

# Recent *Rice stripe virus* Epidemics in Zhejiang Province, China, and Experiments on Sowing Date, Disease–Yield Loss Relationships, and Seedling Susceptibility

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## ABSTRACT

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*Rice stripe virus*, transmitted by the small brown planthopper *Laodelphax striatellus*, has recently reemerged as a major disease in Zhejiang province, eastern China. Intensive surveys during 2003 to 2006 demonstrated how the disease has spread rapidly from the northern to central and eastern regions with increasing incidence each year. In bioassays, the highest proportions of viruliferous vectors were from regions where the disease was most severe. The greatest disease incidence was in the earliest sown plants, and substantial control could be achieved by delaying planting from late May to mid-June. In experiments where different proportions of infected plants were established (by inoculation or varying the sowing date), average yield losses were 0.8% for every 1% increase in disease incidence. In inoculation experiments, young seedlings, particularly those at the three- to five-leaf stage, were the most susceptible, whereas  $\leq 1\%$  of plants inoculated at or after the elongation stage developed symptoms. Recent epidemics appear to have resulted from large populations of viruliferous vectors colonizing rice seedlings at the most susceptible stage. This is probably because of changes in cropping practice, recent warmer winters in Zhejiang province, and the development of resistance or tolerance to the insecticides widely used (triazophos, synthetic pyrethroids, and Imidacloprid).

Additional keyword: epidemiology

Rice stripe disease is one of the most serious viral diseases in rice. It was first discovered in the central part of Japan at the end of 19th century, and caused more than 50% losses in rice production in some fields (1,11). The disease remains today one of the most important viral diseases in Japan. It also occurs in rice-growing areas of Korea and the former USSR and results in significant yield losses in epidemic years (7). The disease is caused by *Rice stripe virus* (RSV), which is reported to infect as many as 80 species of the family Gramineae (14,22). It is transmitted by the small brown planthopper *Laodelphax striatellus* in a persistent manner and can be passed through the egg to about 90% of progeny insects for as many as 40 generations

(5,16). RSV is the type member of the genus *Tenuivirus* (6).

In China, rice stripe disease occurred for the first time in Eastern Zhejiang province, Shanghai city, and Southern Jiangsu province in the early 1960s (24), then spread to eastern and southern China, including Jiangsu, Fujian, and Yunnan provinces. In the end, the epidemic affected about 2,660,000 ha of rice fields in over 20 provinces, with a disease incidence of 10 to 20% in general and 50 to 80% in serious cases, and resulted in great loss of rice yield (13,23). In Yunnan province, the disease usually affects around 67,000 ha and causes annual losses of 4,000 tons of grain (12). However, in eastern China, where the first outbreak had occurred and the disease had been a major constraint on rice production, the disease suddenly and completely disappeared for a decade in the 1990s. Since the beginning of this century, it has reemerged in this region and has spread very rapidly to become the most economically destructive disease of the rice crop. For instance, in Jiangsu province, the disease has expanded rapidly since 1999. According to an estimate by

the Jiangsu Provincial Department of Agriculture and Forestry, the disease affected over 660,000 ha of rice fields during the period 2001 to 2003 and, in some regions where the epidemic was severe, both disease incidence and yield losses exceeded 50%. By July 2004, the disease had spread to 1,530,000 ha in more than 40 counties, of which 1 million ha was severely infected (>20% plants infected); in some of the fields, there was no harvest. Rice is grown as a summer crop in Jiangsu; however, a recent survey also found the disease on winter wheat in several counties, with an incidence in fields ranging from 3 to 70% (20).

In Zhejiang, (the southern neighbor to Jiangsu province), the disease has also become of increasing importance on *japonica* rice in recent years. In the northern part of the province (where the disease has mostly occurred), *japonica* rice is generally grown as a single summer crop (May to October), alternating with winter crops that may include other cereals (such as wheat or barley) or oilseed rape. In response to the emerging disease problem, the Zhejiang Provincial Department of Science and Technology launched a research project involving 16 agricultural research and extension organizations to monitor the disease, investigate its epidemiology, and establish a disease control strategy. We now report the results of the disease and vector survey and some experiments to investigate the effects of planting date on disease incidence, the relationship between disease incidence and yield, and the susceptibility of plants at differing growth stages to infection.

