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ORIGINAL RESEARCH PAPER

Occurrence of *Cicadulina bipunctata* (Hemiptera: Cicadellidae) in southwestern Shikoku, Japan and comparisons of gall-inducing ability between Kyushu and Shikoku populations

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Abstract The maize orange leafhopper *Cicadulina bipunctata* (Melichar) is an insect pest of cereal crops in tropical and subtropical regions of the Old World. This leafhopper induces gall symptoms characterized by stunted growth and swollen leaf veins on various Poaceae. Damage by *C. bipunctata* has been reported from Australia, the Philippines, China, Taiwan, and Japan. In Japan, *C. bipunctata* occurs in the central and southern parts of Kyushu. Because the leafhopper is a potential pest of various cereal crops, we conducted field surveys in Shikoku and the southern part of the Kii Peninsula (a part of Honshu), where the climate seems to be suitable for the establishment of *C. bipunctata*. As a result, we found *C. bipunctata* at some localities in Ehime and Kochi Prefectures. This is the first record of this leafhopper from Shikoku. Our laboratory experiment revealed that a Kochi population of *C. bipunctata* had a gall inducing ability similar to the Kumamoto population. More attention should be paid to assessing the risk of further expansion of *C. bipunctata* populations in Shikoku.

Keywords Distribution · Maize orange leafhopper · Maize wallaby ear symptom · Poaceae

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Introduction

Maize orange leafhopper, *Cicadulina bipunctata* (Melichar), is distributed in tropical and subtropical regions of the Old World (Webb 1987; Matsukura et al. 2009a). In Japan, this species is known to occur in the Bonin Islands (Matsumura 1914), the Ryukyu Islands (Hayashi 2002), and Kyushu (Matsumura et al. 2006; Matsukura et al. 2009a; Kumashiro et al. 2012). The leafhopper feeds on various Poaceae including important crops, such as maize, rice and wheat (Maramorosch et al. 1961; Catindig et al. 1996; Kumashiro et al. 2011). *Cicadulina bipunctata* is a serious pest of maize because feeding by the leafhopper induces gall symptoms characterized by stunted growth and swollen leaf veins, which is referred to as the maize wallaby ear symptom (Grylls 1975; Ohata 1993). The symptoms have been reported from Australia (Grylls 1975), the Philippines (Agati and Calica 1949; Maramorosch et al. 1961), China (Li and Liu 2004; Li et al. 2004), Taiwan (Chen 1991), and Japan (Ohata 1993; Matsumura et al. 2005, 2006).

Although previous studies attributed the symptoms to a leafhopper-transmitted virus (Agati and Calica 1949; Maramorosch et al. 1961; Grylls 1975; Reddy et al. 1976; Boccardo et al. 1980), recent studies have recognized that some chemicals injected by *C. bipunctata* during feeding are responsible for gall induction (Ofori and Francki 1983; Ohata 1993; Kawano 1994; Matsukura et al. 2012a). Galls are induced only on leaves which are newly expanding during leafhopper feeding, but not on leaves already developed or those which expand after the removal of leafhoppers (Ofori and Francki 1983; Ohata 1993; Matsukura et al. 2009b). The degree of gall induction depends on the density and duration of feeding by *C. bipunctata* (Matsukura et al. 2009a; Matsukura and Matsumura 2010; Tokuda et al. 2013).

In Japan, the gall symptom was first reported in Kumamoto and Okinawa Prefectures in the late 1980s (Ohata 1993; Kawano 1994). Heavy damage occurred on the second planting of forage maize, especially in Kumamoto Prefecture (Ohata 1993). According to Matsumura et al. (2005), the total area of forage maize fields damaged by *C. bipunctata* has expanded gradually since 2001 in Kumamoto Prefecture, and outbreaks of the leafhopper were reported in 2004. Thereafter, a resistant maize variety was developed to reduce the damage caused by *C. bipunctata* (Tokuda and Matsumura 2005).

Because *C. bipunctata* has a certain degree of tolerance to high temperatures, such as 31 and 34 °C (Tokuda and Matsumura 2005), the expansion of distribution range following global warming is of concern for this species (Matsumura et al. 2005; Matsukura et al. 2009a, 2012b). *Cicadulina bipunctata* occurs in the southern parts and middle-western parts of Kyushu (Webb 1987; Matsukura et al. 2009a), and our most recent study revealed that the leafhopper is also distributed in the middle-eastern parts, but not in the northern parts, of Kyushu (Kumashiro et al. 2012). Until now, *C. bipunctata* had not been reported from Shikoku or the Kii Peninsula, Honshu, even though the annual mean temperatures of these areas are rather higher than in the middle parts of Kyushu (Japan Meteorological Agency 2011). This implies that the climate in these areas would permit the survival of *C. bipunctata*.

