Journal of Asia-Pacific Entomology 20 (2017) 1145–1149

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

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

Short Communication

Prevalence of rice stripe virus can be altered by temperature and the virus-mediated development of insect vector, *Laodelphax striatellus*, in Korea

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ARTICLE INFO

Keywords:
Spring temperature
Geographical shift
Developmental time
Transmission time
Epidemic model

ABSTRACT

Since the 2000's in Korea, the regional hotspots of rice stripe virus (genus *Tenuivirus*, RSV), transmitted by small brown planthopper (*Laodelphax striatellus* (Fallén), SBPH), have changed from the southeastern to the western coastal regions. The reasons for this are as yet unknown, but recent changes in temperatures in spring, the time at which the vectors migrate from overwintering sites to rice fields, are thought to be an important factor. In this study, we investigated the differences in the development of RSV-infected and RSV-free SBPH populations and further investigated how spring temperatures affect RSV transmission to rice (*Oryza sativa*). Two temperature conditions (15 and 20 °C) were chosen based on the average temperature in April–May and May–June in Korea, respectively, at which the SBPHs begin to migrate from Gramineae weeds to rice during the spring season. The nymphal developmental period in the RSV-infected SBPH was significantly shorter than that in the RSV-free SBPH. The body weight of males was significantly higher in the RSV-infected SBPH than in the RSV-free SBPH at early nymphal stage (1st day of 5th instar). The transmission of RSV to the rice seedlings was higher at 20 °C than at 15 °C. Rapid nymphal development of the RSV-infected SBPH with higher RSV transmission rate at high temperature is considered to be related to the geographical shift of RSV hotspots from the southeastern to the western coastal regions of Korea.

Introduction

Rice stripe virus (*Tenuivirus*, RSV) causes a devastating disease in rice fields in many East Asian countries, including Korea (Chung, 1974; Otuka et al., 2010; Lian et al., 2011). RSV is transmitted to rice by a small brown planthopper (*Laodelphax striatellus* (Fallén), SBPH) through a persistent, circulative-propagative manner (Falk and Tsai, 1998).Typical symptoms of RSV infection are chlorotic stripes or necrotic streaks on leaves and weakness in newly emerged leaves. The plant becomes considerably stunted when infected at the early growth stages (Ou, 1972).It is reported that virus infection at an early stage of rice plants caused 70–80% grain yield losses in Korea and China (Chung, 1974; Qu et al., 1997; Wei et al., 2009). These outbreaks might be due to several reasons such as the monoculture of the virus-susceptible rice cultivars, higher winter temperature, early rice planting, and mass migration of the vector populations from China to Korea (Park et al., 2009; Otuka et al., 2010). These, in turn, may result in changes in the spatial and temporal patterns of RSV outbreaks in response to the different physiological responses of SBPH populations to regional environmental factors.

Until the 1980's, in Korea, the outbreak hotspot areas of RSV had been restricted to the southeastern regions, but in recent decades, the outbreak hotspots have shifted to the western coastal regions. Several attempts have been made to describe why and how these geographical shifts of RSV disease occurred in Korea (Kim, 2002; Kim et al., 2009), but they were not successful because of lack of RSV diagnostic tools (Chung, 1974; Kim et al., 1987) or limited understanding of the RSV-SBPH interaction based on the local environmental conditions (Park et al., 2009; Kang et al., 2010).

Yamamura and Yokozawa (2002) reported out that the complex interaction among three biological entities—the rice plant, RSV, and vector—can determine the geographical occurrence and prevalence of this disease, which suggests that the degree of synchronization of SBPH with the cultivation of rice plants might be the reason for the

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http://dx.doi.org/10.1016/j.aspen.2017.08.012
Received 16 May 2017; Received in revised form 8 August 2017; Accepted 18 August 2017
Available online 18 August 2017

geographical shift of the RSV occurrence. Given the importance of RSV infection at the early growth stages and synchronization of SBPH with its host plant in RSV occurrence and prevalence, changes in spring temperatures at which the vectors migrate from overwintering sites to rice fields may be a major factor determining RSV outbreak. However, to date, no RSV disease models are available that predict the geographical outbreaks based on temperatures of the spring seasons when the overwintered SBPH populations migrate into the rice paddy fields in Korea. Therefore, given the necessity of incorporating regional environmental factors to understand and forecast the recent geographical shift of RSV outbreaks in Korea, there is a need to improve our understanding of how spring temperatures in Korea affect the development of the RSV-infected SBPH and its transmission to host plants.

In this study, we investigated the differences in the life history traits between the RSV-infected and the RSV-free SBPHs, and a rice seedling test was conducted to investigate how temperature affects the transmission rate of RSV-infected and RSV-free SBPHs to their host plants (*Oryza sativa*) at 15 and 20 °C, representing average temperature in the early (April–May) and the late spring (May–June), respectively, at which the SBPH populations change their hosts from Gramineae weeds or barley to rice plants. Based on the findings of this study, we discussed the factors that caused a recent geographical shift of RSV hotspots from the southeastern to the western coastal regions of Korea.

Materials and methods

Plant, virus, and insect

The RSV isolates and SBPH populations were collected from the paddy fields (Buan 35°43.959′ N, 126°44.536′ E and Seocheon 36°4.820′ N, 126°41.010′ E, respectively) in the western coastal region of Korea in 2008, and maintained on 5–7 leaf stage seedlings of the susceptible rice cultivar Chucheong-byeo in a greenhouse compartment by repeatedly introducing SBPH populations. The RSV infection in rice plants was identified by means of typical symptoms of chlorotic stripes and necrotic streaks on the leaves, as described by Iida (1969).

