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# Suitability of poaceous plants for nymphal growth of the pecky rice bugs *Trigonotylus caelestialium* (Hemiptera: Miridae) and *Stenotus rubrovittatus* (Hemiptera: Miridae) in Niigata, Japan

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## Abstract

Under laboratory conditions, we investigated the host suitability of 24 poaceous plants for *Trigonotylus caelestialium* (Kirkaldy) and *Stenotus rubrovittatus* (Matsumura). More than 50 % of the nymphs of both bugs reached the adult stage on *Poa annua* L., *Alopecurus aequalis* Sobol. var. *amurensis* (Komar.), *Poa sphondylodes* Trin., *Hordeum murinum* L., *Agrostis clavata* Trin. ssp. *matsumurae* (Hack. ex Honda), and *Lolium multiflorum* Lam. In addition, a similar number of *S. rubrovittatus* nymphs reached the adult stage on *Dactylis glomerata* L. and *Digitaria violascens* Link. However, a high percentage of *T. caelestialium* adults emerged on both spikelets and leaves of the host plants, whereas a lower percentage of *S. rubrovittatus* adults emerged on leaves than on spikelets. While the numbers of *T. caelestialium* adults that emerged on spikelets and leaves were similar, those reared on spikelets had a shorter developmental period and longer forewings than those reared on leaves, indicating that spikelets were more suitable for growth. In addition, more adults of both species emerged on plants with ears that arise during the spring and early summer than on plants with ears that arise during the summer and fall. Therefore, the development of these bugs is dependent on the season of ear emergence.

**Keywords:** Pecky rice bugs · Heading stage · Nymphal growth · Poaceous weeds

## Introduction

Grain-feeding bugs cause major problems in rice (*Oryza sativa* L.) and other economically important grains on a global scale. When rice plants in paddy fields are attacked by heteropteran bugs, rice grains (at the milk and dough stages) are susceptible to invasion by bacteria and fungi, resulting in spotted rice. These spotted rice grains are termed pecky rice (Douglas and Tullis 1950; Ingram 1927; Ito 1978). Currently, heteropteran bugs are a major problem for rice farmers in Japan because pecky rice reduces the commercial value of the crops. The rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy) (Hemiptera: Miridae), and the sorghum plant bug, *Stenotus rubrovittatus* (Matsumura) (Hemiptera: Miridae), both of which are found throughout Japan, specifically cause pecky rice (Watanabe and Higuchi 2006). Poaceous plants are the primary hosts of these rice bugs (Hachiya 1999; Hayashi 1986). The rice bugs grow on the poaceous plants around the paddy fields and move into the paddy fields during the emergence of rice panicles (i.e., the heading stage).

The identification of plants suitable for the growth of pecky rice bugs might allow their occurrence to be predicted and densities to be suppressed. Monma and Kikuchi (2004) demonstrated that the addition of non-host ground-cover plants could reduce the occurrence of the bugs, while other studies have also indicated that the type of surrounding vegetation can influence the density of bug populations. For example, several studies have suggested that removal of weeds could reduce the occurrence of the bugs (Kikuchi and Kobayashi 2001; Niiyama and Itoyama 2006; Ono

et al. 2007; Yokota and Suzuki 2008), and therefore, it is important to identify the plant species that host them.

