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Field survey and greenhouse evaluation of non-rice host plants of the striped stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae), as refuges for resistance management of rice transformed with *Bacillus thuringiensis* toxin genes

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ABSTRACT

Several thousand tillers of each of the five weedy grasses were dissected over a 13-month period. Only four Chilo. suppressalis larvae or pupae were found: three on E. crus-galli and one on E. colona. Stem borer C. suppressalis generally does not use these hosts in the field. The pink stem borer, Sesamia inferens (Walker) (Lepidoptera: Noctuidae), a minor pest of rice, was frequently collected from E. crus-galli (63 specimens) and occasionally from E. colona, E. indica, and I. rugosum (4-5 specimens each). There was relatively little difference among the six plant species in C. suppressalis larval survival after four days on stem pieces. All C. suppressalis recovered on the weedy grasses after 25 days were third instars, with the exception of four pupae found on E. indica.

Keywords: non-rice host plants, *Chilo suppressalis*, Bt rice, resistance management.

INTRODUCTION

The striped stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), is the most abundant stem borer of rice *Oryza sativa* L. (Poaeceae) in temperate Asia. *Chilo suppressalis* also occurs throughout the tropical rice-growing areas of Asia, where it is generally second in abundance to the yellow stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae) (Pathak & Khan, 1994). Stem borers are the primary target pests for control by rice varieties transformed with toxin genes from *Bacillus thuringiensis* Berliner (Bacillaceae) (Bt). Bt rice is under development in several countries but has not yet been released to farmers (Cohen *et al*., 2000; Ye *et al*., 2001).

The need to implement resistance management strategies to delay the development of pest resistance to Bt toxins in transgenic crops is widely recognized (Gould, 1998; EPA-USDA, 1999; Cohen *et al*., 2000). The most promising strategy entails the use of plants with a high dose of toxin in combination with the maintenance of "refuges," i.e. non-Bt plants that serve to maintain Bt-susceptible insects in the pest population (Gould, 1998). Refuges can consist of non-Bt cultivars of the same species as the Bt crop as well as, for polyphagous pests, alternative host species. Refuges consisting of alternative hosts are an attractive option for Bt rice because it will be difficult to enforce the maintenance of refuges of non-Bt rice by small, low-income farmers.

In contrast to *S. incertulas*, which is restricted to *Oryza* spp., *C. suppressalis* is considered to be polyphagous. The bibliography of Khan *et al*. (1991) reports 41 species of host plants of *C. suppressalis* in six families, primarily wild and cultivated Poaceae. This information suggests that non-rice hosts might serve as refuges for *C. suppressalis*. However, a critical review of the literature reveals that this host list is based on a very limited amount of experimental or field survey data (see Discussion). The objective of

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this study was to survey the abundance of *C. suppressalis* in five grass species that are common in and around rice fields in tropical and subtropical Asia (Soerjani *et al*., 1987) and are listed as alternative hosts of *C. suppressalis* by Khan *et al*. (1991), and to evaluate the suitability of these grasses as hosts of *C. suppressalis* under greenhouse conditions.

MATERIALS AND METHODS

Field survey

Field samples of five weedy grasses, *Echinochloa crus-galli* (L.) P. Beauv. ssp. *hispidula* (Retz.) Honda, *Echinochloa colona* (L.) Link, *Eleusine indica* (L.) Gaertn., *Ischaemum rugosum* Salisb., and *Panicum repens* L. (Poaeceae) were collected during the first week of each month from July 1999 to August 2000, with the exception of December 1999 and January and February 2000. These three months are the coolest of the year in the Philippines and stem borer populations are low. Most plants were collected in and around rice fields of the International Rice Research Institute Experiment Station, Los Baños, Laguna, Philippines. *Chilo suppressalis* routinely infests rice in this area. During months when sufficient numbers of particular plant species were not available at the research station, collections were also made in and around rice fields within a 20 km radius. Depending on the abundance of the plants, 20 or more plants of each species were collected from 3-9 fields. On each sample date, both vegetative and reproductive tillers were collected. The plants were dissected to detect stem or leaf sheath infestation by lepidopterous stem borers. Stem borer larvae or pupae were examined to determine if they were *C. suppressalis.*

Greenhouse experiments

Chilo suppressalis larvae used in the greenhouse experiments were obtained from egg masses laid by moths collected in Laguna Province, Philippines.

