ゲッキツの新葉あるいは成葉で飼育したミカンキジラミ雌成虫(半翅目:キジラミ科)の卵巣発育程度の比較

誌名	果樹研究所研究報告 = Bulletin of the National Institute of Fruit Tree Science
ISSN	13473549
著者名	上地,奈美 岩波,徹
発行元	農業技術研究機構果樹研究所
巻/号	13号
掲載ページ	p. 39-42
発行年月	2012年3月

原著論文

Comparison of the ovarian development in *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in relation to the leaf age of orange jasmine, *Murraya paniculata* (L.) Jack

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Summary

Ovarian development in *Diaphorina citri* females that fed on new leaves of *Murraya paniculata* after eclosion was compared with that of females that fed on mature leaves of *M. paniculata*. At 3 days after eclosion, no ovarian development was observed in either the new-leaf or the mature-leaf group. At 5 days after eclosion, 31 of 39 (79.5%) females in the new-leaf group had developed ovaries with at least 1 mature egg, whereas only 1 out of 39 (2.5%) females had developed ovaries with at least 1 mature egg in the mature-leaf group. The mean number of mature eggs in females in the new-leaf group was also significantly greater than that in the mature-leaf group. A similar trend was observed at 7 days after eclosion. These results suggest that feeding on new leaves promotes ovarian development in *D. citri* females.

Key words: Asian citrus psyllid, orange jasmine, flush foliage, ovarian development, plastic-bagging method

Introduction

The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), an insect vector of the citrus greening disease (Huanglongbing), feeds on the flush foliage of host plants such as *Citrus* spp. and *Murraya* spp. (Rutaceae). *Murraya* spp. are considered favorite hosts of *D. citri* for maintaining a high population density, because they have flush foliage even when the young shoots of citrus trees are not available (Hall et al., 2008; Tsai and Liu, 2000; Tsai et al., 2002) and are often grown as hedges and ornamental trees in residential areas (Staples and Herbst, 2005). An early report suggested that young leaves may be beneficial to immature psyllids (Husain and Nath, 1927). We suspect that young leaves are also beneficial to adults, especially females, with respect to their reproductive maturity. Several observations support this hypothesis. For example, in a field observation in Kagoshima Prefecture during early spring at a grove of *Citrus tankan* Hayata (Rutaceae) surrounded by hedges of orange jasmine, *Murraya paniculata* (L.) Jack, many mature females were found on *C. tankan* trees. It was suspected that these females flew in from the hedges of orange jasmine surrounding the citrus grove, since orange jasmine trees flush earlier than *C. tankan* (Hayashikawa et al., 2007). These

(Received 2 August 2010, Accepted 26 July 2011)

findings suggest that feeding on new flush foliage of orange jasmine accelerates the maturation of *D. citri* females. Although some of the reproductive characteristics of *D. citri* females, such as their reproductive capacity and preoviposition period, have been reported (Tsai and Liu, 2000; Wenninger and Hall, 2007), the effect of leaf age on adult maturity has only been studied by Hayashikawa and Ashihara (2005). These previous studies prompted us to speculate that flush foliage accelerates adult maturity, especially ovarian development. If proven, we can reasonably support the approach in Okinawa and Kagoshima Prefectures that the control of *D. citri* should be focused on early spring, when citrus and orange jasmine trees have many flushes of leaf growth. Therefore, in this study, we attempted to investigate the effect of new leaves on ovarian development in *D. citri* females by comparing the ovarian growth between females reared on new leaves of orange jasmine and those reared on mature leaves of the plant under laboratory conditions.

Materials and Methods

The rearing experiments were carried out from January 19 to March 10, 2009, under natural photoperiod conditions ranging from 10L:14D to 12L:12D (Rika Nenpyo, 2008).

A total of 221 females were used in the experiments, all of which were obtained from a laboratory colony reared on orange jasmine at the NARO Institute of Fruit Tree Science, Tsukuba, Ibaraki Prefecture, Japan, as described by Ashihara (2007).

Newly eclosed females were collected daily and randomly divided into 2 groups, a new-leaf group and a mature-leaf group, and these groups were transferred to new or mature leaves on potted orange jasmine trees. In the former group, orange jasmine leaves that were pale green, not completely hardened, and <10 cm long (Fig. 1A), and in the latter group, leaves that were dark green, fully hardened, and >10 cm long (Fig. 1B), were supplied as food. Subsequently, the insects and leaves were covered with fabricated zip-locked plastic bags (70 mm long, 50 mm wide; Seisannipponsha Ltd., Japan; Fig. 2) and reared in a greenhouse. After 3, 5, and 7 days, females were removed from the leaves and were preserved in 75% ethanol.

Thereafter, these females were dissected in a drop of 75% ethanol on a glass slide using insect pins. The ovaries were observed under a stereomicroscope, and the number of mature eggs, which was used as an index of ovarian development, was recorded (Fig. 3).

Results and Discussion

On day 3, no mature eggs were observed in any of the females (Table 1). On day 5, 31 of 39 (79.5%) females had

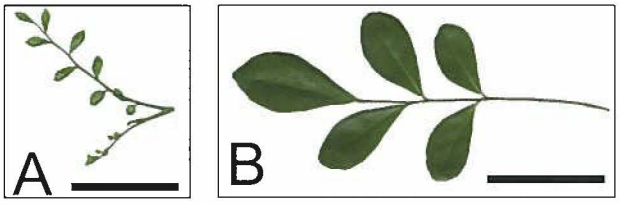

Fig. 1. Leaves of *Murraya paniculata* (L.) Jack used in the experiment. A: new leaves, B: a mature leaf. Scale: 50 mm.