## MATERIALS AND METHODS

**Disease survey.** An intensive survey and systematic monitoring of the emergence of rice stripe disease in Zhejiang province was carried out during the period 2003 to 2006. In all, 27 locations representative of the major *japonica* rice-growing regions in the province were selected by the provincial plant protection service (Fig. 1) and, at each location, six fields within an area of 1 km<sup>2</sup> were chosen for detailed monitoring. Every 5 days throughout the cropping

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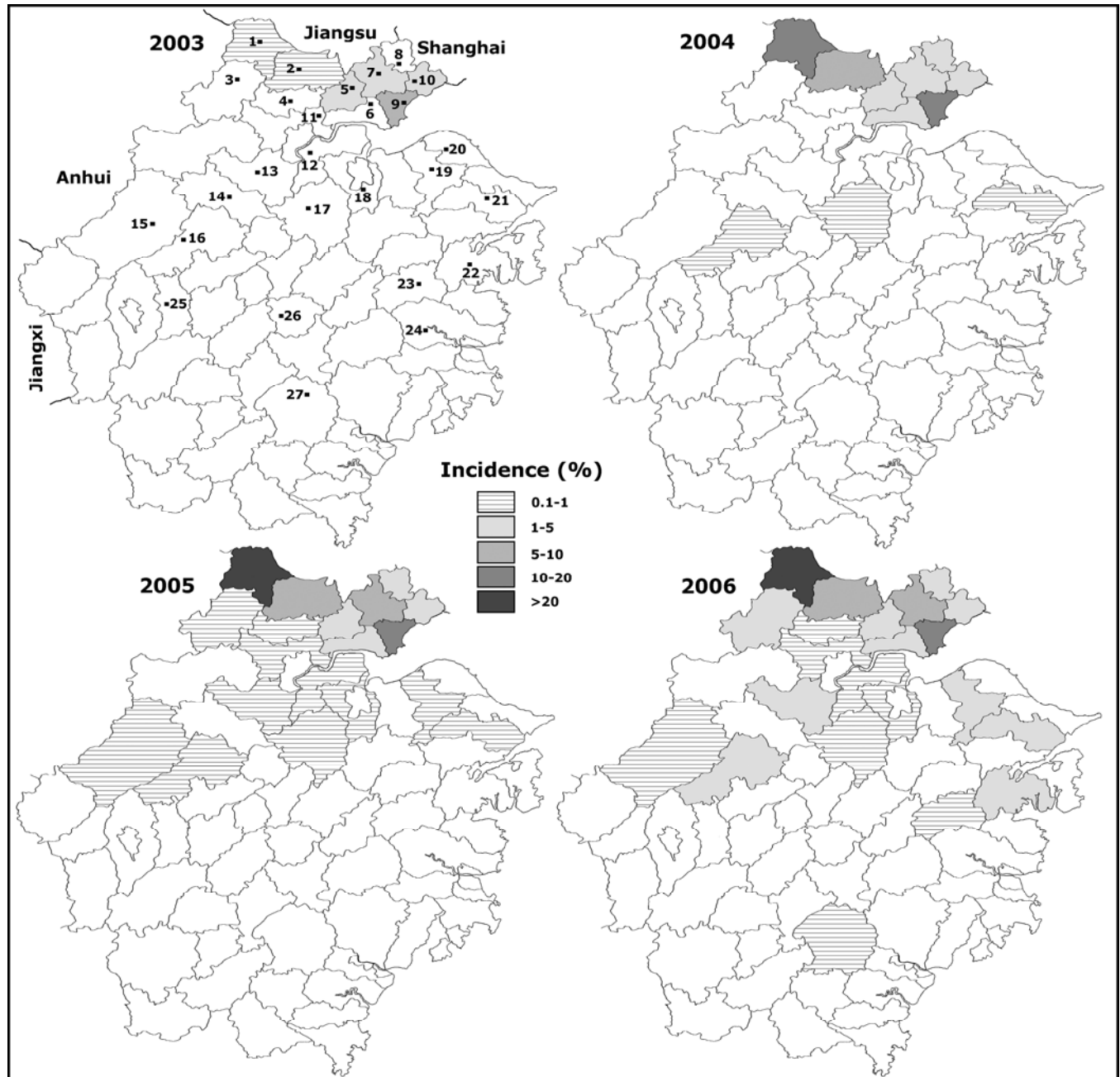
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season each year, 500 plants were examined from each field (100 plants from each of five predetermined sampling positions in different parts of the field) and the numbers of plants with symptoms recorded. In addition, throughout the selected counties and districts, local plant protection technicians reported the location of fields in which disease symptoms had appeared for collation by the Provincial Station of Plant Protection and Plant Quarantine.

