Original Contribution

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., Suppl. 6, 2014: 35-40

Investigations on some Biological Characters of *Pseudococcus cryptus* (Hempel) (Hemiptera: Pseudococcidae) on Four *Citrus* species

Didem Holat[1], M. Bora Kaydan[2], Murat Muştu[3]

[1]Yuzuncu Yil Universtiy, Agriculture Faculty, Plant Protection Department, Van, Turkey
[2] Imamoglu Vocational School, Çukurova Üniversity, Adana, 01330, Turkey: E-mail: bkaydan@cu.edu.tr
[3] Erciyes University, Seyrani Faculty of Agriculture, Plant Protection Department, Kayseri, Turkey.

Abstract: The developmental time, longevity, fecundity, sex ratio, duration of preoviposition, oviposition and postoviposition, number of nymphs and life table parameters of *Pseudococcus cryptus* (Hempel) (Hemiptera: Pseudococcidae) were studied on four citrus (Rutaceae) species (*Citrus paradisi, C. limon, C. unshiu, C. sinensis*), in an acclimatized room at 25±2^{0}C, 60-70% relative humidity and 14:10h (l:d) photoperiod. The results suggest that some of the parameters for *P. cryptus* populations were affected by the species of citrus, and that *C. limon* was the preferred host plant. The population parameters on *C. limon* were determined as r_m = 0.1526 d^{-1}; R_0 = 65.8 offspring/individual and T_0 = 27.4 days.

Key Words : Cryptic mealybug, life table, development time.

Introduction

Pseudococcus cryptus Hempel (Hemiptera: Coccoidea: Pseudococcidae), the cryptic mealybug, is an important pest in citrus orchards and is widely distributed in South East Asia, tropical Africa, mid-eastern Mediterranean and South America (Ben-Dov *et al*. 2013). Although this mealybug is fairly polyphagous (with 90 host plant species recorded), it is a particular pest of citrus in Israel (Ben-Dov, 1988). The cryptic mealybug feeds on the leaves, fruits and branches and produces honeydew on which sooty mold develops. Avidov, Harpaz (1969) mentioned that heavy infestations caused leaf and fruit drop and that the entire tree could become covered in sooty mold.

Citrus is one of the most important crops for both domestic and international markets, with almost 33 billion trees in Mediterranean, Aegean and in the Black sea region in Turkey. The primary production zone in Turkey is Çukurova, which produces 70% of all citrus grown in Turkey, where the main crops in three provinces are (Anonymous 2010):

- Hatay Province (southern part): mainly oranges,
- Adana Province (central part): oranges, tangerines and grapefruit,
- Mersin Province (western part): lemons.

In fact, the citrus mealybug, *P. citri* (Risso), occurs in all citrus growing areas in Turkey and is regarded as the most harmful and most common citrus pest and is therefore frequently chemically controlled, leading to pesticide residue problems. So far, *P. cryptus* is restricted to the Hatay region, where it was recorded on *Citrus* spp. in 2013. Previously it had been erroneously identified as *Pseudococcus viburni* (Signoret) (Dr. Lerzan Erkılıç, personal communication, MBK personal observation) . The presence of *P. cryptus* in the Hatay region can be explained by its restriction to the Levant (i.e. Cyprus, Israel, Jordan, Lebanon, Syria (Aleppo), Palestine and Hatay province) in the East Mediterranean sub-region.

Regardless of the importance and invasiveness of *P. cryptus*, there have been few studies regarding the effect of temperature and host plant species on its development and fecundity (Arai 1996, Kim *et al.* 2008). It is known that this species is ovoviviparous (Kim et al. 2008) and that the developmental threshold temperature has been determined as about 10°C (Arai 1996), although his estimates were based on data collected from experiments conducted between 20 and 30°C. Kim *et al.* (2008) reported that the total development time decreased with increasing temperature and ranged from 54.9 days at 16°C, 17.4 d at 28°C and 19.3 d at 32°C. However, *P. cryptus* produced 111 eggs per female at 28°C but only a mean of 102.7 eggs per female at 32°C.

