African Journal of Agricultural Research Vol. 6(15), pp. 3609-3615, 4 August, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.789
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Effects of intercropping of garlic or lettuce with Chinese cabbage on the development of larvae and pupae of diamondback moth (*Plutella xylostella*)

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Accepted 29 June, 2011

The effects of intercropping on the growth and development of the diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), were investigated over five generations in a laboratory in Fuzhou, China. The treatments included intercropping Chinese cabbage (*Brassica chinensis*) with garlic (*Allium sativum*) (CG) and lettuce *(Lactuca sativa*) (CL), and the monoculture of Chinese cabbage as control (CK). The larval stage was significantly longer for the intercropping treatments compared to CK. Intercropping affected pupal weight but not the percentage of successful larvae population. Pupae tended to be 10 to 15% larger in the intercropping treatments for three of the five generations. The increase in size, pupa mortality was significantly higher with intercropping. Our findings suggest that intercropping can suppress the DBM populations in a long period rather than in a short term.

Key words: *Plutella xylostella* intercropping, diamondback moth, larval development, pupal mortality.

INTRODUCTION

Diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), is a serious pest of crucifer crops worldwide (You and Wei, 2007). In addition to crop losses, the annual management cost for controlling this pest was estimated to be more than US$1.0 billion globally (Grzywacz et al., 2010). DBM has developed resistance to as many as 73 insecticides (Zhao et al., 2002; Phani Kumar and Gujar, 2005). Studies on alternative control methods to ensure environmental and food safety have become an important task for agriculture professionals. Much research has been conducted on the factors regulating DBM populations, including biological control agents (Kadirvel et al., 2011; You et al., 2004). Cultural control through alternations in the species of crop plant (Ulmer et al., 2002; Badenes-Perez et al., 2004), timing of planting (Shankar et al., 2005), and planting of mixed crops (Hooks and Johnson, 2003) may also provide effective means for reducing DBM populations and their associated damage.

Cultural practices such as intercropping and trap cropping are used to manipulate or reduce pest populations with less reliance on chemical means. Intercropping systems generally house a greater diversity of insects, especially natural enemies (Hooks and Johnson, 2003; Cai et al., 2007, 2010), reduce pest populations (Andow, 1991), increase yields (Maluleke et al., 2005; Issac et al., 2010), and deter insect attraction to host plants (Finch and Collier, 2000). In China, garlic intercropped with turnip (*Brassica rapa*) or coriander (*Coriandrum sativum*) has long been adopted for managing problems caused by various pests (Jia, 1982). Garlic and other plants in the Allium family release strong volatiles which can reduce the attraction of phytophagous insects (Renwick, 1999), alter host-finding behaviors (Nottingham, 1987), deter or stimulate some insects' olfactory organs (Calvo-Gómez et al., 2004), and repel or attract predators (Grez and Prado, 2000). Lettuce has

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been reported to be associated with fewer pest outbreaks (Huang et al., 2003) and to reduce oviposition (Ryan et al., 1980). Generally, yields were increased using insecticides (that is, Bt and λ-cyhalothrin) (Ayalew, 2006). Also, monoculture of kale or cabbage supported significantly more DBM larvae or pupae compared to the intercrops, resulting in reduced damage and increased marketable yield (Ogol and Makatiani, 2007). However, no study has examined the indirect effects of these two species on DBM growth and development in associated crops.

Various intercropping treatments have demonstrated reduced DBM population densities (Kianmatee and Ranamukhaarachchi, 2007). Increased abundance of DBM natural enemies was also observed in diversity habitats (for example, intercropping, under-sown non-host plants, and vegetation borders) (Hooks and Johson, 2003). Lower leaf and head damage accompanied with a higher yield were reported in cases of intercropping cabbage with onion and tomato (Asare-Bediako et al., 2010). Most studies were conducted in the fields for one generation due to the generation overlap of DBM. No data on the effects of intercropping on larval development were previously reported. The objective of this study was to determine the effects of intercropping systems on DBM larval growth and pupal development.