Hosts of *T. caelestialium* in Japan include ladino clover (*Trifolium repens* L. var. *giganteum* Lagreze-Fossat), timothy (*Phleum pratense* L.), bentgrass (*Agrostis palustris* Hud.), wheatgrass (*Agropyron cristatum* (L.) Gaertn.), Kentucky bluegrass (*Poa pratensis* L.), bermudagrass (*Cynodon dactylon* (L.) Pers.), rice, Italian ryegrass (*Lolium multiflorum* Lam.), wiregrass (*Eleusine indica* (L.) Gaertn.), southern crabgrass (*Digitaria ciliaris* (Retz.) Koel.), common barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), redtop (*Agrostis gigantea* Roth), annual bluegrass (*Poa annua* L.), orange foxtail (*Alopecurus aequalis* Sobol.), and wheat (*Triticum aestivum* L.) (Hachiya 1999; Kawasawa and Kawamura 1977; Watanabe et al. 2002). Likewise, the hosts of *S. rubrovittatus* include rice, corn (*Zea mays* L.), Sudan grass (*Sorghum sudanense* (Piper) Stapf.), Johnson grass (*Sorghum halepense* (L.) Pers.), pearl millet (*Pennisetum americanum* (L.) Leeke.), sweet sorghum (*Sorghum saccharatum* Pers.), foxtail millet (*Setaria itarica* (L.) P. Beauv.), rescue grass (*Bromus catharticus* Vahl), foxtail grass (*Setaria viridis* (L.) Beauv.), Italian ryegrass, orchard grass (*Dactylis glomerata* L.), *A. aequalis*, *P. annua*, Japanese paspalum (*Paspalum thunbergii* Kunth), wheat, early barnyardgrass (*Echinochloa oryzicola* Vasing.), *D. ciliaris*, and daisy fleabane (*Erigeron annuus* (L.) Pers.) (Hayashi 1986; Hayashi and Nakazawa 1988; Kato and Hasegawa 1950; Kawasawa and Kawamura 1977). However, as this information is based on field

observations or sweep-net sampling, the suitability of these plants for nymphal growth remains unclear. Under laboratory conditions, *T. caelestialium* grows well on *L. multiflorum*, *P. annua*, *A. aequalis*, *A. gigantea*, *P. pratensis*, *Eragrostis ferruginea* (Thunb.) Beauv., and violet crabgrass (*Digitaria violascens* Link) (Kikuchi and Kobayashi 2004). Comparable studies of the suitability of various plant hosts for *S. rubrovittatus* are lacking. Therefore, we investigated the suitability of 24 poaceous plants as hosts for these two species of pecky rice bugs. In addition, we separately tested nymphal growth of the two species on both spikelets and leaves because the presence of ears may influence their growth (Kikuchi and Kobayashi 2004; Sato et al. 2009; Yokota and Suzuki 2007).

## Materials and methods

### Insects

*Trigonotylus caelestialium* and *S. rubrovittatus* were collected from fields at the Hokuriku Research Center, National Agricultural Research Center, Niigata Prefecture, Japan. These insects were successively reared on young wheat seedlings under laboratory conditions (L:D photoperiod, 16:8 h; 25 °C) as described previously (Higuchi and Takahashi 2000; Nagasawa and Higuchi 2008, 2010).

### Plants

We used 24 poaceous plants in this study: *P. annua*, *A. aequalis* var. *amurensis*, *Anthoxanthum odoratum* L., *Poa sphondylodes* Trin., *Hordeum murinum* L., *D. glomerata*, *Festuca arundinacea* Schreb., *Vulpia myuros* (L.) C. C. Gmel., *Agrostis clavata* Trin. ssp. *matsumurae* (Hack. ex Honda) T. Tateoka, *L. multiflorum*, *Elymus tsukushiensis* Honda var. *transiens* (Hack.) Osada, *A. gigantea*, *D. ciliaris*, *Eragrostis multicaulis* Steud., *Setaria faberi* Herrm., *E. crus-galli*, *E. indica*, *Echinochloa crus-galli* (L.) Beauv. var. *aristata* S. F. Gray, *O. sativa* [cultivar Koshiibuki], *Panicum dichotomiflorum* Michx., *Setaria glauca* (L.) Beauv., *E. ferruginea*, *D. violascens*, and *Pennisetum alopecuroides* (L.) Spreng. All plants were growing collected from wild populations growing in or around the experimental fields of the Hokuriku Research Center, except for rice, which was cultivated in the paddy fields of the Hokuriku Research Center. We divided these plants into 2 groups, according to the time in the season when ears appeared and disappeared (Fig. 1). Spring plants, which had ears that appeared before June and disappeared by July, were as follows: *P. annua*, *A. aequalis* var. *amurensis*, *A. odoratum*, *P. sphondylodes*, *H. murinum*, *D. glomerata*, *F. arundinacea*, *A. clavata* subsp. *matsumurae*, *V. myuros*, *L. multiflorum*, *E. tsukushiensis* var. *transiens*, and *A. gigantea*. Summer–fall plants, which had ears that appeared between June and November, were as follows: *D. ciliaris*, *E. multicaulis*, *S. faberi*, *E. crus-galli*, *E. indica*, *E. crus-galli* var. *aristata*, *P. dichotomiflorum*, *S. glauca*, *E. ferruginea*, *D. violascens*, and *P. alopecuroides*.