Compared to resistant or tolerant hosts, pests have greater population growth rate and cause more damage on the more susceptible host plants and it is therefore important to study host plant susceptibility (Kaydan *et al.* 2006, Özgökçe, Atlihan, 2004). There is no information available on the population development of *P. cryptus* on the different citrus species in Turkey. The present study was carried out to determine the developmental time, survival and fecundity of *P. cryptus* on four *Citrus* species (*Citrus paradisi, C. limon, C. unshiu* and *C. sinensis*) at 25±1°C, 65±10% relative humidity and 14:10h (l:d) artificial light conditions.

Materials and Methods

Mealybug source: *Pseudococcus cryptus* used in the experiments were obtained from Hatay on *Citrus* spp. It was initially cultured on potato sprouts and squash at 26±2°C, 65±10% rh and 14:10h (l:d) artificial light conditions in an insect free climatic room. The mealybugs were reared for one generation on each of the *Citrus* species (generally on entire one year seedlings) prior to the actual experiment. Five or six weeks after the initial infestation, newly matured adult females were collected from each *Citrus* species and allowed to mate; these mated adult females were used for the final experiments. The remaining mealybugs were used as the stock culture.

Host source: Four *Citrus* species grown in different regions of Turkey were used in this study (*C. sinensis* Osb. Navelina; *C. limon* Burm. Mayer; *C. unshiu* Marcow Satsuma; *C. paradisi* Macf. Rio-Red) (Rutaceae). Seedlings of each *Citrus* species were obtained from a commercial company in Mersin and grown on as described above.

Experiments

A single adult female which had just started to lay eggs, was transferred onto a leaf disk taken from the middle of each of the four host plants and placed upside down on water agarose in the Petri dishes (9 cm diameter). When the crawlers started emerging, they were collected and transferred onto individual leaf disks taken from the middle of each of the four host plants and placed upside down on water agarose in the Petri dishes (6 cm diameter). The leaf discs were changed every three days and each nymph was checked daily for exuviae and survivorship. Once adult, the duration of the pre-reproductive, reproductive, post-reproductive stages and the total longevity were determined by daily observations. Observations were continued until death. During the reproductive period, all eggs were removed from the test arena after counting. Twenty-five to 60 replicates were conducted on each *Citrus* species, but only those individuals that survived to adulthood were included in the calculations of immature developmental time.

The experiments were conducted in a climatic room at 25±1°C, 65±10% relative humidity and 14:10h (l:d) photoperiod conditions.

Statistical Analyses Data on the developmental time, longevity and fecundity at different host plant species were analyzed by one-way ANOVA followed by LSD Test ($P \leq 0.05$) (capital letters in the tables on the columns).

Population growth rates of *P. cryptus* on the four *Citrus* species were analyzed based on the theory of an age-stage, two-sex life table (Chi, Liu 1985, Chi 1988). The means, variances and standard errors of the life table parameters were estimated using the Bootstrap technique (Efron, Tibshirani 1993, Meyer *et al.* 1986, Huang, Chi 2012). The computer program TWOSEX-MSChart (Chi 2012) was used to analyze the life history raw data. The age-stage specific survival rates (s_{xj}) (where x is the age and j is the stage), age-stage specific fecundity (f_{xj}), age-specific survival rates (l_x), age-specific fecundity (m_x), and population parameters (r=intrinsic rate of increase, λ=finite rate of increase, R_0=net reproductive rate; T=the mean generation time and GRR=gross reproductive rate) were calculated. The intrinsic rate of increase is estimated using the iterative bisection method from the Euler-Lotka formula: $\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$ with age indexed from 0 (Goodman 1982). The mean generation time is calculated as $T = \ln R_0 / r$. The gross reproductive rate (*GRR*) is calculated as $\sum mx$.

