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Spread and yield loss mechanisms of rice stripe disease in rice paddies

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Abstract

Rice stripe disease is an economically important disease of rice caused by the *Rice stripe virus* (RSV), which is transferred by the small brown planthopper (SBPH). The recent rapid increase in damage to rice crops throughout Japan caused by this disease makes it imperative to develop control methods as soon as possible. To obtain basic data for developing such methods, we studied how the disease causes damage and spreads within paddy fields. Our investigations revealed that diseased plants first appear in mid-June to early July, after which the disease spreads from affected plants to adjacent plants. This suggests that SBPH carrying RSV enter paddy fields, where they infect plants as they move about and lay eggs. Subsequently, hatched viruliferous nymphs infect surrounding plants, thereby spreading the disease. Our analysis of the damage caused by rice stripe disease showed that the earlier the onset of disease, the more extensive the damage caused, and that the disease reduces yield by reducing the number of healthy panicles. This suggests that to reduce damage caused by this disease, it is necessary to ensure the growth of a sufficient number of healthy panicles by controlling the vector insect during the crop's early growth period. To be most effective, pest control efforts should be timed to target either the first-generation adults that colonize the paddy fields or the second-generation nymphs and adults that cause the rapid increase in the number of diseased plants within a field.

Key words: damage analysis, rice, rice stripe disease, small brown planthopper, yield loss

1. Introduction

Rice stripe disease is one of the most serious viral diseases affecting rice (*Oryza sativa* L.) crops in Japan, South Korea, and China. The disease is caused by the *rice stripe virus* (RSV, Toriyama 1983), in the genus *Tenuivirus* (Shirako et al. 2011), which is persistently transmitted by the small brown planthopper (SBPH, *Laodelphax striatellus* (Fallén)) and is passed to the next generation by transovarial transmission (Hibino 1996; Toriyama 1983). In Japan, RSV caused widespread damage from the 1960s to the 1980s, but was brought under control from the late 1980s through control of the vector insect, increased use of RSV-resistant rice cultivars, and other measures (Hibino 1996). However, in recent years, rice stripe disease has returned with a vengeance in the Kanto region (the east-central area of Japan's main island), the Kinki region (the west-central area of Japan's main island), and the Kyushu region (southwestern Japan) (Shiba et al. 2016; Yoshida et al. 2014). Serious outbreaks have also been reported in China and South Korea (Jonson et al. 2009; Wang et al. 2008). It is not yet known why this disease has re-emerged in East Asia, but suspected causes include the development of pesticide resistance by SBPH (Sanada-Morimura et al. 2011), climate change (Yamamura and Yokozawa 2002), mass immigration of SBPH from overseas (Otuka et al. 2010, 2012), and changes in the cropping systems and environments surrounding production areas.

Susceptibility to RSV in rice varies widely with growth stage (Adachi and Yamada 1968, Hibino 1996, Wang et al. 2008). Rice in the early vegetative phase (from planting

61 to the early tillering stage) is highly susceptible to RSV. Leaves of tillers infected
62 during this period develop a mosaic of light yellow or yellow-green lesions along their
63 veins, and new leaves curl and droop instead of fanning out. The majority of tillers that
64 show these symptoms wilt without heading. In the late vegetative phase (the late
65 tillering stage), susceptibility to RSV declines, and wilting due to infection does not
66 occur. However, infected tillers cannot head normally; instead, they produce deformed
67 panicles. Plants in the reproductive phase following panicle initiation are less
68 susceptible to infection, and even if they are infected, symptoms are not severe.
69 The typical SBPH life cycle in areas of Japan prone to rice stripe disease is
70 described by Shiba et al. (2016). Nymphs overwinter in patches of grass, and adults of
71 the overwintering generation emerge in spring and move to adjacent wheat fields to
72 propagate. Adults of the next generation (first generation) colonize paddy fields after
73 rice seedlings have been planted. After three or four generations in the paddy fields,
74 adults move to nearby grassy areas during the harvest season to lay eggs, and the next
75 generation overwinters as nymphs. Because wheat is an ideal SBPH food source, SBPH
76 numbers are liable to increase in areas where wheat is grown, and rice stripe disease
77 tends to occur more frequently in these areas.
78 Research on the epidemiology and control of rice stripe disease in Japan was
79 carried out intensively from the 1960s to the 1980s, but since then, factors that affect
80 rice stripe disease epidemiology such as the cultivar, cropping system, and surrounding

environment have changed substantially, rendering much of the knowledge gained in that period inapplicable. With rice stripe disease once more becoming pervasive in Japan, we launched a comprehensive research project to develop control techniques aimed at early containment of outbreaks. We have previously reported that measurements of the effective cumulative temperature can be used to accurately predict the appearance of SBPH in paddy fields (Hirae and Shiba 2016), and that the elimination of rice ratoons and of grass near paddies after harvest is critical to suppressing the disease (Shiba et al. 2016). Here, we report on the mechanism by which rice stripe disease causes damage to infected rice plants, and how the disease spreads through paddy fields. This is essential information to developing effective control techniques against the current outbreak of rice stripe disease.

2. Materials and Methods

2.1 Test plots

From 2012 to 2014, we conducted experiments in Nikinari, a district of Chikusei City, Ibaraki Prefecture, in Japan's Kanto region (36°17′N, 139°58′E), where rice stripe disease occurs every year. We planted seedlings of 'Koshihikari' (which is susceptible to RSV), Japan's most widely grown cultivar of rice, in two paddy fields in each year. In 2012, Fields A and B each covered approximately 3000 m^2 and were 65 m apart at

100 their closest points. In 2013, Fields C and D each covered approximately 7000 m^2 and
101 were 60 m apart at their closest points. In 2014, Fields E and F each covered
102 approximately 3000 m^2 and were 100 m apart at their closest points. The seedlings were
103 planted 24 cm apart in rows 30 cm apart. Each field was planted in mid-May (15 May
104 2012, 17 May 2013, 14 May 2014) and harvested in early to mid-September (12
105 September 2012, 18 September 2013, 9 September 2014). No pesticides were applied
106 during cultivation in each of the test plots. In 2012, we established rectangular plots of
107 30 rows with 73 plants per row in each field, and also selected individual plants within
108 each plot for detailed observation. Every fifth plant in every third row was designated as
109 a fixed-point-survey plant, for a total of 15 such plants per row in 10 rows. Two of those
110 plants in Field A failed to survive. Thus, the fixed-point-survey for Field A included
111 only 148 plants, compared with 150 in Field B. In the same manner, we established
112 rectangular plots of 30 rows with 50 plants per row in each field and designated 99 or
113 100 fixed-point-survey plants within each plot in 2013 and 2014.
114 In the experimental area, first-generation SBPH adults colonized the survey fields
115 in mid-June, second-generation nymphs emerged in the paddy fields from late June to
116 early July, and third-generation nymphs emerged from late July to early August
117 according to estimates based on the measurements of the effective cumulative
118 temperature obtained from JPP-NET (Japan Plant Protection Agency, Tokyo, Japan).
119 Rates of virus-infected first-generation adults of SBPH collected in rice paddies were

