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Life Table and Demographic Parameters of Asian Citrus Psyllid *Diaphorina citri* on Limau Madu *Citrus suhuiensis*

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ABSTRACT

The Asian citrus psyllid *D. citri* is regarded as one of the most important pest of citrus, because it is known to be the most efficient vector of bacterium *Liberobacter asiaticum* that cause citrus greening or huanglongbing. Survivorship from egg to adult emergence and fertility of *D. citri* on *C. suhuiensis* (limau madu) were studied under laboratory condition (29±1°C and 85% RH). Limau madu of height 12 cm with flush leaves were placed in a wooden cage covered with nylon mesh containing citrus plant with *D. citri* for egg laying. Life table was constructed base on population parameters obtained under unlimited food supply and natural enemies free environment. The result showed that highest mortality in 1st instar nymphs (40.23%) with k-value of 0.22 and mortality (25.59%) in 2nd instar nymphs with k-value of 0.13 are the key factors regulating the population size. The sex ratio (proportion of female to male) was 1:0.65. The maximum life span of female was 23.6 days and the trend of oviposition showed a peak at about the 12th day of the female life span with 3.85 mean numbers of eggs per female. The intrinsic rate of natural increase (r_m) was 0.026 per female per day and daily finite rate of increase (λ) was 1.023 per female per day, with a mean generation time (T) of 26.528 days. The net reproductive rate (R_0) of the population was 2.004 and the population Doubling Time (DT) was within 26.456 days. Besides that, the life table showed that, the population of *D. citri* in the present study display type 3 type of survivorship with about 25.971% of the eggs successfully reached adult stage. Above findings on life table and demographic parameters of *D. citri* particularly its capacity for natural increase and mortality rate of the different developmental stages are necessary for development of reliable and sustainable IPM strategy for the pest.

Key words: Citrus greening disease, net reproductive rate, intrinsic rate of natural increase, population doubling time

INTRODUCTION

The Asian citrus psyllid *D. citri* is a host specific plant-sap sucking insect (Dzokou *et al.*, 2009), and regarded as one of the most important pest of citrus globally, because it is known to be the most efficient vector of bacterium *Liberobacter asiaticum* that cause citrus greening or huanglongbing

(Hall *et al.*, 2011). Other damage caused by psyllids includes deformation of leaves, secretion of honeydew which stimulates fungal growth on plant organs (Ndankeu *et al.*, 2011; Lebel *et al.*, 2007) that may lead to reduce photosynthesis. The psyllid was first described from Taiwan in 1907 (Pluke *et al.*, 2005) and a little later it was described in Philippines and India (Aubert, 1987). Although, *D. citri* has been reported to have a restricted host range (Hall, 2008) mainly from the Rutaceae family, but a list of 57 plant species were reported as host of *D. citri* (Halbert and Manjunath, 2004) and now it is widely distributed in several countries and well established in countries that produce citrus in commercial quantities (Da Graca *et al.*, 2008).

D. citri and huanglongbing has been present in Malaysia since 1970 but the disease was overlooked (Abdullah *et al.*, 2009) until when the pest was reported on mandarin tree in Kaula Terla, Cameron highland in 1987 (Garnier and Bove, 1996), also an earlier observation of symptoms of the disease was made in Ringlet area of Cameron highland and in Ulu Tiram in Johor and recent report shows that, most of the cultivated citrus varieties in Peninsular Malaysia including Sabah and Sarawak have been infected by huanglongbing disease (Khairulmazmi *et al.*, 2008).