### Nymphal development

We reared newly hatched *T. caelestialium* and *S. rubrovittatus* nymphs (<24 h after hatching) individually in Petri dishes (height 15 mm, diameter 50 mm). Nymphs were provided with either leaves or spikelets from a single plant species until they either emerged as adults or died. A piece of filter paper was placed at the bottom of the Petri dishes and moistened with water to maintain humidity. The expanded leaves or spikelets were obtained from plants of each

poaceous species during the flowering stage. We cut the leaves or ears into appropriate sizes and exposed the nymphs to the plant parts (1 or 2 pieces) immediately after preparation. We replaced leaves or spikelets with fresh ones every 1–2 days. To estimate body size, we measured the length of the forewing of newly emerged adults by using a stage micrometer on a microscope. Fifty nymphs of each bug species were reared on the leaves of each plant species and 50 nymphs of each bug were reared on the spikelets of each plant species. We conducted all tests under controlled conditions in the laboratory (L:D cycle, 16:8 h; 25 °C).

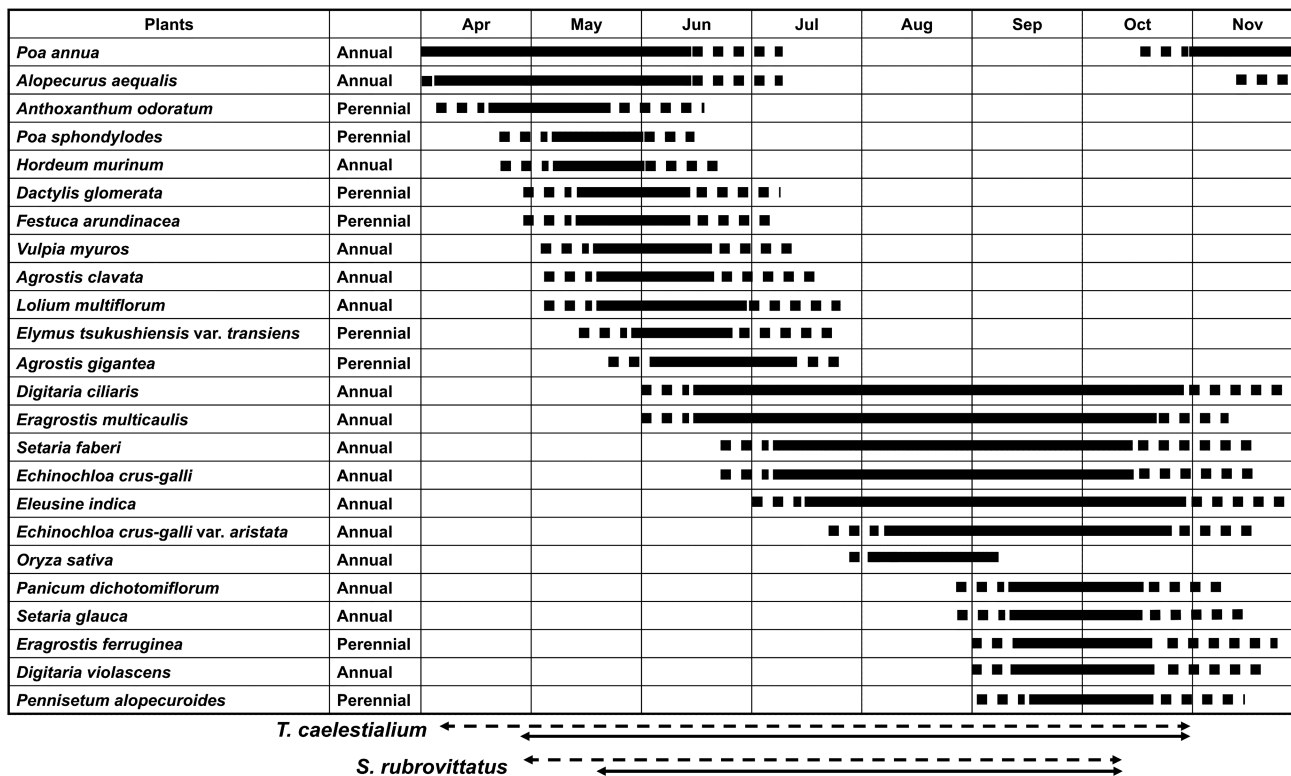
### Statistical analysis

We performed statistical analyses using R version 2.11.1 for Mac OS X (R Development Core Team 2010). We compared the number of adults that emerged on spring and summer–fall plants using the Wilcoxon rank-sum test. To compare adult emergence between plant species, we used the value from the portion of the plant (leaves or spikelets) that produced the greatest number of adults to represent the species. We compared the number of adults that emerged on the leaves and spikelets of the same plant using Fisher's exact test. To compare the performance of the host plant, the duration of the nymphal period (days) and the size (forewing length) of emerged *T. caelestialium* adults on leaves and spikelets were compared using the Wilcoxon rank-sum test. Mean values were significantly different when  $P < 0.05$ .

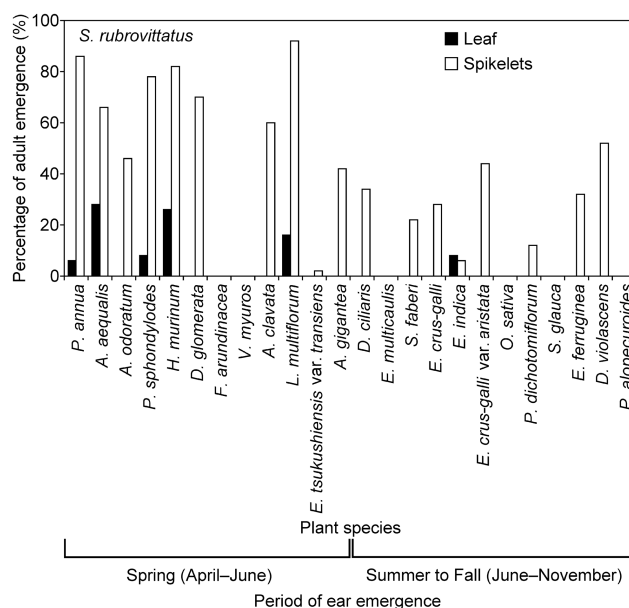
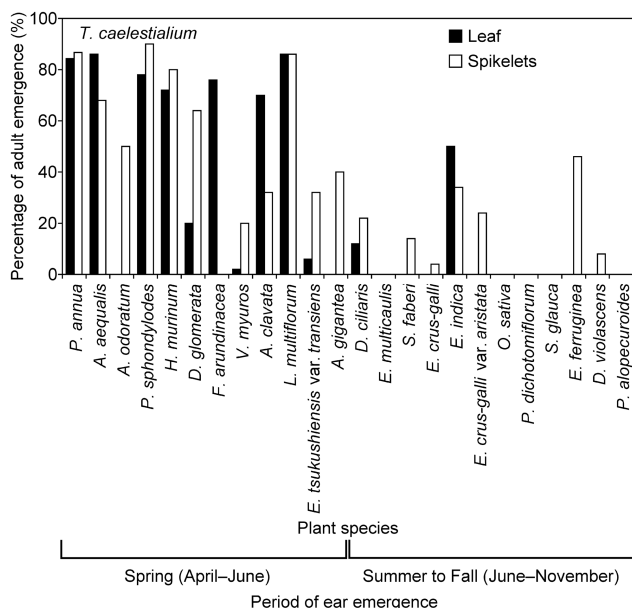
## Results

