Plant Pathology (2009) **58**, 815–825 Doi: 10.1111/j.1365-3059.2009.02091.x

Studies on the epidemiology and yield losses from rice black-streaked dwarf disease in a recent epidemic in Zhejiang province, China

H.-D. Wang[a], J.-P. Chen[b]*, A.-G. Wang[c], X.-H. Jiang[a] and M. J. Adams[d]

[a] *Zhejiang Provincial Station of Plant Protection and Plant Quarantine, Hangzhou 310020;* [b] *Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021;* [c] *Linghai Station of Plant Protection, Taizhou 317000, China; and* [d] *Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK*

The spread of rice black-streaked dwarf disease, which has emerged as a major problem on winter wheat and the two summer rice crops (early *indica* and late *japonica*) grown in central and southern Zhejiang province, China, is documented from 1995 to 2007. The late *japonica* crop suffered the most: up to 64 640 ha were affected with estimated losses of *c.* 120 000 t grain per year. Peak adult numbers of the small brown planthopper vector, *Laodelphax striatellus*, coincided with the seedling stages of both rice crops and the proportion of the insect population carrying virus increased during 1998–2005. Seedlings with three to four leaves were the most susceptible, whereas plants inoculated after the end of tillering developed few or no symptoms. Disease levels were strongly correlated with numbers of viruliferous vectors. In sowing-date experiments with both rice crops, the earliest sowings had the most disease and suffered the greatest yield losses. With the last sowing date (25 days after the first), there were almost no losses. There were yield losses of 0·80% for every 1% increase in disease incidence in early *indica* rice and rather more (0·92%) in the late *japonica* crop. There were large differences in susceptibility between cultivars, indicating the possibility, within currently available germplasm, of using more resistant cultivars to help contain the disease. Changes in cropping practice and in recent winter weather conditions have probably contributed to the emergence of the virus as a major pathogen in eastern China.

Keywords: cultivar susceptibility, disease survey, *Laodelphax striatellus*, *Rice black-streaked dwarf virus*, sowing date

Introduction

Since its first report in Japan (Kuribayshi & Shinkai, 1952) there have been sporadic, but serious, outbreaks of rice black-streaked dwarf disease in China, Japan and Korea. Infected plants are severely stunted with darkened leaves and galls on the leaf blades and sheaths. Huge losses in rice production have been reported in epidemic years (Lee *et al.*, 1977; Ruan *et al.*, 1984; Chen, 1996; Hibino, 1996). The disease is caused by *Rice black-streaked dwarf virus* (RBSDV), a member of the genus *Fijivirus* (family *Reoviridae*) that is mostly transmitted by the small brown planthopper, *Laodelphax striatellus*, in a persistent (propagative) manner, but not via its eggs (Shinkai, 1962, 1967; Shikata, 1974; Lee & Kim, 1985; Bae & Kim, 1994). RBSDV infects not only rice, but also wheat, barley, maize and a number of grass weed species (Shikata, 1974), which provide reservoirs of the virus throughout the year.

In China, the disease was first reported on rice in Linhai county, Zhejiang province in 1963, and the first major outbreak occurred in Linhai, Xianju and Taintai counties of the province in 1965–66 (Hong & Zhang, 1984; Ruan *et al.*, 1984). In the following 30 years, the disease declined greatly and infected plants became very difficult to find. The reasons for this are probably complex, but include the use of insecticidal sprays against the vector and farming practices that removed the weeds on which both virus and vector overwintered. In addition, cultivars grown during this period appear to have been relatively resistant to the virus (Chen, 1996). In consequence, there was little research on the disease for many years. However, since 1996, the disease has re-emerged in this region and expanded very rapidly to become an economically destructive disease in the entire rice-growing area of the middle and eastern Yangtze river basin, including Zhejiang, Shanghai, Jiangsu, Anhui, Jiangxi and north Fujian provinces. In some areas, disease incidence has exceeded 90%. This has highlighted the need for a better understanding of the epidemiology of the disease to help explain its re-emergence and to establish disease control strategies. In Zhejiang, the disease has mostly occurred in

---

*E-mail: jpchen2001@yahoo.com.cn

the central and southern parts of the province, where there are usually two crops of rice a year. The early crop (*indica* rice) is sown in nursery beds in mid-April and transplanted to the fields in May for harvesting by the end of July. The late crop of *japonica* rice is sown in late June, transplanted in late July and harvested by October. Wheat, barley or sometimes oilseed rape are then commonly grown during the winter. The disease affects all cereal crops, but is usually most severe in the late *japonica* rice crop.

In response to the emerging threat, the Zhejiang Provincial Department of Science and Technology launched a major research project via the Provincial Plant Protection Network for the period 1996–2005. This included comprehensive monitoring of the disease, assessments of yield losses and experiments on its epidemiology. This paper reports some of these studies and discusses their implications for disease management in Zhejiang province.

Materials and methods

Disease distribution and severity in Zhejiang province

An intensive survey and systematic monitoring of the emergence of rice black-streaked dwarf disease was carried out by the Provincial Plant Protection Network service during 1995–2007. In the first stage of the survey (1995–99) only Linhai, Xianju and Taintai counties were inspected, but from 2000 onwards the study was extended to all 24 counties of Zhejiang province where the disease had been reported. Local technicians estimated the numbers of cereal fields (winter wheat or barley, early *indica* rice and late *japonica* rice) within their district that were affected by the disease and reported the data (as proportion of fields infected) to the county plant protection station. Each station received reports from hundreds of districts, providing a comprehensive estimate of the areas affected within the county. In addition, five locations (5–10 km apart) were chosen within each county and at each location 5–10 fields of late *japonica* rice were selected and disease incidence recorded from 400–600 rice plants (on two parallel lines from the edge to the middle of a field) at the heading–booting stage of growth (when RBSDV was usually at its maximum). In each year, the county data were then summarized into five severity grades based on the average RBSDV incidence in the fields visited and the proportion of rice fields infected throughout the county: grade 1 (mild) = RBSDV incidence < 1% and < 20% rice fields infected; grade 2 (mild–moderate) = RBSDV incidence 1–5% and < 20% rice fields infected; grade 3 (moderate) = RBSDV incidence 5–10% and < 20% rice fields infected; grade 4 (moderate–severe) = RBSDV incidence 10–15% and < 20% rice fields infected; grade 5 (severe) = RBSDV incidence > 15% and > 20% rice fields infected.