120 3.2% in 2012 (Shiba et al. 2016), 4.7% in 2013 (Shiba et al. 2016), and 16.8% in 2014
121 (Ibaraki Control Station for Pests 2014).
122
123 **2.2 Disease surveys**
124 In 2012, we investigated all plants in the survey plot in Field A to detect the presence of
125 diseased plants on 11 July (the panicle initiation stage), on 8 and 9 August (the
126 flowering stage), and on 4 and 5 September (immediately before harvest). In addition,
127 on the fixed-point-survey plants, we counted the numbers of total, diseased, and healthy
128 panicles during the survey in early August. In Field B, we investigated disease
129 incidence among the fixed-point-survey plants and the surrounding 8 plants on the same
130 dates as the Field A surveys. As in Field A, we also counted the number of total,
131 diseased, and healthy panicles of the fixed-point-survey plants in Field B in early
132 August. We judged plants to be diseased if they showed typical rice stripe disease
133 symptoms, such as wilted new leaves, mottled leaves, or deformed panicles. We
134 categorized diseased plants identified during the early July survey as “mid-June to
135 early-July onset” plants, those newly identified during the early-August survey as “mid-
136 July to early-August onset” plants, and those newly identified during the early
137 September survey as “mid-August to early-September onset” plants. Because the area
138 chosen for this study is almost entirely free of pests and diseases other than rice stripe

139 disease, we ignored the presence of other pests and diseases. In the same manner as in
140 2012, we investigated disease incidence on the fixed-point-survey plants in 2013 and
141 2014. Surveys were conducted on 11 and 12 July, 8 and 9 August, and 29 August 2013,
142 and on 10 July, 7 and 8 August, and 28 August 2014.

143

144 **2.3 Yield survey**

145 In 2012, we harvested all fixed-point-survey plants that developed rice stripe disease up
146 to harvest time, and evaluated the number of total, healthy, and diseased panicles, the
147 brown rice yield, the number of brown rice kernels, and the 1000-kernel weight of each
148 plant. We also randomly harvested half of the disease-free fixed-point-survey plants in
149 each plot and evaluated yield in the same manner. In cases in which a fixed-point-
150 survey plant was unlikely to yield a large enough sample for analysis, we also harvested
151 surrounding plants. The above measurements were taken after harvesting individual
152 plants from the survey fields and drying them naturally for a month inside field cages.
153 In conformity with Japanese survey standards for paddy rice yield (Hosaka 2014), any
154 brown rice grains with a diameter of ≤1.69 mm were excluded from the survey.

155

156 **2.4 Statistical analysis**

157 We conducted two-way ANOVA for brown rice yield, total number of panicles, the

158 number of healthy panicles, and 1000-kernel weight by survey field, disease onset
159 period, and their interaction. When two-way ANOVA showed the disease onset period
160 to have a significant effect, we performed the Tukey–Kramer HSD test as a *post-hoc*
161 test. To analyze the relationship between the number of healthy panicles and brown rice
162 yield, we conducted simple regression analysis of yield on the number of healthy
163 panicles for each disease onset period. We used Pearson's correlation coefficient to
164 analyze the relationship between the number of panicles at the flowering stage and at
165 harvest, and conducted paired *t*-tests to confirm that the difference in the number
166 between flowering and harvest was significant. To investigate how the disease spreads,
167 we performed spatial autocorrelation analysis using join-count statistics (Cliff and Ord
168 1981, Plant 2012) on the data from the 30-row × 73-plants-per-row survey plot in Field
169 A, in which all plants were checked for disease. We used the *spdep* package (Bivand et
170 al. 2013) for version 3.3.3 of the R statistical software (R Core Team 2017) for the join-
171 count statistical analyses, and version 12.2.0 of the JMP software (SAS Institute, Cary,
172 NC, USA) for the other analyses.
173

174 # 3. Results

175 ## 3.1 Change in disease incidence in survey fields

176 Figure 1 shows the change in disease incidence over time among the fixed-point-survey

177 plants in the two study fields from 2012 to 2014. In 2012, disease incidence in Field A
178 increased remarkably, from 6.7% in the early-July survey (at the panicle initiation
179 stage) to 57.3% in the early-August survey (at the flowering stage), to 68.0% by harvest
180 time. Although Field B was less severely affected, disease incidence showed the same
181 trend, rising rapidly from 2.0% in early July to 34.7% in early August and then
182 gradually to 41.3% in early September (at harvest). In 2013 and 2014, the incidences of
183 diseased plants in early July were higher than in 2012 (44.0%, 20.0%, 35.4%, and
184 52.0% in Fields C, D, E, and F, respectively), and the disease spread quickly throughout
185 the test plot by early August (reaching 98.0%, 93.0%, 96.0%, and 96.0% in Fields C, D,
186 E, and F, respectively). As a result, the percentages of diseased plants plateaued in late
187 August (at 100%, 97.0%, 100%, and 97.0% in Fields C, D, E, and F, respectively).
188 Most diseased plants showed typical rice stripe disease symptoms, with new leaves in
189 the early-July survey drooping instead of fanning out, or showing mottle symptoms, and
190 most of the diseased plants newly identified in the early-August and with early-
191 September surveys showing deformed panicles.
192
193 **3.2 Spatial autocorrelation among the plants that developed rice stripe disease**
194 Of the 2181 plants (the total after excluding 9 missing plants) surveyed in Field A in
195 2012, 6.8% were symptomatic in the early-July survey, and 55.8% were symptomatic in

196 the early-August survey (Fig. 2). We conducted spatial autocorrelation tests using join-
197 count statistics to analyze the relationships among the diseased plants found in early
198 July (V), newly diseased plants found in early August (V2), and healthy plants found in
199 early August (H). The number of joins for V and V, for V2 and V2, and for V and V2
200 were significantly higher than the expected values based on the assumption of a random
201 distribution (Table 1). This means that the diseased plants identified in early July tended
202 to be spatially congregated, and that diseased plants newly identified in early August
203 tended to be distributed close to those identified in early July and to each other.
204

205 **3.3 Damage to plants affected by rice stripe disease**

206 We harvested both diseased and healthy plants from the fixed-point-survey plants in
207 Fields A and B to analyze disease damage. Because we were unable to obtain sufficient
208 diseased fixed-point-survey plants for analysis, we also harvested diseased plants
209 around the survey plants. In total, we harvested 146 plants from Field A (including 127
210 fixed-point-survey plants) and 113 plants from Field B (including 93 fixed-point-survey
211 plants). Table 2 shows the brown rice yield, number of brown rice kernels, brown rice
212 1000-kernel weight, number of panicles, and number of healthy panicles on these 259
213 plants for each disease-onset period and survey field.