The percentages of *T. caelestialium* and *S. rubrovittatus* adult emergence are shown in Figs. 2 and 3, respectively. More than 50 % of *T. caelestialium* nymphs emerged as adults when reared on the leaves or spikelets of the following spring plants: *P. annua*, *A. aequalis*, *P. sphondylodes*, *H. murinum*, *D. glomerata*, *A. clavata*, and *L. multiflorum*. However, the number of emerging *T. caelestialium* adults on summer–fall plants was significantly lower than that on spring plants (Wilcoxon rank-sum test,  $P < 0.01$ ; Fig. 2). Among those plant species with >50 % adult emergence, the number of emerging *T. caelestialium* adults on the leaves differed significantly from those on the spikelets of *D. glomerata* and *A. clavata* (Fisher's exact test,  $P < 0.001$ ). However, the number of emerging *T. caelestialium* adults did not differ significantly between the leaves and spikelets of *P. annua*, *A. aequalis*, *P. sphondylodes*, *H. murinum*, and *L. multiflorum* (Fisher's exact test,  $P > 0.05$ ). To compare the leaf and spikelet performance of these 5 suitable plants, the duration of the nymphal period and the length of the forewing of *T. caelestialium* were measured. The duration of the nymphal period on the 5 plant species was significantly shorter on spikelets than on leaves (Wilcoxon rank-sum test,  $P < 0.05$ ; Table 1). The length of the forewing of *T. caelestialium* adults was significantly longer when nymphs were reared on spikelets than on leaves, except for males and females on *A. aequalis* and males on *H. murinum* (Wilcoxon rank-sum test,  $P < 0.01$ ; Table 2).

More than 50 % of all *S. rubrovittatus* nymphs emerged as adults when reared on the spikelets of *P. annua*, *A. aequalis*, *P. sphondylodes*, *H. murinum*, *D. glomerata*, *A. clavata*, *L. multiflorum*, and *D. violascens*, which are all spring plants (except for *D. violascens*). However, the percentage of adult emergence of *S. rubrovittatus* was significantly lower on summer–fall plants than on spring plants (Wilcoxon rank-sum test,  $P < 0.05$ ; Fig. 3). In addition, there were significantly fewer emergent *S. rubrovittatus* adults on



**Fig. 1** The sequence of emergence of pecky rice bugs *Trigonotylus caelestialium* and *Stenotus rubrovittatus* nymphs and adults, and of poaceous plant ears in Joetsu, Niigata, Japan. The solid and broken lines represent the time when most and some plant ears, respectively, are present. The solid and dashed arrows represent the time when pecky rice bug adults and nymphs, respectively, are present on the plants



**Figure 2.** Numbers of eggs laid by cigarette beetles on various extracts of roasted coffee beans. Each extract was provided on filter paper to a pair (male and female) of beetles for six days. Bars with asterisks are different from the control [Dunnett's test after a log ( $x + 0.5$ ) transformation,  $P < 0.05$ ].

**Figure 3.** Numbers of eggs laid by cigarette beetles on fractions of a chloroform extract of roasted coffee beans. was provided on filter paper to a pair (male and female) of beetles for six days. Bars with asterisks are different from the control [Dunnett's test after a log ( $x + 0.5$ ) transformation,  $P < 0.05$ ].

the leaves than on the spikelets of plant species with  $>50\%$  adult emergence (Fisher's exact test,  $P < 0.001$ ; Fig. 3).

### Discussion

The development and ear emergence times of poaceous plants vary depending on the species; therefore, species compositions change

across seasons (Osada 1993). As a result, *T. caelestialium* and *S. rubrovittatus*, which have 4–5 generations per year (Ishimoto 2004; Sato et al. 2009), use a succession of host plants. Our field observations showed the heading stage of potential host plants of two rice bugs (Fig. 1). A high percentage of both *T. caelestialium* and *S. rubrovittatus* emerge as adults when reared on several spring plants. In contrast, a relatively low percentage of these two species