To assess the potential risk of agricultural damage by *C. bipunctata*, information about the detailed distribution of *C. bipunctata* and its gall-inducing ability in local populations will be very important. In this study, we surveyed the distribution of *C. bipunctata* in the southwestern and eastern parts of Shikoku and the southwestern part of the Kii Peninsula, and we compared the gall inducing ability of *C. bipunctata* between Kyushu and Shikoku populations.

Materials and methods

Field survey

Field investigations were conducted in 17 localities in Ehime Prefecture and in 16 localities in Kochi Prefecture, southwestern Shikoku, Japan in November 2011. Additional investigations were performed in 8 localities in Kagawa Prefecture, 11 localities in Tokushima Prefecture, eastern Shikoku and 18 localities in Wakayama Prefecture, southwestern Kii Peninsula in October 2012 (Table 1 and Supplementary Table 1). In each locality, the grasslands where host grassy weeds of *C. bipunctata* such as *Eleusine indica* (L.), *Digitaria ciliaris* (Retz.), and *Setaria viridis* (L.) grew were selected for the census sites and 30 or 25 sweeps using a sweeping net with a diameter of 42 cm were performed at each census site. The census sites were separated from each other by at least 2 km. Collected leafhoppers were kept in glass tubes filled with 99.5 % ethanol, but approximately 40 adults collected from a census site in Kochi (Ohtsu, see Fig. 1; Table 1) were transferred alive to Kyushu Okinawa Agricultural Research Center in Kumamoto and reared on rice seedlings at 25 °C

Table 1 Collection sites and numbers of *C. bipunctata* captured in Ehime Prefecture

No.	Census site	Longitude (N)	Latitude (E)	Male	Female	Total
1	Shimodomari, Mikame Town, Seiyo City, Ehime	33°20′05.7	132°22′41.2	19	7	26
2	Karihama, Akehama Town, Seiyo City, Ehime	33°18′36.9	132°26′46.9	0	2	2
3	Sunokawa, Ainan Town, Ehime	33°01′48.0	132°28′56.1	1	2	3
4	Ohtsu, Tosashimizu City, Kochi	32°45′03.9	132°47′01.0	31	32	63
5	Tsumajiro, Tosashimizu City, Kochi	32°47′16.1	132°52′13.5	1	0	1
6	Nakanohama, Tosashimizu City, Kochi	32°46′22.9	132°57′52.4	1	3	4
7	Ashizurimisaki, Tosashimizu City, Kochi	32°43′17.4	133°00′39.9	1	2	3
8	Kubotsu, Tosashimizu City, Kochi	32°46′57.2	132°59′36.6	3	7	10
9	Ukibuchi, Kuroshio Town, Kochi	32°59′33.4	133°00′25.6	1	0	1
10	Shirahama, Kuroshio Town, Kochi	33°02′26.3	133°05′48.2	18	24	42
11	Okitsu, Shimanto Town, Kochi	33°09′45.9	133°12′24.3	60	74	134

