Journal of Asia-Pacific Entomology 17 (2014) 595–600

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Differences in Rice stripe virus transmission abilities of *Laodelphax striatellus* (Homoptera: Delphacidae) from four geographical populations

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ARTICLE INFO

Article history:
Received 19 January 2014
Revised 9 March 2014
Accepted 4 June 2014
Available online 17 June 2014

Keywords:
Laodelphax striatellus
Rice stripe virus (RSV)
RSV transmission ability
Geographical population
Horizontal transmission
Vertical transmission

ABSTRACT

The small brown planthopper, *Laodelphax striatellus* (Fallén), can transfer Rice stripe virus (RSV) to host plants, which then develop rice stripe disease. Between vectors, there are two paths for RSV transmission. In current study, we examined the horizontal, vertical and compound transmission rates (horizontal and vertical transmissions together) by *L. striatellus* from one non-epidemic area (Fuyang in Zhejiang province) and three epidemic areas (Yizheng and Peixian in Jiangsu province, and Donggang in Liaoning province). RSV acquisition rates for naïve *L. striatellus* from the four populations were not significantly different. RSV transmission rate to healthy rice plants by viruliferous *L. striatellus* from Fuyang population was relatively lower than those of the other three populations. For example, RSV transmission rate in Fuyang population decreased by 1 fold compared to that in Peixian population when the transmission times were 48 and 72 h. It indicated that horizontal transmission ability of Fuyang population was lower. Vertical transmission rate and the compound transmission abilities of infective *L. striatellus* in the first generation did not differ significantly among the four populations. However, the ratio of RSV-positive offspring of an infective mother in the fourth generation of Fuyang population (84.3 ± 2.4%) was lowest, and decreased by 10% compared to that of Peixian population. It meant that compound transmission ability of Fuyang population was significantly lower than the other three populations. The reason for the difference in transmission abilities of *L. striatellus* from different populations was discussed.

Introduction

The small brown planthopper (SBPH), *Laodelphax striatellus* (Fallén) (Homoptera: Delphacidae), is one of the most destructive insect pests on rice in Asia (Wei et al., 2004; Gu et al., 2005; Sanada-Morimura et al., 2011). *L. striatellus* causes damage to rice plants by sucking their sap, especially at high density. Moreover, *L. striatellus* can transfer the Rice stripe virus (RSV) to host plants, which then develop rice stripe disease, even when the vector occurs at a much lower density in rice-growing areas (Shinkai, 1962; Hibino, 1996). Rice stripe disease is a serious rice disease in subtropical and temperate regions and is distributed throughout most parts of Japan, Southern Korea, the ex-USSR and China (Toriyama, 1986; Lin et al., 1991). In China, losses of 20–30% have been commonly observed in regions where *Oryza sativa* var. *japonica* is grown. Severe crop losses caused by rice stripe disease in 2001, 2003, 2004, and 2006 in Jiangsu, Zhejiang, Shandong, Anhui and Henan provinces have been reported (Zhou et al., 2004; Sang et al., 2006; Zhang et al., 2007; Wang et al., 2008).

RSV is a typical member of the genus *Tenuivirus* (Ramirez and Haenni, 1994; Toriyama and Tomaru, 1995) and is transmitted in a circulative/persistent manner by *L. striatellus* (Toriyama, 1986). There are two paths for virus transmission between vectors. One path is vertical transmission from infected parents (transovarial transmission), and the other path is horizontal transmission by sucking into infected plants (Yamamura, 1998).

L. striatellus is able to overwinter as diapause nymphs in various habitats, including wheat and barley fields and gramineous weeds (Gingery, 1988; Yamamura, 1998; Syobu et al., 2011), although it has long-distance dispersal ability (Noda, 1986; Otuka et al., 2010; Sanada-Morimura et al., 2011; Syobu et al., 2011). Hoshizaki (1997) suggested that the long-distance dispersal did not have a large effect on the genetic structure of the *L. striatellus* population. The geographic differences among 11 *L. striatellus* populations from Japan and Taiwan were observed using allozyme polymorphism. Random amplified polymorphic DNA (RAPD) markers were used to analyze *L. striatellus* polymorphisms from different areas of China and Japan. The results indicated that there were genetic differences among the different geographical populations (Wan et al., 2001; Xu et al., 2001). Few studies have been conducted to investigate the effect of geographical populations on the virus transmission by *L. striatellus*.

The epidemic and outbreak of rice stripe disease have close relationship with the outbreak of viruliferous populations of SBPH (Deng et al., 2013). A preliminary study showed that RSV transmission rates of

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http://dx.doi.org/10.1016/j.aspen.2014.06.001

viruliferous *L. striatellus* from two heavy occurrence areas, Changxing and Huzhou, were significantly higher than that from Nanhu, a less seriously-infested area in Zhejiang province. The result indicated that high RSV transmission rate would lead to high incidence of rice stripe disease (Sun et al., 2008). The role of RSV transmission rate in the occurrence and prevalence of rice stripe disease needs to be further studied.

To explore the possible reason for rice stripe disease prevalence, we examined the RSV transmitting abilities of *L. striatellus* from four regions in China. One region was the non-epidemic area, Fuyang in Zhejiang province. The other three regions were the epidemic areas, Yizheng and Peixian in Jiangsu province, and Donggang in Liaoning province.