**Tests for viruliferous vectors among overwintering populations of *L. striatellus*.** In each of the years 2004 to 2006,

adults of the over-wintering generation of *L. striatellus* were collected from locations in Nanhu, Xiuzhou, Jiashan, Pinghu, Haiyan, Haining, Tongxiang, Wuxing, Changxing, and Zhuji counties. At each location, six to nine fields were selected for sampling and 30 insects were randomly collected from different parts of each field by gently shaking them off the plants onto a white tray below. Virus transmission was then tested by the bioassay method. In some samples, the individual plant hoppers were then also tested for RSV by enzyme-linked immunosorbent assay (ELISA) (see below).

**Intensive disease monitoring in 2004 to 2006.** Monitoring locations were selected in Nanhu, Tongxiang, and Haiyan counties in northern Zhejiang province and Zhuji County in the central region of the province. At each location, there were more than 67 ha of rice fields. After sowing, five fields (each 2,000 m<sup>2</sup>) were randomly selected from each location for detailed inspections. Every other day until rice harvest, 500 plants in each field were examined (100 plants from each of five predetermined sampling positions in different parts of the field) and the numbers



**Fig. 1.** Rice stripe disease incidence as assessed by surveys in Zhejiang province during the period 2003 to 2006. The 27 locations chosen are numbered in the 2003 figure as follows: Huzhou city: Changxing (1), Wuxing (2), Anji (3), Deqing (4); Jiaxing city: Tongxiang (5), Haining (6), Nanhu, Xiuzhou (districts of Jiaxing town) (7), Jiashan (8), Haiyan (9), Pinghu (10); Hangzhou city: Yuhang (11), Xiaoshan (12), Fuyang (13), Tonglu (14), Chunan (15), Jiande (16); Shaoxing city: Zhuji (17), Shaoxing (18); Ningbo city: Yuyao (19), Cixi (20), Ningzhou (21), Ninghai (22); Taizhou city: Tiantai (23), Linhai (24); Quzhou city: Longyou (25); Jinhua city: Yongkang (26); Lishui city: Qintian (27). The shading shows the average of the maximum disease incidence recorded from each of six fields at each location.

of plants with symptoms and the total numbers of *L. striatellus* planthoppers were recorded.

**Effects of different numbers of viruliferous *L. striatellus* on subsequent disease incidence and yield.** In an inoculation experiment at Shuangqiao, Xiuzhou district, Jiaying city in 2006, experimental plots with different proportions of infected plants were established by seedling inoculation and subsequently related to yield losses. Rice cv. Xiouhui 09 (from Jiaying Academy of Agricultural Sciences, Zhejiang, China) was sown in seedling beds under nets to protect from incoming insect vectors. At the three-leaf stage, plots of 1 m<sup>2</sup> were inoculated with a total of 0, 10, 25, 50, 100, 150, or 200 plant hoppers (three plots per treatment). Bioassay tests indicated that 9.7% of these hoppers were transmitting virus. Twenty days after inoculation, the rice seedlings were transplanted to the field (1 m<sup>2</sup> of seedlings to 10 m<sup>2</sup> of field as three replicate plots per treatment in a randomized design) for growing and later yield assessments. The plants received two insecticide sprays (Regent at 750 ml/ha and 5% fipronil SC a.i.; Bayer Crop Science, Hangzhou, China) during the growing season (on the third day after transplanting and then 15 days later) to kill any immigrant plant hoppers. Plants were examined to determine the proportion with symptoms every 5 days and, at harvest, the total produce from each plot was harvested by hand. The grains were then threshed and cleaned by machine (STD370 rice manual thresher, Guangxi Nanfang Machine Co.) dried to 13.5% moisture and weighed to determine the grain yield.

**Effects of planting date on disease incidence and yield.** At three separate locations (Xingfeng, Nanhu; Shuangqiao, Xiuzhou; and Longxiang, Tongxiang) in 2006, rice cv. Xiouhui 09 was sown directly into field plots on different dates at 5-day intervals between 25 May and 20 June. At each date, seed were sown to six plots, each of 2 by 5 m with 0.3-m paths between the plots, using different fields for each sowing date (however, all those used at any one location were adjacent to one another). Three plots received four insecticide sprays (as described above and done at 10-day intervals starting 5 days after

sowing) to prevent infection during the growing season and the other three were left unsprayed. Plants were examined to determine the proportion with symptoms every 5 days and, at harvest, total grain yields of each plot were measured as described above.