Sites where *C. bipunctata* was not found are shown in Supplementary Table 1

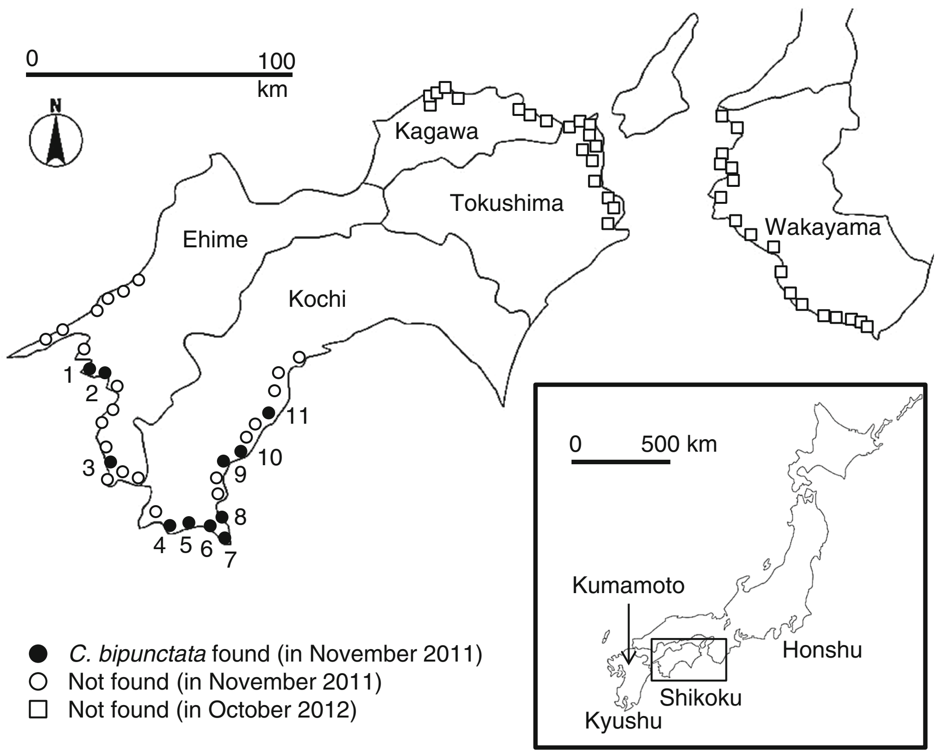

Fig. 1 Census sites for *C. bipunctata* in Shikoku and the Kii Peninsula. Sites where *C. bipunctata* was found are marked by *solid circles with site numbers* shown in Table 1 and those where it was not found are shown with *open circles* or *squares*

under a 16L: 8D photoperiod, as in Matsumura and Tokuda (2004).

Gall-inducing ability

In order to compare the gall-inducing ability of *C. bipunctata* between Kyushu and Shikoku populations, the following two stock cultures, Kumamoto and Kochi populations, of *C. bipunctata* were used in this study. The Kumamoto population was established from approximately 70 adults collected from Shisui, Kumamoto, Kyushu in December 2012 and the Kochi Population was established from ca. 40 adults collected from Ohtsu, Kochi, Shikoku in November 2011 (Fig. 1; Table 1; No. 4). Both populations were reared on rice seedlings (a *japonica* type rice variety Reiho) following the method described in Matsumura and Tokuda (2004). Two maize varieties, 3470 and 30D44 were used as plant hosts. The former is susceptible to feeding by *C. bipunctata* (Matsukura and Matsumura 2010). Though the latter is known as a resistant variety, it suffers severe symptoms when it is infested by *C. bipunctata* at younger stages (1–5 weeks after sowing) (Matsukura and Matsumura 2010).

Both of the maize varieties were individually sown in plastic cups (220 ml) with soil, and kept in a phytotron at 25 °C under a 16L: 8D photoperiod. Seven days after sowing, when seedlings reached the second (var. 3470) or third leaf stage (var. 30D44), zero (=control) or five adult males (less than 7 days after emergence) of either Kumamoto or Kochi populations were released on each seedling. Prior to the release of males, all plants were covered individually with an acrylic cylinder (4.5 cm diameter and 24.5 cm high) with nylon cloth on the top for ventilation. Seven days after the release, all males were removed from the plants and the number of surviving individuals was counted.

As mentioned earlier, galls of *C. bipunctata* are induced on leaves newly expanding during leafhopper feeding. Thus, the length of the third (var. 3470) or fourth (var. 30D44) leaf was measured from the base of plant to the tip of plant (an equivalent measurement to leaf length often used in monocotyledons) 7 days after the removal of males, when seedlings of both varieties reached the fourth and early fifth leaf stages, respectively. Then the symptom scores (Matsumura and Tokuda 2004) of these leaves were evaluated as described below. The gall symptoms were classified into the following three categories according to its severity: (0) no visible symptoms; (1) leaf veins partially rising; and (2) leaf veins heavily swollen (Matsumura and Tokuda 2004). Ten replications were conducted.

Statistical analysis

The number of surviving *C. bipunctata* males was analyzed by analysis of variance (two-way ANOVA) followed by the Tukey HSD test. To compare the effect of feeding by *C. bipunctata* on the degree of stunted growth, the leaf length was analyzed by planned comparisons (Sokal & Rohlf 1995) between control and treatments (Kumamoto and

Kochi populations of *C. bipunctata*) for each maize variety, followed by the comparison between the two populations. The differences in the mean symptom scores between the leafhopper populations were assessed by Wilcoxon rank-sum test for each maize variety. All analyses except the planned comparisons were performed with R software, version 2. 12. 2 (R Development Core Team 2011).