Results

The results show that total developmental time of female *P. cryptus* was significantly affected by the *Citrus* species ($F_{(3,\ 146)}$= 4.10; P<0.0001) (Table 1). The longest development time (25.25 days) was recorded on *C. sinensis*, significantly longer than that on *C. limon* and *C. unshiu*. The same trend was observed with the males ($F_{(3,146)}$= 2.18; P<0.005) (Table 2). When the development time of males and females was compared, male development time was always longer than that for the female. Total mortality of the *P. cryptus* nymphs was generally low on all four host species, but the highest mortality rate was observed on *C. sinensis* (7.6%) (Table 3)

The pre-reproductive time of individuals reared on the four host plant species was not significantly different at around 15 days (Table 4) ($F_{(3,\ 169)}$=4.86; P>0.05). Postoviposition values were also not significantly different ($F_{(3,\ 169)}$= 0.19; P>0.05). However, the shortest reproductive times were observed for populations reared on *C. unshiu*, and the longest were for those reared on *C. sinensis* and *C. limon* (Table 4) ($F_{(3,\ 169)}$= 3.47; P<0.005). Female longevity was also significantly affected by host species (Table 4). (F=2.16; df=159; P=0.032), with greatest adult longevity on *C. sinensis* and shortest longevity on *C. unshiu* (Table 4).

Host species also affected fecundity. The fewest eggs were produced on *C. unshiu*, significantly fewer than on the other *Citrus* species tested (Table 4); whilst the most eggs were laid on *C. limon* ($F_{(3,\ 182)}$=47.78; P<0.005) (Table 4).

Life table parameters for *P. cryptus* varied on the different citrus species (Table 5). The highest intrinsic rate of increase (r) was for populations reared on *C. limon* (0.1526 d^{-1}), significantly higher than those on the other host plant species such as on *C. unshiu* (0.1181 d^{-1}). Similarly, the highest finite rate of increase (λ) was estimated for populations reared on *C. limon* (1.1649 d^{-1}) and the lowest were for populations reared on *C. unshiu* (1.1254 d^{-1}). The net reproduction rate (R_0) was greatest on *C. limon* (65.8 offspring/individual) and lowest on C. *unshiu* (28.8 offspring/individual). Mean generation time (T) values on the different host-plant species were not significantly different. The highest gross reproductive rate (GRR) was for populations reared on *C. limon* (91.2 offspring/individual) while, the lowest was for those on *C. unshiu* (42.9 offspring/individual).