For assays using cut stems, pieces ca. 7 cm in length were cut from vegetative tillers of field-collected plants of the five weedy grasses and from greenhouse-grown plants of rice line IR68011-15-1-1 (a breeding line moderately susceptible to *C. suppressalis*; hereafter referred to as IR68011). The stem pieces were placed singly in 500-ml plastic cups lined with moistened filter paper. There were ten replicates of each species. Ten neonate *C. suppressalis* larvae were released per cup. The cups were covered and arranged in a completely randomized design in an air-conditioned laboratory where the temperature ranged from approximately 22 to 30°C and relative humidity from 70 to 100%. The numbers of dead, live, and unrecovered larvae were recorded four days after infestation (DAI). The experiment was conducted twice (August 1999 and June 2000).

For whole plant assays, seedlings or stem cuttings of the five wild species were collected from fields within the IRRI experiment station and transplanted into pots. Seedlings of rice line IR68011 were also transplanted into pots. The pots were arranged in a completely randomized design in a greenhouse under natural temperature, humidity, and lighting conditions. At flowering stage, the plants were infested with five larvae per tiller and covered with clear plastic cages with nylon mesh tops and side windows. In trial 1 (October 1999) there were eight pots of each species; in trial 2 (April 2000) there were ten. In both trials, half the pots were dissected 14 days after infestation and half after 25 days. The numbers of dead, alive and unrecovered larvae and the insect instar or growth stage were recorded.

Larval survival was calculated as the number of larvae recovered alive divided by the number of larvae used to infest each replicate; these values were arcsin-square root transformed prior to analysis. Data were analyzed using the PROC GLM procedure of the SAS package (SAS Institute, 1989).

RESULTS

Several thousand tillers of each of the five weedy grasses were dissected over a 13-month period (table 1). Only four *C. suppressalis* larvae or pupae were found: three on *E. crus-galli* and one on *E. colona*. In all four cases, the *C. suppressalis*-infested plants were collected from within rice fields at the ripening stage and it was possible that the larvae had moved from rice to the *Echinochloa* plants as the rice plants were reaching maturity.

The only common stem borer found on any of the weedy grasses was *Enosima* sp. (Lepidoptera: Pyralidae), which was frequently collected from *Echinochloa* (60-70 specimens from each species) and occasionally from *P. repens* (5 specimens). The pink stem borer, *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae), a minor pest of rice, was frequently collected from *E. crus-galli* (63 specimens) and occasionally from *E. colona*, *E. indica*, and *I. rugosum* (4-5 specimens each).

There was relatively little difference among the six plant species in *C. suppressalis* larval survival after four days on stem pieces (table 2). In both trials, survival was numerically highest on rice and lowest on *P. repens*. In the whole plant assays, survival was significantly higher on rice than on the other species at both 14 and 25 days after infestation (table 3). Survival was low on all the weedy grasses, ranging from 1 to 17% after 14 days and 1 to 4% after 25 days. *Chilo suppressalis* reached the final (fifth) larval instar or pupation on rice after 25 days. All *C. suppressalis* recovered on the weedy grasses after 25 days were third instars, with the exception of four pupae found on *E. indica*.

Table 1. Field survey of five weedy grasses as alternative hosts of *Chilo suppressalis*.

Year	Month	No. of tillers dissected				
		Echinochloa colona	*Echinochloa crus-galli*	*Eleusine indica*	*Ischaemum rugosum*	*Panicum repens*
1999	July	799	191	264	242	898
	August	875	217	175	81	152
	September	998	387	436	410	783
	October	423	191	136	680	711
	November	242	336	260	183	492
2000	March	240	702	559	959	265
	April	2901	1367	1121	931	1046
	May	2321	1065	1601	656	649
	June	2091	1035	779	645	499
	July	2654	1607	843	991	545
	August	1795	1946	685	928	208
Total		15339	9044	6859	6706	6248

Table 2. Percent larval survival of *Chilo suppressalis* four days after infestation on six plant species in cut stem assays.[1]