Results

Distribution of *C. bipunctata* in Shikoku and the Kii Peninsula

Cicadulina bipunctata was collected from three out of 17 sites (17.6 %) in Ehime and eight out of 16 sites (50.0 %) in Kochi Prefecture (Fig. 1; Table 1). More than 40 individuals of *C. bipunctata* were collected from each of three census sites in Kochi Prefecture (Fig. 1; Table 1; Nos. 4, 10, and 11), but less than 30 individuals from each of the other sites. *Cicadulina bipunctata* was found at all census sites on Cape Ashizuri in Kochi (Fig. 1; Table 1; Nos. 6, 7, and 8). In contrast, the leafhopper was not collected from northern census sites of Ehime Prefecture (Fig. 1). In addition, no *C. bipunctata* was collected from all 19 sites in eastern Shikoku or 18 sites in the southwestern Kii Peninsula (Fig. 1 and Supplementary Table 1).

Gall-inducing ability

Both maize varieties and leafhopper populations did not affect the number of surviving *C. bipunctata* (two-way ANOVA, variety $F_{1,36} = 0.69$, $p = 0.40$; population $F_{1,36} = 0.69$, $p = 0.40$; variety × population $F_{1,36} = 0.12$, $p = 0.72$; Fig. 2).

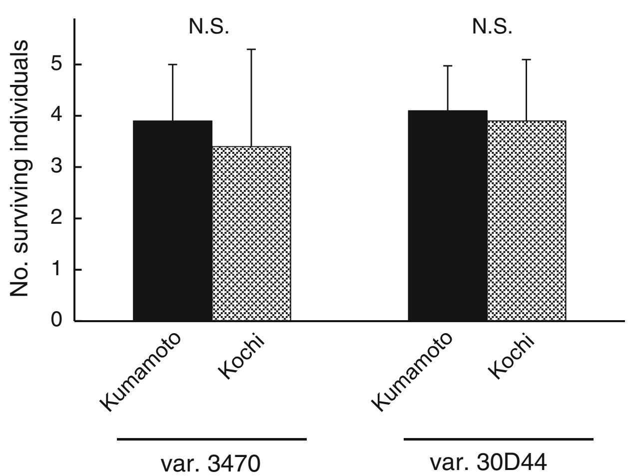

Fig. 2 The number of surviving *C. bipunctata* of Kumamoto and Kochi populations 7 days after the release onto susceptible (3470) and resistant (30D44) maize varieties. *Vertical bars* show the standard deviation

Both the Kumamoto and Kochi populations induced gall symptoms on susceptible and resistant maize varieties. On the susceptible variety 3470, the length of the third leaf infested by *C. bipunctata* was significantly shorter than that of the control ($F_{1,27} = 13.67$, $p < 0.005$; Fig. 3), but the severity of stunting was not significantly different between the two populations ($F_{1,27} = 3.04$, $p > 0.05$; Fig. 3). On the resistant variety 30D44, leaves infested by *C. bipunctata* were significantly shorter than the control

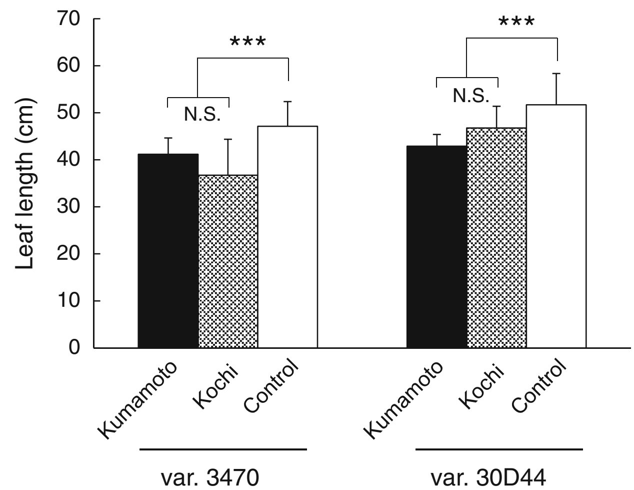

Fig. 3 Effect of feeding by Kumamoto and Kochi population on growth stunting of susceptible (3470) and resistant (30D44) maize varieties. *Vertical bars* show the standard deviation. *Asterisks* indicate significant differences (planned comparisons, *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$)

