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REVIEW

Application of vetiver grass *Vetiveria zizanioides*: Poaceae (L.) as a trap plant for rice stem borer *Chilo suppressalis*: Crambidae (Walker) in the paddy fields

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Abstract

Rice is an important staple food in China and it is at risk of attack by rice striped stem borer *Chilo suppressalis*, which occurs in most rice growing areas. In recent years, severe *C. suppressalis* outbreaks have been observed in China mainly due to changes in the rice cultivation systems, wide adoption of hybrid varieties and resistance to the dominant insecticides. Management relies primarily on chemical insecticides and resistance is an important contributing factor in these outbreaks. As a result, food safety of agricultural produce is reduced and the ecological and environmental integrities are threatened as well. Recently, environmentally friendly pest management measures, such as trap plants have been introduced for *C. suppressalis* management and this method can greatly reduce insecticide use. Our previous results indicated that the vetiver grass (*Vetiveria zizanioides*) is a dead-end trap plant that can effectively attract the adult females of *C. suppressalis* to lay eggs on it but where larvae are unable to complete their life cycle. This paper further explored the application of vetiver grass as a trap plant to manage *C. suppressalis* in the paddy fields. This environment-friendly tool can not only reduce *C. suppressalis* populations, it can also increase the diversity and abundance of natural enemies that can provide better environmental conditions for rice production.

Keywords: rice, vetiver, trap plants, *Chilo suppressalis*, control, application

1. Introduction

The striped stem borer *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) is an economically important rice pest in Asia, northern Africa, and southern Europe. In China, this pest is particularly serious because of widespread rice cultivation and the adoption of hybrid varieties (Qu *et al.* 2003; Peng 2016). Insecticides remain the major method to manage *C. suppressali* and this has led to rapid development of insecticide resistance. In addition, the huge amounts of insecticides used have brought about negative impacts on the environment, ecosystem services and human health. In the past few years, chlorantraniliprole has been widely used to control *C. suppressalis*; however, field control efficacy has greatly decreased, leading to control failures in some areas in 2016 (Lu *et al.* 2017c). The Ministry of Agriculture of China introduced 'green plant protection' as a sustainable strategy to use less insecticides for managing

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stem borers and was gradually accepted by farmers (Lu *et al*. 2012; Xia 2010). The use of vetiver grass as a trap crop will contribute significantly towards this strategy.

Studies have shown that most herbivores have significant preferences for certainly plants, crop varieties and crops in particular growing stages (Wang *et al.* 2014). These preferred plants might be used as the trap plants. Small areas of trap plants that attract the target pests for oviposition might be planted around or in crop fields, thus preventing the pests from reaching the main crops so as to protect the crops (Lu *et al.* 2008). Using trap plants to control pests can significantly reduce the amount of pesticides needed. When the trapping effect is strong enough to control pests well, pesticides for the trap plant's target pests could be avoided, which would benefit farmers and field ecosystem from reducing insecticide spraying costs, conserving natural biological control and reducing environmental pollution (Mitchell *et al.* 1997, 2000). The use of trap plants has recently attracted more attention as authorities become aware of the hazards that pesticides bring about and the need for sustainability in agricultural production. The trap plant strategy is also playing an important role in integrated pest management (IPM) (Charleston and Kfir 2000; Hilje *et al*. 2001; Horsfield *et al*. 2002; Liang *et al*. 2015). In this paper, we discuss concepts in the use of trap plants, their advantages, and their roles in biodiversity conservations.