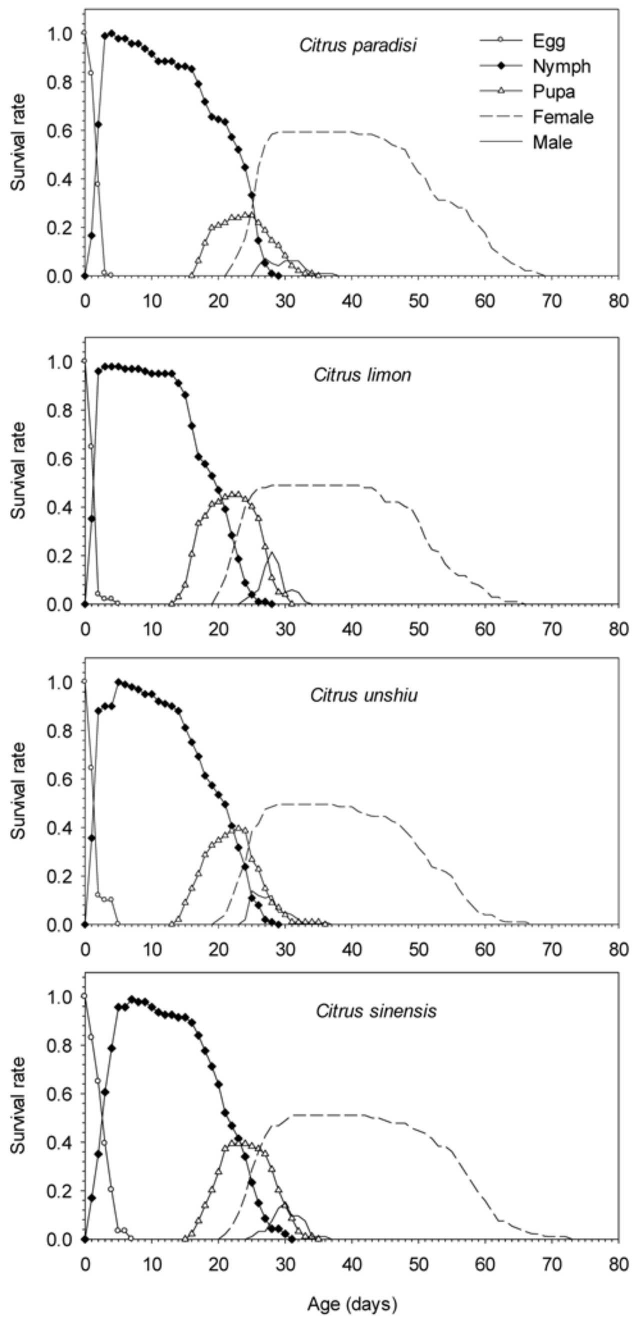

Fig. 1. Age-specific survival rate (*sxj*) of *P. cryptus* on different *Citrus* species

The *sxj* value of *P. cryptus* (Fig. 1) indicates the probability of a newly laid egg surviving to age *x* and stage *j*. These curves also show the survivorship and stage differentiation and overlapping between life-stages. It shows that female nymphs became adult latest on *C. paradisi* but that they lived longest on *C. sinensis*.

Figure 2 shows l_x, f_{x3} (the adult is the third female life stage), m_x (the age-specific fecundity), and $l_x m_x$ (age-specific maternity) of *P. cryptus.* The age-specific survival rate (l_x) is a simplified curve for all the preadult stages of both sexes. The highest peaks for f_{xj}, were observed on *C. limon* and *C. sinensis* while the highest peaks for m_x and $l_x m_x$ were on *C. limon* and *C. paradisi* (Fig. 2).

Discussion

The results show that the performance of *P. cryptus* and, consequently, its population increase, is affected by the host species. Of the citrus species currently grown in Turkey, *C. limon* appears to be the most susceptible to the cryptic mealybug and it is therefore likely that *P. cryptus* will spread to the Mersin Province which presumably has the same environmental conditions and where *C. limon* trees are common.

Although there have been a few studies on the effect of environmental factors on insects in general, few of them discuss the effect of host plant species on development and reproduction parameters (Kaydan *et al*. 2006, Mataş, Kaydan 2013, Özgökçe, Atlihan, 2004, Polat *et al*. 2008). In the present studies, the net reproduction rate (R_0) and intrinsic rate of increase (r), were both good indicators of the effect of host plant on development, survival and fecundity, particularly on *C. unshiu*, where these values were lower than on the other citrus species tested (i.e. the plant was less suitable as a host). These lower values were due to a lower daily birthrate and a later peak in reproduction. Because of the lower intrinsic rate of increase (r) on *C. unshiu*, gross reproductive rate (GRR) was also longer than on the other host species. Lower r and GRR values on *C. sinensis* showed that this species was a least favorable host for the mealybug of the four citrus species tested.

The results, especially the intrinsic rate of increase (r), indicate that *P. cryptus* did not perform well on *C. unshiu*. It is well known that even a small reduction in the intrinsic rate of increase can result in great changes on population sizes of the pest species (Goundoudaki et al. 2003) Knowing life history parameters of pest species in commercial citrus production is important in predicting population growth in these citrus growing regions.