214 ***3.3.1 Relationship between disease onset period and yield***

The earlier a plant developed disease symptoms, the lower was its yield. Two-way ANOVA showed that the disease onset period significantly affected brown rice yield (df = 3, SS = 5996.28, F = 27.53, $P < 0.001$), but that the survey field (df = 1, SS = 7.40, F = 0.10, $P = 0.750$) and its interaction with the disease onset period (df = 3, SS = 463.09, F = 2.13, $P = 0.097$) did not. *Post-hoc* Tukey–Kramer HSD tests showed that the brown rice yield of the early-July onset plants was significantly lower than that of plants that developed symptoms later and of plants that remained healthy, and that the yield of mid-July to early-August onset plants was higher than that of early-July onset plants but lower than that of healthy plants. No significant difference in brown rice yield was found between mid-August to early-September onset plants and plants that showed no symptoms (Fig. 3).

3.3.2 Relationship between disease onset period and 1000-kernel weight

Two-way ANOVA indicated that the survey field had a significant effect on the 1000-kernel weight (df = 1, SS = 6.89, F = 27.62, $P < 0.001$), but that the disease onset period (df = 3, SS = 0.02, F = 0.031, $P = 0.993$) and its interaction with the survey field (df = 3, SS = 0.86, F = 1.14, $P = 0.332$) did not.

3.3.3 Relationship between disease onset period and panicle numbers

Two-way ANOVA showed that the disease onset period had a significant effect on the total number of panicles (df = 3, SS = 653.14, F = 6.69, $P < 0.001$), whereas the survey

234 field (df = 1, SS = 36.99, F = 1.14, $P = 0.288$) and the interaction (df = 3, SS = 64.08, F
235 = 0.66, $P = 0.580$) did not. The *post-hoc* Tukey–Kramer HSD test showed that the total
236 number of panicles in the early-July onset plants was significantly lower than that of
237 plants that developed symptoms at other times, and that there was no significant
238 difference in the total number of panicles between mid-July to early-August onset
239 plants, between mid-August to early-September onset plants, and between plants
240 showing no symptoms (Fig. 4).

241 Two-way ANOVA showed that the disease onset period had a significant effect on
242 the number of healthy panicles (df = 3, SS = 3066.0, F = 27.17, $P < 0.001$), whereas the
243 survey field (df = 1, SS = 2.38, F = 0.06, $P = 0.802$) and its interaction with the disease
244 onset period (df = 3, SS = 108.28, F = 0.96, $P = 0.413$) did not. *Post-hoc* Tukey–
245 Kramer HSD tests confirmed that the earlier a plant developed symptoms, the lower the
246 number of healthy panicles it produced, and indicated that there was no significant
247 difference in the number of healthy panicles between mid-August to early-September
248 onset plants and plants that showed no symptoms (Fig. 4).

249 ***3.3.4 Relationship between the number of healthy panicles at harvest and brown rice***
250 ***yield***

251 Because the relationships between the disease onset period and brown rice yield or the
252 number of healthy panicles were unaffected by the survey field, we combined data from
253 both fields to conduct a simple regression of yield on the number of healthy panicles for

each disease onset period. This analysis confirmed that, regardless of the disease status or disease onset period, a greater number of healthy panicles at harvest time was associated with a greater brown rice yield (for mid-June to early-July onset plants: df = 1, SS = 5904.98, F = 757.47, $P < 0.001$; for mid-July to early-August onset plants: df = 1, SS = 6118.19, F = 512.35, $P < 0.001$; for mid-August to early-September onset plants: df = 1, SS = 1148.09, F = 136.75, $P < 0.001$; for plants with no symptoms: df = 1, SS = 2509.40, F = 134.13, $P < 0.001$). The resulting coefficients of determination for the regression equations were 0.943 for the mid-June to early-July onset plants, 0.804 for the mid-July to early-August onset plants, 0.825 for the mid-August to early-September onset plants, and 0.725 for plants that showed no symptoms, demonstrating that brown rice yield can be adequately explained solely on the basis of the number of healthy panicles at harvest, regardless of the disease status and disease onset period (Fig. 5).

3.4 Relationship between the number of panicles at flowering and at harvest

We used data for the 220 fixed-point-survey plants surveyed up to harvest (127 in Field A, 93 in Field B) to analyze the relationship between the number of panicles at flowering and at harvest: neither the total number of panicles nor the number of healthy panicles differed by survey field. Thus, we combined the data from both fields for this

273 analysis. Pearson's correlation coefficient for the relationship between the number of
274 healthy panicles at flowering and at harvest was 0.920 (95% confidence interval [CI] =
275 0.897 to 0.938), that for the number of diseased panicles at flowering and at harvest was
276 0.889 (95% CI = 0.857 to 0.914), and that for the total number of panicles at flowering
277 and at harvest was 0.870 (95% CI = 0.833 to 0.899), indicating strong and significant
278 positive correlations between the number of panicles at flowering and at harvest for
279 healthy, diseased, and total panicles (Fig. 6). The mean number of healthy panicles was
280 23.81 at flowering and 23.72 at harvest, versus 2.54 at flowering and 2.68 at harvest for
281 diseased panicles and 26.35 at flowering and 26.40 at harvest for the total number of
282 panicles. Paired *t*-tests showed that there was no significant difference between the
283 mean number of panicles at flowering and at harvest for healthy panicles (df = 219, t =
284 0.47, P = 0.638), diseased panicles (df = 219, t = –1.36, P = 0.175), and total panicles
285 (df = 219, t = –0.390, P = 0.697).
286

287 ## 4. Discussion

288 In 2012, rice plants infected with rice stripe disease started to appear in mid-June to
289 early July, after which the disease spread rapidly during the following month. In 2013
290 and 2014, the disease spread rapidly throughout the test plot by early August, and as a
291 result, the percentages of diseased plants in early August were much higher than those

292 in 2012. The reason for the high incidence of the disease in 2013 and 2014 was likely
293 the large number of first-generation adults of SBPH that migrated into the rice paddies
294 in mid-June. Even under such conditions, the patterns of spread of the disease
295 resembled that in 2012: diseased plants started to appear in mid-June to early July, and
296 then the number increased during the following month. As paddy-colonizing first-
297 generation SBPH adults appear in mid-June, second-generation nymphs appear in late
298 June to early July, and third-generation nymphs appear in late July to early August in
299 the study area, and as symptoms of rice stripe disease appear 10 to 15 days after a plant
300 has been infected with RSV (Shinkai 1962), we conclude that the diseased plants
301 observed in the early July were infected mainly by the first-generation SBPH adults,
302 and that the subsequently identified diseased plants, which increased rapidly in number
303 from mid-July to early August, were infected mainly by the second-generation nymphs
304 and adults. The third generation contributed little to the increase of diseased plants
305 because rice had entered its reproductive growth phase before these insects emerged in
306 the field, when rice is less susceptible to RSV. Furthermore, spatial autocorrelation
307 analysis using the detailed data from field A revealed that the mid-June to early-July
308 onset plants tended to be distributed close to each other, and that the mid-July to early-
309 August onset plants were congregated around the early-July onset plants. These
310 observations suggest that rice stripe disease spreads within a paddy field through the
311 following two-stage process: (i) first-generation adult SBPH carrying RSV colonize