Plant species	Trial 1	Trial 2
Echinochloa colona	88.0 ± 3.1ab	68.3 ± 9.6bc
Echinochloa crus-galli	87.8 ± 4.2ab	77.3 ± 6.0abc
Eleusine indica	75.0 ± 4.0bc	88.8 ± 3.3ab
Ischaemum rugosum	66.5 ± 8.7c	76.3 ± 7.9abc
Panicum repens	48.6 ± 8.7d	64.8 ± 9.9c
Oryza sativa	92.3 ± 3.1a	92.5 ± 2.3a

[1] Mean ± SE, n=10. Means within a column sharing the same letter are not significantly different at $P_{0.05}$, LSD test.

Table 3. Percent larval survival of *Chilo suppressalis* 14 and 25 days after infestation on six plant species in whole plant assays.[1]

Plant species	Trial 1		Trial 2	
	14 days	25 days	14 days	25 days
Echinochloa colona	1.4 ± 0.6c	0.8 ± 0.2b	13.1 ± 3.1b	1.4 ± 0.2b
Echinochloa crus-galli	10.3 ± 3.8b	1.7 ± 0.5b	11.2 ± 2.8b	4.3 ± 1.6b
Eleusine indica	4.8 ± 2.2bc	6.2 ± 2.6b	17.1 ± 3.5b	3.5 ± 0.4b
Ischaemum rugosum	9.0 ± 1.3b	0.5 ± 0.2b	11.4 ± 1.3b	0.6 ± 0.1b
Panicum repens	0.7 ± 0.1c	0.5 ± 0.1b	1.7 ± 0.4c	1.0 ± 0.1b
Oryza sativa	75.0 ± 9.2a	26.6 ± 10.0a	89.2 ± 3.1a	66.5 ± 6.9a

[1] Mean ± SE, n=4 (trial 1) or 5 (trial 2). Means within a column sharing the same letter are not significantly different at $P_{0.05}$, LSD test.

DISCUSSION

The results of the greenhouse studies demonstrate that the five weedy grasses examined are poor hosts for *C. suppressalis*, and the survey results indicate that *C. suppressalis* generally does not use these hosts in the field. These results are similar to those of Rothschild (1971), who found no *Chilo* larvae in large samples of grasses and sedges collected near rice fields infested with *C. suppressalis* in Sarawak, Malaysia.

Chilo suppressalis is considered to be polyphagous, but there are few published experiments or quantitative field surveys to support this conclusion. Cuong (2001) provided a critical review of most of the 33 references concerning alternative hosts cited in the bibliography of Khan *et al*. (1991). Six references were not available, all of which were published prior to 1922 and concern observations from India. Twelve references provided host lists compiled from earlier papers and contained no original data on host range. Four references were incorrectly cited in Khan *et al*. (1991) and/or earlier review articles, e.g. the references reported data on species other than *C. suppressalis*. Two papers reported results from greenhouse experiments. Nine papers reported original field observations of *C. suppressalis* feeding on non-rice hosts, but none provided quantitative data. Several of these papers described unusual events, such as three dicotyledenous species adjacent to rice fields that were found infested during a *C. suppressalis* outbreak (Kiritani & Oho, 1962).

There are numerous reports in the older literature of cultivated grasses, including maize, sugarcane, sorghum, and millet, as hosts of *C. suppressalis* (Khan *et al*., 1991). However, a search of the electronic version of the Entomology Abstracts (National Information Services Corporation, Baltimore, Maryland, USA) covering the period from 1978 to August 2001 found only one paper, a review (Neupane, 1990), reporting *C. suppressalis* as a pest of any of these four crops. This suggests that *C. suppressalis* occurs in these crops only at low levels, if at all.

Two wild *Oryza* species are abundant in some rice-growing areas of Asia, including southern Vietnam and eastern India: *O. rufipogon* Griff. and *O. nivara* Sharma et Shastry (both close relatives of *O. sativa*) (Vaughan, 1994). Surprisingly, these two wild rices are not listed as hosts of *C. suppressalis* in Khan et al. (1991). However, both species can support *C. suppressalis* development under greenhouse conditions (Romena & Heinrichs, 1989) and it is probable that small numbers of *C. suppressalis* occur on *O. rufipogon* and *O. nivara* in the field.