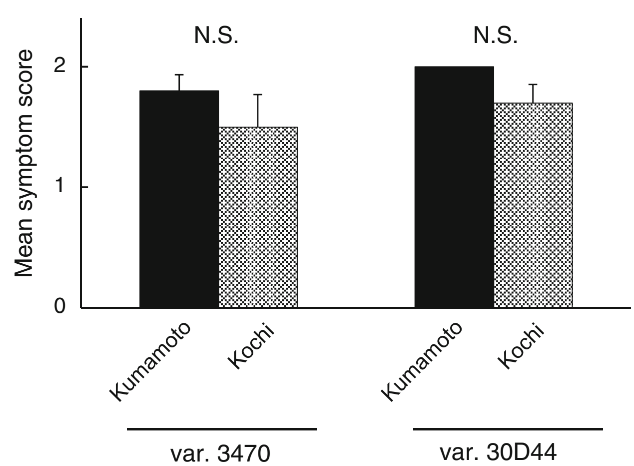

Fig. 4 Effect of feeding by the Kumamoto and Kochi populations of *C. bipunctata* on the leaf vein swelling of susceptible (3470) and resistant (30D44) maize varieties. The degree of leaf vein swelling was assessed by the symptom score (*0* no visible symptoms, *1* leaf veins partially swelling, *2* leaf veins heavily swelling). *Vertical bars* show the standard errors

($F_{1,27} = 13.11$, $p < 0.005$; Fig. 3). The leaf length was not significantly different between the Kumamoto and Kochi populations ($F_{1,27} = 3.11$, $p > 0.05$; Fig. 3).

As shown in high symptom scores (Fig. 4), maize leaf veins were heavily swollen by infestation in both varieties. The degree of leaf vein swelling was not significantly different between Kumamoto and Kochi populations on both 3470 ($N = 10$, Wilcoxon rank-sum test, $W = 43$, $p = 0.51$) and 30D44 ($N = 10$, Wilcoxon rank-sum test, $W = 35$, $p = 0.076$) (Fig. 4). No leaf vein swellings were induced on control plants (Data not shown).

Discussion

Our field survey revealed that *C. bipunctata* occurs in Ehime and Kochi Prefectures. This is the first collection record of this species from Shikoku. In this study, *C. bipunctata* was found only in southwestern Shikoku, the area closest to central Kyushu (Fig. 1). On the other hand, the leafhopper was not collected from eastern Shikoku or the southwestern Kii Peninsula, far from Kyushu (Fig. 1). These results suggest that the *C. bipunctata* found in southwestern Shikoku are possibly due not to human-mediated long-distance dispersal but to natural short-distance dispersal such as wind-mediated and flight dispersal. Indeed, some species of the genus *Cicadulina* other than *C. bipunctata* are known to have the ability to fly continuously over several hours (Rose 1972).

Other invasive pest species such as *Liriomyza trifolii* (Burgess) (Saito 1992; Ozawa 2002) and *Aleurocanthus camelliae* Kanmiya & Kasai (Kasai et al. 2010) have dispersed throughout Japan within 10 years. This rapid dispersal is attributed to the human transportation of infested host plants (Saito 1992; Kasai et al. 2010). In contrast, the maize plants inhabited by *C. bipunctata* are seldom transported alive by people. Thus, *C. bipunctata* would not disperse as rapidly as the above mentioned species. Indeed, the distribution of *C. bipunctata* has been restricted to small areas even a hundred years after they were first detected in central Kyushu (Matsumura 1914; Matsukura et al. 2009a). More extensive investigation, however, will be required to determine the distribution range of *C. bipunctata*.

The warm climate in Shikoku and the Kii Peninsular (similar to that in central Kyushu) would allow the colonization and reproduction of *C. bipunctata* in these areas once it invades or is introduced there. In particular, the eastern part of Shikoku is at risk of *C. bipunctata* invasion from neighboring source habitats. Further expansion of *C. bipunctata* towards the northern and eastern part of Japan, therefore, should be monitored carefully.

As mentioned earlier, *C. bipunctata* is recognized as a serious pest of the second planting of forage maize in central Kyushu, Japan (Ohata 1993; Matsumura et al. 2006). Although damage caused by *C. bipunctata* on cereal crops has not yet been reported in Shikoku, the present results clearly indicate that *C. bipunctata* has a potential to become a serious pest of poaceous crops in Shikoku as well as in Kyushu. In addition, the present results suggest that the Kochi population is similar to the Kumamoto population in the ability to induce growth stunting and leaf vein swellings on susceptible and resistant varieties (Figs. 3, 4). We will need to pay further attention to the possible distribution, expansion, and growth of *C. bipunctata* populations in Japan.

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