312 paddy fields, where they infect rice plants as they move about and lay eggs; and (ii)
313 second-generation nymphs and adults emerging within the paddy field infect plants
314 adjacent to the previously infected plants. Most of the regions in Japan that are currently
315 affected by rice stripe disease share many characteristics with our study site in terms of
316 climate, cultivars, and cropping systems. Thus, the process by which rice stripe disease
317 spreads and that was elucidated in this study should prove useful when pest control
318 timing and methods are considered in other regions where this disease is prevalent.
319 The magnitude of the damage caused by rice stripe disease differs greatly with the
320 timing of disease onset: earlier onset results in significantly lower brown rice yield.
321 Similarly, earlier onset leads to a greater reduction in the total number of panicles and
322 the number of healthy panicles. The decreases in the number of healthy panicles and
323 brown rice yield were particularly dramatic in plants that developed disease symptoms
324 in mid-June to early July. Susceptibility to RSV in rice has been reported to vary widely
325 with growth stage (Hibino 1996, Wang et al. 2008), and our results confirm these earlier
326 results. Plants that develop the disease before panicle initiation not only suffer
327 considerable decreases in yield, but also become the starting points of new infections.
328 To reduce the damage caused by this disease, pesticide-based control must be used to
329 target the first-generation adults that are responsible for disease onset during this period.
330 The diseased plants that were newly identified in the early-August survey (i.e., that
331 developed the disease after the panicle initiation stage) suffered much less damage in

terms of the number of healthy panicles and brown rice yield than plants that developed the disease before the panicle initiation stage. The panicle initiation stage represents a midpoint in the growth of rice plants between vegetative and reproductive growth. Our results suggest that disease onset has less impact on yield once plants have entered the reproductive phase, which confirms previous results. However, even if the damage per plant is slight, the overall damage may be considerable because plants that develop disease symptoms during the reproductive phase account for a significant proportion of the total number of diseased plants in a field. Accordingly, pest control aimed at reducing damage caused by this disease should also target the second-generation nymphs and adults that cause disease onset after the panicle initiation stage. Diseased plants that were newly identified in the early-September survey after the flowering stage suffered even less damage, and no significant difference from healthy plants was observed in terms of the total number of panicles, the number of healthy panicles, or the brown rice yield. In addition, few plants develop disease after the flowering stage. Thus, we conclude that instances of the disease that developed after the flowering stage have no major impact on total rice yield. Pest control that targets plants after the flowering stage would therefore not be cost-effective and appears to be unnecessary.

We analyzed the relationship between brown rice yield and the number of healthy panicles. Our analysis demonstrates that yield can be adequately explained solely in terms of the number of healthy panicles at harvest, regardless of the disease status or

352 onset period. Furthermore, the 1000-kernel weight remained fairly consistent regardless
353 of the disease status or onset period. These results indicate that (i) a decrease in the
354 number of rice kernels associated with a decrease in healthy panicles is the direct cause
355 of decreased yield; (ii) damage caused by rice stripe disease can be estimated by
356 evaluating the number of diseased panicles at harvest time; and (iii) measures to
357 minimize the number of diseased panicles are vital to mitigating damage from the
358 disease.

359 The numbers of healthy and diseased panicles, and the total number of panicles,
360 changed very little from the flowering stage onward. Because rice does not produce new
361 tillers after the tillering stage, it is reasonable to expect that the total number of panicles
362 at flowering and at harvest will be the same. The numbers of healthy and diseased
363 panicles also changed very little from the flowering stage onward. This is likely due to
364 the rapid decline in susceptibility of rice to RSV after the panicle initiation stage. These
365 results indicate that the total number of panicles and the numbers of healthy and
366 diseased panicles at harvest can be predicted with a high degree of accuracy by
367 conducting surveys at the flowering stage. This means that although the damage caused
368 by rice stripe disease can be estimated by counting diseased panicles at the harvest
369 stage, the same assessment could be carried out earlier, at the flowering stage.

370 Controlling rice stripe disease requires integrated pest management that combines
371 pesticide-based control of insect vectors, planting of RSV-resistant cultivars, and paddy

372 field management designed to interrupt the infection cycle of the disease. RSV-resistant
373 cultivars can be developed by means of marker-assisted selective breeding using the
374 rice stripe disease resistance gene *Stvb-i* (Hayano-Saito et al. 1998, Sugiura et al. 2004).
375 In terms of paddy field management, elimination of rice ratoons by plowing paddy
376 fields after harvest and removal of grass from the banks of paddy fields have proven
377 effective in curbing rice stripe disease (Shiba et al. 2016). Our results suggest that in
378 addition to these measures, pesticide-based control that targets first-generation SBPH
379 adults that colonize paddy fields and the second-generation nymphs and adults born in
380 the paddy fields would also be effective in mitigating damage. Controlling the first-
381 generation adult vectors can be done by applying pesticides to seedling trays when
382 sowing the seeds or when transplanting the seedlings. This method can also be effective
383 against the second-generation nymphs and adults. However, since the effectiveness of
384 pesticides may be lost if pesticides with a short residual effect are used, it would be
385 advisable to apply additional pesticide as needed. We are now conducting field
386 demonstrations in various regions of Japan of integrated pest management based on the
387 ideas revealed in this study.
388

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397

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Figure legends

Fig. 1. Seasonal changes in the distribution of plants with rice stripe disease in the rice paddies. Each colored cell represents fixed-point-survey plants in each test plot. Rice plants were surveyed in early July (panicle initiation stage), early August (flowering stage), and late August or early September (full maturity). The numbers of surveyed plants were 148 in Field A, 150 in Field B, 100 in Field C, 100 in Field D, 99 in Field E, and 100 in Field F.

Fig. 2. Detailed distribution of plants with rice stripe disease in the test plot of Field A in 2012. Each cell represents one plant.

Fig. 3. Effect of disease onset period on brown rice yield per plant (g). Boxes show the median, 25th, and 75th percentiles; × shows the mean; ends of whiskers extend to the furthest point within the 1.5 interquartile range from the box; ○ outliers. Boxes marked with the same letter do not differ significantly (Tukey–Kramer HSD test, $P < 0.05$). The numbers of samples were 48 for mid-June to early-July onset plants, 127 for mid-July to early-August onset plants, 31 for mid-August to early September onset plants, and 53 for plants showing no symptoms.

