

Dispersal of adult Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae), the vector of citrus greening disease, in artificial release experiments

Youichi Kobori · Tadafumi Nakata ·
Yasuo Ohto · Fugo Takasu

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Abstract Artificial release of marked adult *Diaphorina citri*, the insect vector of citrus greening disease, was performed in July 2007 (10,000 adults released, experiment 1) and October 2008 (1,000 adults released, experiment 2) to determine the association of the dispersal pattern with disease invasion risk. During the experimental periods, in experiment 1, the mean dispersal distances from the release point were 5–6 m and in experiment 2 they were 6–12 m. Further examination of the relationship between dispersal distance and season is needed. The number of released *D. citri* declined with increasing distance from the release point and did not change markedly with time. The center of distribution moved little during the experimental period. These results suggest that the adult *D. citri* barely moved once they had colonized a host plant within a few days after their release. Our results also suggest that the released *D. citri* moved with the wind. The diffusion coefficient was estimated at 7.23 from experiment 1, but random diffusion might be a poor descriptor of the dispersal of adult *D. citri*.

Keywords Huanglongbing · Psyllid vector · Citrus orchard · Risk management · Epidemiology

Introduction

Citrus greening disease (huanglongbing, HLB) critically limits sustainable citrus production (Bové 2006). HLB is an untreatable disease that makes trees wither and die. In Southeast Asia, this disease is caused by the *Candidatus Liberibacter asiaticus*, and the bacterium is transmitted by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Halbert and Manjunath 2004). In Japan, the distribution of *D. citri* was until recently limited to south of Amami Island (Kohono et al. 2002); however, in 2002, the distribution area began to disperse northward as far as Yakushima Island (Inoue 2004). Furthermore, one recent study reported the occurrence of *D. citri* in the city of Ibusuki in Kagoshima Prefecture (Inoue 2007). Disease-free trees can be also infected by the grafting of infected branches (van Vuuren 1993; Bové et al. 1996). For HLB management, it is therefore necessary to control the primary and secondary infection risks posed by the transmission by *D. citri* that invade, reproduce in, and move around disease-free fields, as well as to remove the human factors that bring infected young trees or vector insects into such fields. The risk of disease transmission by *D. citri* is the product of the invading vector insect's frequency of occurrence multiplied by the efficiency of transmission; therefore, it is important to know how the invasion frequency of the vector insect varies with distance from an infected field. We investigated the dispersal patterns of adult *D. citri* by tracking the distribution of artificially released populations in order to estimate the risk of HLB infection by the vector insect.

Y. Kobori (✉) · T. Nakata · Y. Ohto
Tropical Agriculture Research Front,
Japan International Research Center for Agricultural Sciences,
Ishigaki, Okinawa 907-0002, Japan
e-mail: koboriy@affrc.go.jp

F. Takasu
Department of Information and Computer Sciences,
Nara Women's University, Kita-Uoya Nishimachi,
Nara 630-8506, Japan

Materials and methods

Location and conditions of the experimental field

The experiments were performed in an experimental field at the Tropical Agriculture Research Front (TARF, 24°20'N, 124°09'E) of the Japan International Research Center for Agricultural Sciences in the city of Ishigaki in Okinawa Prefecture, Japan. The experimental field was flat. One thousand eighty-nine pots of *Citrus depressa* Hayata were set out in a grid (33 × 33 pots at 2.5-m intervals). The height of the *C. depressa* was approximately 80 cm. Within the experimental periods, we tried to remove all predators of *D. citri* (i.e., ladybirds and spiders), although complete removal was not possible. After each experimental period, fenitrothion (emulsion, dilution rate 1,000) was sprayed all over the field to ensure that the *D. citri* were killed.

Released adult *D. citri*

The *D. citri* used in this experiment were kept on orange jessamine, *Murraya paniculata* (L.) Jack, the host plant in the glasshouse, where the temperature was kept at 27 ± 4°C. Adult *D. citri* 5–8 days after emergence were marked by the method used in our previous study (Nakata 2008), i.e., with micronized fluorescent powder (FZ-2803). Previously, we reported that there was no significant difference in mortality between two groups (marked and unmarked) over a 40-day period of observation after marking (Nakata 2008). Two days after marking the adults, twigs of *M. paniculata* with marked *D. citri* were cut from the trees. These twigs were placed at the bottom of a pot in the center of the experimental field.

Experiment 1

Ten thousand adult *D. citri* were released on 19 July 2007. The numbers of adults on each *C. depressa* tree were counted visually 1, 3, 5, 7, 11, 14, 18, and 20 days after release. According to wind data from an observatory located 4 km from TARF, a southerly wind at 2–4 m/s was reported on all but a few days (Japan Meteorological Agency, <http://www.data.jma.go.jp/obd/stats/etrn/index.php>). The maximum temperature in the field was 33°C, minimum 24.5°C, and average approximately 30°C. During the 20-day experimental period, rainfall of 12 mm (day 8 after release), 3 mm (day 17), 12 mm (day 18), and 23 mm (day 19) was observed.