**Susceptibility of rice plants at different growth stages to RSV infection.** To determine the susceptibility of rice plants at different stages of growth, seed of rice cv. Xiouhui 09 were germinated at 8- to 15-day intervals, sown to pots (50/pot), covered by netting to avoid infection by viruliferous planthoppers, and grown in the field under natural conditions until inoculation. At inoculation, plants were at nine different stages of growth (two-leaf, three-leaf, four-leaf, five-leaf, early tillering, end of tillering, elongation, booting, and heading). Planthoppers collected from Changxing were used to inoculate five replicate pots of each growth stage (100 insects per pot), after which plants were examined daily for symptoms. There were also non-inoculated controls at each growth stage. The experiment was performed in each of the years 2004 to 2006.

**Bioassay to test planthoppers for RSV.** Planthoppers collected from field sites were placed individually on rice plants (cvs. Jia 991 and Xiouhui 110 at the three-leaf stage) and removed by hand after 24 h. The plants were then grown in a glasshouse at approximately 25°C and grown and examined for RSV symptoms after 28 days. Preliminary experiments had shown that this was sufficient time for all infected plants to develop symptoms (*data not shown*).

**Detection of RSV in plants and planthoppers by ELISA.** ELISA was done using a modification of the method described by Wang et al (17). Leaf sap was prepared from freshly collected samples (usually within 6 h and never more than 24 h after sampling) by grinding 0.1 g of leaf tissue in 1 ml of phosphate-buffered saline (PBS), clarified by a few seconds' centrifugation, and then diluted 1:10 in PBS. Whole insects were ground in 200 µl of PBS and the suspension clarified as before. For each sample, duplicate wells of microtiter plates were coated with 100 µl of leaf or insect sap and incubated overnight at 4°C. After three washes with PBS, 200 µl of PBS containing 2% bovine serum albumin

was added to each well and incubated for 1 h at 37°C. After three washes with PBS, 100 µl of a 1:1000 dilution of RSV monoclonal antibody in PBS was added to each well and incubated for 2 h at 37°C. The monoclonal antibody was a gift from Professors Y. Zhou (Jiangsu Academy of Agricultural Sciences) and X. Zhou (Zhejiang University). Its sensitivity is 0.78 to 3.10 ng ml<sup>-1</sup> and its titer with infected leaf sap is in the range of 1:80,000 to 1:512,000 (17). Then, after three washes with PBS, 50 µl of a 1:2000 dilution of alkaline phosphatase-labeled anti-mouse antibody (Sigma, Shanghai, China) in PBS was added to each well and incubated for 2 h at 37°C. After three washes with PBS, the substrate (p-nitrophenyl phosphate at 1 mg/ml) was added and the plates incubated for 30 min at 37°C, after which values for the optical density at 405 nm were determined using a spectrophotometer (Biorad, Hangzhou, China). A sample was considered positive if its optical density value was at least three times the average of the healthy controls (0.112); in most cases, infected samples greatly exceeded this threshold.

**Statistical analyses.** Data were analyzed by analysis of variance (ANOVA),  $\chi^2$  contingency, or regression analysis using the functions in Microsoft Office Excel 2003.

## RESULTS

**Disease incidence in Zhejiang province, 2003 to 2006.** Results of the survey during 2003 to 2006 are summarized in Figure 1. The disease emerged first in Changxing, Wuxing, and Haiyan counties in 2003 (presumably from Jiangsu province to the north) and spread rapidly from the north of the province to the central and eastern parts with increasing incidence each year. It reached the Hang-Jia-Hu Plain, covering an area of approximately 66,000 ha in 2004, and, by 2005, 18 counties in all had reported the disease while, in the two northern cities of Huzhou and Jiaying, it was estimated that about 23,000 ha (14% of fields) of the *japonica* rice-growing region was affected. Tiantai and Qingtian counties, in the middle and southern regions of the province, also reported the disease in 2006, while the average disease incidence in Huzhou and Jiaying increased further. In the whole province, an estimated 100,000 ha of the rice crop was infected, of which one-third in the north was severe (Changxing, Wuxing, and Haiyan, where average incidence exceeded 10%). In Changxing alone the disease affected 23,300 and 21,500 ha in 2005 and 2006, respectively, representing 57 to 58% of the total rice-growing area in the county and causing substantial loss of rice production. Moderate levels of disease (average of 1 to 5% plants infected) occurred in Nanhu, Xiuzhou, Tongxiang, Pinghu, Haining, and Jiashan while else-

**Table 1.** Comparative results for the presence of *Rice stripe virus* (RSV) in three different samples of the vector *Laodelphax striatellus* assessed by two different methods

Source <sup>a</sup>	ELISA <sup>b</sup> test for RSV, positive/total planthoppers tested		Transmission bioassay, infected/total plants inoculated		ELISA/transmission
	Number	Percent	Number	Percent	
1	33/312	10.6	37/399	6.8	1:0.64
2	9/219	4.1	7/258	2.7	1:0.66
3	9/240	3.8	7/291	2.4	1:0.66

<sup>a</sup> Planthoppers were collected from three of the field sites used for disease monitoring: 1, Changxing (cv. Xiaoshui 110); 2, Nanhu (cv. Jia 991); and 3, Nanhu (cv. Xiouhui 110).