MATERIALS AND METHODS

Intercropping treatments

There were three treatments in this study: (a) The Chinese cabbage monoculture as control (CK), (b) the Chinese cabbage intercropped with garlic (CG), and (c) the Chinese cabbage intercropped with lettuce (CL). Garlic seeds were obtained from the Vegetable Research Institute of Fuzhou, while the Chinese cabbage and lettuce seeds were from the Choi Hing Lee Seed Company Limited. All seeds were conventional products.

In May 2005, the accompanying crops were planted two weeks prior to the cabbage because Chinese cabbage grows faster and has a shorter growth period (Lv, 2001). Plant density was approximately 30 cabbage plants/m^2 in the monoculture and 15 garlic or lettuce plants with 15 cabbage plants/m^2 in each of the intercropping treatments. Three replicates of each treatment were used, resulting to nine plots (size was 2.0 × 1.2 m) per treatment. To protect the plants against insect damage, plants were grown inside a 2.0 × 1.2 × 1.5 m fine nylon cage (mesh size: <1 mm). All plants were fertilized and regularly watered. Chinese cabbage leaves were collected from each plot every day to feed DBM larvae in the laboratory.

Insect colony

Sixty DBM pupae were collected from the fields around the Fujian Agriculture and Forestry University campus in Fuzhou, China (26.05°N, 119.16°E). They were placed in sealed plastic bottles to allow adult emergence, to mate, and to oviposit. All pupae were kept in the rearing room at 26±1°C, 70 to 80% Relative humidity, and 14 h light: 10 h dark. Twenty pupae were collected and placed in each of the three bottles. The top of the bottle was covered with a cotton stopper dipped in 20% honey water to provide nutrition for the adults. After the adults emerged and mated, females laid eggs on the walls of the bottles. The bottom of the bottle was punctured with numerous small holes (diameter = 1mm) to allow newly hatched larvae to crawl out. These larvae were used in the experiments and arbitrarily labeled as the first generation.

Larval growth and development

The first instar larvae of the first generation were transferred with a fine brush into a petri dish (1 larva/petri dish). Each petri dish (diameter = 8.5 cm) was lined with filter paper and a newly cut cabbage leaf disc (diameter = 5.0 cm) from each of the three treatments. The end of each leaf petiole was wrapped with wet cotton to prevent the leaves from withering and the leaf discs were replaced daily. To obtain 90 pupae in each generation for later experiments, approximately 50 larvae were reared in each replicate to produce at least 100 larvae in each treatment. Data from 90 larvae per treatment were calculated and analyzed. To determine the time of pupation, larvae were observed daily. Larval mortality (%) and duration (days) of larval development were quantified (Mean± standard deviation (SD) days).

Pupal duration and weight

Thirty pupae from each replicate of each treatment were placed on petri dishes. After two days of pupation, pupae were individually weighed to the nearest 0.1 mg (mg/pupa) using electrical balance. Pupae were observed daily for adult emergence.

Data analyses

DPS software (Tang and Feng, 1997) was used to analyze the data. The mean duration of egg, larval and pupal stage (number of days) and mean pupal weight were compared among the intercropping treatments and the monoculture control using one-way ANOVA for each generation (Tang and Feng, 1997) at the P = 0.05 level. Data on mortality of larva and pupa were transformed to normalize the residuals using a log(x+1) before being subjected to ANOVA. When multiple comparisons of means were required, Bonferonni inequality was used to adjust a–values to control type I errors (Jones, 1984). Although all tests of significance were based on the transformed data, only untransformed data were presented in the figures in this study.

RESULTS

Larval duration and mortality

The duration of larval development differed significantly among the three treatments in F1 and F5 (P≤0.05). Compared with CK, the duration of the larval stage was significantly longer in the CG treatment in FI, F2, F4 and F5 (P≤0.05), but not in F3 (P>0.05) (Figure 1A). Figure 1A also illustrates that compared with CK, the duration of the larval stage was significantly longer for the CL treatment in F1 (P≤0.05), F2, F4, and F5 but not in F3 (P>0.05). The duration of the larval stage was also significantly longer in the CG treatment in F1 (P = 0.0015), but shorter in F5 (P≤0.05) than that in CL (Figure 1A). No significant difference was found in CG in F2, F3, and F4 (P>0.05) (Figure 1A).

