to investigate the interactions between the weevil density versus the parasitoid density and hairy vetch weight versus the weevil density. All statistical analyses were conducted using JMP 6.0 for Windows (SAS Institute, 2008).

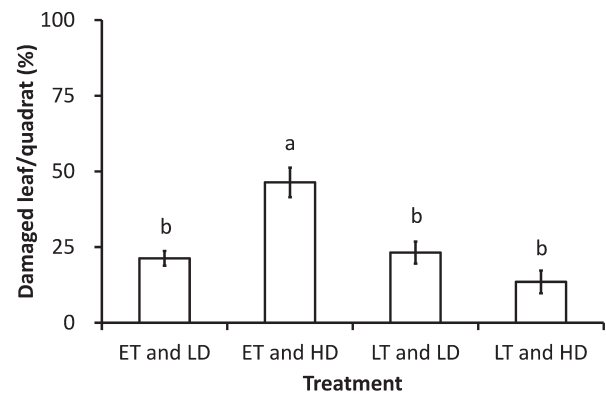
## RESULTS

The densities of the emerged alfalfa weevil *H. postica* adults, parasitoid *B. anurus* cocoons, and host alfalfa weevils were significantly affected by the interactions between the timing and density of hairy vetch seeding at  $P=0.05$  with  $df=1$  (emerged alfalfa weevil adults,  $F=7.5209$ ; parasitoid cocoons,  $F=9.6459$ ; weevil density,  $F=10.5323$ ). The average densities of the emerged alfalfa weevil adults, parasitoid cocoon span and host alfalfa weevils were significantly higher with the early timing and high density seeding (ET and HD) treatment compared with the other three treatments (Tukey HSD multiple comparison test:  $P<0.05$ , Fig. 2). However, the parasitism of *B. anurus* on *H. postica* was not significantly affected by the timing and density of hairy vetch seeding and their interaction at  $P=0.05$  with  $df=1$  (seeding timing,  $F=0.6099$ ; seeding density,  $F=0.6535$ ; interaction,  $F=0.1910$ ; Fig. 3). Leaf damage rate of hairy vetch by *H. postica* was significantly affected by the interaction of the hairy vetch seeding timing and density ( $df=1$ ,  $F=18.3043$ ,  $P<0.05$ ). Leaf damage rate was significantly higher with the ET and HD treatment compared with the other three treatments (Tukey–Kramer multiple comparison test:  $P<0.05$ , Fig. 4). Hairy vetch weight per quadrat was correlated significantly with the host alfalfa weevil density per quadrat ( $n=40$ ,  $P<0.05$ ,  $R^2=0.70$ ). The density of *H. postica* per quadrat was correlated significantly with the density of *B. anurus* per quadrat ( $n=40$ ,  $P<0.05$ ,  $R^2=0.99$ ).

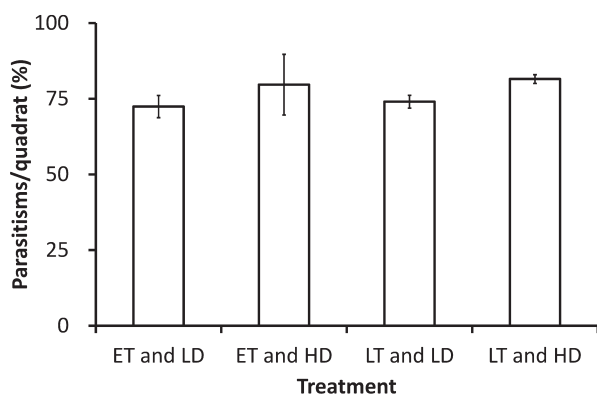
Weight of the hairy vetch *V. villosa* and the narrow-leaved vetch *V. sativa* subsp. *nigra* was affected significantly by the interactions between the timing and density of hairy vetch seeding, with  $df=1$  (hairy vetch,  $P=0.0494$ ,  $F=4.1349$ ; narrow-leaved vetch,  $P=0.0192$ ,  $F=6.0119$ ). However, the weights of other plants were not affected significantly by the timing and density of hairy



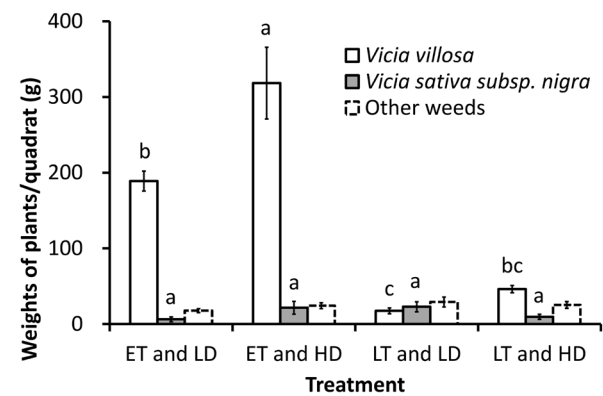
**Fig. 2.** Density (mean  $\pm$  standard errors) of emerged alfalfa weevil adults, parasitoid cocoons and host alfalfa weevils with each treatment. The bars capped with different letters differed significantly among treatments with each density ( $P < 0.05$ ), according to Tukey–Kramer multiple comparison tests. Abbreviations: ET, early timing seeding; LT, late timing seeding; LD, low density seeding; HD, high density seeding.