Understanding population dynamics of specie rely heavily on understanding life table development of the specie (Farooq-Ahmad, 2001), as such construction of life table is regarded as one of the most significant tools in entomological research (Win *et al.*, 2009) and a most for understanding the population dynamics of an insect. Life table may be used for the quantification of mortality caused by natural enemies and the life table parameters such as intrinsic rate of natural increase (r_m) which is define as rate of increase of a species under specific condition in unlimited environment (Fouly *et al.*, 2011) and finite rate of increase (λ) define as population multiplication in unit time (Shahi *et al.*, 2007; Al-Shammery, 2010) are regarded as the best available description of the population growth of specie under a given condition (Abdel-Salam, 2000). The r_m of specie determine by its developmental time and reproductive rate has been used widely in insect population study to compare specie under different environmental conditions and also as an index of population rate response to selected pray (Abdel-Salam, 2000). Nevertheless, information on demographic parameters which are important in measurement of population growth capacity, predicting pest outbreak, estimating extinction probabilities of population (Amiri *et al.*, 2010) and development of pest management strategy are lacking for *D. citri* in Southeast Asian countries where the psyllid remain the major threat to citrus production.

Therefore, the aims of this study were to construct the life tables for *D. citri* on citrus (limau madu) plants for demographic analysis and to determine the survivorship and rate of increase of *D. citri*.

MATERIALS AND METHODS

Sourcing and rearing of insects: Laboratory colonies of Asian citrus psyllid *D. citri* were established on potted ornamental orange jasmine plants, *Murraya paniculata* (L.) Jack with nymphs and adults collected from *M. paniculata* plant in Universiti Putra Malaysia Masjid using aspirator or cutting of shoots containing the adults or nymphs and quickly placing it in glass vial with ventilated cover. Thereafter ten or more nymphs or adults of *D. citri* were released into a screen cage measuring 70×70×70 cm containing one jasmine plant with new flush of vegetative shoots to multiply. The colonies were maintained in a screened cages in glasshouse and the plants were regularly pruned and fertilized to promote production of new growth that is preferred for oviposition by adult female (Weathersbee and McKenzie, 2005) and glasshouse conditions were maintained during the rearing.

Life table: Survivorship from egg to adult emergence and fertility of *D. citri* on *C. suhuiensis* (limau madu) were studied under laboratory condition ($29\pm 1^\circ\text{C}$ and 85% RH) using three different cohorts comprising of 150, 143 and 127 eggs each. *D. citri* eggs for the study were obtained by placing limau madu of height 12 cm with flush leaves in a wooden cage covered with nylon mesh containing *M. paniculata* plant with *D. citri* for 24 h. Eggs laid on the limau madu were then group in to batches of more than 20 eggs and observed for hatching. After egg hatching the developmental time of the nymphal stages was measured as time in days within each stage. Observation on survival and mortality of eggs, nymphs and adults was made and recorded daily.

Also, from adults that emerged in survival and mortality study, three pairs of male and female *D. citri* were transferred to another screen cage containing limau madu plant for mating and egg laying. Eggs laid by each female were counted and recorded daily and the adults transferred to another limau madu plant in cage until when the female died.

Life table parameters such as net reproductive rate (R_0), intrinsic rate of natural increase (r_m), finite rate of increase (λ), intrinsic birth rate (b), intrinsic death rate (d), Generation Time (GT) and Doubling Time (DT) were measured on the basis of population parameters obtained under unlimited food supply and natural enemies free environment according to the procedure described by Birch (1948), Southwood (1978) and Islam and Shunxiang (2007).

RESULTS AND DISCUSSION

The survivorship of *D. citri* reared on limau madu is shown in Fig. 1. Egg hatchability of *D. citri* in cohort 1 was about 84.7% and the first nymph emerged on day five, however the survivorship of individuals start a sharp drop on day six and lasted until day nine (Fig. 1). Highest number of within stage mortalities occurred on day six, seven and nine with a total of about 27 (78.7%), 12 (88%) and 11 (87%) individuals, respectively while from day 12 mortalities incurred in the cohort gradually decreases throughout the life span. All surviving nymphs underwent four moults and the first emerging adults occurred on days 20 and live for up to 24 days, whereas the maximum life span (from hatching egg to death of adult) was 40 days for cohort 1.