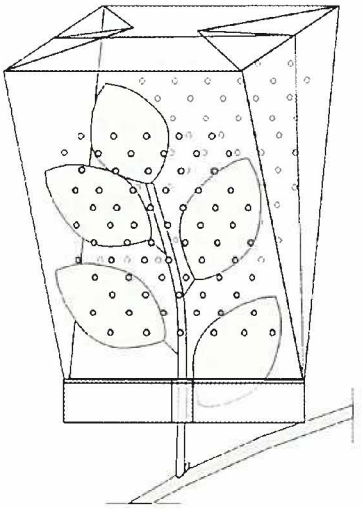

Fig. 2. Illustration of a zip-locked plastic bag used in this study. Bottom (topside in this figure) corners of the bag were sealed and folded into a sterical shape, and on the lateral sides, small holes were punctured with a pair of pin holders for ventilation.

Fig. 3. Ovary of a *Diaphorina citri* female that contains mature eggs. Scale: 0.5 mm.

at least 1 mature egg in the new-leaf group, compared with only 1 of 39 (2.5%) females in the mature-leaf group (Table 1). Thus, the number of mature females (i.e., those with at least 1 mature egg) in the new-leaf group was significantly greater than that in the mature-leaf group (chi-square test, chi-square value = 47.7, d.f. = 1, $p < 0.01$). The mean (± SE) number of mature eggs in the new-leaf group (17.4 ± 1.8) was also significantly greater than that (4.0) in the mature-leaf group (Mann-Whitney U test, $p < 0.01$; Table 1). Similarly, on day 7 after eclosion, 38 of 43 (88.4%) and 10 of 30 (33.3%) females had matured their ovaries in the new- and mature-leaf group, respectively (Table 1). This difference was significant (chi-square test, chi-square value = 23.8, d.f. = 1, $p < 0.01$). The mean (± SE) number of mature eggs in the new-leaf group was 19.9 ± 2.3, which was significantly greater than that (4.6 ± 1.1) in the mature-leaf group (Mann-Whitney U test, $p < 0.01$; Table 1). These results suggest that oocytes mature within 4 or 5 days after eclosion in females that feed on new leaves, but not in females that feed on mature leaves. It is surmised that new leaves may accelerate ovarian development.

On the other hand, mature eggs were observed in some females reared on mature leaves (Table 1). This result suggests that new leaves are not strictly necessary for ovarian development, although they are beneficial. In future experiments, it would be interesting to elucidate how many females feeding only on mature leaves can develop their ovaries and lay their eggs. Another interesting study would be to investigate whether females that have been fed only on mature leaves for a long time can accelerate their oocyte development after transfer to new leaves.

However, besides the leaf age, several environmental factors such as temperature, day-length, and illumination, as well as the density of *D. citri* may have an influence on ovarian development. Mating also accelerates ovarian development (Dossi and Cônsoli, 2010). Future experiments are needed to determine clearly whether these factors have an effect on ovarian development in *D. citri* females. When all significant factors have been accounted for, a model can be developed that precisely estimates the reproductive capacity of *D. citri* females.

Acknowledgements

We thank the members of the citrus greening disease research team (NARO Institute of Fruit Tree Science, Japan) and the members of the Okinawa Prefectural Agricultural Research Center (Okinawa, Japan) for their valuable inputs. We also thank Dr. Shinji Kawano (Okinawa Prefectural Agricultural Research Center, Okinawa, Japan) for providing us with detailed information on the plastic-bagging technique and Dr. Susan E. Halbert for improving our manuscript. This study was partly supported as "Research and Development Projects for Application in Promoting New Policy of Agriculture Forestry and Fisheries" (No. 21087).

Table 1. The number of mature females[z] and the mean number of mature eggs in *Diaphorina citri* females on days 3, 5, and 7 after rearing on new and mature leaves of *Murraya paniculata*.

Age of females	Group	Number of females in the group	Number of mature females		Mean (±SE) number of eggs per mature female	
3 days	new leaf	41	0 (0%)		0	
	mature leaf	33	0 (0%)		0	
5 days	new leaf	39	31 (79.5%)	**	17.4 ± 1.6	††
	mature leaf	39	1 (2.5%)		4. 0	
7 days	new leaf	43	38 (88.4%)	**	19.9 ± 2.2	††
	mature leaf	30	10 (33.3%)		4.6 ± 0.6	

[z] "mature female" means a female that has at least 1 mature egg.

** indicates a significant difference in the number of mature females between new and mature leaf groups ($p < 0.01$, chi-square test).

†† indicates a significant difference in the mean number of mature eggs per mature female between new and mature leaf groups ($p < 0.01$, Mann–Whitney U test).

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摘　要

ゲッキツの新葉上で飼育したミカンキジラミ雌成虫の卵巣発達程度を，成葉で飼育した雌成虫のそれと比較した．3日齢の雌成虫においては，いずれの処理区においても成熟卵を持つ個体はいなかった．5日齢では，新葉上の雌成虫の39頭中31頭（79.5%）が成熟卵を持っていたのに対し，成葉上の雌成虫は39頭中1頭（2.5%）だけであった．1雌あたりの平均成熟卵数も新葉上の成虫の方が有意に多かった．また，7日齢の雌成虫においても，成熟卵を持つ個体数および成熟卵数は，成葉上よりも新葉上の成虫で多い傾向がみられた．これらの結果から，新葉上での吸汁は雌成虫の卵巣発育を促すことが示唆された．