Seasonal fluctuations in insect vector populations and RBSDV incidence

Detailed studies were made of the populations of *L. striatellus* and the incidence of RBSDV in fields at Datian town, Linhai county in each of the years 2000–06. In each year, inspections were made at monthly intervals in seven selected fields of winter wheat and every 2 weeks in 13 nurseries or fields of early *indica* rice and 16 nurseries or fields of late *japonica* rice. Populations of *L. striatellus* were estimated according to standard protocols adopted by the Chinese Ministry of Agriculture: five sampling points were randomly selected within each field or nursery and insects were collected from 0·11 m^2 (in rice seedling nursery) or 20 plants (for fields) by gently patting the plants so that the insects dropped onto a tray beneath, where they were counted. RBSDV disease incidence was estimated visually in wheat by examining 500–1000 plants on a line from the edge to the middle of the field. In rice crops, 400–600 plants on two parallel lines from the edge to the middle of the field were examined.

Relationships between natural populations of viruliferous vectors and subsequent disease incidence

At five fields at each of two locations (Shuanggang, Linhai county and Xiage, Xianju county) in each of the 10 years 1998–2007, overwintering and second-generation insect populations were assessed at the end of April and in late July/early August, respectively (see above). Two-hundred insects were then collected from each field and tested by bioassay (see below) to determine the proportion of insects that was viruliferous. RBSDV incidence in the following rice crops was also assessed as described previously.

Effects of inoculation with different numbers of viruliferous *L. striatellus* on subsequent disease incidence and yield

In inoculation experiments at the Linhai Pest and Disease Monitoring Station, Datian town, Linhai city in each of the years 2004–06, experimental plots with different proportions of infected plants were established by seedling inoculation and subsequently related to yield losses. Early *indica* rice cv. Zhe 733 was sown in seedling beds under nets to protect it from incoming insect vectors. At the three-leaf stage, plots of 1 m^2 were inoculated with a total of 0, 2, 3, 4, 5, 7, 9, 10, 15, 20, 25, 30, 50 or 100 plant hoppers (three plots per treatment). Bioassay tests (see below) indicated that 11·6% (2004), 15·7% (2005) and 9·8% (2006) of these hoppers were transmitting virus. Similar experiments were also established later each season using the late *japonica* cv. Shanyou 10 with inoculations of 10, 20, 30, 60, 100, 120 or 150 plant hoppers, of which 10·2% (2004), 17·7% (2005) and 13·6% (2006) were transmitting virus. Twenty days after inoculation, the rice seedlings were transplanted to the field (1 m^2 seedlings to 10 m^2 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^{-1}; 5% fipronil SC a.i., Bayer Crop Science) 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 (5TD370 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

In experiments at the Linhai Pest and Disease Monitoring Station, Datian town, Linhai city in each of the years 2004–06, early *indica* rice (cv. Zhe 733) was sown directly into field plots on different dates at 5-day intervals between 10 April and 5 May. Similar experiments were also established later using late *japonica* rice (cv. Shanyou 10) sown between 15 July and 10 August. At each date, seeds were sown to six plots each of 2 × 5 m with 0·3-m-wide paths between the plots using different fields for each sowing date (but all those used at any one location were adjacent to one another). Three plots received four insecticide sprays (as described above and applied 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 RBSDV infection

To determine the susceptibility of rice plants at different stages of growth, seeds of hybrid *japonica* rice cv. Shanyou 10 were germinated at 8- to 15-day intervals, sown to pots (20 per 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 eight different stages of growth (two-, three-, four- or five-leaf, early tillering, end of tillering, elongation and booting). Planthoppers collected from Datian were used to inoculate three replicate pots of each growth stage (60 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 2000–06.

Cultivar susceptibility

Ten rice cultivars were sown in separate seedling beds at Datian town, Linhai city on 20 June in each of the years 2004–07. The cultivars used were the hybrids Shanyou 10, Xieyou 2, Xieyou 46, Xieyou 92, Xieyou 914 and Xieyou 963, together with Zhe 733 (early *indica*) and the late *japonica* cultivars Xiushui 63, Yuegeng 2 and Shaonuo 119. Seedlings were transplanted to fields 30 days after sowing, in a randomized block design with three plots of 30 m^2 for each cultivar. Plots received standard field management, but without insecticidal sprays. Disease incidence was estimated visually every 5 days after transplanting on 20 July and, when the disease had reached a stable maximum, 500 plants from each plot were examined to obtain a detailed record of disease incidence. At the same time, vector numbers were also assessed by collecting and counting insects from ten 0·25-m^2 areas within each plot.

Bioassay

Insect vectors collected from field sites were placed individually on rice plants (cv. Zhe 733 or Shanyou 10 at the three-leaf stage) and removed by hand 36 h later. The plants were then grown in an insect-proof glasshouse at *c*. 25°C and grown and examined for virus symptoms after 28 days. Preliminary experiments had shown that this was sufficient time for all infected plants to develop symptoms.

Statistical analyses

Regression analyses and analysis of variance (ANOVA), with transformation to normalize the variance where necessary, were performed using the functions in Microsoft® Excel 2002.

Results

Disease distribution and severity in Zhejiang province

The disease emerged in late *japonica* rice (cv. Shanyou 10) in Shuangong town, Linhai, in 1989, and during the 1990s the area affected expanded, with four major outbreaks (in 1992, 1996, 1997 and 1998). In Linhai county, the disease spread from two towns to 23 towns (and from *c*. 1200 to *c*. 8600 ha) in 1992 and 1998, respectively. Similarly, the diseased area in Xianju county expanded from *c*. 330 ha in 1996 to *c*. 4000 ha in 1998, and in Tiantai county, from *c*. 1800 ha in 1996 to *c*. 6000 ha in 1998. In addition to late *japonica* rice, the disease also infected early *indica* rice, barley, wheat and maize. Thus, in 1998 in Linhai, *c*. 530 ha of barley and wheat were infected with an average incidence of 30% (range 4–80%) and *c*. 800 ha of early *indica* rice were infected with an average incidence of 24% (12–58%). In Tiantai county in the same year, *c*. 667 ha of barley and wheat and *c*. 1800 ha of early *indica* rice were infected (average incidence 10%). The disease became a serious threat to rice production in these counties.