**Table 1** Nymphal period (days) of *Trigonotylus caelestialium* on spikelets and leaves of 5 spring host plants

Plant part	Plant species				
	<i>P. annua</i>	<i>A. aequalis</i>	<i>P. sphondylodes</i>	<i>H. murinum</i>	<i>L. multiflorum</i>
Spikelets	10.5 ± 0.1 [39] <sup>a</sup>	11.0 ± 0.1 [34] <sup>a</sup>	10.7 ± 0.1 [45] <sup>a</sup>	11.3 ± 0.1 [40] <sup>a</sup>	10.9 ± 0.1 [43] <sup>a</sup>
Leaves	11.2 ± 0.1 [43] <sup>a</sup>	11.6 ± 0.1 [46] <sup>a</sup>	11.7 ± 0.2 [39] <sup>a</sup>	11.7 ± 0.1 [36] <sup>a</sup>	12.1 ± 0.1 [43] <sup>a</sup>
	**	**	**	*	**

Statistically significant difference between spikelets and leaves (\*\* $P < 0.01$ , \* $P < 0.05$ ; Wilcoxon rank-sum test)

<sup>a</sup>Mean ± standard error [number of adults]

**Table 2** The forewing length (mm) of emergent *Trigonotylus caelestialium* adults on 5 host plants

Plant part	Plant species					
	<i>P. annua</i>	<i>A. aequalis</i>	<i>P. sphondylodes</i>	<i>H. murinum</i>	<i>L. multiflorum</i>	
Female	Spikelets	4.56 ± 0.04 [18] <sup>a</sup>	4.39 ± 0.04 [18] <sup>a</sup>	4.58 ± 0.03 [27] <sup>a</sup>	4.53 ± 0.05 [20] <sup>a</sup>	4.63 ± 0.05 [16] <sup>a</sup>
	Leaves	4.31 ± 0.05 [18] <sup>a</sup>	4.36 ± 0.04 [16] <sup>a</sup>	4.23 ± 0.05 [18] <sup>a</sup>	4.27 ± 0.04 [17] <sup>a</sup>	4.12 ± 0.04 [15] <sup>a</sup>
		**	ns	**	**	**
Male	Spikelets	3.95 ± 0.04 [21] <sup>a</sup>	3.81 ± 0.05 [16] <sup>a</sup>	3.90 ± 0.07 [18] <sup>a</sup>	3.91 ± 0.05 [20] <sup>a</sup>	3.98 ± 0.02 [27] <sup>a</sup>
	Leaves	3.76 ± 0.03 [25] <sup>a</sup>	3.76 ± 0.03 [29] <sup>a</sup>	3.64 ± 0.04 [21] <sup>a</sup>	3.78 ± 0.05 [19] <sup>a</sup>	3.64 ± 0.03 [28] <sup>a</sup>
		**	ns	**	ns	**

Statistically significant difference between spikelets and leaves (\*\* $P < 0.01$ ; Wilcoxon rank-sum test)

<sup>a</sup>Mean ± standard error [number of adults]

emerge as adults when reared on summer–fall plants. This indicates that overwintering insects and early generations of these insects that occur during the heading stage of spring plants will perform very well on highly suitable host plants, while the growth of subsequent generations will be poorer and on less suitable host plants. This is consistent with previous studies that showed that densities of both insects decrease in summer (Sato et al. 2009; Takita 2005).

Our results demonstrate that the development of *S. rubrovittatus* depends on the presence of spikelets of poaceous plants. Removing ears of weedy grasses will therefore limit the growth of *S. rubrovittatus*. These results are consistent with those of several previous studies, which demonstrate that removing grass weeds around paddy fields reduces the occurrence of pecky rice bugs (Kikuchi and Kobayashi 2001; Niiyama and Itoyama 2006; Ono et al. 2007; Yokota and Suzuki 2008). However, our results also show that *T. caelestialium* can grow on poaceous plants, regardless of whether ears are present. As a result, the cutting of grass weeds may have a limited effect on the development of *T. caelestialium* because the leaves of the host plants will still be present. The percentage adult emergence of *T. caelestialium* was similar on leaves and spikelets. However, spikelets provide more nourishment than leaves, as indicated by the shorter period of nymphal development and longer length of adult forewings when nymphs were raised on the spikelets than when they were raised on the leaves of host plants. Forewing length is positively associated with survival time and the number of eggs in *T. caelestialium* adults (Shintani 2009). Therefore, the presence of grass ears will also promote *T. caelestialium* nymphal growth and adult fecundity.

