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Subcellular localization of the stripe disease-specific protein encoded by rice stripe virus (RSV) in its vector, the small brown planthopper, *Laodelphax striatellus*

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Abstract **The stripe disease-specific protein (SP) encoded by the rice stripe virus (RSV) was successfully used as a localization signal of the virus in its vector, the small brown lanthopper, *Laodelphax striatellus* Fallen. Immunogold particles in large numbers were detected in various parts of the viruliferous females: the ovum, surface of chorion, the midgut lumen, and the columnar cells. Whereas there was none of these particles in the non-viruliferous females and males, and testis of viruliferous males. Endosymbionts (mycetocytes) were abundant, harboring ovaries of both viruliferous and non-viruliferous females, but none in the testis of males. The results provided us with the direct proof that RSV is a ciruculative and propagative plant virus and it was transovarially transmitted alongside with endosymbionts of its vector. Therefore, we deem it a nice lead for future studies on the mechanism of RSV transmission and functioning of its viral proteins.**

Keywords: ***Laodelphax striatellus*, rice stripe virus, midgut, ovary, immunogold labeling, immunoelectron microscopy.**

Rice stripe disease is a major disease of rice in China. It also causes heavy losses in the Southeastern Asian countries. The etiology of the disease is the rice stripe virus (RSV), that transmits persistently by an insect vector, the small brown planthopper, *Laodelphax striatellus* Fallen[1,2]. RSV infects only the Graminaceae with a host spectrum of 37 species, including rice, maize and wheat[2]. The disease may cause significant losses in yield during its prevalence[3]. In China it was first found in Zhejing and Jiangsu provinces in 1962, and has since been found consecutively in at least 16 provinces. So far, there are no measures for its effective control except application of insecticides, crop rotation practices and breeding of hopper-resistant rice varieties.

RSV particle is noneveloped, of filamentous shape, with a diameter of ca. 3—10 nm, and different lengths. The genome is ssRNA, consisting of four segments. Progress in the studies of RSV molecular biology has only recently been reviewed[4]. Complete sequence of the genome has been determined[5—7], and it is featured by utilizing an ambisense coding strategy for gene expression[8,9]. RSV have since been studied by biochemical analysis, immunological assay, and electron microscopy, and viral proteins are detected both in the host plant and its vectors by Western blotting. However, information on the virus/rice and virus/vector interrelationships is fragmentary, especially on the virus structure and function, multiplication, circulation route in the host plant and the vector, and mechanisms of transmission and infection. An understanding of the virus transmission mechanism is vital to the development of effective strategies in interrupting the virus-vector connections and thus alleviating losses in the yield of rice[11].

The aim of this study is the localization of Stripe Disease-Specific Protein (SP) encoded by RSV in *L. striatellus* with immunogold-labeling electron microscopy and thus provides direct proofs of virus multiplication and circulation in the vector. It is the first report on ultrastructure of internal organs in *L. striatellus* and immunological localization of SP product in the midgut and the gonads.

1 Materials and methods

(i) Insect, diseased rice plants, and antiserum. *L.*

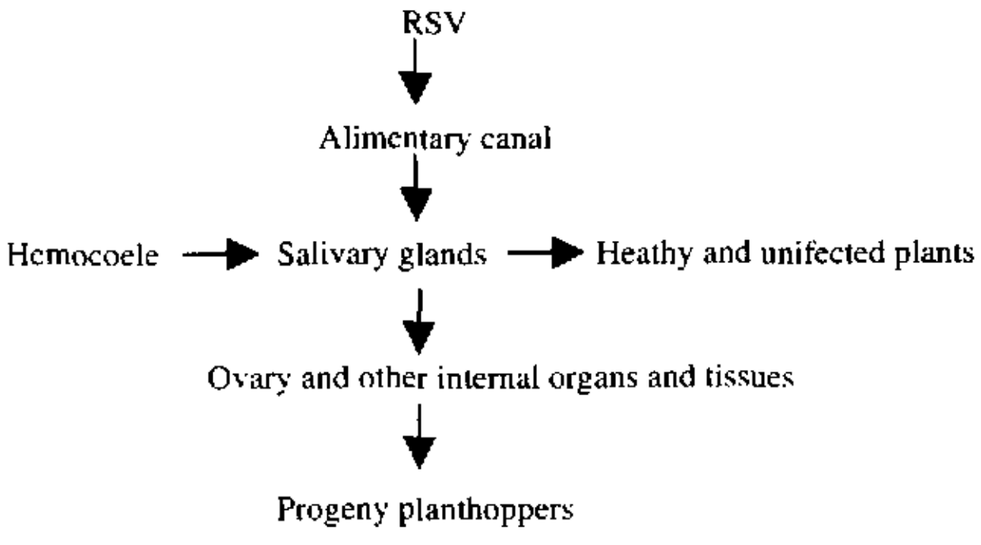
was oblong in shape and ensheathed in the chorion. Mycetocytes were present in the ovum and the ovarian tissues (fig. 2(b)). In males, the spermatogonia were also of different developing stages, but the mycetocytes were absent in the mature sperm (fig. 2(c), (d)). It indicated therefore that the transmission of mycetocytes was closely associated with the maturation of eggs in *L. striatellus*. This was additional histochemical evidence for transovarial transmission of mycetocytes in the hopper vectors[14].