It is likely that many of the non-rice host records for *C. suppressalis* are attributable to misidentification of other species of *Chilo*, and to cases in which *C. suppressalis* larvae had dispersed from rice plants of declining quality to nearby plants of other species. The only plant in addition to *O. sativa* that is clearly a regular host of *C. suppressalis* is *Zizania latifolia* (Griseb.) Turcz. ex Stapf (Poaeceae).

One paper cited by Khan *et al*. (1991) (Hachiya, 1981), as well as several more recent papers, report field data on *Z. latifolia* as a host of *C. suppressalis* in China and Japan. In some temperate rice-growing areas of these countries, *Z. latifolia* is an abundant wild plant and is also grown as a vegetable. This species may provide an important non-Bt refuge for *C. suppressalis*. However, there is evidence of reproductive isolation between *C. suppressalis* from rice and *Z. latifolia* (Konno & Tanaka, 1996), and this question should be further investigated.

Farmers in the USA who grow Bt corn or cotton must plant a refuge of approximately 4-20% of their land to non-Bt cultivars, to serve as a source of Bt-susceptible insect pests (EPA-USDA, 1999). Based on the literature review and experimental results reported in the present study, it appears unlikely that alternative wild or cultivated hosts in tropical or subtropical rice-growing areas will support sufficient numbers of *C. suppressalis* to serve as refuges for Bt rice. Therefore, fields of non-Bt rice cultivars will have to be maintained as refuges for resistance management of *C. suppressalis* and the monophagous *S. incertulas*. Cohen *et al*. (2000) suggest policies that can be implemented to help maintain sufficient areas of non-Bt rice after Bt rice cultivars are released.

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SUMMARY IN VIETNAMESE

Nghiên cứu ký chủ phụ của sâu đục thân sọc nâu sử dụng như là vùng trú ẩn cho chiến lược quản lý tính kháng của sâu đối với giống lúa chuyển nạp gen Bt

Một trong những chiến lược triển vọng nhằm quản lý tính kháng của sâu đối với giống lúa chuyển nạp gen Bt là biện pháp kết hợp việc sử dụng nồng độ cao và vùng trú ẩn (high dose/refuge strategy). Vùng trú ẩn (refuge) được định nghĩa là vùng cây trồng không chứa gen Bt nhằm để duy trì nguồn sâu nhiễm Bt trong quần thể sâu hại. Vùng trú ẩn có thể bao gồm cả những cây trồng cùng loài với cây chuyển nạp gen hoặc là những cây ký chủ phụ đối với loài sâu đa tạp. Vùng trú ẩn bao gồm phổ ký chủ phụ của sâu đục thân được xem như là một biện pháp triển vọng đối với giống lúa Bt bởi vì sẽ rất khó khuyến cáo những nông dân có diện tich nhỏ, thu nhập thấp duy trì ruộng lúa nhiễm sâu như là một vùng trú ẩn trong biện pháp quản lý tính kháng của sâu hại. Kết quả đánh giá khả năng sống sót và phát triển của sâu đục thân trên năm loại cỏ hoà bản: *Echinochloa crus-galli* (L.), *E. colona* (L.), *Eleusine indica* (L.) Gaerth, *Ischaemum rugosum* Salisb., và *Panicum repens* (L.), cho thấy rất có ít sâu non và nhộng của sâu đục thân được tìm thấy trên hai loại cỏ lồng vực: *E. crus-galli* and *E. colona* qua thí nghiệm điều tra ngoài đồng. Kết quả thí nghiệm lây nhiễm trên đoạn thân cho thấy sâu non có thể sống sót trên cả 5 loại cỏ cho đến 4 ngày sau khi lây nhiễm. Nhưng cho thấy tỷ lệ chết cao và kéo dài thời gian sinh trưởng của sâu trong thí nghiệm toàn cây trong điều kiện nhà lưới. Điều này chứng tỏ rằng những loại cỏ này không cung cấp đủ mật số sâu cần thiết để được xem như là vùng trú ẩn (refuge) trong biện pháp nồng độ cao kết hợp với vùng trú ẩn (high dose/refuge strategy) trong chiến lược quản lý tính kháng của sâu đục thân.