<sup>b</sup> ELISA = enzyme-linked immunosorbent assay.

where the disease was mild, with average incidence lower than 0.1%.

**Tests for viruliferous vectors among overwintering populations of *L. striatellus*.** A comparison between the bioassay and ELISA tests for the presence of RSV within the overwintering *L. striatellus* is shown for one field at Changxing and two at Nanhu (Jiaxing city) in Table 1. There was an average incidence of transmission in bioassays of about 4%, whereas tests of the same insects by ELISA indicated that about 6% contained RSV, indicating that the serological test had detected virus in some insects that did not transmit ( $\chi^2$ , 1 df = 10.3;  $P = 0.0014$ ). The proportions of viruliferous overwintering vectors in 10 counties during 2004 to 2006 are shown in Figure 2. Although there were some variations between sites, there was a strong tendency for the proportion of viruliferous vectors to increase with time. The regions with the highest proportions of viruliferous vectors (e.g., Changxing) were also those where the disease was most severe (Fig. 1).

**Virus and vector dynamics.** An intensive field survey in four counties of northern Zhejiang indicated that there were two obvious peaks of rice stripe disease (Fig. 3A). The first peak was from the middle of June to early July when rice was at the seedling stage as a result of the first generation *L. striatellus* transmitting RSV from other cereals and grasses to rice seedlings in the nursery. Following a decline in disease incidence as severely infected plants died, there was then a second peak of infection from the middle to the end of July. This occurred when rice was at the tilling stage, usually 15 to 25 days after transplanting of the seedlings, as a result of RSV transmission by the second generation of the insect; nymphs emerged in early to middle June and became adults in late June to early July, transmitting virus to the newly transplanted seedlings. There also appeared to be a smaller, third peak at booting stage (mid- to late August), presumably resulting from transmission by the third generation of the insect. Follow-

ing the initial invasion of the fields, planthopper populations declined slightly and a peak corresponding to the second generation was evident (Fig. 3B). In the period from late July to August when the temperatures were very high, the population of the insect decreased dramatically; however, numbers then increased greatly under the more favorable temperatures of September to early or middle October. At this time, rice was at the booting stage and virus transmission was of no consequence to the maturing crop; however, these large populations subsequently moved to other cereals and grass weeds, contributing to the overwintering population.

**Effects of different numbers of viruliferous *L. striatellus* on subsequent disease incidence and yield.** Regression analysis showed that there was a highly significant linear relationship between vector numbers applied at the seedling stage and the subsequent incidence of disease after transplanting, which was reflected in the grain yields obtained at harvest (Table 2). Differences between the treatments (planthopper numbers) were highly significant in ANOVA and, in the most severely affected plots (20.7% plants infected), yield losses were 17.2%.

**Effects of planting date on disease incidence and yield.** The insecticide sprays

completely prevented disease development and, therefore, provided appropriate controls at each sowing date. At most dates and sites, ANOVA showed significant yield losses resulting from RSV infection. The earliest sown rice had the largest disease incidence and suffered the most yield loss (Table 3). Yields of treated plots were similar from the first four sowing dates and there was evidence of only the very slightest yield reduction (1 to 2%) from sowing on or after 15 June.

**Relationship between disease incidence and yield loss.** Individual plot data from the experiments where different numbers of vectors were introduced at the seedling stage or where rice was sown on different dates were used to examine the relationship between disease incidence and yield loss (Fig. 4). Using linear regression analysis, the relationship appeared to be linear over the disease levels obtained in these experiments and was highly significant ( $R^2 = 0.949$ ). The relationship was similar at all sites and indicates an average yield loss of 0.8% for every 1% increase in disease incidence.