A 4–5 leaf stage seedling of the rice plant was used for all the bioassays. The RSV-infected and virus-free SBPH populations were maintained separately in the rearing chambers (Sanyo, Japan) at 23 ± 1 °C with a 16-h light/8-h dark photoperiod. The RSV-infected SBPH adults were isolated from the RSV-infected rice plants and mated among themselves for more than two generations before being used in the transmission assays.

Effects of RSV-infection on the life history traits of SBPH

To assess the effects of RSV-infection on SBPH populations, the development and life history differences between the RSV-infected and the RSV-free SBPH populations were determined and compared at 20 ± 1 °C in a growth chamber with a 16-h light/8-h dark photoperiod. Life history parameters investigated were development, fecundity, longevity, and sex ratio. Female adults from the virus-free and RSV-infected SBPH populations were inoculated individually on the rice seedlings (3–4 leaf stage) in test tubes (26 × 200 mm) and allowed to lay eggs for two days. A total of 45 individuals for the RSV-infected SBPHs and 54 for the virus-free SBPHs were tested. After removing all the adults from the seedlings, the eggs on the seedlings were maintained over 10 days at the same conditions. As soon as the eggs were hatched, individual nymph was transferred to a healthy seedling and reared until death. The insects were checked on a daily schedule.

The nymphal period and female oviposition periods in days were determined and compared between the RSV-infected and RSV-free SBPH populations. The oviposition period was calculated by averaging from the first to the last oviposition days. The longevity was determined by sex. Total fecundity was estimated as the numbers of nymphs found on the seedling because of the difficulty in isolating and counting inserted eggs in the midrib of the leaf sheath. The sex was determined on the 2nd day of the adult period, and the sex ratio was expressed as a proportion of females in the individuals studied. The body weights (10^{-4} g/individual) of the insects were compared according to the sex and virus infection at the 1st and 3rd days of the 5th nymphal stage, and the 2nd day of the adults.

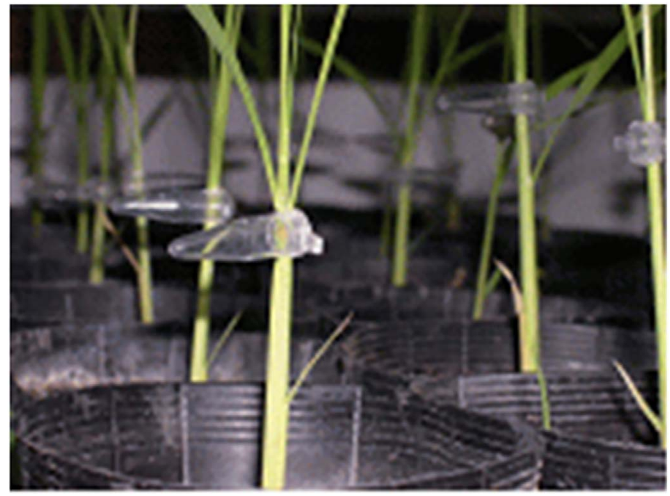

Fig. 1. Inoculation of small brown planthopper using an e-tube (0.5 ml in volume) onto the node of 4–5 leaf stage of rice seedling. The upper and lower body of the e-tube at the lid was grooved, and then the lid was closed and fixed onto the rice seedling.

Effects of temperature and viral propagation period on RSV transmission to rice plants

A rice seedling assay was conducted to investigate the effects of temperature and virus propagation periods on RSV transmission by SBPH at15 and 20 °C with a 16-h light/8-h dark photoperiod. A 4–5 leaf stage rice seedling was used throughout the assays. A single female adult was confined at the RSV-infected rice node with a 0.5 ml e-tube (Fig. 1).This e-tube confining method of SBPH individual can increase virus acquisition without limiting the behavior of the vector. at 15 and 20 °C in separate incubators with a photoperiod of 16 h:8 h (L:D), respectively.

In each temperature treatment, RSV-free female adults were fed on the RSV-infected seedlings for 1 h (acquisition period), and then were incubated at 15 and 20 °C in a separate incubator with a 16-h light/8-h dark photoperiod for 0, 0.5, 1, 3, 6, 9, 12, 24, 48, and 72 h (virus propagation period) to allow the RSV to proliferate in the infected SBPH individuals. After each propagation period, each individual adult was transferred to the healthy seedlings individually, and allowed to feed on the rice seedling for 1 h. After removing the individual adults, each seedling was maintained at the same environmental conditions for two weeks. The seedlings and the vectors were stored at −70 °C until the RSV diagnosis. Ten plants were tested for each propagation period. The whole experiment was replicated twice at different dates.

A reverse transcriptase polymerase chain reaction (RT-PCR) method was used to confirm the presence of RSV in SBPHs and seedlings, and the DAS-ELISA method was used to detect the presence of RSV in seedlings in case of mass sample being available (Clark and Adams, 1977). RSV diagnosis in SBPHs and rice plants was conducted using a reverse transcriptase polymerase chain reaction (RT-PCR) method. Total RNA from the short cut (3–4 cm) of RSV-infected rice leaf and one individual SBPH was extracted using the TRI reagent (Sigma-Aldrich, USA) according to the manufacturer's instruction. The following primers used to detect viruses by RT-PCR were derived from the coat protein (CP) gene sequences in RNA3 aligned from eight strains from China and Japan published in GenBank (Kakutani et al., 1991; Zhu