

## Ecological Fitness of Non-vector Planthopper *Sogatella furcifera* on Rice Plants Infected with Rice Black Streaked Dwarf Virus

HE Xiao-chan<sup>1,2</sup>, XU Hong-xing<sup>1</sup>, ZHENG Xu-song<sup>1</sup>, YANG Ya-jun<sup>1</sup>, GAO Guang-chun<sup>1</sup>, PAN Jian-hong<sup>1</sup>, LU Zhong-xian<sup>1</sup>

<sup>1</sup>*Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China;*  
<sup>2</sup>*Department of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China)*

**Abstract:** Rice black streak dwarf virus (RBSDV) transmitted by the small brown planthopper (SBPH), *Laodelphax striatellus* (Fallen), causes great rice yield loss in east China. White-backed planthopper (WBPH), *Sogatella furcifera* (Horvath), a non-vector of RBSDV, usually shares its host plants with SBPH. We evaluated the effects of RBSDV-infested rice plants on the ecological parameters and its relevant defensive and detoxification enzymes of WBPH in laboratory for exploring the relationship between RBSDV and non-vector insect. The results showed that nymph survival rate, female adult weight and fecundity, and egg hatchability of WBPH fed on RBSDV infested rice plants did not markedly differ from those on healthy plants, while the adult longevity and egg duration significantly shortened on diseased plants. Furthermore, significant higher activities of defensive enzymes (dismutase, catalase and peroxidase) and detoxification enzymes (acetylcholinesterase, carboxylesterase and glutathione S-transferase) were found in WBPH adults fed on infected plants. Results implied that infestation by RBSDV increased the ecological fitness of non-vector planthopper population.

**Key words:** ecological fitness; rice black streaked dwarf virus; non-vector; white-backed planthopper

Rice black streak dwarf virus (RBSDV) transmitted by the small brown planthopper (SBPH), *Laodelphax striatellus* (Fallen), was found fragmentarily in rice fields in east China in the early of 1960's, and occurred frequently during the 1990's in Zhejiang, and damaged increasingly and caused greatly yield loss in Zhejiang, Jiangsu and Shandong Provinces in east China since this century (Chen and Zhang, 2005), especially in 2008. More than 267, 000 hectare of rice fields were infested by RBSDV in Jiangsu Province (Ji et al, 2009). Furthermore, the maize rough dwarf disease and dwarfing disease occurred in cereal crops and gramineae weeds have been predicatively attributed to RBSDV (Fang et al, 2001; Bai et al, 2002).

Ecological interactions between vector-insect, virus and host plants were complex in the process of evolution (Sisterson, 2008; Froissart et al, 2010; Hodge et al, 2010; Mauck et al, 2010), and it had been demonstrated that virus had a positive or negative influence on the increase of vector insect population by direct or indirect effects (Belliere et al, 2005; Whitfield et al, 2005; Colvin et al, 2006; Stout et al, 2006; Luan and Liu, 2010). Vector insects carried with virus could prolong their longevities and improve their fecundities (Belliere et al, 2005; Stout et al, 2006). In contrast, some negative effects were reported on the vectors which reduced adult longevities and reproductive abilities compared to those maintained on virus-free plants (Rubinstein and Czosnek, 1997; Jiménez-Martínez et al, 2004; Maris et al, 2004; Sinisterra et al, 2005). However, the potential effects of plant virus on the non-vectors and their ecological and physiological mechanisms are still unclear.

White-backed planthopper (WBPH) *Sogatella fucifera* (Horvath), a non-vector of RBSDV, always shared its host rice plants with SBPH in east China, and became one of economically important insect pests since high yield hybrid rice being released widely in China (Cheng, 2009), and was strongly exacerbated by misuse of pesticides (George et al, 2010). In this paper, we explore the ecological fitness of WBPH fed on rice plant infected with RBSDV for optimizing the practical strategies to effectively manage planthopper populations and control spread of RBSDV in rice-based ecosystem.