Fig. 4. Effect of disease onset period on the total number of panicles and the number of healthy panicles. Boxes show the median, 25th, and 75th percentiles; × shows the mean; ends of whiskers extend to the furthest point within the 1.5 interquartile range from the box; ○ outlier. Boxes marked with the same letters do not differ significantly (Tukey–

493 Kramer HSD test: $P < 0.05$; A, B: total; a, b, c: healthy). The numbers of samples were
494 48 for the mid-June to early-July onset plants, 127 for the mid-July to early-August
495 onset plants, 31 for the mid-August to early September onset plants, and 53 for plants
496 showing no symptoms.
497 **Fig. 5.** Relationship between the number of healthy panicles and brown rice yield (g) in
498 four rice stripe disease onset periods. The numbers of samples were 48 for the mid-June
499 to early-July onset plants, 127 for the mid-July to early-August onset plants, 31 for the
500 mid-August to early September onset plants, and 53 for plants showing no symptoms.
501 **Fig. 6.** Correlations (Pearson's r) between the number of panicles at flowering and at
502 harvest. The number of samples in each plot was 220.
503

504

Table 1. Results of join-count analysis to assess spatial autocorrelation of healthy and diseased plants based on data shown in Figure 2.

	Number of joins [b]				
Combination [a]	Expected	Variance	Observed	*Z*-value	*P*-value [c]
H:H	1971.89	625.37	2323	14.040	<0.001
V:V	38.88	34.21	64	4.294	<0.001
V2:V2	1671.06	597.62	1893	9.079	<0.001
H:V	555.93	308.72	407	–8.476	1.000
H:V2	3634.09	1843.14	3076	–12.999	1.000
V:V2	511.80	299.69	630	6.828	<0.001

[a] H: healthy plant; V: mid-June to early-July onset plants; V2: mid-July to early-August onset plants.

[b] Number of joins in eight directions (orthogonal and diagonal directions) were counted for each combination listed. Expected: expected number of joins based on the null hypothesis of no spatial autocorrelation; Variance: variance of expected number of joins; Observed: observed number of joins.

[c] Weighted *P*-values from Bonferroni procedure for multiple tests of significance.

505

506

Table 2. Brown rice yield, brown rice kernel number, brown rice 1000-kernel weight, number of panicles, and number of healthy panicles on diseased and healthy rice plants.

Disease onset period	n [a]	Brown rice yield/plant (g) [b]	SE	Brown rice kernel No./plant	SE	brown rice 1000-kernel weight/plant (g)	SE	No. of panicles/ plant	SE	No. of healthy panicles/ plant	SE
Field A											
Mid-June – early July	29 (10)	24.68	2.07	1187.93	98.24	20.76	0.12	22.45	1.26	15.72	1.36
Mid-July – early Aug	78 (78)	35.87	0.91	1720.72	43.37	20.82	0.06	27.12	0.66	23.47	0.72
Mid-Aug – early Sept	16 (16)	36.34	1.47	1730.56	71.45	20.97	0.15	26.25	1.20	23.94	1.12
No symptoms	23 (23)	44.09	1.60	2104.91	80.01	20.91	0.12	28.30	1.28	28.30	1.28
Field B											
Mid-June – early July	19 (4)	27.51	2.80	1291.68	132.02	21.31	0.16	22.47	1.04	16.58	1.74
Mid-July – early Aug	49 (49)	34.81	1.05	1636.10	50.14	21.28	0.05	25.84	0.74	22.98	0.73
Mid-Aug – early Sept	15 (10)	38.24	2.00	1808.93	97.05	21.15	0.08	26.67	1.47	25.33	1.45
No symptoms	30 (30)	38.87	1.44	1837.87	69.27	21.22	0.07	25.67	1.06	25.67	1.06

[a] Values in parentheses show the number of fixed-point-survey plants.

[b] We evaluated brown rice yield, brown rice kernel number, and brown rice 1000-kernel weight of filled grains with a grain diameter of ≥1.70 mm at 15% moisture content.