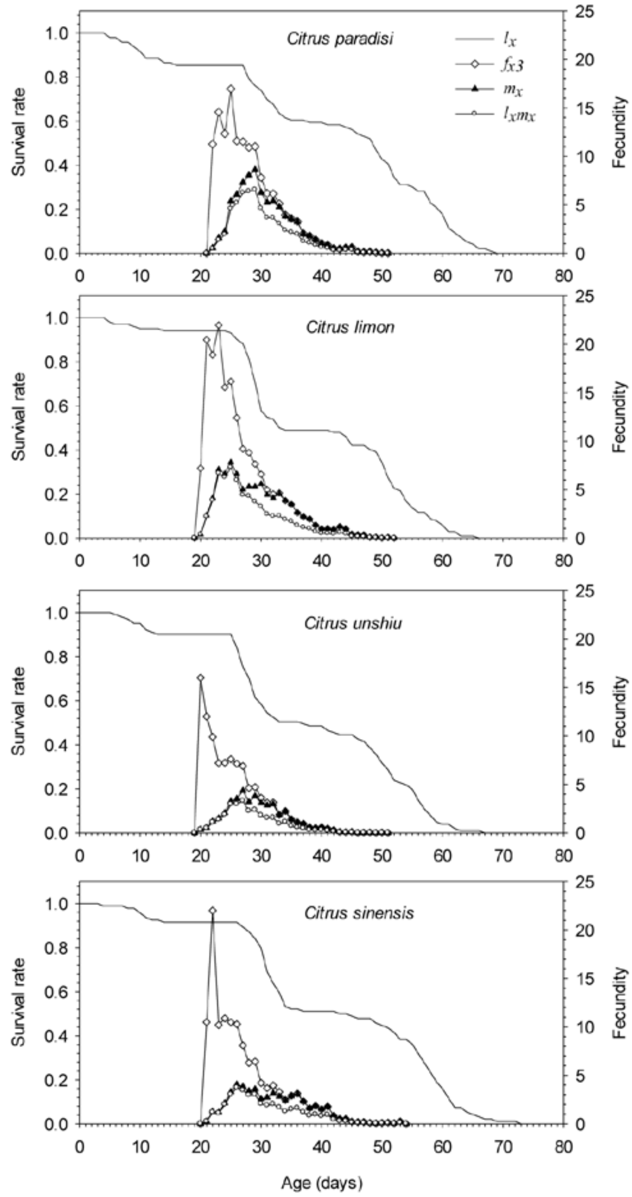

Fig. 2. Age-specific survival rate (l_x), female age-specific fecundity (f_{x3}), age-specific fecundity (m_x), and age-specific maternity ($l_x m_x$) of *P. cryptus* on different *Citrus* species

Acknowledgements: The authors are very grateful to Dr. Lucia Zappalà (University of Catania, Department of Agri-Food and Environmental Systems Management (DiGeSA), Italy) for reviewing early draft of the manuscript. This study was supported by YYU-BAP (2010-FBE-YL-155), we would like to thank them for their financial support.

Table 1. Developmental time of the preadult stages of *P. cryptus* females on different *Citrus* species (Mean±SE)

Host Plant		Instars (Day)				Egg to adulthood
	n	Egg	First	Second	Third	
Citrus paradisi	57	1.89±0.14B*	11.22±0.16A	5.94±0.13A	6.14±0.09A	25.21±0.22A
Citrus limon	50	1.28±0.14C	10.12±0.18C	5.58±0.12A	5.58±0.9C	22.56±0.29C
Citrus unshiu	50	1.54±0.16BC	10.50±0.15BC	5.92±0.13A	5.94±0.11AB	23.90±0.32B
Citrus sinensis	48	2.87±0.22A	10.66±0.18B	5.91±0.13A	5.79±0.11BC	25.25±0.37A

*Within columns means followed by the same letter do not differ statistically (LSD; $P \leq 0.05$).