## MATERIALS AND METHODS

### Insects and rice plants

Adults of WBPH were collected in experimental farm of China National Rice Research Institute (CNRRI), Hangzhou, China, and were maintained on susceptible rice TN1 at 26±1 °C and photoperiods 12L: 12D for one generation.

Rice variety Jiayou 5 used in this experiment was recently bred by Jiaying Academy of Agricultural Science Research (JAASR), China and was transplanted with regular agricultural practices in JAASR farm. Healthy rice plants and plants with obvious symptom infected with RBSDV which were 45-60 day-old were uprooted and transplanted in clay pots (Diameter =15 cm) for recovering, then individual plants were molecularly identified with the RT-PCR methods developed by Ji et al (2009) to confirm the diseased plants scientifically.

### Effects of RBSDV on ecological parameters of WBPH

Individual rice plants removed outer sheathes and inactive

**Received:** 2 November 2011; **Accepted:** 12 February 2012

**Corresponding author:** LU Zhong-xian ([luzxmh@yahoo.com.cn](mailto:luzxmh@yahoo.com.cn))

roots were cleaned carefully in tap water and put into a test tube (Diameter = 1.5 cm, Height = 18 cm) with 1.5 cm deep Kimura B nutrient solution. Ten 1<sup>st</sup> instar nymphs hatched in 12 h were introduced into a prepared tube sealed with medical purified cotton, and then all test tubes were kept in climate chamber with 26±1 °C and relative humidity (RH) 75-90%. Fresh rice plants at the same age with tested ones and Kimura B nutrient solution were replaced properly. Each treatment with healthy or diseased rice plants was replicated ten times. Survival nymphs and adults of WBPH were recorded daily, and the female adults newly emerged in 12 h were weighed individually and then kept under -70 °C for measuring the activities of defensive and detoxification enzymes.

The same individual rice plants were prepared and put into a test tube (Diameter = 1.5 cm, Height = 30 cm) with 1.5 cm deep Kimura B nutrient solution. One pair of adults newly emerged in 12 h was introduced into a prepared tube with medical purified cotton sealed, and all test tubes were kept in climate chamber with 26±1 °C and RH 75-90%. Survival adults were recorded daily, while fresh rice plants and Kimura B nutrient solution were added properly. Newly hatched nymphs were counted and removed everyday until no nymphs observed for three days, and unhatched eggs were dissected under microscope. Each treatment with healthy or diseased plants was replicated ten times.

#### Measurement of activities of defensive enzymes and detoxifying enzymes

Ten mg fresh brachypterous female adults of WBPH fed on healthy or RBSDV infested rice plants, respectively, were homogenized with 600 µl 0.2 M phosphate buffer at pH 7.8 in a homogenizer under ice bath. The homogenate was centrifuged for 10 min at 4 °C and 3000 rpm. Then the supernatant fluid was fetched for measurement. The activities of defensive enzymes, peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT), and detoxifying enzymes, acetylcholinesterase (AChE), carboxylesterase (CAE), and glutathione S-transferase (GST), were detected according to the instruction of matched test kit provided by Jiancheng Bioengineering Institute, Nanjing, China. The concentration of protein was measured with Bradford's Coomassie Brilliant Blue G-250 method (Bradford, 1976) and the calibration curve was performed with bovine serum albumin.

#### Data analysis

Values in percentages were transformed by arcsine SQRT before analysis performed using SPSS 13.0 for windows. The differences of ecological and physiological parameters on healthy and RBSDV- infested rice plants were analyzed by analysis of variance (ANOVA).

## RESULTS

#### Effects of RBSDV on ecological parameters of WBPH

Ecological parameters including nymph survival rate and duration, and weight of female adults on rice plants infested with RBSDV were slightly higher than those on healthy rice plants, but not significant (Table 1).