507

508

Figure1

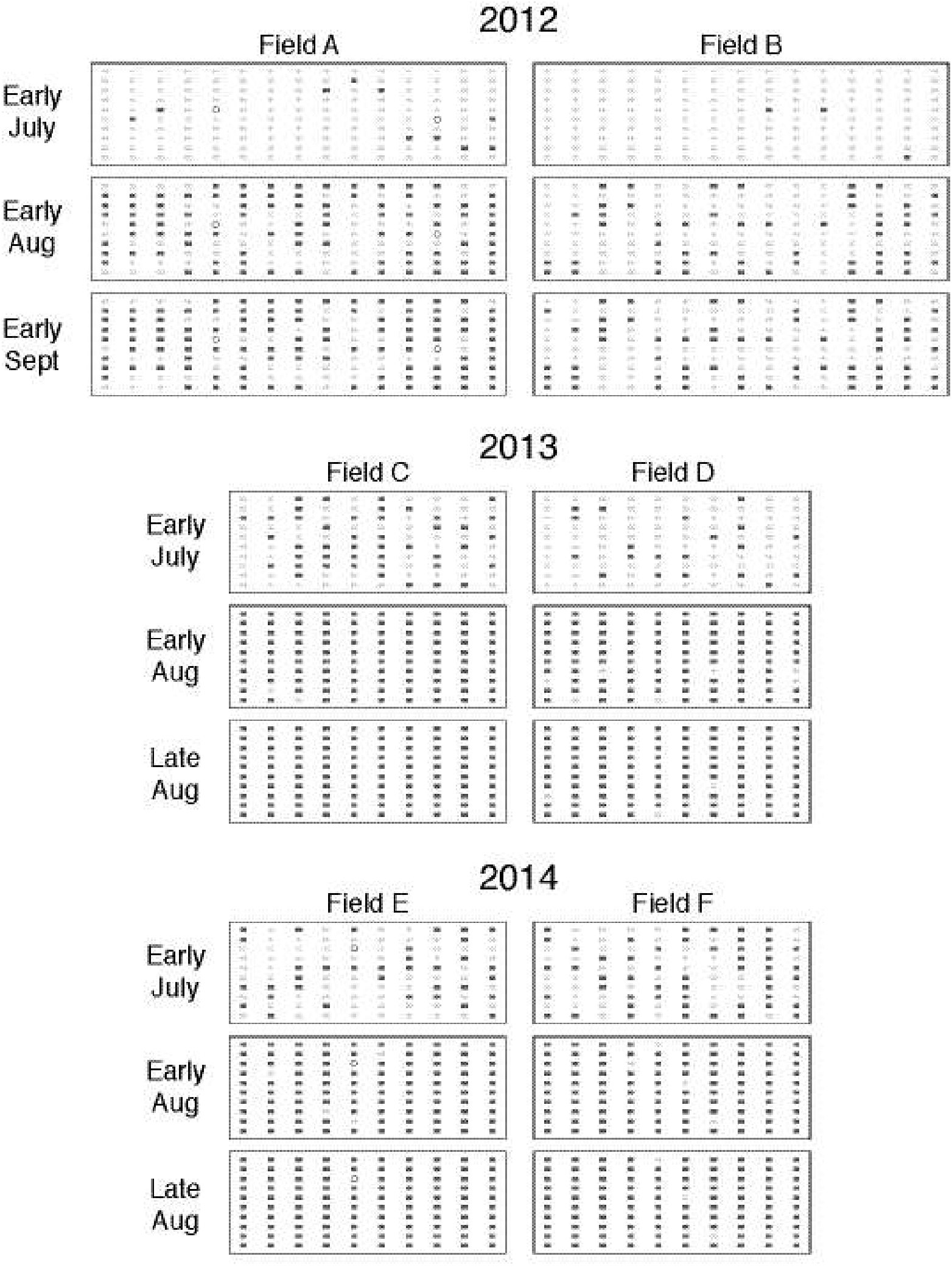

2012
Field A
Field B
Early July
Early Aug
Early Sept
2013
Field C
Field D
Early July
Early Aug
Late Aug
2014
Field E
Field F
Early July
Early Aug
Late Aug
Diseased plants
Healthy plants
Plant missing

Figure2

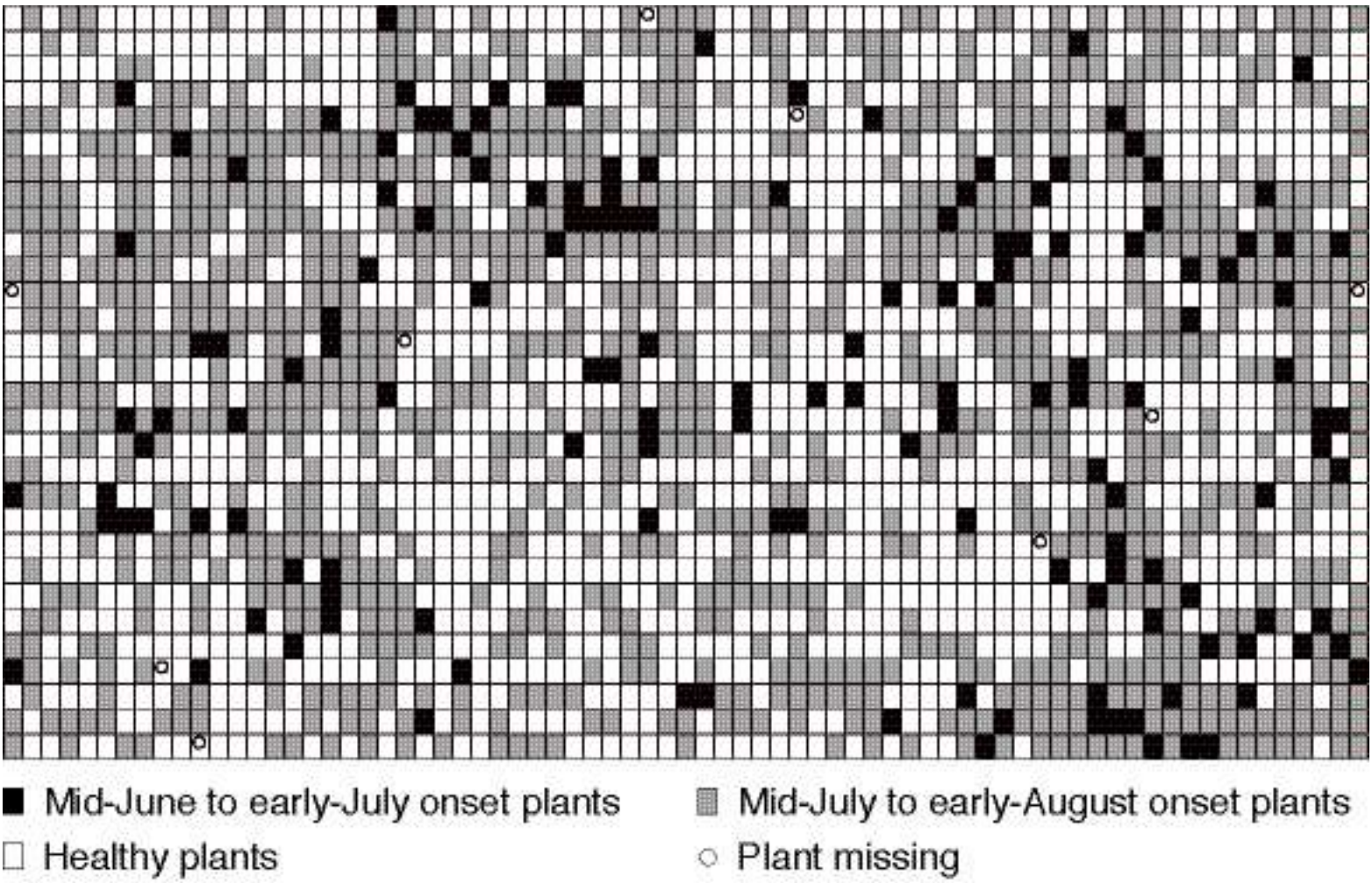

Mid-June to early-July onset plants
Mid-July to early-August onset plants
Healthy plants
Plant missing