In the second stage of the survey (2000–07), late *japonica* rice was monitored in all affected counties of Zhejiang province and results are summarized in Fig. 1. During this period, the disease spread rapidly from the east part of the province (Linhai, Xianju and Taintai counties) to the central and southern parts. The total area of rice affected throughout Zhejiang province rose from about 26 000 ha to a maximum of 64 640 ha in 2005. In one severely affected area, Tianzhou city, the disease affected 55 000 ha (66% of the total rice-growing area). Most crops had 10–30% incidence, with up to 80% incidence

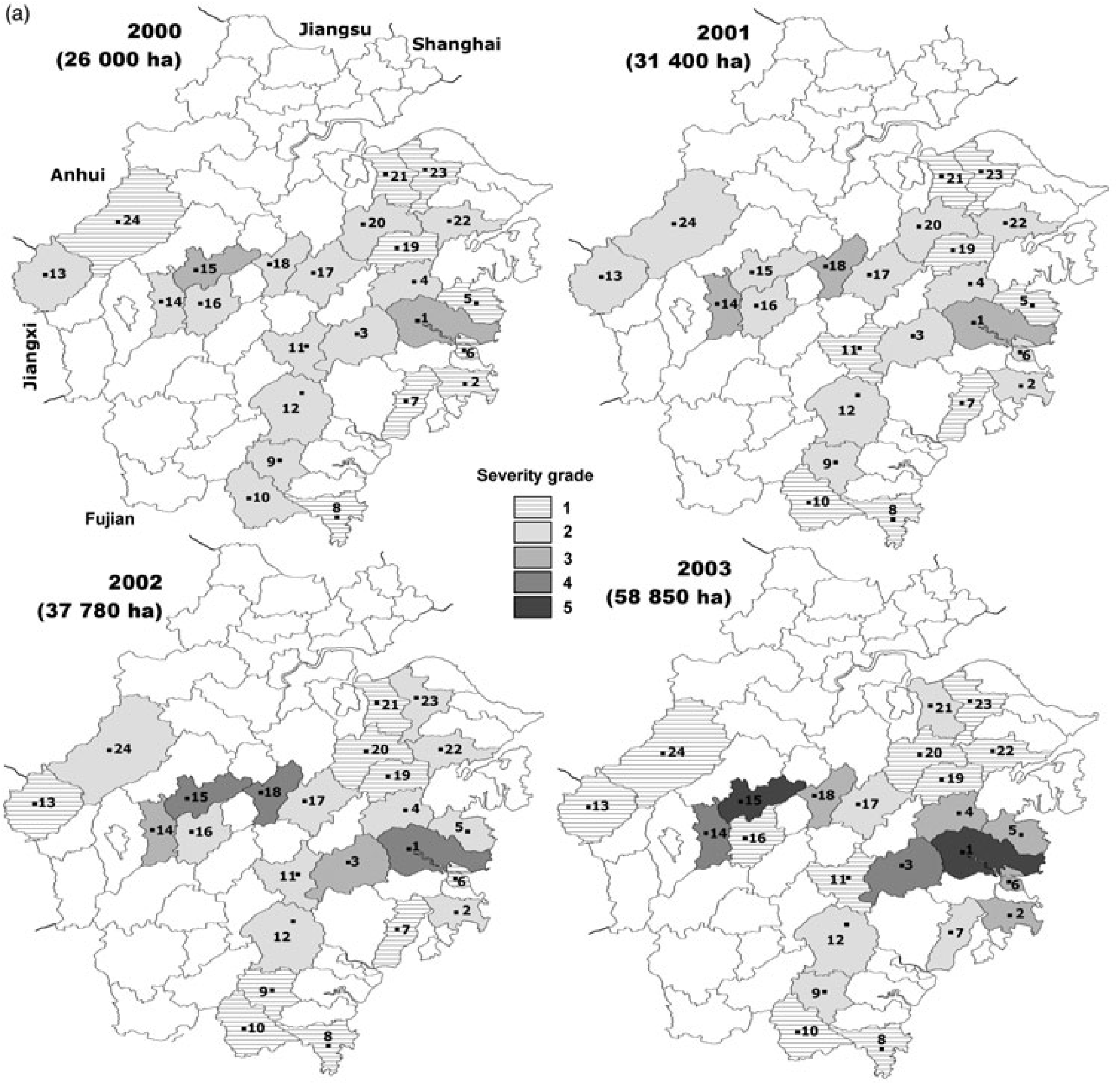

Figure 1 Rice black-streaked dwarf disease severity as assessed by surveys in Zhejiang province during the period 2000–07. The 24 counties surveyed are numbered as follows: 1, Linhai; 2, Wenlin; 3, Xianju; 4, Tiantai; 5, Shanmen; 6, Jiaojiang; 7, Yueqing 8, Channan; 9, Wencheng; 10, Taishun; 11, Jinyun; 12, Qingtian; 13, Kaihua; 14, Longyoun; 15, Lanqi; 16, Wucheng; 17, Dongyang; 18, Yiwu; 19, Xianchang; 20, Shenzhou; 21, Shangyu; 22, Fenghua; 23, Yuyao; 24, Chunan. The shading shows the average disease score (see text) in each county at the early heading stage in the late *japonica* rice crop (early September), when the disease had usually reached its maximum severity. The estimated total rice-growing area affected in the entire province is also shown for each year.

in the worst cases, sometimes leading to complete crop loss. Linhai, Wenlin, Xianju, Tiantai, Jiaojiang, Yueqing, Channan, Longyoun, Lanqi and Yiwu were the most seriously affected counties. The data show evidence of a decline in 2006 and 2007, believed to result from the adoption of improved control measures.

Seasonal fluctuations in insect vector populations and RBSDV incidence

Results of detailed monitoring of vector populations and virus incidence throughout each of the years 2000–06 at Datian town (Linhai county) are shown in Fig. 2. Disease patterns were similar in each year, with the greatest RBSDV incidence (usually 10–15%) in the late *japonica* rice crop. There were three major summer peaks in vector numbers, often reaching about 60 m^{-2}. These represented different adult generations, the first of which occurred on the *indica* rice seedlings soon after transplanting, while the third coincided with the early stages of the *japonica* rice crop. Vector numbers increased again during the autumn, mostly on weeds in the young winter cereal crop, but were substantially decreased (usually < 5 m^{-2}) by weed control and cold weather in December–January.