Female adult longevity ( $P = 0.05$ ) and egg duration ( $P = 0.033$ ) on rice plants infected with RBSDV were significantly shorter than those on healthy plants, while female fecundity and egg hatchability obviously increased on RBSDV-infected rice plants (Table 2).

#### Effects of RBSDV on activities of defensive and detoxifying enzymes of WBPH

Activities of all defensive and detoxification enzymes increased significantly in WBPH adults after feeding on rice plants infected with RBSDV ( $P < 0.05$ ). Compared to those on healthy rice plants, defensive enzymes CAT, SOD and POD in WBPH on infested plants increased 61.04%, 16.51% and 17.84%, respectively; meanwhile detoxification enzymes AChE, GST and CAE increased 26.96%, 20.75%, 29.61%, respectively (Table 3).

## DISCUSSION

Rice is the most important grain crop for Chinese food security with an annual  $2.87 \times 10^8$  hectare of paddy fields and about 40% of total grain production (FAOSTAT, 2007; Peng et al, 2009). Rice planthoppers, mainly included SBPH, WBPH and brown planthopper (BPH) *Nilaparvata lugens*, have been the economically destructive insect pests of rice in China since 1970's and resulted in more than 13.33 million hectare of rice

**Table 1. Nymph survival and duration, weight of female adults on healthy and rice black streak dwarf virus (RBSDV) infected plants.**

Rice plant	Nymph survival rate (%)	Nymph duration (d)		Weight per female adult (mg)
		♀	♂	
Healthy	95.8 ± 2.8	13.5 ± 0.3	12.6 ± 0.4	1.5 ± 0.1
Infected	96.8 ± 2.1	13.6 ± 0.2	13.0 ± 0.2	1.6 ± 0.0
<i>P</i>	0.867	0.989	0.380	0.637

**Table 2. Adult longevity and fecundity, egg duration and hatchability on healthy and rice black streak dwarf virus (RBSDV) infected plants.**

Rice plant	Adult longevity (d)	Eggs laid per female	Egg duration (d)	Hatchability (%)
Healthy	13.4 ± 1.2	31.3 ± 4.7	11.8 ± 1.2	49.9 ± 4.0
Infected	10.1 ± 0.6	33.3 ± 5.1	9.3 ± 0.5	65.4 ± 5.4
<i>P</i>	0.033	0.777	0.050	0.079

**Table 3. Activities of defensive enzymes and detoxification enzymes of white-backed planthopper (WBPH).**

Rice plant	Defensive enzymes			Detoxification enzymes		
	CAT ( $\times 10^{-8}$ mol/s·mg)	SOD ( $\times 10^{-7}$ mol/s·mg)	POD ( $\times 10^{-7}$ mol/s·mg)	AChE ( $\times 10^{-9}$ mol/s·mg)	GST ( $\times 10^{-7}$ mol/s·mg)	CAE (mol·OD/min)
Healthy	4.1 ± 1.6	13.7 ± 0.6	4.4 ± 0.3	7.5 ± 0.3	32.3 ± 0.3	3.3 ± 0.1
Infected	10.5 ± 2.1	16.5 ± 0.7	5.3 ± 0.2	10.2 ± 0.7	40.8 ± 0.6	4.7 ± 0.2
<i>P</i>	0.013	0.007	0.011	0.005	0.001	0.001