Immunogold labeling in *L. striatellus* had shown that the immunogold particles were present inside the eggs and outside around the chorion (fig. 2(e), (f)), whereas these were absent in the control. In testes from both viruliferous and the control males no immunogold particle was observed. Previously, it was reported by Toriyama's group[15] that, upon injection of RSV into the hemocoel of the vector, virus particles are found later by the conventional electron microscopy in various organs of viruliferous hoppers, such as the abdominal region and gonads of both sex, and that fluorescent signals were detected in various organs by the fluorescent antibody staining technique[15]. However, since both the techniques used and route of virus introduction are different between Toriyama's group and ours, no single conclusion can be drawn by simple comparison, thus remaining grounds for further study.

There is no doubt also from the above that the ovary plays an important role in *in vivo* circulation of RSV in *L. striatellus*, and it is highly possible that the ovary and various tissues and organs are also sites of its multiplication and the virus is transovarially (or vertically) transmitted from generation to generation in the vector.

Both results from conventional electron microscopy and immunogold labeling also testify that the mature eggs could be infected by RSV by the same route as the mycetocytes and transmitted to the next generations[15]. This provides a direct proof that non-viruliferous female mating with viruliferous male does not transmit RSV to its progeny.

Taking into full consideration the nature of persistent propagative transmission in RSV by its vector *L. striatellus* and the results obtained in our study, we would like to propose the vertical and horizontal transmission of RSV by its vector *L. striatellus* as the following scheme:



So far, we have not yet detected any RSV particles in *L. striatellus*, and further study on its circulation and multiplication is currently under way.

Little is known about other proteins encoded by RSV genome with the exception for virus genes of coat protein and RNA polymerase. The present note is the first study on RSV localization *in vivo* by SP immunogold labeling, and the results could be a lead for the elucidation of RSV localization and functioning of other proteins encoded by the virus.

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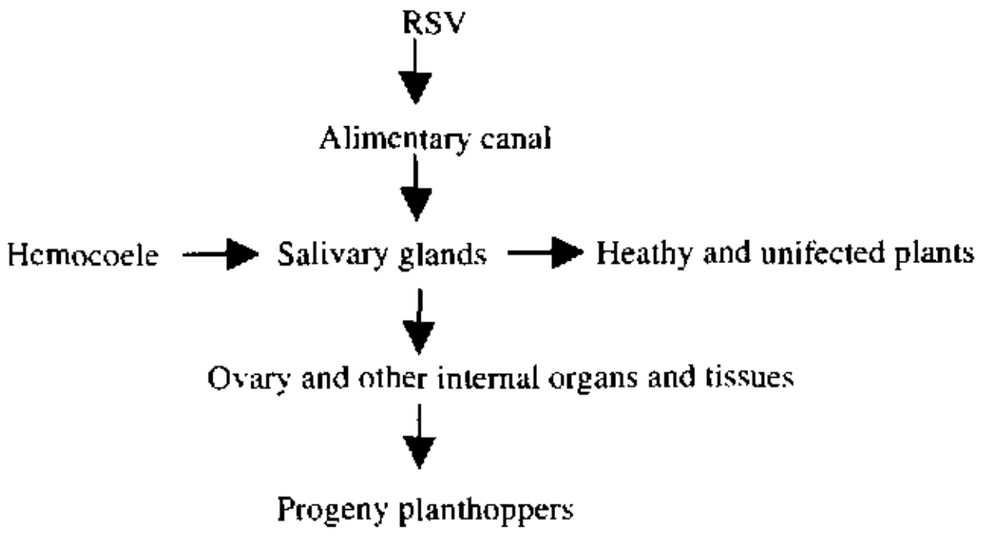

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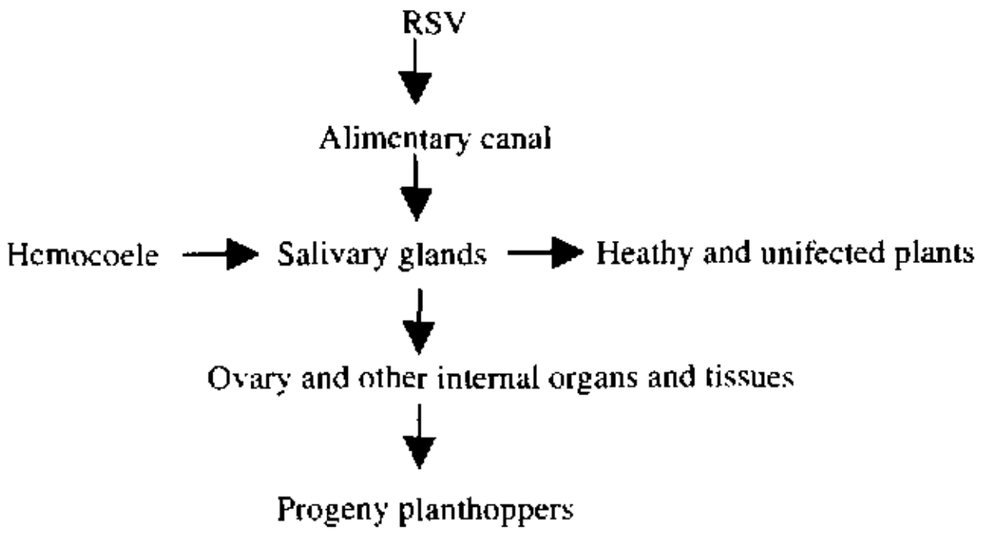

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