Table 2. Developmental time of preadult stages and longevity of *P. cryptus* males on different *Citrus* species (Mean±SE)

Host Plant	Instars (Day)						Egg to adulthood	Adult male
	n	Egg	First	Second	Prepupae	Pupa		
Citrus paradisi	25	1.89±0.14B*	10.88±0.24A	5.80±0.29A	1±0	9.16±0.19A	29.16±0.51A	1.76±0.10A
Citrus limon	46	1.28±0.14C	9.97±0.15C	5.15±0.15B	1±0	9.63±0.28A	27.23±0.29B	1.82±0.08A
Citrus unshiu	41	1.54±0.16BC	10.14±0.22AC	5.60±0.17AB	1±0	8.46±0.15B	26.70±0.41B	1.65±0.8A
Citrus sinensis	38	2.87±0.22A	10.73±0.23AB	5.60±0.17AB	1±0	8.46±0.15B	29.47±0.34A	1.78±0.06A

*Within columns means followed by the same letter do not differ statistically (LSD; $P \leq 0.05$).

Table 3. Egg hatching rate, proportion of females and immature survival rates of *P. cryptus* on different *Citrus* species (Mean±SE)

Host Plant	n	Egg hatching rate (%)	Proportion of females (%)	Immature survival rates (%)*
Citrus paradisi	30	96	70.0	5.3
Citrus limon	30	100	85.5	7.0
Citrus unshiu	30	100	77.9	4.3
Citrus sinensis	30	99	70.9	7.6

Table 4. Preoviposition, oviposition, postoviposition, longevity and fecundity of *P. cryptus* females on different *Citrus* species (mean±se)

Host plant	n	Pre-oviposition	Oviposition	Post-oviposition	Female longevity	Fecundity
Citrus paradisi	45	15.60±0.50A	14.62±0.80AB	1.77±0.35A	30.19±0.94B	116.02±5.64B
Citrus limon	43	14.53±0.43A	15.65±0.91A	1.95±0.23A	30.06±0.80B	152.68±9.24A
Citrus unshiu	40	15.82±0.42A	12.28±0.82B	1.91±0.20A	28.56±0.86C	59.28±2.91D
Citrus sinensis	45	15.40±0.36A	15.65±0.79A	1.70±0.15A	32.22±0.80A	83.20±4.14C

*Within columns means followed by the same letter do not differ statistically (LSD; $P \leq 0.05$).

Table 5. Population parameters intrinsic rate of increase (*r*), finite rate of increase (λ), net reproductive rate (R_0), mean generation time (*T)* and gross reproductive rate (*GRR*) of *P. cryptus* on different *Citrus* species (Mean±SE)

Host plant	r	λ	R	T	GRR
Citrus paradisi	0.1369±0.0039	1.1467±0.0045	59.8±6.7	29.8±0.3	81.8±7.3
Citrus limon	0.1526±0.0049	1.1649±0.0057	65.8±8.4	27.4±0.3	91.2±9.6
Citrus unshiu	0.1181±0.0042	1.1254±0.0048	28.8±3.3	28.4±0.4	42.9±3.5
Citrus sinensis	0.1226±0.0047	1.1304±0.0053	38.8±4.7	29.8±0.5	54.9±4.8

References

ANONYMUS 2010. http://gain.fas.usda.gov/Recent%20GAIN%20 Publications/Citrus%20Annual_Ankara_Turkey_12-30-2011.pdf

ARAI T. 1996. Temperature-dependent developmental rate of three mealybug species,Pseudococcus citriculus (Green), Planococcus citri (Risso), and Planococcus kraunhiae (Kuwana) (Homoptera: Pseudococcidae) on citrus. – *Japanese Journal of Applied Entomology and Zoology*, 40, 25-34. (In Japanese with English summary).

BEN-DOV Y., D.R. MILLER and G. A. P. GIBSON 2013. ScaleNet: A database of the scale insects of the world. Available from http://www.sel.barc.usda.gov/scalenet/scalenet.htm. Accessed May 2013.