fields occurring the disease and around 1500 million kilogram grains lost yearly (Cheng, 2009). With the gradual expansion of the growing area of hybrid rice which susceptible to WBPH, the population of WBPH increased year by year and became the most serious insect pest of rice in China (Cheng et al, 2008). Planthoppers not only damaged to rice plants directly by sucking phloem sap, but also transmitted rice virus, then caused more grain loss indirectly. In 2001, a new rice virus transmitted by WBPH was firstly found in Guangdong Province, south China, and was officially identified as a novel fijivirus and named as the south rice black streaked dwarf virus (SRBSDV) in 2008 (Zhang et al, 2008; Zhou et al, 2008). About  $3.33 \times 10^5$  hectare of rice were infested by SRBSDV, resulting in  $6.67 \times 10^3$  hectare with no harvest in 2009 (Liu et al, 2010). Rice ragged stunt virus (RRSV) transmitted by BPH frequently outbreaks in Vietnam and affected seriously rice production and food supply in 2006. RRSV occurred increasingly in parts of Guangdong and Fujian Provinces and its damaged area increased in recent years (Zheng et al, 2008). Another BPH vectoring virus, rice grassy stunt virus (RGSV), has been found in South China (Lin et al, 2003; Zhang et al, 2001). Planthoppers, WBPH, BPH and SBPH often occur in spatiotemporal coincidence and share the same host rice plants in rice fields, so it is necessary to explore the influence of rice virus on the ecological fitness of vector and non-vector planthopper for optimizing the practical strategies to effectively manage planthopper population and control spread of rice virus in rice-based ecosystem.

There were numerous reports on the effects of plant viruses on the vector insects. Cucumber mosaic virus (CMV) had significantly affected the survival and fecundity of vector aphids *Myzus persicae* (Sulzer) and resulted in decrease of aphids survival rate (Ma et al, 2007). The activities of AChE, CAE, SOD and CAT in aphids on diseased tobacco plants with CMV were markedly higher than those on healthy tobacco plants (Zhang et al, 2007). Tomato spotted wilt virus (TSWV) also can improve host suitability for its vector *Frankliniella occidentalis*, pepper plants infected with TSWV increased the population growth of vector thrips, thereby accelerated the vector development in the next generations (Jiu et al, 2007; Maris et al, 2004; Whitfield et al, 2005). Leafhopper (*Nephotettix virescens*) infected with rice tungro spherical virus (RTSV) slowed growth, and declined fecundity and increased mortality, however, leafhoppers preferred initially to feeding on infested rice plants, meanwhile the majority of them obtained virus from infested plants reselected to the healthy plants after 24 h, thereby promoted the spread of the virus (Khan et al, 1985). SBPH carried with rice stripe virus (RSV) prolonged its egg duration, and increased nymph and adult survival rate of  $F_1$  generation (Zhou, 2009). Former findings have demonstrated that another non-vector BPH on infested plants with RBSDV

increased significantly nymph survival rate and population development, and activities of endogenous defensive enzymes SOD and POD (unpublished data). The data in this paper on WBPH showed the similar results with BPH, however, the ecological mechanisms on the impacts of RBSDV in the non-vector insects and their natural enemies are still unclear.

Plant viruses maybe affect their non-vector insects directly or indirectly. Non-vector insects would be affected physiological and biochemical characters directly by the plant viruses that have been fed into insect body, and they also would be affected population growth indirectly by the changes of nutritional compounds and secondary chemicals in the host plants infested by virus. In this experiment, we could not recognize the direct influence of RBSDV on WBPH, however, the contents of free amino acids and soluble sugar increased by 31.1% and 300.0%, respectively, in the diseased plants compared to the healthy plants (unpublished data), implied that the improvement of ecological fitness of WBPH on infested rice plants should be related closely to the increase of nutritional chemicals by RBSDV infestation. The direct influence and the capacity of indirect influence of RBSDV on non-vector WBPH need further study.

## ACKNOWLEDGEMENTS

This study was supported by National Basic Research Program of China (973 Program) (Grant No. 2010CB126200), the Agro-Industry R&D Special Fund of China (Grant Nos. 200903051 and 201003031). The authors would like to thank GRC of International Rice Research Institute (IRRI) for donating rice seeds of TN1, and Mr. LU Qiang of Jiaying Academy of Agricultural Science Research for his technical assistance in obtaining rice plants infested by RBSDV in fields.