2. Trap plants

2.1. Concepts of using trap plants

Trap plants have been defined as "plant stands grown to attract insects or other organisms like nematodes to protect target crops from pest attacks, or to prevent pests from reaching the crop or concentrating them in a certain parts of the field where they can be easily destroyed" (Hokkanen 1991; Shelton and Badenes-Perez 2006). As a biological control system, trap plants can attract pests and protect the main crops. Pyke *et al.* (1987) conceived trap plants as a strategy for insect pest management in Australia. Trap plants have been used to manipulate the distribution of *Helicoverpa* spp. in cotton, thereby reducing reliance on insecticides. This control method can be simple and easy to operate, does not pollute the environment and can be a powerful tool for management of important pests (Hokkanen 1991; Boucher *et al.* 2003; Liang *et al.* 2015). In recent years, the widespread use of chemical pesticides have caused severe damages to the agricultural ecosystems, reduced natural enemy diversity and abundance, induced insecticide resistance in pests, environmental pollution and increased pesticides residues in agricultural products (Morales 2001; Mathewsa *et al.* 2003; Mu *et al.* 2003; Kumar and Kumar 2004; Zheng *et al.* 2008). Trap plants can serve as an important and environmentally alternative for pest management.

2.2. Classification of trap plants

Trap plants may be classified into three types: traditional trap plants, lethal trap plants and genetically modified trap plants. Traditional trap plants are enduring plants which are planted beside the main crops. The trap plants become a food source and lure pest species to lay eggs on them (Mensah and Khan 1997). This cultivation pattern has been adopted by traditional agricultural countries and industrialized countries with large-scale agricultural operations. Among them, a highly successful example is in California where growers used alfalfa as a trap plant to trap *Lygus lucorum* in the 1960s (Stern 1969; Godfrey and Leigh 1994). Lethal trap plants refer to the plants that have strong attraction to pest species but once the pests eat or oviposit on these plants, they or their off springs are unable to survive (Shelton and Nault 2004). Lethal trap plants as the habitat for pests can effectively prevent the damage on the main crops caused by the offspring (Badenes-Perez *et al.* 2004). Sana as a lethal trap plant can trap *Maruca testulalis* in cowpea cultivation (Jackai and Singh 1983). Lepidopteran pests with high preferences for ovipositing on these lethal non-host plants are unable to survive (Thompson 1988; Thompson and Pellmyr 1991). Transgenic plants are different from traditional trap plants in that they depend on the efficacy of a toxin encoded by target gene inserted into their genome. The importance of the transgenic plants as trap crops may reveal itself in the future (Hoy 1999).

2.3. Advantages of trap plants

As an organic component of IPM, trap plants have many advantages including being environmentally friendly and safe for natural enemies. This not only improves biological diversity in the farmlands and orchards, but also promotes natural enemies. The use of pesticides can be reduced, cost saved and the productivity can be increased. Using trap plants to control pests has significant ecological and economic benefits (Xu *et al.* 2005).

Environment safety Use of trap plants as a tool in pest management may partly or completely replace chemical pesticides in the main crops. They are safe for the environment and natural enemies and have other potential ecological benefits. For example, the population number of diamondback moth was controlled below the economic threshold by planting kale in cabbage fields. The yields of cabbage were comparable to that of the chemically controlled fields but with 75–100% pesticide reduction as well as other ecological benefits (Tara 1999).

Enhanced biological diversity Organisms in nature live through interdependent and complementary ways in the agricultural ecosystem. Higher species richness in the ecosystem tends to lead higher complexity and often stability. An important issue for agricultural systems to be sustainable is to maintain high biological diversity. The practice of intercropping different plants is often considered as a conventional method of increasing farmland biological diversity. This is an important component of sustainable farmland ecosystem and an effective way to control pests. For example, planting corn trap fields in cotton fields would increase the species diversity of the arthropod community. Stability maybe improved, resulting in reduced frequency of pest outbreaks (Cui *et al.* 2001).