Figure3

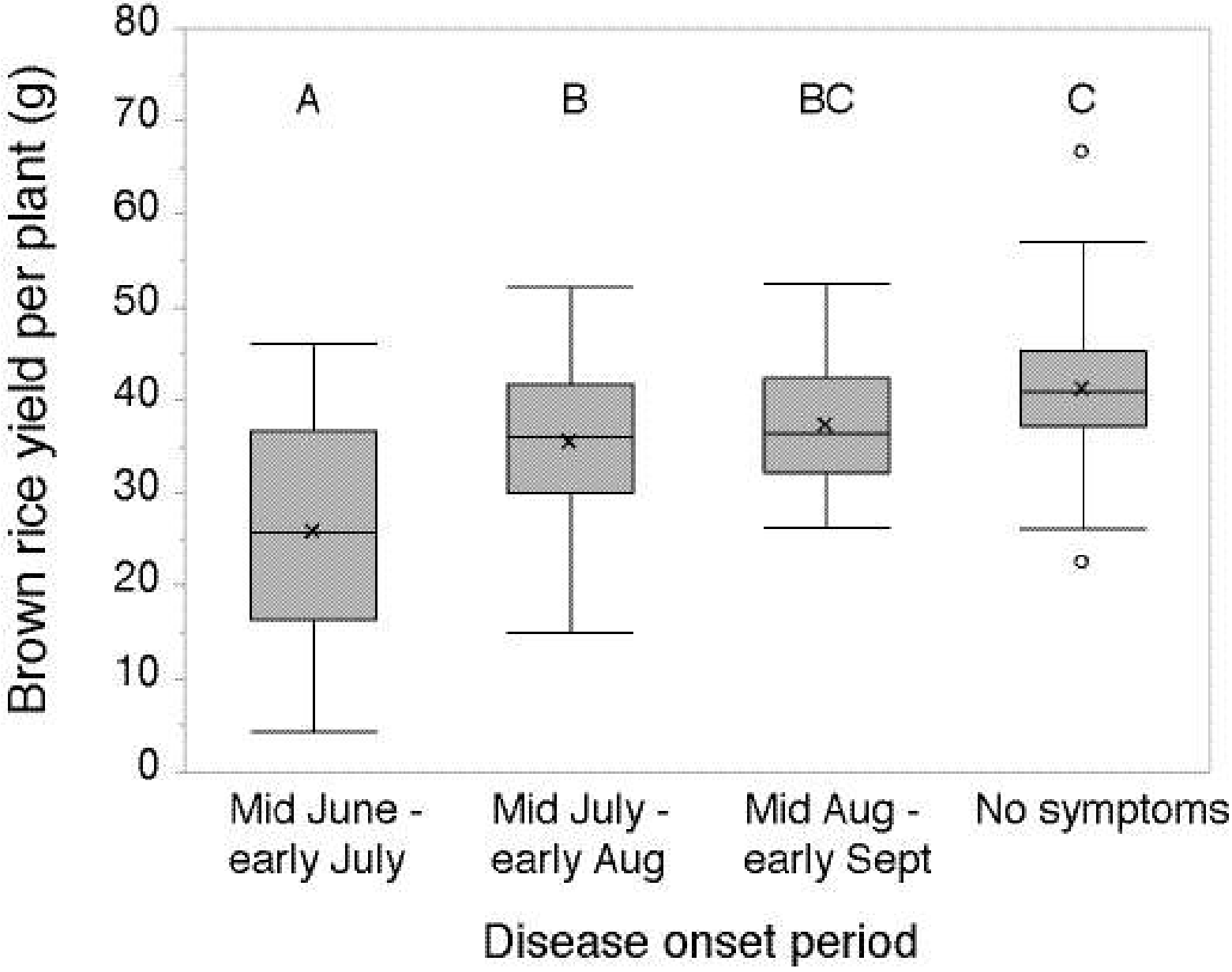

Brown rice yield per plant (g)
80
70
60
50
40
30
20
10
0
A
B
BC
C
Mid June - early July
Mid July - early Aug
Mid Aug - early Sept
No symptoms
Disease onset period

Figure4

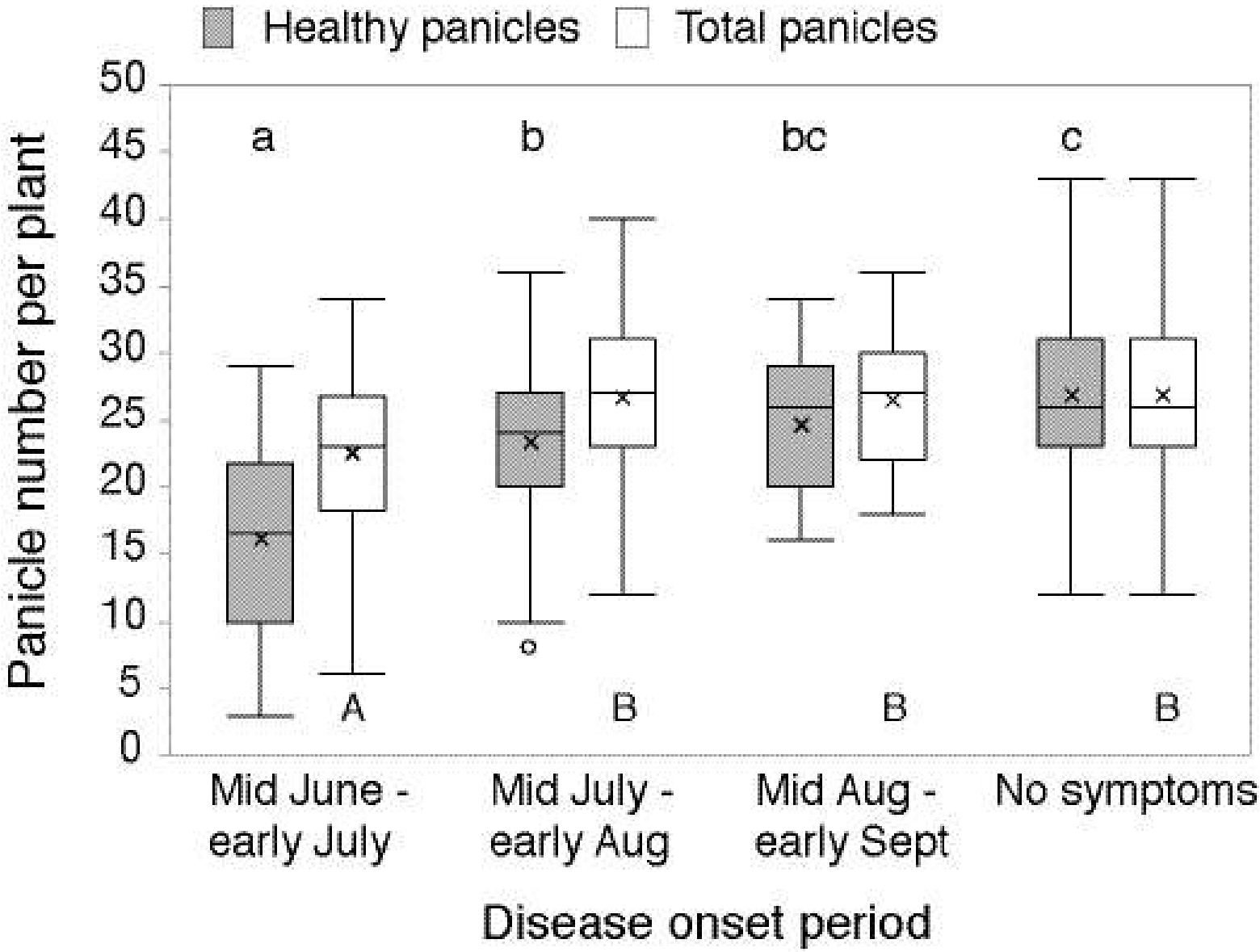

Healthy panicles
Total panicles
Panicle number per plant
50
45
40
35
30
25
20
15
10
5
0
a
b
bc
c
A
B
B
B
Mid June - early July
Mid July - early Aug
Mid Aug - early Sept
No symptoms
Disease onset period