The time of year (season) can influence the growth of host plants. Shintani (2009) found that the nutritional quality of host plants such as *L. multiflorum* and *D. ciliaris* exhibits seasonal variation, and that this results in reduced development of *T. caelestialium* nymphs during the summer. Therefore, further research is required to determine the effect of seasonal variation on the nutritional quality of other *T. caelestialium* and *S. rubrovittatus* host plants. In addition, differences between the developmental stages of host plants may affect nymphal growth. Acquisition of this knowledge may improve our understanding of how pecky rice bugs use host plants.

The plant preferences of phytophagous insects for oviposition are important. Because some insects exhibit asymmetry between plants preferred for oviposition and those suitable for nymphal development (Digweed 2006; Eben and López-Carretero 2008; Gratton and Welter 1998; Roininen and Tahvanainen 1989), the host plants used by *T. caelestialium* and *S. rubrovittatus* cannot be determined by measuring nymphal development alone. Furthermore, selection of oviposition sites by adults greatly limits the range of host plants used by immature stages that are less mobile (Bernays and Chapman 1994; Schoonhoven et al. 2005; Wennström et al. 2010). To date, field investigations have revealed that *T. caelestialium* oviposits both on the ear and leaf sheaths of poaceous plants, whereas *S. rubrovittatus* oviposits only on the ear (Nagasawa 2007). These observations correspond with the suitability of plant parts for nymphal growth that was demonstrated in the present study. Because *S. rubrovittatus* nymphs particularly depend on spikelets, oviposition on spikelets will ensure the presence of food for the offspring on the plants. It remains unclear whether host plant selection for oviposition corresponds with the suitability of food plants for the nymphal growth of these bugs.

The nymphs of both bugs failed to grow on either the leaves or spikelets of rice plants in the present study. Previous studies (Ishimoto 2008; Ishimoto and Sato 2006) have indicated that, when reared on rice plant seedlings, *S. rubrovittatus* was unable to grow and a low percentage of *T. caelestialium* emerged as adults. This is in accordance with our results and indicates that rice plants without ears have little value as food for these bugs. The nymphs of these bugs are reported to grow on hulled rice (Ishimoto 2008; Ishimoto and Sato 2006). In addition, Ishimoto (2007) reported that survival rates of *T. caelestialium* nymphs greatly depend on the presence of split hulls. This indicates that growth of the nymphs depends on opportunity to suck the contents of the rice grains from splits in the hulls. While *T. caelestialium* lay eggs on rice plants (Nagasawa et al. 2012), *S. rubrovittatus* adults lay few eggs on these plants (Ishimoto 2011; Nagasawa et al. 2012). This results in few *S. rubrovittatus* nymphs emerging on rice plants. Therefore, in paddy fields, emergence of *S. rubrovittatus* nymphs necessitates oviposition on plants other than rice (e.g., Poaceae or Cyperaceae plants). Removal

of these weeds in paddy fields may be effective in preventing the occurrence of nymphs on rice plants. Although *T. caelestialium* is able to emerge on rice plants, it cannot grow on rice without split-hull paddies. Therefore, removal of suitable host plants around paddy fields may also be effective in preventing growth of *T. caelestialium* nymphs.

In conclusion, we demonstrate that the seasonal abundance of host plants affect the development of the pecky rice bugs *T. caelestialium* and *S. rubrovittatus*. We identified poaceous plants that enhance the growth of the rice bugs. Preventing the growth of these plants around rice paddies could help to suppress the occurrence of pecky rice. In order to apply this knowledge, we need to know how the abundance of these host plants, and their distance from paddy fields, is correlated with the occurrence of pecky rice. Our results may be important for developing effective management strategies to predict and control pecky rice bugs and improve rice production in Japan.

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