A breeding ground for natural enemies Trap plants can attract and increase populations of natural enemies of pests which increase the biological control ecosystem services (Hokkanen 1991). It has been found that mixed croplands have lower insect pest populations compared with monocultures (Anon 1990). Since the pests of trap plants do not need to be controlled and the nectar and pollen of trap plants provide rich diet and nutrition for natural enemies. The trap plants can also serve as breeding sites for natural enemies and thus improve biological control (Andow 1991). The smell of trap plants may have an attractive effect on natural enemies. Zhou *et al.* (2013) reported that a creeping variety of mung bean could significantly improve the parasitism rate of *Ostrinia furnacalis* eggs in maize. Wang *et al.* (2000) indicated that the volatile matter of the mung bean leaves strongly stimulated the directional behavior of *Trichogramma*. In addition, trap plants provide shelter for natural enemies, especially in hot summers. Sorghum planted in cotton fields provided shelter for the natural enemies of *Nezara viridula* (Glynn and Ben 2003).

Saving cost, enhancing benefits Using trap plants to control pests not only significantly reduced the use of pesticides, but also saved production costs and increased ecological benefits. Studies have shown that the net income for farmers planting trap plants grew by an average of 10–30% (Hokkanen 1991). Planting lupins in cotton fields increased the population density of ladybugs and fire ants saving 60% of the pesticides. Moreover, lupins were able to replace the nitrogen fertilizer required for cotton, equivalent to saving US$35 per 100 m^2 (Preston 2001).

3. Trap plants for *C. suppressalis*

Trap plants are a traditional tool of rice pest management that has increased considerably in recent years (Shelton and Badenes-Perez 2006; Zheng *et al.* 2009; Liang *et al.* 2015; Lu *et al.* 2015, 2017a). Numerous studies reported that several grasses have been used or attempted to be used as trap plants in rice insect pest management (Haile and Hofsvang 2002; van den Berg 2006; Khan *et al.* 2006, 2007). *Vetiveria zizanioides*, also called vetiver grass, has been suggested as a lethal trap plant for control of *C. suppressalis* (Zheng *et al.* 2009). *V. zizanioides*, a perennial herb, belonging to the family Poaceae is known for its fragrant roots. It originates from India, and is mainly distributed in Southeast Asia, Africa and other subtropical regions. China has no natural vetiver but since Chairman of International Vetiver Network, Dick Grimshaw, introduced vetiver into the World Bank China Southern Red Soil Development Project in 1988, vetiver has spread widely in the southern provinces. The biological and ecological characteristics of vetiver, the cultivation and management of vetiver, the effect of hedgerows on soil fertility, erosion and crop yield have been studied. The results showed that the hedgerow of vetiver is beneficial to the soil remediation and riparian solidification of red soil areas, effectively reducing the surface runoff and soil erosion. The temperature and humidity of soil can be adjusted by a covering of vetiver grass on the surface. In some places, vetiver is used to protect tea trees and *Camellia oleifera*.

4. Application of vetiver in the control of *C. suppressalis*

Several studies have shown that vetiver attracts female adults of *C. suppressalis* which lay eggs on the plants (Zheng *et al.* 2009; Xia and Sun 2012). The strategy of planting a certain proportion of vetiver in rice fields has been developed to control *C. suppressalis*. It has been found that the effect of using trap plants for rice borers is not only related to the planting period in the rice field, but also has a close association with distribution and planting density. Results of our lab have revealed that the best planting period of vetiver in rice fields is from late March to early April with the planting area accounting for 6–10% of the total area (Liang *et al*. 2015). Sets with about 3-4 tillers per knot are transplanted. A total of 750 kg of calcium, magnesium and phosphate fertilizers per hectare are applied as the base fertilizer. If no rain falls within 3 days, irrigation is applied to the knots. After transplanting of the rice crop, the water and fertilizer management is consistent with the conventional management of rice. Vetiver can remain in the fields after the harvest of early rice and doesn't need to be re-planted in the late rice period and the following year. The key to trapping rice borer with vetiver is 1) the egg numbers and pest numbers on vetiver during the incubation periods; 2) seize the favorable time to kill eggs and rice borers intensively, aiming to alleviate the damage of rice by rice borers (Chen *et al*. 2007, 2016). If the damage by rice borers is under the economic threshold, the application of pesticides can be avoided (Zheng *et al.* 2008).