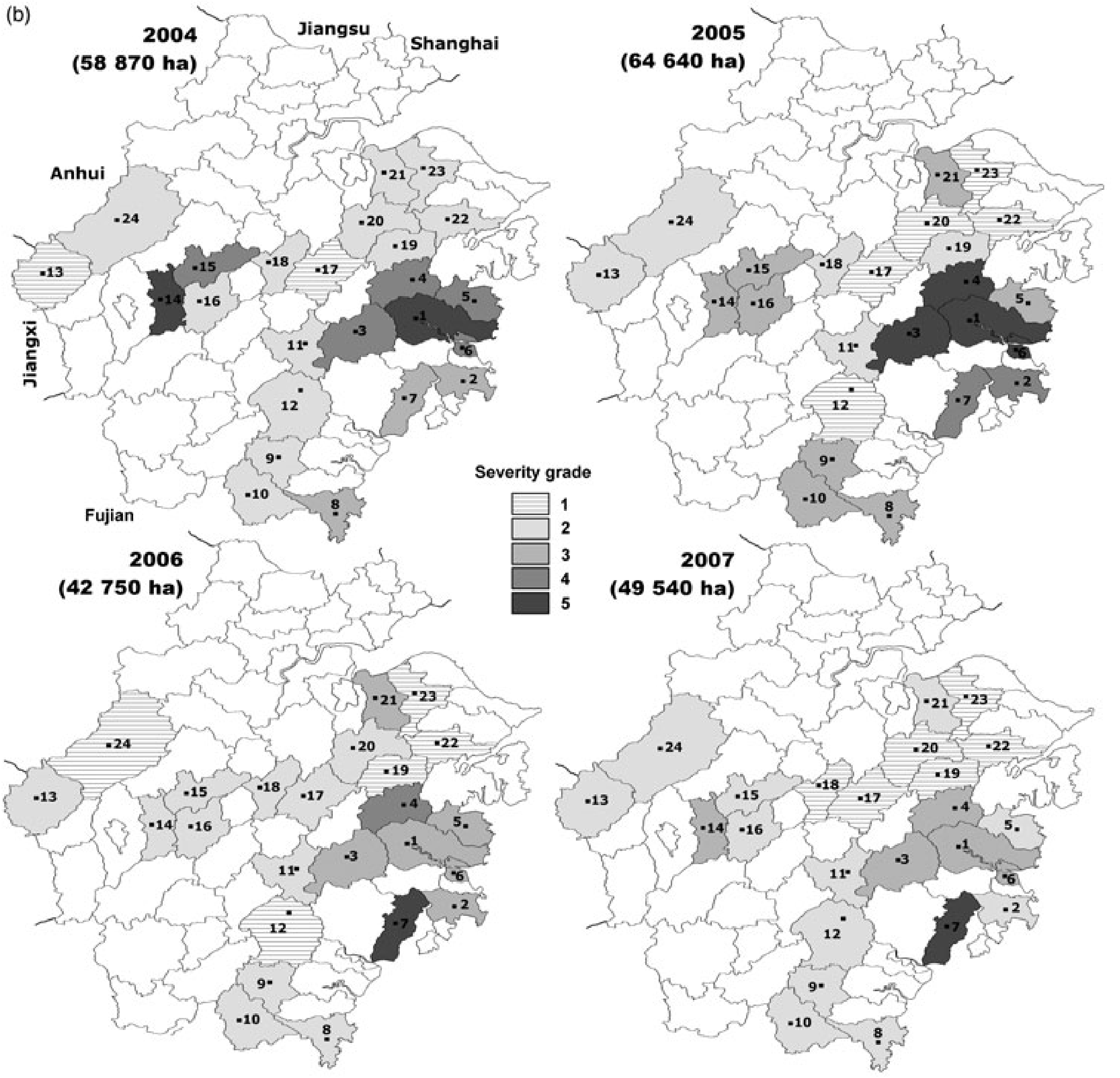

Figure 1 *Continued.*

Relationships between natural populations of viruliferous vectors and subsequent disease incidence

Results from two locations in each of 10 years showed that total numbers of overwintering vectors did not differ greatly between years, but that the proportion carrying virus increased substantially from low levels (2–4%) in 1998 and 1999 to a maximum in 2004 (9% at Xiage) or 2005 (17% at Shuanggang) (Table 1). At Shuanggang, this was positively correlated with the incidence of RBSDV in the winter wheat crop. RBSDV incidence in the early *indica* rice crop was strongly and positively correlated with the numbers of viruliferous overwintering vectors at both sites. By the end of the *indica* crop, the numbers of second-generation vectors (and the proportions that were viruliferous) were greater (usually by a factor of at least two) than the overwintering population. Numbers were greater at Shuanggang (usually > 500 m^{-2}) than at Xiage, and differences between years were less pronounced than earlier in the season. At both sites, RBSDV incidence in the late *japonica* rice was strongly and positively correlated with numbers of viruliferous second-generation vectors (Table 1).

Effects of inoculation with different numbers of viruliferous *L. striatellus* on subsequent disease incidence and yield

Inoculation experiments in which different numbers of viruliferous vectors were applied to experimental plots gave similar results each year. Regression analysis of the combined data showed that there were highly significant

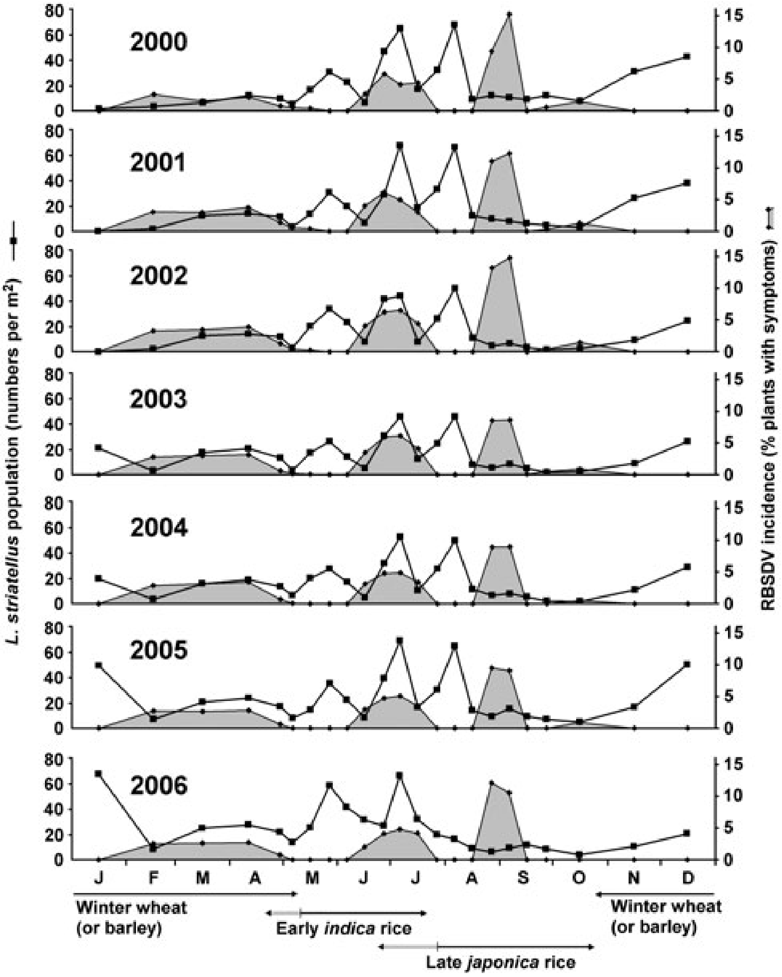

Figure 2 Diagram showing incidence of *Rice black-streaked dwarf virus* (RBSDV) (shaded areas below diamond symbols) and population densities of *Laodelphax striatellus* (squares) estimated at different dates throughout each of the years 2000–06. Results are from Datian, Linhai county, and are the average of observations made in several fields (see text for details). Cropping patterns are also indicated (grey bars indicate the stages when rice seedlings were in nursery beds prior to transplanting).