**Susceptibility of rice plants at different growth stages to RSV infection.** Disease developed only on inoculated seedlings and ANOVA showed that there were big differences in disease incidence de-

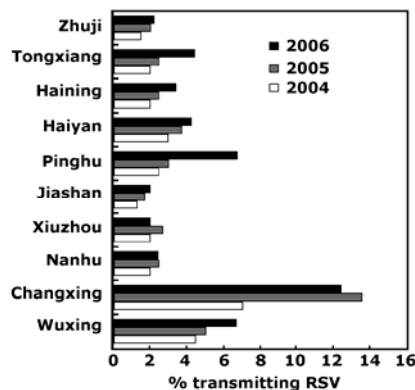


Fig. 2. Percentage of overwintering *Laodelphax striatellus* planthoppers from 10 counties in northern Zhejiang province that transmitted *Rice stripe virus* (RSV) in bioassay experiments.

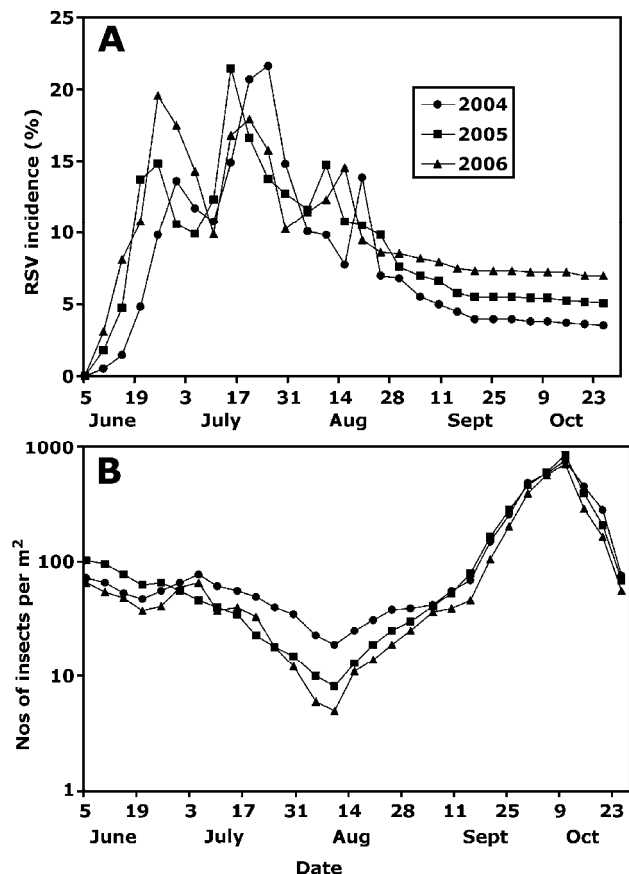


Fig. 3. Changes in **A**, the incidence of rice stripe disease and **B**, the population of *Laodelphax striatellus* planthoppers in northern Zhejiang (2004 to 2006). Results are averages of five fields from each of four counties in each year.

pending on the plant growth stage when they were inoculated. Young seedlings, particularly those at the three- to five-leaf stage, were the most susceptible, whereas plants inoculated after the elongation stage developed almost no symptoms (Table 4).

## DISCUSSION

The data presented here document the recent spread of rice stripe disease in Zhejiang province and show that the annual epidemic is expanding progressively south. Although the disease was severe only in the northernmost parts of the province by 2006, the pattern of spread over the 4 years of the survey suggests that the epidemic will become more widespread and destructive in future years. This expanding epidemic and increasing inoculum is associated with increasing populations of viruliferous vectors early in the season and these are clearly the primary source of the epidemic. Experiments confirmed that

rice was most susceptible at the seedling stage (three to five leaves) and, in the field, plants are often at this stage in mid-June to early July, coinciding with the emergence of the first generation *L. striatellus* vectors. Experiments in Taiwan have indicated that yield losses were greater the earlier that rice plants were inoculated with viruliferous vectors (4) and our data on disease incidence lead to a similar conclusion. Epidemics in Japan were triggered by earlier transplanting of rice seedlings at a time which coincided with the peak migration of the vector (8,16), and experiments in Japan and Korea showed that disease incidence was much reduced if sowing was delayed from early or mid-May until mid-June (2,9). Similarly, in our experiments, disease incidence and, therefore, yield loss were much less when the rice was sown later, thus avoiding exposure to these vectors. The traditional sowing date for rice in Zhejiang province is around 25 May but

our results suggest that delaying sowing until 10 June would greatly reduce RSV infection and the resulting yield losses. There are no known disadvantages to this delay in sowing, which also reduces the risk of damage from the stem borer *Chilo suppressalis* that results from eggs laid on rice plants by the overwintering generation.