4.1. The working principles of vetiver grass

Attractive mechanism of vetiver on *C*. *suppressalis* adults It has been reported that the volatile oil of vetiver contains a range of terpenoids that are strong attractants and oviposition stimulants for female borers on vetiver (Li *et al.* 2006). We have identified the volatiles of stems and leaves from different rice varieties and vetiver by dynamic headspace analysis with gas chromatography-mass spectrometer (GC-MS). The results showed that most of the volatiles were common to both rice and vetiver and the relative levels were similar. We used pure synthetic compounds and electroantennography (EAG) to determine which of the vetiver volatiles were active. Our results indicated that the responses of *C*. *suppressalis* adult antennae from males and females to the different compounds varied widely. The compounds which elicited strong EAG responses in female antennae were subsequently selected for further development of an attractant volatile formula (Lu *et al.* 2017b). We then measured the field trapping effect of 450 formulae (based on the single compounds or combinations of volatiles from vetiver) and finally screened 17 formulae which have the greatest trapping effect for *C*. *suppressalis* (Fig. 1). Among them, the average moth number in each trap using different formulas was greater than 15. The control was the *C*. *suppressalis* sex pheromone attractant.

Lethal mechanism of vetiver on *C. suppressalis* larvae Fraction 1 (Fr1) and fraction 5 (Fr5) isolated from Sherwood oil extract of vetiver were incorporated into artificial diet (0.05 g mL^{-1}) and 3rd instar *C*. *suppressalis* larvae were exposed for 3 days. Mortalities were 85.00 and 67.67%, respectively (Lu *et al.* 2017a).

The contents of total protein, cellulose, total sugar, amino acid and other nutrient contents in vetiver were significantly

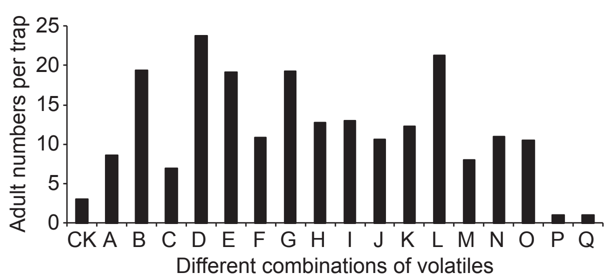

Fig. 1 The field trapping effect of different combinations of volatile matters. CK, sex attractant of *Chilo suppressalis* (Newcon Inc., Ningbo); A–Q represent different combinations of volatiles.

lower than those in rice. In addition, 3 days after feeding, the activity of digestive enzymes such as protease, amylase, trehalase and sucrase in the larvae of *C*. *suppressalis* feeding on vetiver was significantly lower than that of *C*. *suppressalis* feeding on rice. After 6 days, cytochrome P450 activity in the larvae of *C*. *suppressalis* feeding on vetiver was significantly lower than that of *C*. *suppressalis* feeding on rice. After 9 days, the carboxylesterase (CarE) activity in the larvae of *C*. *suppressalis* feeding vetiver was significantly lower than that of *C*. *suppressalis* feeding on rice. The results indicated that the lethal effect of vetiver on *C*. *suppressalis* larvae focused on two aspects. Firstly, vetiver contained toxic-active substances which have a lethal effect on *C*. *suppressalis* larvae. Larvae gradually lost the metabolic detoxification capacity by inhibiting the activity of detoxification enzymes CarE and P450 in larvae and ultimately led to death. Secondly, *V*. *zizanioides* is deficient in nutrients compared with rice. The *C*. *suppressalis* larvae showed nutritional imbalance after feeding vetiver, impacting the activity of digestive enzymes, resulting in digestive disorders and eventually death. The study provided the theoretical basis for the development and identification of insecticidal active substances in vetiver and the establishment of a green management strategy for rice borer based on vetiver (Lu *et al.* 2017a).