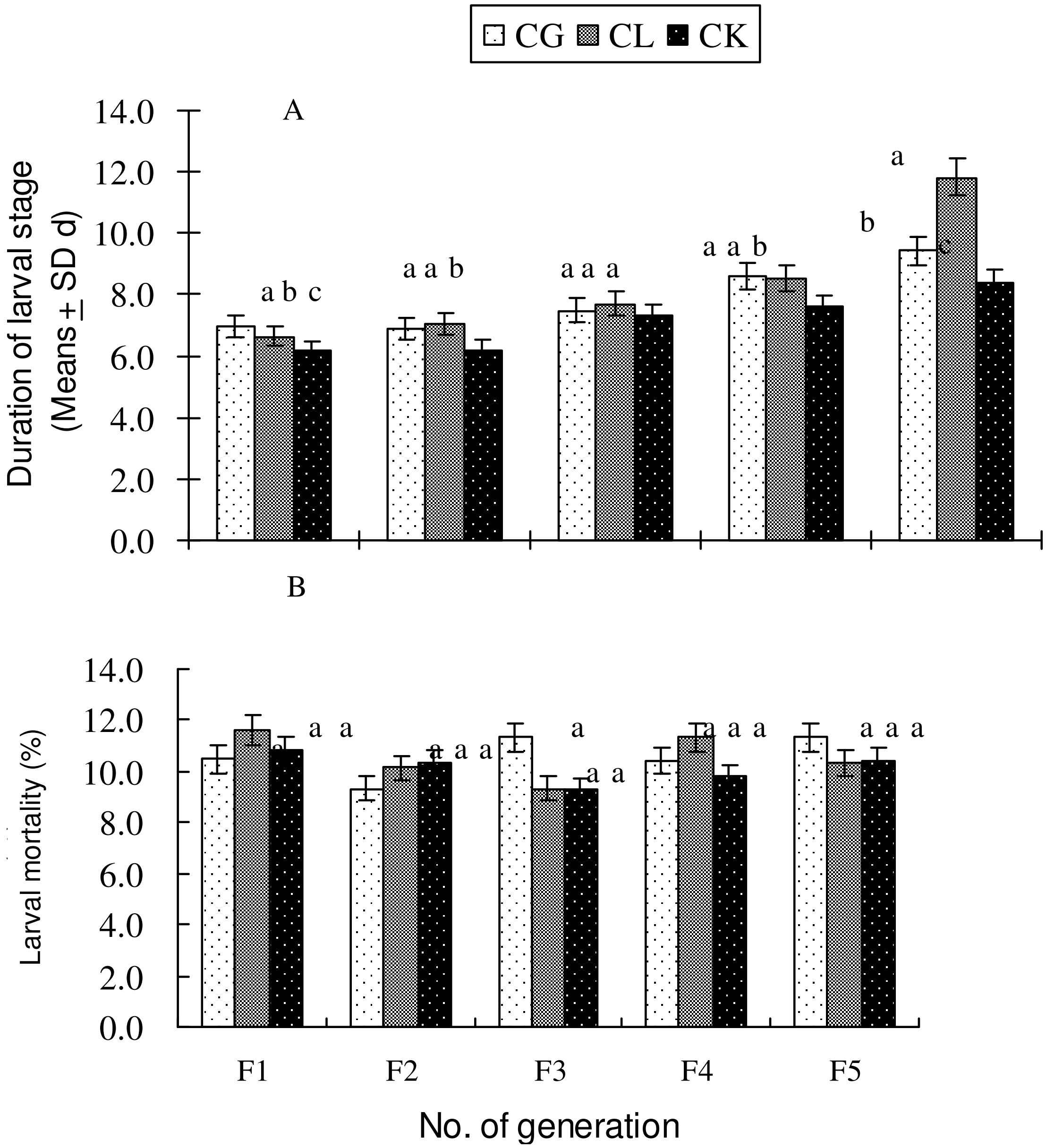

Figure 1. Duration of larval stage (day) (A) and larval mortality (%) (B) of *Plutella xylostella* reared for five generations on Chinese cabbage grown under treatments of intercropping with garlic (CG), with lettuce (CL), or Chinese cabbage monoculture (CK) in Fuzhou, Fujian, China in 2005. Means were calculated from 90 larvae per treatment; within each generation, means marked by the same letters were not significantly different at the 5% level of significance, as determined by ANOVA Duncan's test.