## REFERENCES

- Bai F W, Yan J, Qu Z C, Zhang H W, Xu J, Ye M M, Shen D L. 2002. Phylogenetic analysis reveals that a dwarfing disease on different cereal crops in China is due to rice black streaked dwarf virus (RBSDV). *Virus Genes*, **25**(2): 201–206.
- Belliure B, Janssen A, Maris P C, Peters D, Sabelis M W. 2005. Herbivore arthropods benefit from vectoring plant viruses. *Ecol Lett*, **8**(1): 70–79.
- Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*, **72**: 248–252.
- Chen S X, Zhang Q Y. 2005. Advance in researches on rice black-streaked dwarf disease and maize rough dwarf disease in

- China. *Acta Phytophyl Sin*, **32**(1): 97–103. (in Chinese with English abstract)
- Cheng J A, Zhu J L, Zhu Z R, Zhang L G. 2008. Rice planthopper outbreak and environment regulation. *J Environ Entomol*, **30**(2): 176–182. (in Chinese with English abstract)
- Cheng J A. 2009. Rice planthopper problems and relevant causes in China. In: Heong K L, Hardy B. Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia. Los Baños (Philippines): International Rice Research Institute: 157–178.
- Colvin J, Omongo C A, Govindappa M R, Stevenson P C, Maruthi M N, Gibson G, Seal S E, Muniyappa V. 2006. Host-plant viral infection effects on arthropod-vector population growth, development and behavior: Management and epidemiological implications. *Adv Virus Res*, **67**: 419–452.
- Fang S, Yu J, Feng J, Han C, Li D, Liu Y. 2001. Identification of rice black-streaked dwarf fijivirus in maize with rough dwarf disease in China. *Arch Virol*, **146**(1): 167–170.
- FAOSTAT. 2007. FAO Statistical Databases. www.fao.org. FAO. Rome.
- Froissart R, Doumayrou J, Vuillaume F, Alizon S, Michalakakis Y. 2010. The virulence-transmission trade-off in vector-borne plant viruses: A review of (non-) existing studies. *Phil Trans R Soc B*, **365**: 1907–1918.
- George W L, Heong K L, Johnson D, Savary S. 2010. Rice pest management: Issues and opportunities. In: Pandey S, Byerlee D, Dawe D, Dobermann A, Mohanty S, Rozelle S, and Hardy B. Rice in the Globe Economy: Strategic Research and Policy Issues for Food Security. Los Baños (Philippines): International Rice Research Institute: 297–332.
- Hodge S, Powell G. 2010. Conditional facilitation of an aphid vector, *Acyrtosiphon pisum*, by the plant pathogen, pea enation mosaic virus. *J Insect Sci*, **10**: 155.
- Ji Y H, Ren C M, Cheng Z B, Zhou T, Zhou Y J. 2009. Preliminary identification of a newly occurred rice stunt disease in Jiangsu Province. *Jiangsu J Agric Sci*, **25**(6): 1263–1267. (in Chinese with English abstract)
- Jiménez-Martínez E S, Bosque-Pérez N A, Berger P H, Zemetra R S. 2004. Life history of the bird cherry-oat aphid, *Rhopalosiphum padi* (Homoptera: Aphididae), on transgenic and untransformed wheat challenged with barley yellow dwarf virus. *J Econ Entomol*, **97**(2): 203–212.
- Jiu M, Zhou X P, Tong L, Xu J, Yang X, Wan F H, Liu S S. 2007. Vector-virus mutualism accelerates population increase of an invasive whitefly. *PLoS ONE*, **2**(1): 1–8.
- Khan Z R, Saxena C. 1985. Behavior and biology of *Nephotettix virescens* on tungro virus-infected rice plant: Epidemiology implications. *Environ Entomol*, **14**(3): 297–304.