4.2. Other factors impact on the effectiveness of vetiver

The control effects of vetiver on *C*. *suppressalis* were affected by planting ages (Table 1). The dead tillering rates of rice in the field planted with annual vetiver and biennial vetiver were both lower than those in the control field. Biennial vetiver had a stronger controlling effect on *C*. *suppressalis* than annual vetiver. The seedling protection rate of planting biennial vetiver with every 5, 3 or 1 m on early rice season rice were 63.6, 47.5 and 69.7%, respectively. The annual vetiver with every 5 m showed no seedling protection effect. Similar results were also found in late rice.

The control effects of vetiver strips on *C*. *suppressalis* varied with the spacing distance in fields are shown in Table 2. In the tillering stage of early rice when the damage of *C*. *suppressalis* was relatively severe, planting vetiver on the ridges showed a substantial controlling effect on *C*. *suppressalis*. If the rice field is 5 and 10 m away from the vetiver strips, the controlling effect varied with the spacing distance of vetiver and showed a gradation of 1 m>3 m>5 m. For rice rows 15 and 20 m away from the vetiver strips, the controlling effect of 1, 3 and 5 m spacing along the vetiver rows had the similar seedling protection effect. Planting vetiver on the ridges provided seedling protection effect for early rice at tillering stage. When the vetiver planting

Table 1 Effect of vetiver at different planting vetiver types on rice dead tillering rate (%) (Zheng *et al.* 2017b)

Growth stages of rice	Vetiver type	Spacing distance of vetiver (m)			Control	Covariance parameter
		5	3	1		
Filling stage of early rice	Annual vetiver	0.73±0.03 a	0.51±0.06 b	0.52±0.06 b	0.73±0.03 a	*F*=6.726, *P*=0.001
	Biennial vetiver	0.36±0.08 c	0.52±0.04 b	0.30±0.03 b	0.99±0.08 a	*F*=34.587, *P*<0.001
Yellow ripening stage of late rice	Annual vetiver	0.55±0.11 a	0.33±0.03 b	0.34±0.02 b	0.54±0.04 a	*F*=11.704, *P*<0.001
	Biennial vetiver	0.34±0.02 b	0.24±0.06 b	0.27±0.09 b	0.54±0.04 a	*F*=4.424, *P*=0.011

Data were average value±standard deviation. The same lowercase letter in the same line means the one-way ANOVA results between different treatments had no significant difference (*P*<0.05).

Table 2 Effects of vetiver grass cluster intervals and its distance to rice plants on rice dead sheath rate caused by stem borers (Zheng *et al.* 2017b)

Growth stages of rice	Distance between sampling site and vetiver grass stripe (m)	Rice dead sheath rate (%) under various vetiver grass cluster intervals			Control	Covariance parameter
		5 m	3 m	1 m		
Filling stage of early rice	5	2.08±0.06 b	1.48±0.09 c	0.68±0.12 d	2.85±0.11 a	*F*=82.014, *P*<0.001
	10	1.93±0.10 b	1.62±0.05 b	0.91±0.07 c	3.61±0.17 a	*F*=114.73, *P*<0.001
	15	1.35±0.03 b	1.54±0.12 b	1.40±0.17 b	2.90±0.21 a	*F*=26.141, *P*<0.001
	20	1.88±0.08 b	2.24±0.13 b	1.95±0.16 b	3.81±0.11 a	*F*=53.169, *P*<0.001
Yellow ripening stage of late rice	5	0.16±0.02 b	0.06±0.01 c	0.06±0.01 c	0.60±0.02 a	*F*=266.005, *P*<0.001
	10	0.49±0.06 b	0.10±0.02 c	0.06±0.01 c	0.90±0.08 a	*F*=57.885, *P*<0.001
	15	0.62±0.07 a	0.42±0.09 a	0.66±0.11 a	0.66±0.11 a	*F*=1.453, *P*=0.298
	20	0.93±0.14 a	0.38±0.06 b	0.30±0.03 b	1.08±0.11 a	*F*=16.588, *P*=0.001

Data were average value±standard deviation. The same lowercase letter in the same line means the one-way ANOVA results between different treatments had no significant difference (*P*<0.05).

distance was 1 m, the protection effect reached 70 and 50% for the rice row 5–10 and 15–20 m away from the vetiver row, respectively. The seedling protection effect of vetiver planting distance of 5 m was 27.0–53.4%.