Larval mortality did not significantly differ among the three treatments in any generation (P>0.05) (Figure 1B).

Pupal duration and weight

The difference in duration of the pupal stage varied inconsistently among treatments for all generations, except F4. No significant difference was found among the treatments in F4 (P>0.05). The length of pupal stage was significantly shorter in CG than CK in F1, longer in F2 (P≤0.05), and had no difference in F3, F4 or F5 (P>0.05) (Figure 2A). It was not significantly different between CL and CK in F1 and F4 (P>0.05), but had a significant increase in CL in F2, F3, and F5 (P≤0.05) (Figure 2A). The length of the pupal stage was significantly longer in CL than CG in three generations: F1, F3, and F5 (P≤0.05) (Figure 2A).

No significant difference in mean pupal weight was observed between CG and CK in the first two generations,

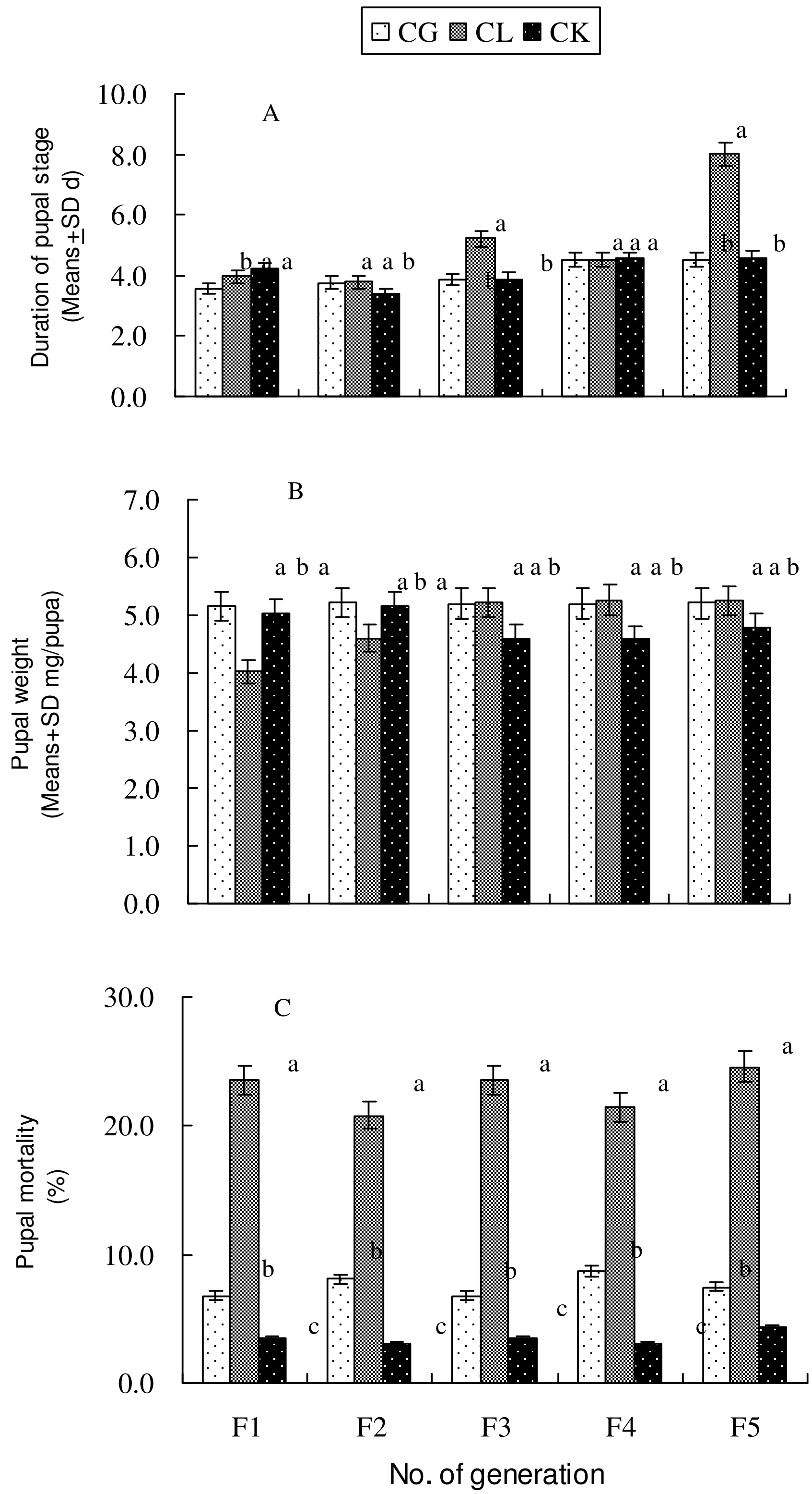

Figure 2. Duration of pupal stage (day) (A), pupal weight (mg) (B), and pupal mortality (%) (C) of *Plutella xylostella* reared for five generations on Chinese cabbage grown under treatments of intercropping with garlic (CG), with lettuce (CL), or Chinese cabbage monoculture (CK) in Fuzhou, Fujian, China in 2005. Means were calculated from 90 pupae per treatment; within each generation, means marked by the same letters were not significantly different at the 5% level of significance, as determined by ANOVA Duncan's test.

Table 1. Comparisons of DBM development between intercropping systems.