Experiment 2

One thousand adult *D. citri* were released on 1 October 2008. They were counted in the same way as in experiment 1.

Counting was performed 3, 7, 14, and 21 days after release. According to the Japan Meteorological Agency, a northerly wind of 2–5 m/s was reported on all but a few days. The maximum temperature was 31°C, minimum 21°C, and average approximately 28°C. During the 21-day experimental period, rainfall of 5 mm (day 8 after release), 3 mm (day 9), 5 mm (day 10), 2 mm (day 17), 1.5 mm (day 18), and 4.5 mm (day 19) was observed.

Data analysis

The mean dispersal distance from the release point of the adult *D. citri* was calculated every observation day. The number of *D. citri* found at each point as a proportion of the total number found in the field was plotted against the distance from the release point on a one-dimensional transect from south to north in the field. The plotted points were joined by spline interpolation. In experiment 1, we calculated the proportions on days 3, 7, 14, and 20. In experiment 2, we calculated the proportions on every observation day. In experiment 1, the diffusion coefficient was estimated from data taken 3 days after release using the method of Ito and Yamamura (2005). The mean value of the squared distances from the release point was divided by the number of days after release (in this case 3) multiplied by four. The mean of individual positions was also calculated to estimate the center of balance. This value was calculated in two dimensions (north–south and east–west) on each observation day. All values were calculated using Mathematica 7.0.1 (Wolfram Research 2009).

Results

Experiment 1

Three days after release, 3,306 adult *D. citri* were observed in the experimental field. The mean dispersal distances from the release point were about 5 m over the whole observation period (Table 1). The proportions of *D. citri* found at each point on days 3, 7, 14, and 20, plotted against the distance from the release point, were similar (Fig. 1a). By day 20, of the individuals found in the field, about 85% were found <7.5 m from the release point. More than 90% of *D. citri* that had moved >7.5 m were found in the northern area, on the lee side of the release point. The centers of distribution were plotted in two dimensions on each observation day (Fig. 2). During the experimental period, the values were within 1 m of the release point. The mean values of the squared distances from the release point were 98.14 (day 1), 86.72 (day 3), 85.20 (day 5), 79.06 (day 7), 72.69 (day 11), 66.42 (day 14), 66.93 (day 18), and

Table 1 Mean dispersal distances of adult *Diaphorina citri* from the release point

Conditions	Days after release	Mean dispersal distance from the release point (m) ^a
Experiment 1 (10,000 adults released on 19 July 2007)	1	5.83 ± 0.02
	3	5.53 ± 0.02
	5	5.53 ± 0.03
	7	5.58 ± 0.03
	11	5.68 ± 0.03
	14	5.43 ± 0.02
	18	5.28 ± 0.02
	20	5.35 ± 0.02
Experiment 2 (1,000 adults released on 1 October 2008)	3	6.10 ± 0.23
	7	11.40 ± 0.28
	14	12.13 ± 0.71
	21	11.89 ± 0.53

^a Value ± standard error of the mean

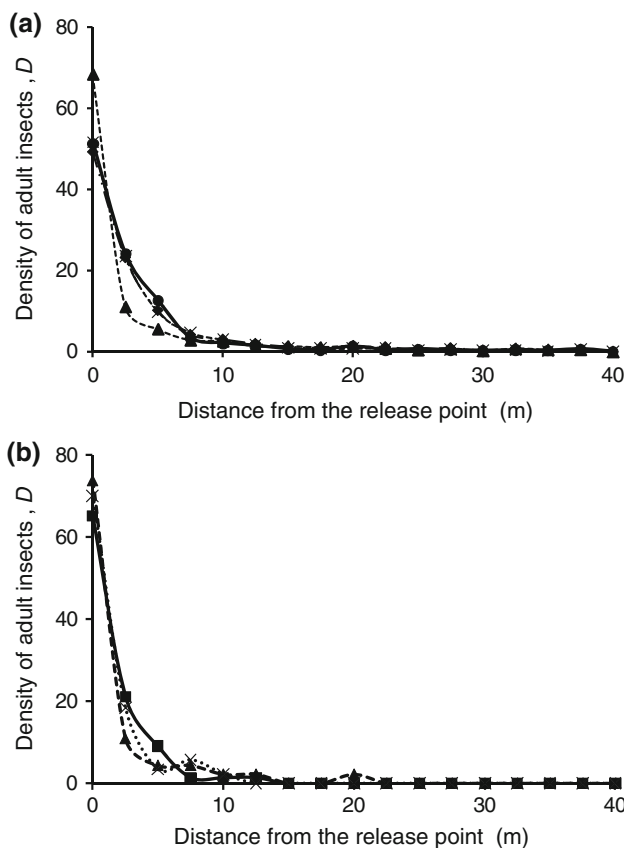


Fig. 1 The density of *Diaphorina citri* (*D*) found at different time periods was plotted against distance from the release point. **a** Experiment 1, **b** experiment 2. These values were calculated in a one-dimensional cross-section from south to north in the field. *D* = (number of individuals/total number of the individuals in field × 100). Filled triangles 3 days, filled squares 7 days, times symbol 14 days, filled circles 20 days (a), 21 days (b) after release