Different vetiver spacing distances along the rows have a good seedling protection effect on late rice at tillering stage 5 and 10 m away, but a relatively poor effect at a distance of 15 and 20 m. It was concluded that biennial vetiver is effective in controlling *C. suppressalis* in rice for up to 20 m from the planted row.

The vetiver was planted on the ridges to achieve a distribution of vetiver that would control the population density of overwintering *C. suppressalis* (Table 3). The density of overwintering *C. suppressalis* at different distances in rice fields of ecological control areas were all significantly lower than that in the control field. The reduction rate of *C. suppressalis* varied from 76.2 to 92.6% depending on the distance from the vetiver rows. The reduction rate of *C. suppressalis* in the rice fields 25 m from the vetiver strips was also around 80%. The average insect reduction rate reached 84.2%, indicating that in addition to the technological measures of biodiversity conservation, the controlling effect of *C. suppressalis* was effective with vetiver rows at 50 m spacing and vetiver clusters at 4 m spacing along the rows.

4.3. Application and controlling effects of vetiver

The population of overwintering *C. suppressalis* in the vetiver planting area with 5 to 25 m of the distance from the ridge were lower than that in the control field. The average density of overwintering *C. suppressalis* larvae in vetiver field was 13.8 per 100 clusters while that in control field was 85.4 per 100 clusters. The reduction rate of *C. suppressalis* was 83.8% (Fig. 2).

The survey results on the natural enemies showed that the number of egg parasitoids of rice stem borers *Telenomus* and Trichogrammatidae and other larval stage wasps in vetiver planting field were significantly higher than that in the control field (Fig. 3). Among them, the main parasitic wasps in Scelionidae included *Telenomus sesamtae* and *Telenomus chilocolus*. The main wasps in Trichogrammatidae included *T. japonicun* and *T. chilonis*, and the others included *Aceratoneuromyia indica* from Eulophidae, *Apanteles baoris* and *Apanteles flavipes* from Braconidae, and etc. The number of *Telenomus* and Trichogrammatidae, other parasitic wasps in vetiver fields was 2.8, 1.7 and 0.8 times higher than in control field.

5. Perspective

In recent years, the incidence and degree of *C. suppressalis* infestation in rice in China has increased. The two basic reasons are high level of insecticide resistance in *C. suppressalis* to bisamides (Gu *et al*. 2016) and the other is associated with mechanical harvesting. Tall rice stubbles are generally left in the field after machine harvesting and these provide

Table 3 The effect of vetiver on population density of overwintering *Chilo suppressalis* (Zheng *et al.* 2017b)

Distance from vetiver strips (m)	Population density of *C. suppressalis* (no. per 100 clusters)			Covariance parameters
	Biological test field 1	Biological test field 2	Control	
5	8.61±2.47 b	7.22±4.01 b	96.67±9.62 a	*F*=68.661, *P*<0.001
10	14.17±2.68 b	15.56±4.34 b	100.56±17.46 a	*F*=22.194, *P*=0.002
15	17.22±11.40 b	12.20±4.34 b	72.23±12.52 a	*F*=10.882, *P*=0.010
20	13.34±0.96 b	17.25±6.26 b	80.56±11.88 a	*F*=15.930, *P*=0.004
25	15.83±3.37 b	13.89±5.63 b	77.21±10.81 a	*F*=24.307, *P*=0.001

Data were average value±standard deviation. The same lowercase letter in the same line means the one-way ANOVA results between different treatments had no significant difference (*P*<005).