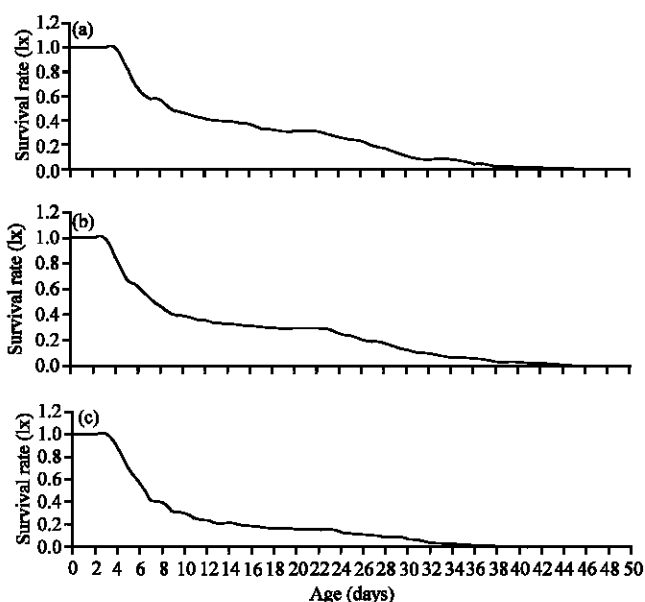


Fig. 1(a-c): Patterns of survivorship curve (lx) of *D. citri* for the (a-c) three cohorts

Table 1: Pooled life table of *D. citri* on limau madu

Age (days)	I_x	L_x	d_x	100 q_x	S_x	T_x	e_x	k-value
Eggs	412	382.5	59	14.32	85.68	1292	3.38	0.07
Nymph								
Instar 1	353	282.0	142	40.23	59.77	909.5	3.23	0.22
Instar 2	211	184.0	54	25.60	74.4	627.5	3.41	0.13
Instar 3	157	147.0	20	12.74	87.25	443.5	3.02	0.06
Instar 4	137	129.0	16	11.68	88.32	296.5	2.30	0.05
Instar 5	121	114.0	14	11.57	88.43	167.5	1.47	0.05
Adult	107	53.5						

In cohort 2 the survivorship follow a similar pattern to cohort 1 with high within stage mortality occurring during nymphal growth particularly in the 1st instar nymphs 30 (73.7%) and in 2nd instar nymphs 9 (87.5%), which subsequently decreases gradually throughout the life span of the population (Fig. 1). Egg hatchability in cohort 2 was about 85.1% with the first nymph emerging on day four, while the first adult emerged on day 20 and also spend a maximum life span of about 24 days. Similarly, in cohort 3, also high mortality occurred during the early nymphal stages with number of individual within stage mortality being 40 (64.3%) in 1st instar and 16 (70.4%) in 2nd instar. Egg hatchability was about 88.2% and the first nymph emerging on day four, while the first adult emerged on day 22 and spend a maximum life span of about 18 days.

The pattern of survivorship observed in the three cohorts indicate a soaring rate of mortality during early nymphal stage and a subsequent gradual reduction as it approached adulthood, given rise to a type III survivorship curve following the classification of Schowalter (2006), this early nymphal mortality may be caused due to sensitivity of the immature to overcrowding and their inability to move and locate a suitable flush leave for feeding. Type I survivorship curves represent species in which high mortality concentrated near the end of their maximum life span, while type II survivorship curves represent species in which the mortality is uniform throughout the life span of the specie.

Table 1 shows the pooled life table of *D. citri* for the three cohorts, it reveals that, from the total of 412 eggs of *D. citri* in the three cohorts, the bulk of mortality occurred during early nymphal stages as obtained in most insects. Highest mortality occurred in 1st instar nymphs (40.23%) with k-value of 0.22 followed by mortality (25.59%) in 2nd instar nymphs with k-value of 0.13 and in egg stage (14.32%) with k-value of 0.07, nevertheless about 25.97% of the 412 *D. citri* eggs had successfully emerged as adults. This high mortality in the early nymphal stages may be attributed due to overcrowding of the nymphs on flush leave and their inability to feed on stem and matured leaves and this mortalities recorded in the two nymphal stages (1st and 2nd instar) may be regarded as the key stages or factors regulating the population size.