Table 1 Incidence of *Rice black-streaked dwarf virus* (RBSDV) in winter wheat, early *indica* rice and late *japonica* rice and numbers of overwintering and second-generation vectors carrying virus at two sites in Zhejiang province in each of the years 1998–2007; data are averages of five fields at each location

	Shuanggang, Linhai county					Xiage, Xianju county			
	RBSDV[a] winter wheat	Vector[b] overwintering	RBSDV[a] *indica*	Vector[b] 2nd gen	RBSDV[a] *japonica*	Vector[b] overwintering	RBSDV[a] *indica*	Vector[b] 2nd gen	RBSDV[a] *japonica*
Year	A	B1/B2	C	D1/D2	E	F1/F2	G	H1/H2	I
1998	0·2	3·2/150·0	1·0	58·4/656·0	16·0	8·4/201·9	2·4	31·4/466·2	10·6
1999	0·4	3·1/141·9	1·0	62·7/670·8	18·5	6·0/159·5	2·0	35·8/478·4	12·2
2000	0·6	6·6/209·9	2·5	72·9/693·6	19·0	12·5/221·3	3·6	39·1/438·3	13·0
2001	0·7	8·9/190·4	3·0	75·1/608·4	19·4	14·3/285·3	3·7	58·4/431·0	17·7
2002	1·1	17·0/190·1	5·8	84·1/578·6	23·4	16·4/253·1	4·0	39·9/383·7	13·2
2003	1·5	23·7/214·1	9·0	88·3/564·2	24·1	18·5/218·0	5·7	55·7/355·2	18·6
2004	2·0	24·5/177·9	9·5	99·6/525·3	26·8	21·3/236·0	6·8	56·3/397·1	18·0
2005	2·6	32·1/189·0	12·7	105·2/521·4	29·6	16·9/215·1	5·0	45·2/410·0	14·6
2006	2·4	22·8/181·7	9·0	91·6/541·7	25·8	13·4/200·4	4·0	28·1/346·7	9·5
2007	1·7	22·4/211·4	7·5	56·2/467·4	15·0	11·9/220·4	3·7	25·5/339·8	8·1
Regression statistics		B1 v A	C v B1	D1 v C	E v D1		G v F1	H1 v G	I v H1
slope		11·5 ± 1·30	0·4 ± 0·02	3·3 ± 0·91	0·3 ± 0·02		0·3 ± 0·03	5·3 ± 2·26	0·3 ± 0·01
intercept		1·4 ± 2·00	–0·4 ± 0·30	59·0 ± 6·55	–0·5 ± 1·32		–0·2 ± 0·44	20·0 ± 9·73	1·0 ± 0·63
R^2		0·907	0·988	0·628	0·974		0·928	0·404	0·982

[a] Percentage of plants with virus symptoms.

[b] Numbers of vector *Laodelphax striatellus* transmitting RBSDV from bioassay tests/total population estimates (m^{-2}).

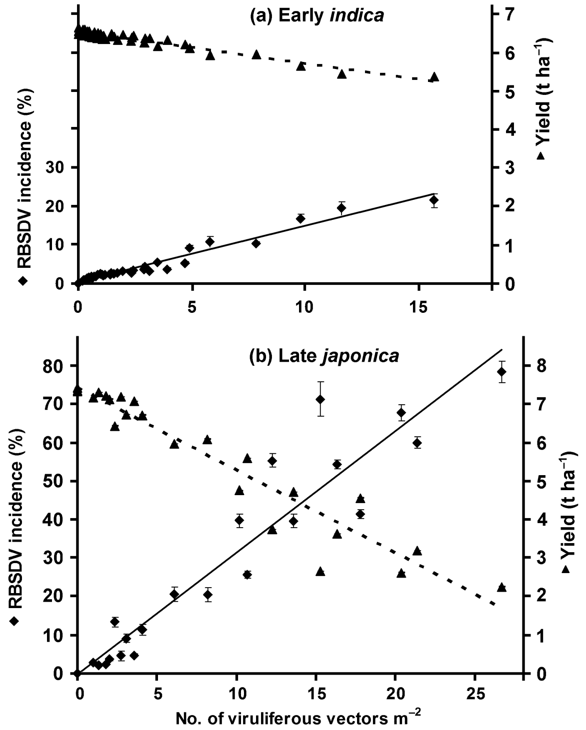

Figure 3 Diagrams showing incidence of *Rice black-streaked dwarf virus* (RBSDV) (diamonds and solid line) and yield (triangles and broken line) in plots of rice inoculated at the three-leaf stage with different numbers of viruliferous vectors. Results are from experiments at Linhai with (a) early *indica* (cv. Zhe 733) or (b) late *japonica* (cv. Shanyou 10) rice and show the combined data from each of the years 2004–06. Bars show the standard errors of each mean.

linear relationships between numbers of viruliferous vectors applied at the seedling stage and the subsequent incidence of disease after transplanting (early *indica*: $y = 1{\cdot}4498x + 0{\cdot}3688$; $R^2 = 0{\cdot}9601$; late *japonica*: $y = 3{\cdot}1627x - 0{\cdot}297$; $R^2 = 0{\cdot}9101$), which was reflected in the grain yields obtained at harvest (Fig. 3). With similar densities of viruliferous vectors, considerably more disease developed in the late *japonica* rice than in the earlier crop. Yields decreased in proportion to disease levels, falling from around 7 t ha^{-1} in the absence of vectors to 3 t ha^{-1} or less in the most severely affected late *japonica* crops.

Effects of planting date on disease incidence and yield

In each of the years 2004–06, sowing date had a large effect on disease incidence and subsequent grain yields (Fig. 4). In both the early *indica* and the late *japonica* crops, the non-treated plots from the earliest sowing dates had the most disease (often around 30%) and suffered the greatest yield losses (usually ≥ 20% and always highly significant in ANOVA tests). Insecticide sprays completely prevented disease development and so provided appropriate controls at each sowing date and in both rice crops. Disease incidence and yield losses decreased as sowing was delayed; at the last sowing date (25 days after the first), disease incidence was < 4% and losses were always < 2% (and sometimes statistically insignificant). Within each year and rice crop, yields from the treated plots did not differ from one another, indicating that there was no yield penalty from the later sowing dates.