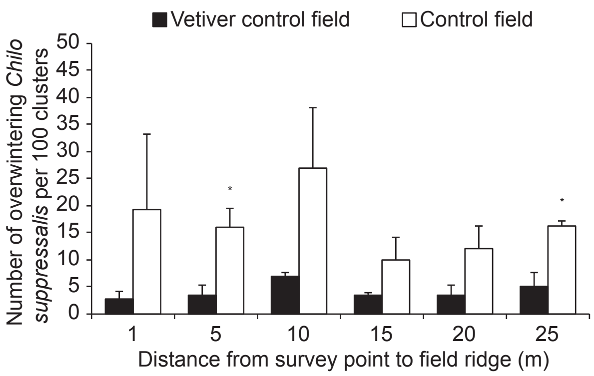

Fig. 2 Control effect of vetiver on amount of overwintering *Chilo suppressalis* (Zheng *et al.* 2017a). *, significant difference (*P*<0.05). Bars indicate SE.

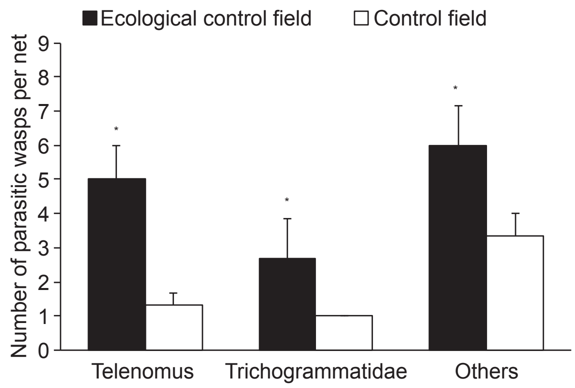

Fig. 3 Protection and improvement of planting vetiver on natural enemy parasitic wasps (Zheng *et al.* 2017a). *, significant difference (*P*<0.05). Bars indicate SE.

the overwintering sites for *C. suppressalis*, resulting in the significantly increased field populations (Xie *et al*. 2016). The use of vetiver trap plants is an important measure for sustainable management of *C. suppressalis* in rice. This paper summarized the principles of trap plants, the lethal mechanism of vetiver and the application of vetiver in the control of rice *C. suppressalis*. This technique can continuously reduce damages of *C. suppressalis* and significantly increase parasitoid wasp populations that provide biological control.

Trap plants protect the main crops by affecting the oviposition and feeding of herbivorous insects (Shelton and Badenes-Perez 2006). Vetiver attracts the *C. suppressalis* female adults to oviposit intensively on it, so the larvae cannot survive. Therefore, vetiver is a typical "lethal trap plant" for *C. suppressalis* (Zheng *et al.* 2009). Long-term field experiments have shown that planting vetiver results in 30–70% control of *C. suppressalis* in rice. Vetiver shows great potential as one of the most important measures for green management of *C. suppressalis* in rice. At the same time, trap plants play the role of the natural enemy bank (Emana *et al.* 2003; Tillman and Mullinix 2004). *C. suppressalis* eggs in vetiver provide abundant hosts for its natural enemies, especially egg parasitic wasps. Survey results have demonstrated that the abundances of egg parasitic wasps, including *Telenomus* and Trichogrammatidae, in vetiver planted fields were 2.8 and 1.7 times higher than those in the farmer autonomous area, respectively. Vetiver plays a complex role in trapping and biological control of natural enemies.

Using vetiver as a trap plant to control rice *C. suppressalis* has been widely applied in southern China, which reduced insecticide usage to some content. Although most vetiver planting sites showed high control efficiency of vetiver on *C. suppressalis*, there are several sites indicated that *C. suppressalis* was not controlled by vetiver. It is not surprising that different geographical populations of *C. suppressalis* have different response to volatiles from vetiver grass. Thus, a trail should be tested before vetiver widely applied in a new area and control efficiency should be observed after long-term application.

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