Age-specific fertility life table for *D. citri*: The survivorship and fecundity of *D. citri* are shown in Fig. 2 based on the detailed data in Table 2. The first female emerged on day 20 and the first death in the cohort was recorded on day 22. The survival from egg to adult emergence was about 0.26 (Table 1). The proportion of male to female recorded was 0.65:1.00. The last females died on the 44th day. The females remained alive for a maximum of 22 days. Females started laying eggs from the 23rd days or about 3 days from their first emergence. The numbers of eggs deposited were low in the early period and raised gradual than decline towards the final period of life span. The highest number of eggs were laid on day 28 or 7 days post emergence with number of eggs per female being 3.94.

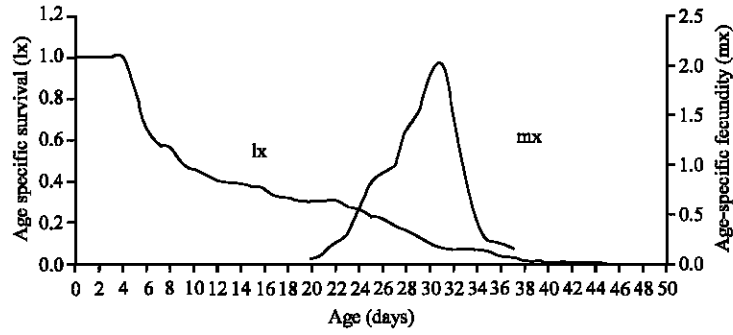


Fig. 2: Daily age-specific survival (l_x) and fecundity (m_x) of female *D. citri* on limau madu

Table 2: Life and age-specific fecundity table of *D. citri* on limau madu

Age (days)	l_x	Egg/female	m_x	$l_x m_x$	$\sum l_x m_x$	$e^{-r_n x} (l_x m_x)$
0	1					
1	1					
2	1					
3	1					
4	1					
5	0.846667					
6	0.666667					
7	0.586667					
8	0.566667					
9	0.493333					
10	0.466667					
11	0.44					
12	0.413333					
13	0.4					
14	0.393333					
15	0.38					
16	0.366667					
17	0.333333					
18	0.326667					
19	0.313333					
20	0.313333	0.106383	0.055319	0.017333	0.814667	0.01553
21	0.313333	0.212766	0.110638	0.034667	1.629333	0.03106
22	0.313333	0.425532	0.221277	0.06933	3.25866	0.06212
23	0.286667	0.581395	0.302326	0.086667	3.726667	0.071042
24	0.266667	1.025	0.533	0.142133	5.685333	0.10838
25	0.24	1.555556	0.808889	0.19413	6.9888	0.133228
26	0.226667	1.764706	0.917647	0.208	7.072	0.134814
27	0.193333	1.724138	0.896552	0.17333	5.02666	0.095824
28	0.173333	2.576923	1.34	0.23226	6.03893	0.115121
29	0.14	2.904762	1.510476	0.211467	4.4408	0.084655
30	0.106667	3.625	1.885	0.201067	3.217067	0.061327
31	0.086667	3.846154	2	0.17333	2.25333	0.042956
32	0.08	2.916667	1.516667	0.121333	1.456	0.027756
33	0.08	1.666667	0.866667	0.069333	0.832	0.015861

Table 2: Continued

Age (days)	l_x	Egg/female	m_x	$l_x m_x$	$\sum l_x m_x$	$e^{-r_m x} (l_x m_x)$
34	0.08	0.833333	0.433333	0.034666	0.416	0.00793
35	0.066667	0.5	0.26	0.017333	0.173333	0.003304
36	0.046667	0.428571	0.222857	0.0104	0.0728	0.001388
37	0.04	0.333333	0.173333	0.00693	0.0416	0.000793
38	0.026667	0	0	0	0	0
39	0.026667	0	0	0	0	0
40	0.02	0	0	0	0	0
41	0.02	0	0	0	0	0
42	0.013333	0	0	0	0	0
43	0.013333	0	0	0	0	0
44	0.006667	0	0	0	0	0
45	0	0	0	0	0	0
Total			14.05398	2.003733	53.144	1.01309