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. In both rice crops, the relationship was linear over the disease levels obtained in these experiments (Fig. 5) and was highly significant in regression analyses. The fitted regression lines for y (percentage yield loss) against x (RBSDV incidence) were:

$$\text{early } \textit{indica} \text{ rice}: y = 0\cdot80(\pm0\cdot014)x - 0\cdot03(\pm0\cdot147); \quad R^2 = 0\cdot949$$

$$\text{late } \textit{japonica} \text{ rice}: y = 0\cdot92(\pm0\cdot087)x - 0\cdot65(\pm0\cdot269); \quad R^2 = 0\cdot990$$

The slopes of these lines differed significantly ($P < 0{\cdot}001$), suggesting that at similar disease levels, the late crop suffered a slightly higher yield penalty (0·92% for every 1% increase in disease incidence) than the early one (0·80%).

Cultivar susceptibility

Results of the cultivar field tests are shown in Table 2. There were fewer vectors and less disease in 2007 than in the earlier years. There were no significant differences in vector numbers between the cultivars in any year, showing that differences in disease incidence were not the result of selective feeding by the planthopper vectors. Cultivars showed highly significant differences in susceptibility to the virus ($P < 0{\cdot}001$ in ANOVA). The late *japonica* cultivars were consistently the most susceptible, with virus incidence exceeding 20% in 2005 and 2006, whereas the hybrid cultivars were much more resistant, with Xieyou 963 always having the least disease (< 4%).

Susceptibility of rice plants at different growth stages to RBSDV infection

In the pot experiments in which seedlings were inoculated at different growth stages, disease developed only on inoculated seedlings and not on control plants. There were large and statistically highly significant differences in disease incidence depending on the plant growth stage when they were inoculated. Young seedlings, particularly those at the three- to four-leaf stage, were the most susceptible (disease incidence > 50%), while plants inoculated after the end of tillering developed few or no symptoms (Table 3).

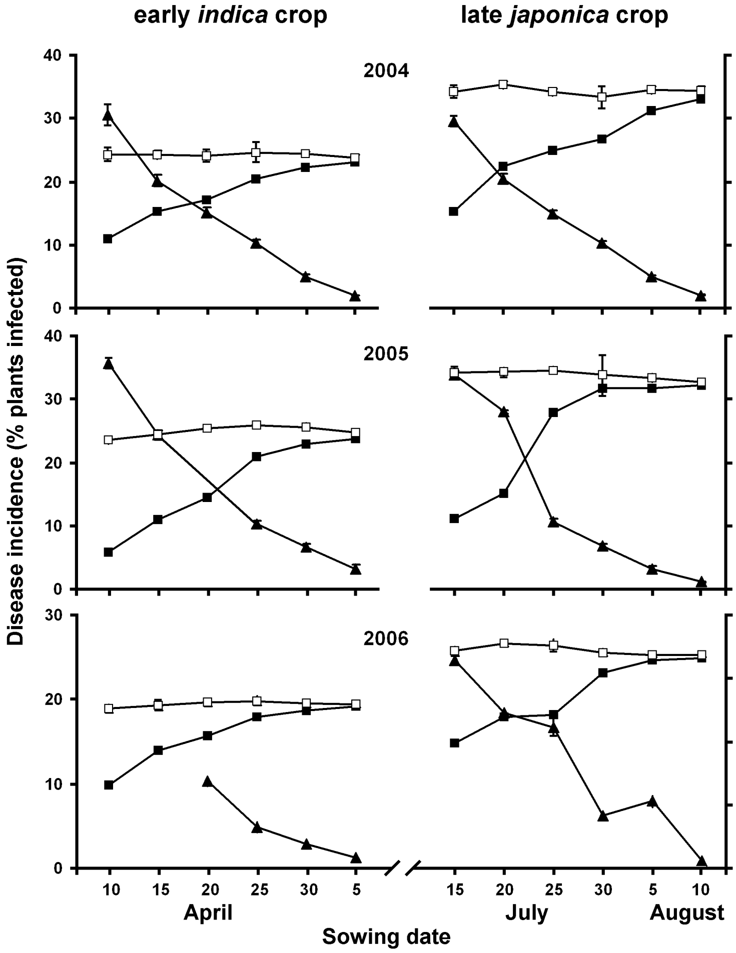

Figure 4 Diagrams showing incidence of *Rice black-streaked dwarf virus* (RBSDV) (diamonds) on non-treated rice crops (early *indica* cv. Zhe 733 or late *japonica* cv. Shanyou 10) sown on different dates at Linhai in each of the years 2004–06. Yields of insecticide-treated (open squares) and non-treated (solid squares) crops are plotted on the secondary axis. Bars show the standard errors of each mean.

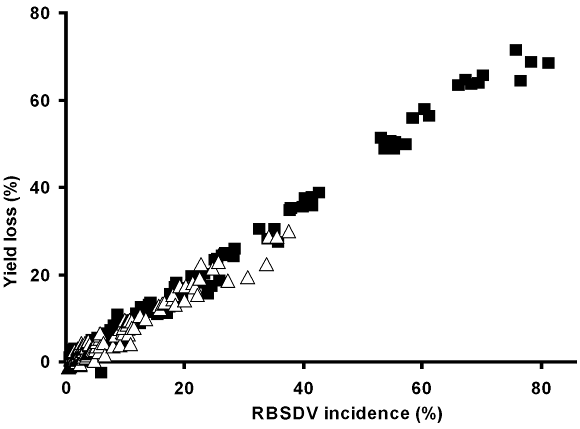

Figure 5 Relationships between *Rice black-streaked dwarf virus* (RBSDV) incidence and yield loss in plots of early *indica* (cv. Zhe 733, open triangles) or late *japonica* (cv. Shanyou 10, solid squares) rice from sowing-date and vector-inoculation experiments at Linhai in each of the years 2004–06.