Materials and methods

Rice variety and culture

The rice variety used in the experiment was *japonica* rice Wuyujing No. 3 (which was previously identified as a variety susceptible to *L. striatellus*) (F. Liu et al., 2007a). In virus acquisition rate evaluation, the plants were sown in batches in cement tanks (60 × 100 × 200 cm). After 2 weeks, 10 seedlings of similar sizes were transplanted into a plastic bucket (with a height of 13 cm and a diameter of 11 cm) and plants with two leaves were used for the experiment. In the experiments for evaluating the virus transmission rate involved in horizontal viral transmission, vertical viral transmission rate and compound transmission rate, the plants were sown and grown in soil in a plastic cup (with a height of 8 cm and a diameter of 5 cm) and 20 day old rice seedlings were used for the experiment.

Insect rearing

The *L. striatellus* used in this study were collected from 4 different regions in China (Table 1). Yizheng, Peixian and Donggang populations were from epidemic areas (Sang et al., 2006; Wang, 2006). These three populations were chosen because they were from different rice cultivation regions, and were geographically isolated from each other. Fuyang population was from non-epidemic area. Each population was reared separately at 25 ± 1 °C, 60 – 80% RH, and 14:10 h (L:D) photocycle. The plants were grown in a plastic cup (with a height of 8 cm and a diameter of 5 cm) and were covered with a transparent cylindrical plastic cage (with a height of 50 cm and a diameter of 6 cm), which was enclosed with nylon mesh after the insects had been introduced onto the 2–3 cm tall rice seedlings. *L. striatellus* were transferred to fresh seedlings every 10–14 days to assure sufficient nutrition. Each population would be re-collected if it was reproduced more than 4 generations in the laboratory.

Screenings of viruliferous (RSV-infected) and naïve (noninfected) L. striatellus

Viruliferous (RSV-infected) and naïve (noninfected) *L. striatellus* were screened from field populations with the method described by H.J. Liu et al. (2007b). Within 24 h after emergence, a pair of *L. striatellus* was kept on the plant in a plastic cup for 2 days to ensure that the female mated. The mated female was subsequently numbered and raised individually in a plastic cup containing 2–3 cm tall rice seedlings to lay eggs in the plant. After the female died, each female was collected for RSV detection by the dot immunobinding assay (DIBA) described by Zhou et al. (2004). If a mother was naïve, its progeny was considered naïve and was used as naïve insects for the experiments. If a mother was viruliferous, its progeny was considered viruliferous. The offspring of viruliferous *L. striatellus* were then reared separately. Viruliferous individuals used in the experiment were selected from viruliferous colonies.

Table 1
Population information of tested *Laodelphax striatellus*.

Populations	Location	Lat/Long	Cultivation regions
Fuyang	Fuyang, Zhejiang, China	N 30°03′, E 119°57′	Cultivation region for double-cropping rice in Central China
Yizheng	Yizheng, Jiangsu, China	N 32°23′, E 119°26′	Cultivation region for double-cropping rice in Central China
Peixian	Peixian, Jiangsu, China	N 34°15′, E 117°11′	Cultivation region for single-cropping rice in North China
Donggang	Donggang, Liaoning, China	N 40°15′, E 124°22′	Cultivation region for early single-cropping rice in Northeast China

Experimental protocols

Three sets of experiments were performed to investigate the viral transmission abilities of *L. striatellus* from four geographical populations (see Table 1). Experiment 1 investigated the horizontal viral transmission rate of *L. striatellus*. The ability of naïve *L. striatellus* to acquire RSV from infected rice leaves and the ability of viruliferous *L. striatellus* to transmit the virus to healthy rice plants were measured. Experiment 2 evaluated the vertical transmission rate of *L. striatellus* from four populations and concerned transovarial transmission abilities. Experiment 3 studied the compound transmission rate of *L. striatellus* from four populations, which involved in both horizontal and vertical viral transmission rates.

Experiment 1. An evaluation of the horizontal viral transmission rates of L. striatellus from four populations

The horizontal viral transmission included two courses: the viral acquisition of naïve *L. striatellus* from infected rice leaves and the viral transmission by viruliferous *L. striatellus* to healthy rice plants. The transmission abilities involved in the different courses were evaluated separately.

An evaluation of naïve L. striatellus virus acquisition rate from infected rice leaves

A field population of *L. striatellus* from Yangzhou, Jiangsu province, China was placed into a plastic bucket that contained 10 healthy rice plants for 2 days to prepare the RSV-infected plants. *L. striatellus* were then removed from the experimental set-up. The plants were maintained under natural conditions until rice stripe disease occurred. Each bucket was thinned to 5 plants that showed the typical stripe symptoms.

For each population, 15 naïve *L. striatellus* nymphs (third–fourth instars) that had been starved for 5–6 h were placed into a bucket containing 5 plants showing the typical stripe symptoms to acquire the virus. Virus acquisition time lasted 4, 8, 12, 24, 48 and 72 h, respectively. *L. striatellus* nymphs were moved out for RSV detection after virus acquisition; a 7-day test-feeding period followed to pass the virus through a circulative period. The nymphs were then individually subjected to detect RSV by the DIBA method (Zhou et al., 2004). The RSV acquisition rate was defined as the ratio of viruliferous *L. striatellus* to the total number of tested *L. striatellus* (15). Every 15 naïve *L. striatellus* nymphs were placed into a bucket and considered a replication. Each treatment had three replications.

An evaluation of viruliferous L. striatellus's ability to transmit RSV to healthy rice plants

Each plastic cup containing approximately 5 healthy rice plants, each with two leaves, was thinned to 2 plants. For each *L. striatellus* population, a single *L. striatellus* nymph from viruliferous offspring (third–fourth instars) was placed into the cup that contained healthy plants for RSV transmission. Viral transmission time lasted 4, 8, 12, 24, 48 and 72 h, respectively. *L. striatellus* nymphs were removed after viral