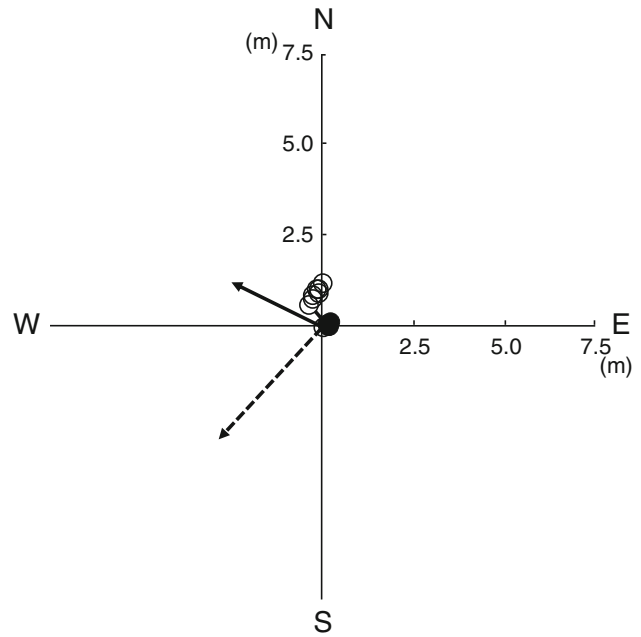


Fig. 2 Centers of distribution during the experimental period of insects released at the zero coordinate. Open and closed circles pertain to experiments 1 and 2, respectively. The two arrows (solid experiment 1; dashed experiment 2) indicate the composite wind vectors during the experimental periods, as calculated from the daily dominant wind directions. The average velocities were 2.42 m/s in experiment 1 and 3.98 m/s in experiment 2

99.01 (day 20). The diffusion coefficient estimated from data collected 3 days after release was 7.23.

Experiment 2

Three days after release, 602 adult *D. citri* were observed in the experimental field. The mean dispersal distance from the release point changed from 6.1 m on observation day 3 to 11.4 m on observation day 7. From then on, the mean values remained at about 11–12 m (Table 1). The proportions of *D. citri* found at each point on days 3, 7, 14, and 21, plotted against the distance from the release point, were similar to each other and to those in experiment 1 (Fig. 1b). More than 90% of *D. citri* that had moved >7.5 m were found in the southern area, on the lee side of the release point. The center of distribution showed a similar trend to that in experiment 1. The values were within 2 m of the release point during the experimental period (Fig. 2).

Discussion

The poor flight ability of this species has been reported in a flight mill experiment (Arakawa and Miyamoto 2007). Boina et al. (2009) reported that a portion of *D. citri* moved bidirectionally between separated unmanaged and managed

groves. We used field data in an attempt to perform a more quantitative evaluation of the relationship between the dispersal distance from the release point and arrival frequency. The proportions of *D. citri* found on different days in the experimental periods declined with similar trends with increasing distance from the release point and did not change substantially with time (Fig. 1). These results suggest that once the adult *D. citri* arrived on the host plants, they hardly moved again. The estimated diffusion coefficient was 7.23. Assuming that adult *D. citri* moved randomly, the mean value of the squared distances from the release point was expected to be proportional to the number of days after release; however, our results did not show this proportionality. Random diffusion might therefore be a poor descriptor of the dispersal of adult *D. citri*, and approximation by diffusion coefficient was not well suited to the dispersal.

The center of distribution would be expected to move with wind direction if each *D. citri* repeated its random direction movement because the same wind direction was observed throughout most of each experiment period; however, the center of distribution stayed around the release point during the experimental period (Fig. 2). Individuals that moved >7.5 m from the release point were found on the lee side. These results also suggest that once *D. citri*, carried by the wind, had arrived on the host plants, they hardly moved again. We consider that *D. citri* not found in the experimental field were killed by natural enemies. Otherwise, in light of a report by Boina et al. (2009) that adult *D. citri* may move 60–100 m within 3 days, it was possible that the adults had flown out of the experiment field; however, there was insufficient evidence to support this hypothesis.

We speculated the reason for the difference in the mean distances from the release point in experiments 1 and 2 (Table 1). Wakamura et al. (1990) reported that the flying distance of the adult male *Spodoptera litura* Fabricius changed seasonally. Further examination of the relationship between movement distance and season is therefore needed. The results shown here should be useful for establishing a method to estimate the risk of HLB invasion by *D. citri* transmission. By considering these results, we can speculate that risk declined with distance from the infected orchards or trees. We can also speculate that HLB invasion risk is higher in orchards on the lee side of an infected orchard than in those on the windward side. To examine the risk more precisely, it will be necessary to develop a risk estimation model based on knowledge of the dispersal of *D. citri* and the frequency of transmission of *C. Liberibacter asiaticus* by each individual.

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