BEN-DOV Y. 1993. *Pseudococcus cryptus* Hempel in Israel. (In Hebrew; Summary In English). – *Alon Hanotea*, **47** (4): 271-272.

BIRCH L. C. 1948. The intrinsic rate of natural increase in an insect population. – *Journal of Animal Ecology*, **17**: 15-26.

CHI H. (1988). Life-table analysis incorporating both sexes and variable development rates among individuals. – *Environmental Entomology*, **17**: 26-34.

CHI H., H. LIU 1985. Two new methods for the study of insect population ecology. – *Acad. Sin. Bull. Inst. Zool.*, **24**: 225-240.

CHI H. 2012. TWOSEX-MSChart: computer program for age stage, two-sex life table analysis. Available from: <http://140.120.197.173/ecology/>.

EFRON B., R. J. TIBSHIRANI. 1993. An Introduction to the Bootstrap. Chapman & Hall, New York, USA.

GOUNDOUDAKI S., J. A. TSITSIPIS, J. T. MARGARITOPOULOS, K. D. ZARPAS, and S. DIVANIDIS 2003. Performance of the tobacco aphid *Myzus persicae* (Hemiptera: Aphididae) on Oriental and Virginia tobacco varieties. – *Agricultural and Forest Entomology*, **5**; 285-291.

HU L. X., H. CHI, J. ZHANG, Q. ZHOU, and R.J. ZHANG 2010. Life-Table Analysis of the Performance of *Nilaparvata lugens* (Hemiptera: Delphacidae) on Two Wild Rice Species. – *Journal of Economical Entomology*, **103** (5): 1628-1635

HUANG H., Y. BING, H. CHI 2012. Life Tables of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae): with a Mathematical Invalidation for Applying the Jackknife Technique to the Net Reproductive Rate. Available from Nature Proceedings <http://dx.doi.org/10.1038/npre.2012.7070.1>

KAYDAN M. B., R. ATLIHAN, S. TOROS 2006. Effects of Tobacco Varieties on Eidonomy and Life table Parameters of Aphid Species *Myzus persicae* (Hemiptera:Aphididae). – *Entomologia Generalis*, **29** (1): 061-070.

KIM S. C., J. B. SONG, D. S. KIM 2008. Effect of temperature on the development and fecundity of the Cryptic Mealybug, *Pseudococcus cryptus*, in the laboratory. – *Jurnal of Asia-Pasific Entomology*, **11**: 149-153.

LESLIE P. H. 1945. On the use of matrices in certain population mathematics. – *Biometrika*, **33**: 183-212.

LEWIS E. G. 1942. On the generation and growth of a population. – ***Sankhya***, 6: 93-96.

MATAŞ M., M. B. KAYDAN 2013. Investigations on biological characteristics of *Pseudococcus comstocki* (Kuwana) (Hemiptera: Pseudococcidae) on different two mulberry species and different temperature. – *Turkish Journal of Entomology*, (in press).

MEYER J. S., C. G. INGERSOLL, L. L. MCDONALD, M. S. BOYCE 1986. Estimating uncertainty in population growth rates: jackknife bootstrap techniques. – *Ecology*, **67** (5): 1156-1166.

ÖZGÖKÇE M. S., R. ATLIHAN 2004. Biological Features and Life Table Parameters of the Mealy Plum Aphid *Hyalopterus pruni* on Different Apricot Varieties. – *Phytoparasitica*, **33** (1):7-14

POLAT F., S. ULGENTURK, M. B. KAYDAN 2008. Developmental biology of citrus mealybug, *Planococcus citri* (Risso), (Hemiptera: Pseudocccidae), on ornamental plants. – In: BRANCO M., J. C. FRANCO, and C. HODGSON (Eds.). Proceedings of the Xl international symposium on Scale Insect Studies, Lisbon, Portugal, 24-27 September 2007, Oeiras, Portugal. ISA Press, Lisbon, Portugal, 177-184.