Discussion

The outbreak of rice black-streaked dwarf disease in China since about 1996 appears to have been the most serious and extensive that has been reported to date. This study documented its spread within Zhejiang province, but it is also becoming serious in neighbouring provinces, with about 200 000 ha affected in Jiangsu (Y. Zhou, Jiangsu Academy of Agricultural Sciences, Nanjing, China, personal communication), 35 000 ha in Jiangxi and 20 000 ha in Fujian. In the most severely affected years in Zhejiang province it was estimated that *c.* 120 000 t rice grain were lost. Because of its sporadic occurrence, it has not been the most extensively studied virus disease in rice and there are few reports of its epidemiology published in the English language. The data presented here demonstrate just how damaging the disease can be. Studies of the disease-incidence–yield-loss relationship suggest that RBSDV is even more damaging to *japonica* rice yields than *Rice stripe virus* (RSV) (0·92% in this study versus 0·80% for RSV reported by Wang *et al.* (2008) for every 1% increase in disease incidence), showing the importance of a better understanding of the various factors that contribute to epidemics.

It has already been recognized that the epidemiology of the disease, like that of other rice viruses, is closely linked to the life-cycle of its planthopper vector (Hibino, 1996). As temperatures increase in spring, plant hopper numbers on the winter cereal crop increase, mainly because

Table 2 Numbers of vectors and incidence of *Rice black-streaked dwarf virus* (RBSDV; percentage of plants infected) in unsprayed plots of 10 rice cultivars sown at Linhai in each of the years 2004–07

		Year											
		2004			2005			2006			2007		
Cultivar	type	Vector nos m^{-2}	RBSDV %	Arcsin (%)[a]	Vector nos m^{-2}	RBSDV %	Arcsin (%)[a]	Vector nos m^{-2}	RBSDV %	Arcsin (%)[a]	Vector nos m^{-2}	RBSDV %	Arcsin (%)[a]
Xieyou 963	hybrid	4·8	2·4	9·0	6·7	3·4	10·5	4·5	2·8	9·5	3·3	1·9	7·9
Xieyou 2	hybrid	5·2	4·6	12·4	6·4	6·3	14·5	4·4	5·9	14·1	3·7	3·7	11·2
Xieyou 92	hybrid	4·7	6·7	15·0	6·0	7·9	16·3	4·3	8·1	16·6	3·1	5·7	13·8
Shanyou 10	hybrid	4·7	7·6	16·0	6·0	7·7	16·1	4·3	8·4	16·9	3·2	5·8	13·9
Zhe 733	*indica*	5·6	7·6	16·0	6·5	10·2	18·6	4·1	9·2	17·7	3·7	5·5	13·6
Xieyou 46	hybrid	5·2	8·6	17·0	6·1	10·1	18·5	5·1	9·8	18·2	3·2	7·0	15·3
Xieyou 914	hybrid	5·6	12·6	20·8	6·7	15·0	22·8	5·5	14·4	22·3	3·3	8·6	17·1
Xiushui 63	*japonica*	5·1	18·6	25·5	6·5	22·2	28·1	4·7	20·2	26·7	3·7	13·9	21·9
Yuegeng 2	*japonica*	5·5	25·1	30·1	7·2	25·2	30·1	4·7	29·0	32·6	3·6	16·5	24·0
Shaonuo 119	*japonica*	5·1	26·0	30·7	6·7	27·2	31·4	4·8	35·2	36·4	3·9	14·5	22·4
SED[b]		0·40		0·55	0·46		0·61	0·25		0·56	0·30		0·58

[a]Means of transformed data [arcsin(√%)] to normalize the variance.
[b]From ANOVA for comparisons between cultivars, 18 d.f.

Table 3 Incidence of rice black-streaked dwarf disease developing on rice hybrid *japonica* cv. Shanyou 10 inoculated with viruliferous *Laodelphax striatellus* at different plant growth stages in each of the years 2000–06

	2000		2001		2002		2003		2004		2005		2006	
Plant growth stage	%	Arcsin (%)[a]	%	Arcsin (%)[a]	%	Arcsin (%)[a]	%	Arcsin (%)[a]	%	Arcsin (%)[a]	%	Arcsin (%)[a]	%	Arcsin (%)[a]
Two-leaf	43·3	41·2	40·0	39·1	33·3	35·3	38·3	38·2	38·3	38·2	38·3	38·2	30·0	33·2
Three-leaf	66·7	54·8	60·0	50·8	55·0	47·9	53·3	46·9	61·7	51·8	60·0	50·8	56·7	48·9
Four-leaf	60·0	50·8	58·3	49·8	53·3	47·0	50·0	45·0	56·7	48·9	58·3	49·8	51·7	46·0
Five-leaf	53·3	47·0	50·0	45·0	48·3	44·0	46·7	43·1	50·0	45·0	43·3	41·2	38·3	38·2
Start of tillering	16·7	24·0	15·0	22·6	13·3	21·1	13·3	21·3	15·0	22·8	16·7	23·7	10·0	18·0
End of tillering	5·0	10·5	3·3	8·6	3·3	6·1	3·3	8·6	3·3	6·1	5·0	10·5	6·7	14·8
Elongation	1·7	4·3	0·0	0·0	1·7	4·3	0·0	0·0	0·0	0·0	1·7	4·3	1·7	4·3
Heading	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
SED[b]		3·14		3·02		3·49		2·47		2·69		3·27		2·43

[a]Means of transformed data [arcsin(√%)] to normalize the variance.
[b]From ANOVA for comparisons between treatment means, 16 d.f.

overwintering insects migrate from weed hosts. Eggs are laid in early to mid-April, resulting in a peak of nymphs in early May at a time when the early *indica* rice is at a young seedling stage in nurseries or fields. The adult peak of the first generation was consistently around 20 May (Fig. 2) and RBSDV transmission at this time is responsible for the symptoms that appear in the rice after transplanting. A second generation of planthoppers at the end of June (peaking in mid-July), spread virus within the early crop and into the seedling nurseries of late *japonica* rice. Seedlings of late *japonica* rice, particularly the hybrid *japonica* types, remain in nurseries longer than those of *indica* rice; seeds are usually sown on about 18 June and seedlings transplanted in late July–early August. This provides many opportunities for large numbers of planthoppers to migrate from the early *indica* rice at heading stage to the nurseries before transplanting, and symptoms may appear at this stage. The numbers of planthoppers declines in the hot weather of July–August, but the insects become more active and numbers increase as temperatures decrease in late September to October. After harvesting of the late *japonica* rice, the vectors migrate first to grass weeds and volunteer rice plants and then to barley and wheat in November.

As reported before (Ruan *et al.*, 1981; Hibino, 1996), the second rice crop is the most severely affected by the disease. This is probably the result of a combination of factors, including the build up of virus inoculum on the first crop, the large number of vectors occurring in mid-summer, the planting of *japonica* seedling nurseries at a time that allows migration of large numbers of viruliferous vectors from the *indica* crop and the greater susceptibility of many of the *japonica* cultivars. In Asia and the Americas it is the *japonica* rice types that are mostly susceptible to the prevalent viruses, whereas in Africa, where the most serious disease is caused by *Rice yellow mottle virus* (genus *Sobemovirus*), the *indica* types are more severely damaged (Abo & Sy, 1998).