**Fig. 4.** Rate of hairy vetch leaf damage (mean  $\pm$  standard errors) with each treatment. The bars capped with different letters differed significantly among treatments ( $P < 0.05$ ), according to the Tukey–Kramer multiple comparison tests. Abbreviations: ET, early timing seeding; LT, late timing seeding; LD, low density seeding; HD, high density seeding.



**Fig. 3.** Parasitism (mean  $\pm$  standard errors) of *B. anurus* on *H. postica* with each treatment. The treatments did not significantly affect the arcsin-transformed parasitism of *B. anurus* ( $P > 0.05$ ), according to the general linear model analysis. Abbreviations: ET, early timing seeding; LT, late timing seeding; LD, low density seeding; HD, high density seeding.



**Fig. 5.** Weight (mean  $\pm$  standard errors) of each plant category with each treatment. The bars capped with different letters for *V. villosa* and *V. sativa subsp. nigra* differed significantly among treatments ( $P < 0.05$ ), according to the Tukey–Kramer multiple comparison tests. The treatment did not significantly affect the weights of other weeds ( $P > 0.05$ ), according to the general linear model analysis. Abbreviations: ET, early timing seeding; LT, late timing seeding; LD, low density seeding; HD, high density seeding.

vetch seeding and their interaction, with  $df=1$  (seeding timing,  $P=0.1866$ ,  $F=1.8124$ ; seeding density,  $P=0.7698$ ,  $F=0.0869$ ; interaction,  $P=0.2635$ ,  $F=1.2905$ ; Fig. 5). Average density of the hairy vetch was significantly higher with the ET and HD treatment compared with the other three treatments (Tukey–Kramer multiple comparison test:  $P < 0.05$ , Fig. 4). However, the average density of the narrow-leaved vetch was not significantly different among the four treatments (Tukey–Kramer multiple comparison test:  $P > 0.05$ , Fig. 5).

## DISCUSSION

Density control of the alfalfa weevil *H. postica* and the parasitoid *B. anurus* was achieved successfully by the bottom-up effect of hairy vetch seeding methods. The ET and HD treatment significantly increased the

density of *B. anurus* compared with other treatments (Fig. 2), indicating that the ET and HD treatment could lead to efficient field production of *B. anurus* during hairy vetch cultivation. However, we must mention the effects of human disturbance on the production of *B. anurus* because there is a negative interaction between the annual biology of *B. anurus* and human disturbance in the hairy vetch fields. During spring in Japan, *B. anurus* develops from eggs to larvae, and then enters its summer diapause in cocoons on the ground surface (Shobu *et al.*, 2005). During autumn, the summer diapause ends, and the parasitoids pupate to become adults, although remain in their cocoons and enter the winter diapause (Shobu *et al.*, 2005). Thus, *B. anurus* passes the spring on hairy vetch and the summer and autumn in cocoons

on the ground surface. However, majority of *B. anurus* on the ground are killed by soil cultivators and/or weed control during summer and autumn. To avoid human disturbances on *B. anurus* production, the products should be collected until early summer or maintained without soil cultivation and weed control. These avoidance methods will be easier in weed lands than rice paddy fields because the soil cultivation required for rice production begins during early summer.

The amount of hairy vetch can be controlled by the seeding timing and density because seeding significantly affected the weight of hairy vetch, whereas the ET and HD treatment significantly increased the weight of hairy vetch compared with the other three treatments (Fig. 5). The weight of hairy vetch was also highly correlated with the density of *H. postica* ( $R^2=0.70$ ), indicating that the density of *H. postica* can be manipulated by the control of hairy vetch. We also observed a high density interaction between *H. postica* and *B. anurus* ( $R^2=0.99$ ) and similar parasitism among the four treatments (Fig. 3). Therefore, we concluded that control of the *B. anurus* density was caused by a strong indirect interaction between *B. anurus* and hairy vetch and a strong direct interaction between *B. anurus* and *H. postica*. In this tri-trophic interaction, *B. anurus* is a species-specific parasitoid of *H. postica*, whereas *H. postica* can feed on various leguminous host plants (Radcliffe and Flanders, 1998; Shobu *et al.*, 2005). The difference in the  $R^2$  values for *B. anurus* versus *H. postica* and *H. postica* versus hairy vetch may be attributable to the differences in the food ranges of *B. anurus* and *H. postica*. In fact, we observed other host plants in addition to hairy vetch in the experimental area (Fig. 5). This co-occurrence of various host plants may have negative effects on the intensity of the interaction between *H. postica* and hairy vetch.

In our study, the ET and HD treatment significantly increased the density of *B. anurus* compared with other treatments due to the strong indirect effect of seeding methods and the strong direct effect of the *H. postica* density on the *B. anurus* density in this tri-trophic interaction. The results indicated that the ET and HD treatment will lead to efficient field production of *B. anurus* by the bottom-up effect. However, the ET and HD treatment significantly increased the density of *H. postica* and the damage to hairy vetch (Figs. 2 and 4), which indicated that this treatment risked heavy infections in the next year. Therefore, it is necessary to col-

lect *B. anurus* and *H. postica* from hairy vetch fields and to release only *B. anurus* into non-colonized areas to mitigate and avoid any infection risks in the fields and non-colonized areas.

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