- Lin L M, Wu Z J, Xie L H, Lin Q Y. 2003. Cloning, sequence analysis and prokaryotic expression of the vRNA3 NS3 gene in rice grassy stunt virus Shaxian isolate. *J Agric Biotech*, **11**(2): 187–191. (in Chinese with English abstract)
- Liu W C, Liu Y, Guo R. 2010. Warning to outbreak of southern rice black-streaked dwarf virus. *China Plant Prot*, **30**(3): 17–18. (in Chinese with English abstract)
- Luan J B, Liu S S. 2010. Roles of vector-virus-plant interactions in biological invasions. *Biodiv Sci*, **18**(6): 598–604. (in Chinese with English abstract)
- Ma L N, Liu Y H, Zhang L, Zhou L F, Li X Z. 2007. Effects of tobacco plants inoculated by different viruses on development and reproduction of *Myzus persicae* (Sulzer). *Acta Phytophyl Sin*, **34**(1): 10–14. (in Chinese with English abstract)
- Maris P C, Joosten N N, Goldbach R W, Peters D. 2004. Tomato spotted wilt virus improves host suitability for its vector *Frankliniella occidentalis*. *Phytopathol*, **94**(7): 706–711
- Mauck K E, De Moraes C M, Mescher M C. 2010. Deceptive chemical signals induced by a plant virus attracting insect vectors to inferior hosts. *PNAS*, **107**(8): 3600–3605.
- Peng S, Tang Q, Zou Y. 2009. Current status and challenge of rice production in China. *Plant Prod Sci*, **12**(1): 3–8.
- Rubinstein G, Czosnek H. 1997. Long-term association of tomato yellow leaf curl virus with its whitefly vector *Bemisia tabaci*: Effect on the insect transmission capacity, longevity and fecundity. *J Genl Virol*, **78**(10): 2683–2689.
- Sinisterra X H, McKenzie C L, Hunter W B, Powell C A, Shatters J R G. 2005. Differential transcriptional activity of plant-pathogenic begomoviruses in their whitefly vector (*Bemisia tabaci*, Gennadius: Hemiptera Aleyrodidae). *J Gen Virol*, **86**(5): 1525–1532.
- Sisterson M S. 2008. Effects of insect-vector preference for healthy or infected plants on pathogen spread: Insights from a model. *J Econ Entomol*, **101**(1): 1–8.
- Stout M J, Thaler J S, Thomma B P H J. 2006. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annu Rev Entomol*, **51**: 663–689.
- Whitfield A E, Ullman D E, German T L. 2005. Tospovirus-thrips interaction. *Annu Rev Phytopathol*, **43**: 459–489.
- Zhang C M, Wu Z J, Lin L M, Xie L H. 2001. Sequence analysis of RNA6 of the RGSV isolate from Shaxian. *Acta Phytopathol Sin*, **31**(4): 301–305. (in Chinese with English abstract)
- Zhang H M, Yang J, Chen J P, Adams M J. 2008. A black-streaked dwarf disease on rice in China is caused by a novel fijivirus. *Arch Virol*, **153**(10): 1893–1898.
- Zhang L, Liu Y H, Chen Z Y. 2007. Activities of glutathione S-transferases and carboxylesterases in *Myzus persicae* (Sulzer) fed with CMV infected tobacco plants. *Tobacco Sci & Tech*, (3): 61–64. (in Chinese with English abstract)
- Zheng L P, Xie L Y, Lian L L, Xie L H. 2008. Research progress on rice ragged stunt virus (RRSV). *J Agric Sci & Tech*, **10**(5): 8–12. (in Chinese with English abstract)
- Zhou G H, Wen J J, Cai D J, Li P, Xu D L, Zhang S G. 2008. Southern rice black-streaked dwarf virus (SRBSDV), a novel virus of fijivirus (Phytoreovirus). *Chin Sci Bul*, **53**(20): 2500–2508. (in Chinese with English abstract)
- Zhou Y J. 2009. Rice Stripe Virus Disease. Nanjing: Jiangsu Science and Technology Publishing Company: 57–59. (in Chinese)