Recent changes in farming practices in eastern China are almost certainly a contributory factor to the recent increases in populations of viruliferous vectors and, thus, the reemergence of the disease in Zhejiang province. Wheat (or barley) and rice rotations, minimal tillage, and an increasing area of winter fallow have all provided increasing opportunities for the insect vector to overwinter on grass weeds or other cereals. Thus, investigations at the Jiaying Station for Pest and Disease Monitoring indicated that total leaf hopper numbers during 2005 were two to three times those in 2002. Results reported in this article also indicate that an increasing proportion of these insects are viruliferous, no doubt because the grasses and other cereals on which the vectors overwinter are also reservoirs of RSV (14,20,22). According to experience in Japan, no important infestations of RSV were observed when 1 to 2% of the vector population was carrying the virus; however, the disease tended to become serious, resulting in significant losses of rice, when >3% of the vector population was viruliferous (10). This threshold is now regularly exceeded in northern Zhejiang. It is also likely that the warmer conditions in recent years have provided more favorable conditions for the insect vectors; this is likely to happen by increasing survival rates over the winter

**Table 2.** Effect of inoculating seedlings with different numbers of viruliferous *Laodelphax striatellus* on subsequent incidence of rice stripe disease and grain yield

Number <sup>a</sup>	Incidence (%)	Incidence (logit) <sup>b</sup>	Yield (t/ha)
0 (control)	0.0	...	7.57
10	1.1	-2.081	7.51
25	2.6	-1.739	7.41
50	5.5	-1.383	7.25
100	10.5	-1.052	6.86
150	16.2	-0.810	6.65
200	20.7	-0.665	6.27
SED <sup>c</sup>	...	0.0287	0.028
Slope <sup>d</sup>	0.10 ± 0.028	...	-0.01 ± 0.0002
R <sup>2</sup>	0.987	...	0.987

<sup>a</sup> Numbers of viruliferous planthoppers.

<sup>b</sup> Means of transformed data [ $\text{logit} = 0.5 \log_e(\%/[100 - \%])$ ] to normalize the variance.

<sup>c</sup> Standard error of the difference between means. From analysis of variance for comparisons between treatment means, 14 df.

<sup>d</sup> Slope of linear regression line against vector numbers, using individual plot data.

**Table 3.** Effect of sowing date at three sites in 2006 on rice stripe disease and grain yield

Sowing date	Disease incidence (%) <sup>a</sup>	Yield (t/ha)			P <sup>c</sup>	Yield loss (%)
		Nontreated	Treated	SED <sup>b</sup>		
Xingfeng						
25 May	15.3 ± 2.52	6.70	7.61	0.042	0.001	11.9
30 May	7.4 ± 2.09	7.19	7.62	0.036	0.001	5.7
5 June	6.6 ± 1.98	7.26	7.61	0.093	0.01	4.6
10 June	4.9 ± 0.95	7.27	7.59	0.028	0.01	4.2
15 June	3.4 ± 0.64	7.34	7.57	0.023	0.01	3.0
20 June	1.6 ± 0.56	7.34	7.53	0.043	0.05	1.1
Shuangxiao						
25 May	13.5 ± 2.96	6.76	7.61	0.036	0.001	11.2
30 May	8.8 ± 1.91	7.13	7.71	0.027	0.001	7.5
5 June	6.9 ± 1.86	7.42	7.72	0.080	0.01	3.9
10 June	3.3 ± 0.39	7.47	7.64	0.019	0.05	2.3
15 June	1.6 ± 0.23	7.46	7.58	0.030	0.05	1.6
20 June	1.0 ± 0.17	7.49	7.54	0.038	n.s.	0.7
Longxiang						
25 May	12.2 ± 0.97	6.85	7.64	0.061	0.001	10.4
30 May	9.6 ± 0.99	7.02	7.64	0.102	0.001	8.1
5 June	7.9 ± 0.79	7.13	7.68	0.041	0.001	7.1
10 June	3.7 ± 0.54	7.39	7.62	0.030	0.01	3.1
15 June	1.5 ± 0.27	7.50	7.59	0.036	n.s.	1.2
20 June	0.8 ± 0.08	7.50	7.54	0.030	n.s.	0.6

<sup>a</sup> In nontreated plots; no disease developed in treated plots.

<sup>b</sup> From analysis of variance for comparisons between treated and nontreated means, 4 df.

<sup>c</sup> Significance of difference between treated and nontreated plots; n.s. = not significant.

and also by increasing the number of generations during the season (3,21).