Specie stage	Intercropping systems[a]	Number of generation[b]				
		F1	F2	F3	F4	F5
Larval duration (days)	CG	6.96±0.08[a]	6.91±0.14[a]	7.49±0.12[a]	8.57±0.09[a]	9.43±0.10[b]
	CL	6.64±0.05[b]	7.01±0.06[a]	7.69±0.12[a]	8.53±0.13[a]	11.82±0.04[a]
	CK	6.19±0.03 c	6.20±0.11[b]	7.3±0.15[a]	7.57±0.10[b]	8.36±0.09[c]
Larval mortality (%)	CG	10.46±0.58[a]	9.33±2.82[a]	11.34±1.47[a]	10.44±2.81[a]	11.34±1.46[a]
	CL	11.63±1.05[a]	10.12±0.58[a]	9.31±2.63[a]	11.34±1.15[a]	10.33± 3.52[a]
	CK	10.82±1.57[a]	10.33±3.52[a]	9.3±0.58[a]	9.78± 1.46[a]	10.43±1.57[a]
Pupal duration (days)	CG	3.57±0.04[b]	3.77±0.12[b]	3.87±0.08[b]	4.51±0.11[a]	4.52±0.17 [b]
	CL	3.97±0.13[a]	3.79±0.04[a]	5.22±0.31[a]	4.51±0.07[a]	8.01±0.11[a]
	CK	4.2±0.19[a]	3.39±0.08[a]	3.89±0.08[b]	4.56±0.05[a]	4.58±0.10[b]
Pupal weight (ug/pupa)	CG	5.16±0.07[a]	5.22±0.08[a]	5.20±0.02[a]	5.21±0.03[a]	5.21±0.01[a]
	CL	4.02±0.08[b]	4.60±0.05[b]	5.22±0.02[a]	5.26±0.03[a]	5.25±0.02[a]
	CK	5.04±0.09[a]	5.15±0.06[a]	4.60±0.04[b]	4.59±0.01[b]	4.78±0.0 [b]
Pupal mortality (%)	CG	6.75±0.91[b]	8.06±1.29[b]	6.75±0.91[b]	8.73±0.23[b]	7.47±0.10[a]
	CL	23.55±1.12[a]	20.77±1.02[a]	23.55±1.13[a]	21.44±1.4[a]	24.58± 1.17[b]
	CK	3.52±0.97[c]	3.09±1.62[c]	3.52±0.97[c]	3.05±1.63[c]	4.30±1.53[c]

(a). Chinese cabbage grown under treatments of intercropping with garlic (CG), with lettuce (CL), or Chinese cabbage monoculture (CK) in Fuzhou, Fujian, China in 2005. (b). Means marked by the same letters were not significantly different at the 5% level of significance as determined by ANOVA Duncan's test.