Figure5

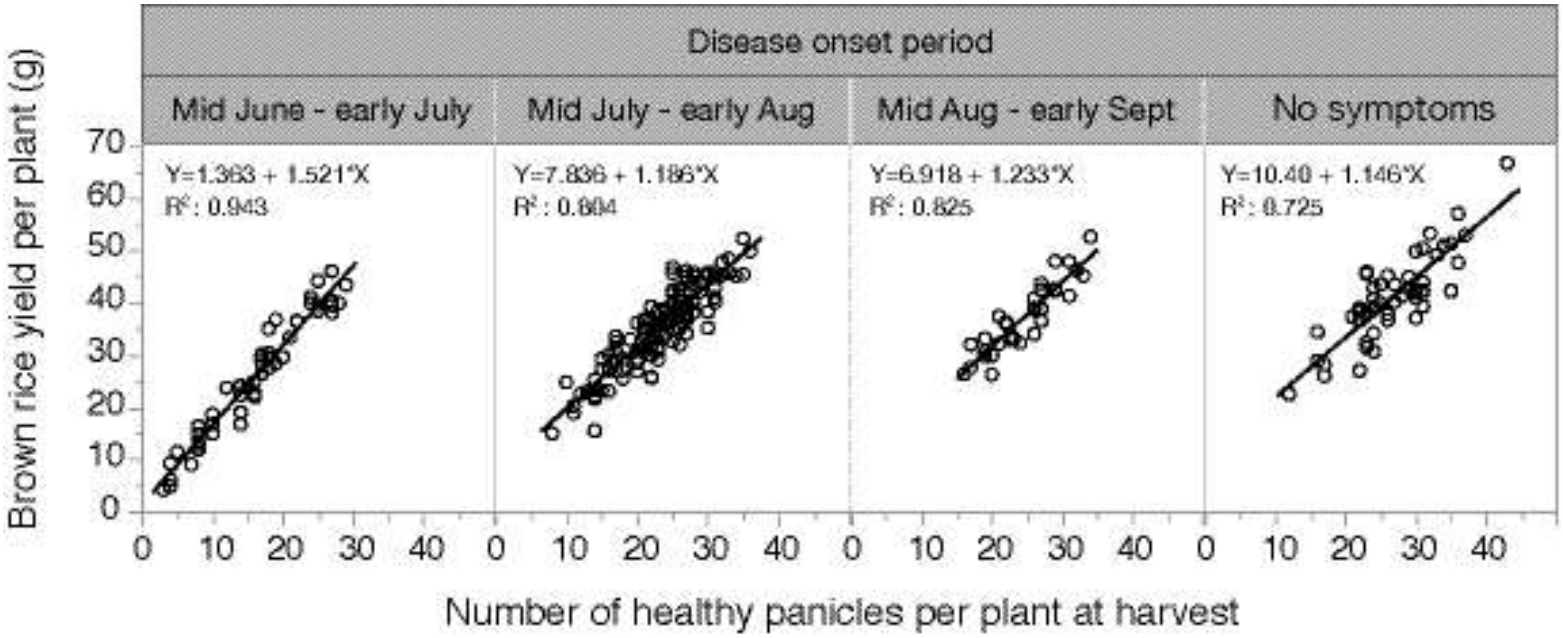
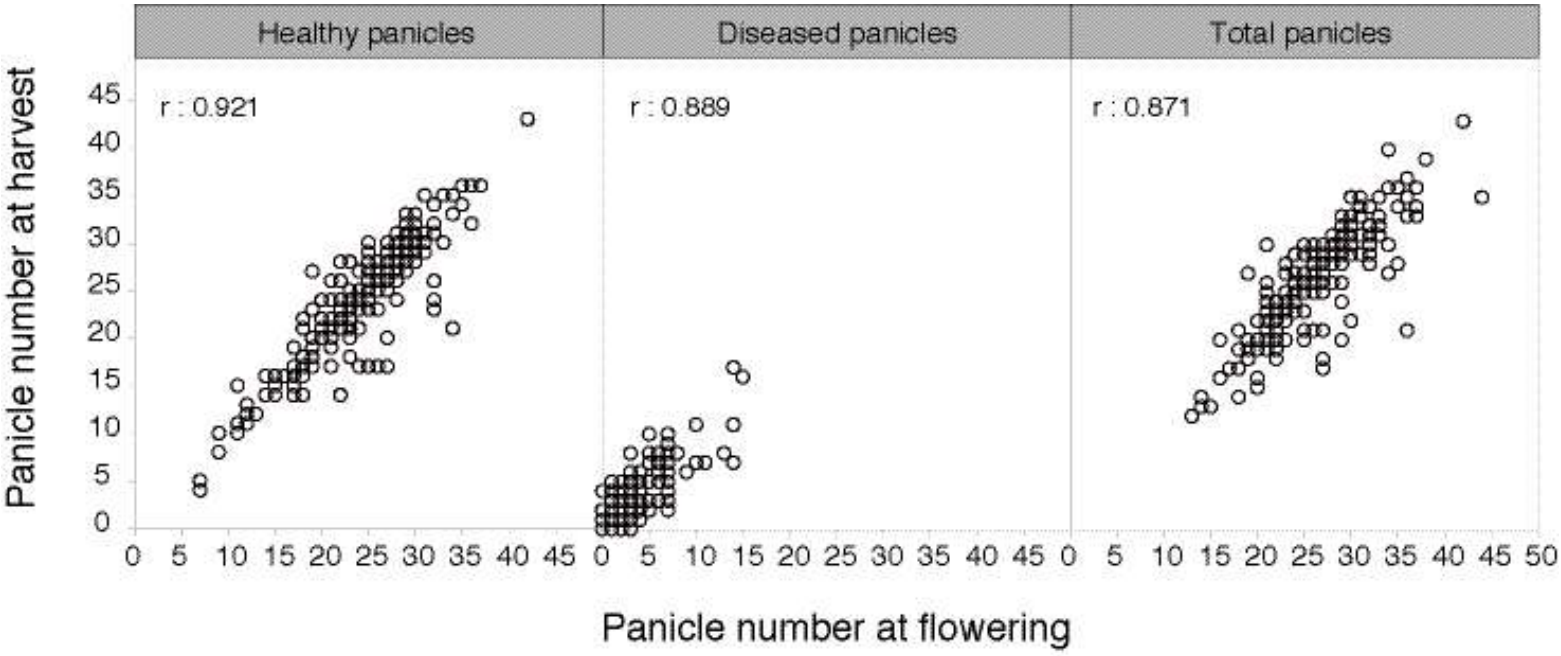

Disease onset period
Mid June - early July
Mid July - early Aug
Mid Aug - early Sept
No symptoms
Y=1.363 + 1.521*X
R²: 0.943
Y=7.836 + 1.186*X
R²: 0.804
Y=6.918 + 1.233*X
R²: 0.825
Y=10.40 + 1.146*X
R²: 0.725
Brown rice yield per plant (g)
Number of healthy panicles per plant at harvest
0
10
20
30
40
50
60
70

Figure6


Healthy panicles
Diseased panicles
Total panicles
r : 0.921
r : 0.889
r : 0.871
Panicle number at harvest
Panicle number at flowering
0 5 10 15 20 25 30 35 40 45
0 5 10 15 20 25 30 35 40 45
0 5 10 15 20 25 30 35 40 45 50