Vector numbers, in general, have undoubtedly been increased because winter barley and wheat are now often sown directly into rice stubble without killing many of the weeds that are hosts of the vector. Economic pressures have also decreased the use of pesticides on the relatively low-value rice crop. In Zhejiang province, the recent practice of cultivating vegetables and flowers in plastic tunnels during the winter has also provided a good environment where insects can overwinter on grass weeds and then migrate to barley and wheat in the spring. Analysis of the meteorological data indicates that in recent years the December–January period was warmer and July was cooler than previously. This may explain why the overwintering populations of planthoppers in barley and wheat fields have been 10–20 times higher than those recorded in the 1980s. These factors have undoubtedly also contributed to the epidemic of RSV (genus *Tenuivirus*, also vectored by *L. striatellus*) which has affected the northern part of Zhejiang province during the same period (Wang *et al*., 2008). In this region, there is usually only one crop of (*japonica*) rice grown during the summer and it is interesting that the two viruses have flourished separately under different cropping systems. It is likely that the much greater resistance of *indica* rice cultivars to RSV provides a break in cropping for this virus and has resulted in its restriction to the northern parts of Zhejiang.

Laodelphax striatellus is not generally a long-distance migrant, but completes its life cycle locally on a variety of graminaceous plants. Many of these are also susceptible to RBSDV and therefore the disease tends to build up within an area once an infection has been established. This was clearly seen in the correlations between disease levels and numbers of viruliferous vectors in successive crops (Table 1). Viruliferous vectors may first be introduced to a region by flooding or by the transport of infected seedlings (e.g. for plant breeding).

The experiments in this paper demonstrated that rice plants at different stages of growth differ greatly in their susceptibility to RBSDV. Those at the two- to five-leaf stage were most susceptible, and both the *indica* and *japonica* rice crops reach this stage at times when the vector population is at its largest and most active. For this reason, a delay in sowing of either crop can substantially avoid infection. This would clearly be a very simple and effective method of control and does not appear to carry any yield penalty. For similar reasons, adjusting the sowing date has also been shown to have benefits in decreasing losses from RSV in a single rice cropping system using dry sowing in Korea (Bae & Kim, 1994) and with transplanted rice in northern Zhejiang province (Wang *et al*., 2008).

Since the late 1980s, new cultivars Zhe 733, Jiayu 293 (*indica* rice), Shanyou 10 and Yeyou 46 (hybrid *japonica* rice) have been released and grown on a large scale. These have proved to be more susceptible to RBSDV than older cultivars such as Guangluai 4 and Erjoufeng (*indica* rice) and Shanyou 6 (hybrid *japonica* rice). This has no doubt contributed to the recent epidemic, but there are clearly possibilities within currently available germplasm to use more resistant cultivars to help contain the disease (Table 2). The deployment of resistant cultivars generally offers the best prospects for the control of most rice virus diseases (Calvert *et al*., 2003).

The severe epidemic in Zhejiang province has led to an increased awareness of the disease and renewed efforts to control it. A combination of measures is being used, including the adoption of more resistant cultivars, improved weed control and the application of insecticides. The results of this study also suggest that delayed sowing could make an important contribution. As a result, there is now evidence that the epidemic has passed its peak, but continued vigilance will be necessary.

Acknowledgements

This work was financially supported by the State Basic Research Program of China (2006CB708209), the International Science and Technology Cooperation Project of the Ministry of Science and Technology of China (2007DFB30350), the State High-Tech Research and Development Program of China (2007AA10Z414), the National Natural Science Foundation of China (30771402) and the Natural Science Foundation of Zhejiang Province (Y307169). Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

References

Abo ME, Sy AA, 1998. Rice virus diseases: epidemiology and management strategies. *Journal of Sustainable Agriculture* **11**, 113–34.

Bae SD, Kim DK, 1994. Occurrence of small brown planthopper (*Laodelphax striatellus* Fallen) and incidence of rice virus disease by different seeding date in dry seeded rice. *Korean Journal of Applied Entomology* **33**, 173–7.

Calvert LE, Koganezawa H, Fargette D, Konate G, 2003. Rice. In: Loebenstein G, Thottappilly G, eds. *Virus and Virus-like Diseases of Major Crops in Developing Countries*. Dordrecht, the Netherlands: Kluwer Academic Press, 269–94.

Chen SX, 1996. Occurrence and research progress on rice viral diseases. *Zhejiang Agricultural Sciences 1996*, 41–2.

Hibino H, 1996. Biology and epidemiology of rice viruses. *Annual Review of Phytopathology* **34**, 249–74.

Hong JM, Zhang ZS, 1984. *Control of Pests and Diseases of Rice in Zhejiang*. Hangzhou, China: Zhejiang Science and Technology Press.

Kuribayshi K, Shinkai A, 1952. On the new disease of rice, black-streaked dwarf. *Annals of the Phytopathological Society of Japan* **16**, 41.

Lee J, Lee S, Chung B, 1977. Studies on the occurrence of rice black-streaked dwarf virus in Korea. *Korean Journal of Plant Protection* **16**, 121–5.

Lee K, Kim S, 1985. The effects of rice black-streaked dwarf virus on vector *Laodelphax striatellus* and host plants. *Korean Journal of Plant Pathology* **1**, 190–4.

Ruan YL, Chen SX, Lin RF, Jiang WL, Jin DD, 1984. Studies on rice black-streaked dwarf disease. *Zhejiang Agricultural Sciences 1984*, 185–7.

Ruan YL, Chiang WL, Lin RF, 1981. Studies on the rice vector small brown planthopper *Laodelphax striatellus* Fallén. *Acta Entomologica Sinica* **24**, 283–90.

Shikata E, 1974. *Rice black-streaked dwarf virus*. Wellesbourne, UK: Association of Applied Biologists: CMI/AAB Descriptions of Plant Viruses No. 135.

Shinkai A, 1962. Studies on insect transmission of rice virus diseases in Japan. *Bulletin of the National Institute of Agricultural Sciences, Series C* **14**, 1–112.

Shinkai A, 1967. Transmission of stripe and black-streaked dwarf viruses by a new planthopper. *Annals of the Phytopathological Society of Japan* **33**, 318.

Wang H-D, Chen J-P, Zhang H-M *et al*., 2008. Recent *Rice stripe virus* epidemics in Zhejiang province, China, and experiments on sowing date, disease–yield loss relationships, and seedling susceptibility. *Plant Disease* **92**, 1190–6.