F1 and F2 (P>0.05), but there was a significant increase in CG in F3, F4 and F5 (P≤0.05) compared with CK (Figure 2B). Similarly, the mean pupal weight was initially lower in CL than CK in the first two generations, F1 and F2 (P≤0.05), but became significantly higher in the latter generations: F3, F4 and F5 (P≤0.05) (Figure 2B). Compared with CL, pupal weight was significantly greater for CG in F1 and F2 (P≤0.05), but no significant differences were found in the following generations F3, F4 and F5 (P>0.05) (Figure 2B).

Significant differences in pupal mortality were found among all treatments in all generations (P≤0.05) (Figure 2C). Higher mortality was found in CG and CL compared with CK (Figure 2C). A significantly higher mortality rate was found in CL than in CG (Figure 2C).

DISCUSSION

Onion and garlic intercropping systems were also found to have repellent effects on DBM to reduce pest's populations because the company plants act as physical barriers to the movement of the insect pest, natural enemies are more abundant and/or the chemical or visual communication between DBM and the cabbage is disrupted (Asare-Bediako, et al., 2010). However, no report was previously documented on the effects of lettuce as accompanying crops in a cabbage intercropping on populations of DBM. Some research has demonstrated that different development and growth of insects including bertha armyworm (*Mamestra configurata*), annam stick insect (*Medauroidea extradentata*), and DBM were found when different host plant species were used, possibly due to the different nutritional quality of the plants (Muhamad et al., 1994; Badenes-Perez et al., 2004; Boucher and Varady-Szabo, 2005). Similarly, Wei et al. (2004a, 2005) indicated that some substances from non-crucifer plants (such as labiate herb, tobacco, lettuce and pepper) can regulate the growth of DBM or reduce the feeding of DBM. Reddy and Urs (1991) reported that cabbage leaves treated with acetone extract of the xerophytic perennial plant (*Agave cantala* Roxb.) prolonged the larval and pupal periods in DBM. Based on the results, garlic and lettuce intercropped with Chinese cabbage significantly prolonged the duration of the pupal stages and increased pupal mortality (Table 1).

In this study, higher pupal mortality was found in the intercropping treatments than in the monoculture counterpart for every generation of DBM. The CL treatment tended to result in significantly smaller DBM pupae in the first two generations (Table 1). Higher pupal weights were found in both CG and CL intercropping treatments than in CK (Table 1). Effects of intercropping on DBM pupal weight and mortality received only limited attention. Factors affecting the development of pupae may include host plant cultivars, sub-lethal insecticides, transgenic crops, and non-host plant extracts; all of which were found to increase or reduce the pupal weight of DBM (Chen et al., 1996; Josan and Singh, 2000). For example, the sub-lethal insecticide Cypermethrin was demonstrated to reduce (Abro et al., 1993) whereas *Bt* transgenic crops increased the pupal weights in DBM (Sayyed et al., 2003). There are currently no reports on using living non-crucifer plants as accompanying crops to increase pupal mortality or depress pupal weight of DBM but some studies indicate that a secondary substance extracted from non-crucifer plants could inhibit pupal development. Chen et al. (1996) found that application of the Chinaberry extracts (Chinaberry) reduced the weight of DBM pupae when newly-hatched larvae were continuously reared on treated rape seedlings. Wei et al. (2004b) found that the ethanol extracts of non-crucifer plants such as *Centella asiatica, Mangifera indica, Broussonetia papyrifera*, and *Eucalyptus tereticornis* significantly decreased the pupal weight and survival rate of DBM due to the action of secondary substances, such as steroidal aglycones and glycosides (Weissenberg et al., 1996). Therefore, intercropping garlic or lettuce with cabbage to reduce pupal weight can result in lower adult populations and subsequently reduce the next generation, making it a plausible approach for the pest insect control.

Our observations demonstrate that intercropping may prolong DBM life cycle and consequently reduce the number of generations per year. Within intercropping systems, plant competition may influence plant quality and thereby alter the growth rates of herbivores (Bukovinszky, 2004). Crop varieties, sowing timing, or field manipulation influence pest behaviors and enhance natural enemies (Lü and Liu, 2008). Future work is required to investigate the metabolic and nutritional status of host plants in the intercropping system. For the pest, possible behavioral responses or metabolic adaptations to changing food resources may also play an important role and should thus be studied further.

ACKNOWLEDGMENTS

This study was supported by the National Key Project of Fundamental Scientific Research ("973" Program, No. 2011CB100404)

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