Table 3: Population and reproductive parameters of *D. citri* on limau madu

No.	Parameter	Formula	Values
1	Approximate generation time (T_c), (days)	$\sum l_x m_x x / \sum l_x m_x$	26.519
2	Corrected generation time (T), (days)	$\ln R_0 / r_m$	26.528
3	Innate capacity for increase (r_c)	$\ln R_0 / T_c$	0.026
4	Intrinsic rate of natural increase (r_m)	$\sum e^{-r_m x} l_x m_x = 1$	0.0262
5	Finite rate of increase (λ)	e^r	1.023
6	Doubling time (DT), (days)	$\ln 2 / r$	26.456
7	Intrinsic birth rate (b)	$1 / \sum e^{-r_m x} l_x$	38.164
8	Intrinsic death rate (d)	$b - r_m$	38.142
9	Gross reproduction rate	$\sum m_x$	14.05
10	Net reproduction rate (R_0)	$\sum l_x m_x$	2.004

The population and reproductive parameters of *D. citri* are summarized in Table 3. The intrinsic rate of natural increase (r_m) of *D. citri* was 0.0262 per female per day and the daily finite rate of increase (λ) was 1.023 female offspring per female per day with mean generation time (T_c) of 26.519 days. The net reproduction rates (R_0) of the population was 2.004. The Doubling Times (DT) occurred within 26.456 days. The intrinsic rate of natural increase, as a measure of animal population growth rate, was first applied to insect populations by Birch (1948) and has since been used on several phytophagous insects. The intrinsic rate of increase, mean generation time, net reproductive rate and population doubling times were useful indices of population growth under a given set of growing conditions (Singh and Yadav, 2009; Siswanto *et al.*, 2008). The population parameters ($T_c = 26.519$, $R_0 = 2.004$, $r_m = 0.0262$ and $\lambda = 1.023$) of *D. citri* fed on limau madu under insectary condition in the present study were lower than the population studied by Nava *et al.* (2007) on two different varieties of citrus (Rangpur lime $T_c = 36.16$, $R_0 = 86.03$, $r_m = 0.1232$ and $\lambda = 1.023$; Sunki mandarin $T_c = 44.42$, $R_0 = 92.15$, $r_m = 0.1018$ and $\lambda = 1.107$) and one ornamental plant (Orange jasmine $T_c = 37.55$, $R_0 = 13.70$, $r_m = 0.0697$ and $\lambda = 1.077$). These variations in population parameters might probably be related to the use of different hosts, temperature and insect strains. Insect fecundity, development and life span could be influenced by their food sources and environmental factors (Siswanto *et al.*, 2008) as observed in *Earias vittella* (Lepidoptera: Noctuidae) fed on different host plants (Satpute *et al.*, 2005) and *Diaphorina citri* fed on four different host plants and on *Acalymma vittatum* (Coleoptera: Chrysomelidae) in

cucurbits (Ellers-Kirk and Fleischer, 2006) and *Nasonovia ribisnigri* (Homoptera: Aphididae) in lettuce (Diaz and Fereres, 2005).

Despite the fact that, this psyllid has a comparatively large number of hosts in the Rutaceae family, they cannot develop completely in all of them (Aubert, 1987), however, the result on intrinsic rate of increase (r_m) and finite rate of increase (λ) obtained in this study were near to those reported by Nava *et al.* (2007) on Sunki mandarin.

CONCLUSION

The life table analysis of *D. citri* fed on limau madu indicated that the survivorship of *D. citri* was a type III with a high mortality occurring during the early nymphal (1st and 2nd instar) stages and a subsequent gradual reduction as it approached adulthood. These (high) mortalities recorded in the two nymphal stages may be regarded as the key stages or factors regulating the population size. Above findings and demographic parameters of *D. citri* particularly its capacity for natural increase and mortality rate of the different developmental stages are necessary for development of reliable and sustainable IPM strategy for the pest.

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