Our results demonstrate that good control of the vector and, therefore, the disease can be achieved by insecticide application. Nevertheless, the present epidemic has undoubtedly been promoted by control practices that have been applied poorly or which have proved inadequate to control the disease. Triazophos and synthetic pyrethroid insecticides were widely used but their use is now declining because triazophos insecticides resulted in the insect vector producing larger numbers of eggs, while the pyrethroids have become ineffective as a result of resistance to the insecticide and effects on the natural enemies of the vector (18). Imidacloprid, currently the main pesticide used, is becoming less efficient, probably because the insects are developing resistance or tolerance (19). In addition, individual farmers prepare their own rice seedlings on a small scale (sometimes in close proximity to fields that are sources of the vector); the timing of rice transplanting varies, providing additional opportunities for the vector to bridge between crops; and some local farmers lack knowledge of the disease or appropriate

technical support. Therefore, improvement (and improved availability) of advice on pesticide management could be an important factor in helping to contain the effects of the epidemic.

An additional important factor in the recent epidemics is the susceptibility to RSV of almost all *japonica* rice cultivars which are grown in Zhejiang province. Although there are no data for cultivars used in the past, the cultivar that is currently most popular, Jia 991, is particularly suitable both for the insect vector to feed upon and for development of virus symptoms. The use of resistant cultivars would be a most attractive control option, not only because insecticide application carries environmental risks and is not always effective (see above) but also because farm sizes are small and uniformity and coordination of insecticide application between neighboring farmers is unlikely. Recent experiments have shown promising resistance in some existing cultivars (e.g., HZ05-29, Zheda 631, Shaonuo 05-19, and Zheda 614) and these are now being recommended to farmers (15). A spectrum of resistance to both RSV and to the vector was also identified in breeding germplasm

lines although the resistances to the virus and the vector do not seem to be correlated (*unpublished results*). Research groups are also exploring the opportunity to exploit antiviral strategies in transgenic plants.

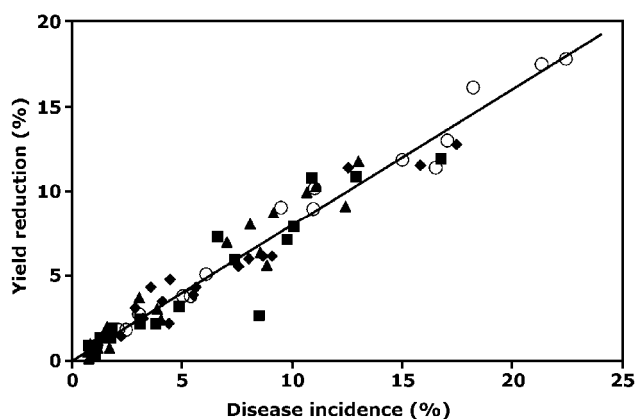
The data obtained in this study has helped explain the current epidemics and indicates some opportunities for future control strategies. It may be possible, for example, to apply measures to control the overwintering vector population, while mathematical models are being developed to provide forecasts of risk and to help provide advice on optimum timing of control measures.

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**Fig. 4.** Relationship between incidence of rice stripe disease and yield losses from individual plots in four different experiments in Zhejiang province. The open circles are plots from the experiment on vector inoculation at Shuangqiao and solid symbols are plots from experiments on sowing date (diamond, Xinfeng; square, Shuangqiao; triangle, Longaiong). The fitted regression line has a slope of  $0.80 \pm 0.022$  ( $R^2 = 0.949$ ).

**Table 4.** Incidence of rice stripe disease developing on rice inoculated with viruliferous *Laodelphax striatellus* at different plant growth stages in each of the years 2004 to 2006

Plant growth stage	2004		2005		2006	
	%	Arcsine (%) <sup>a</sup>	%	Arcsine (%) <sup>a</sup>	%	Arcsine (%) <sup>a</sup>
Two-leaf	30.8	33.7	30.0	33.2	27.6	31.7
Three-leaf	54.4	47.5	68.8	56.1	54.8	47.8
Four-leaf	51.2	45.7	60.8	51.3	56.4	48.7
Five-leaf	43.2	41.1	38.8	38.5	40.8	39.7
Early tillering	14.8	22.5	12.8	20.9	18.8	25.6
End of tillering	4.0	11.2	4.8	11.3	3.6	9.6
Elongation	0.0	0.0	1.2	3.9	1.2	4.9
Booting	0.0	0.0	0.0	0.0	0.4	1.6
Early heading	0.0	0.0	0.0	0.0	0.0	0.0
SED <sup>b</sup>	...	1.18	...	1.70	...	1.65

<sup>a</sup> Means of transformed data [arcsine( $\sqrt{\%}$ )] to normalize the variance.

<sup>b</sup> Standard error of the difference between means. From analysis of variance for comparisons between treatment means, 36 df.

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