Life Table Parameters of the Predatory bug *Orius laevigatus* (Fieber) [Hemiptera: Anthocoridae] Preying upon the Tobacco Whitefly *Bemisia tabaci (*Gennadius) [Homoptera: Aleyrodidae] on Eggplant Host Plant

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Abstract

The current study described the life table characteristics of the predatory bug Orius laevigatus preying upon the tobacco whitefly Bemisia tabaci infestation offered on eggplant leaf discs under laboratory constant conditions of 26±1°C, 75±5% R.H. and 16L: 8D photoperiod regime. Average life table parameters of O. laevigatus were calculated for two successive generations as: intrinsic rate of increase (Rm): 0.038; gross reproductive rate (GRR): 18 insect/female/generation, net reproduction rate (Ro): 2.6 females/female/generation, finite rate of increase (λ): 1.038 females/female/day, mean generation time (T): 25.2 days, and doubling time (DT): 18.2 days. Those parameters indicated that O. laevigatus has the potential to be used as predator against B. tabaci and that this predator would likely be able to overcome populations of B. tabaci on eggplant plantation in greenhouses under the conditions similar to those used in this study. This appears to be the first publication recording the life table parameters of O. laevigatus when used as natural enemy against B. tabaci infestation of eggplant.

Keywords: life table, *Orius laevigatus*, *Bemisia tabaci*, eggplant, biological control

1. Introduction

Generalist predatory insects are capable of attacking a diverse spectrum of prey species (Eubanks & Denno, 2000), because they have the necessary phenotypical plasticity to adjust their biology to different food sources (Mendes *et al*., 2002). However different types of prey can substantially alter the development and reproduction of predators, which in turn affect population dynamics. Species in the genus Orius (Hemiptera: Anthocoridae) are generalist predators that attack eggs and immature stages of various arthropods, or small soft-bodied adult arthropods, including numerous important agricultural pest species (Reitz *et al*. 2006; Butler & O'Neil 2007). Although they are polyphagous, *Orius* spp. show a preference for attacking larval and adult thrips (Thysanoptera) over other available prey (Arno` *et al*., 2008; Xu & Enkegaard, 2009). Consequently, they are considered promising and effective as biological control agents and have been used successfully in biological control programs in greenhouse and open-field cropping systems against various thysanopteran pests. In particular, *Orius insidiosus* (Say) has been released into sweet pepper and cucumber greenhouses in Europe to successfully control *Thrips tabaci* (Lindeman) and the invasive *Frankliniella occidentalis* (Pergande) (Sabelis & van Rijn, 1997; Perdikis *et al*., 2008).

In its area of origin, conservation of *O. insidiosus* is a key component of integrated pest management (IPM) programs for thrips in field grown crops (Funderburk, 2009). *Orius laevigatus* (Fieber) is another species that has been widely employed in successful biological control programs in Europe (Coll *et al*., 2007). For these reasons, a number of *Orius* spp., in particular *O. laevigatus* and *O. insidiosus*, are mass produced for augmentative release by various commercial insectaries in Europe and North America (van Lenteren *et al*., 1997; Lattin, 1999), where they are routinely reared on eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Bonte & De Clercq, 2008). In Europe, several common pests of orchards or greenhouses are preyed upon by different *Orius* species. *Orius vicinus* develops on spider mites and aphids (Fauvel, 1971; 1972; Heitmans *et al.*, 1986), O. *majusculus* attacks many psyllids (Alauzet *et al.*, 1990), and O. *laevigatus* could be a good biological control agent of the western flower thrips, *Frankliniella occidentalis* (Pergande). This polyphagous pest is very difficult to control and its outbreaks are very damaging (Chambers & Long, 1993).

Before using this predator in biological control or in integrated pest management, it is essential to study its biological characteristics including the life table parameters under experimental conditions. The intrinsic rate of increase (***rm***) is a basic parameter which an ecologist may wish to establish for an insect population. Birch (1948) defined the intrinsic rate of increase as the rate of increase per head under specified physical conditions, in an unlimited environment where the effect of increases in density do not need to be considered. Jervis and Copland (1996) reviewed the use of life table analysis both by ecologists and by biological control workers. They indicated that, in a biological control programme, when faced with a choice of candidate parasitoid species, in the absence of other criteria, the selection would be for the species with the greatest value for the intrinsic rate of natural increase. Once the value of ***lx*** (survival) and ***mx*** (fecundity) were tabulated, the following population parameters can be calculated (Messenger, 1964):

a) The intrinsic rate of increase (***rm***) can be measured in female/female/day.
b) Gross reproductive rate (*GRR*) is the mean total number of eggs produced by a female over its life-time, measured in females/female/generation.
c) The net reproductive rate (or basic reproductive rate) (*Ro*) is the number of times a population will multiply per generation, measured in females/ female/ generation.
d) The capacity for increase (*rc*) is an approximation for *rm*.
e) The finite capacity for increase (λ) is the number of times the population will multiply itself per unit of time, measured in females/ female/day.
f) The cohort generation time (Tc) defined as the mean age of maternal parents in the cohort at birth of the female offspring.
g) The mean generation time (T). The comparison of two or more populations by means of their net reproductive rates may be quite misleading unless the mean length of the generation is the same.
h) The doubling time (DT) is the time required for a given population to double its numbers, measured in days.

2. Materials and Methods

This study was conducted to assess the fertility life table parameters of *O. laevigatus* fed on *B. tabaci* stages offered on eggplant leaf discs under standard conditions of 26 ± 1°C, 75 ± 5% R.H. and 16:8 L:D photoperiod.

2.1 Insect Cultures

To establish a stock culture of *B. tabaci* to be used in this study, plant leaves infested with *B. tabaci* were collected from infested eggplant plants from a nearby greenhouse during fall 2009, and placed on eggplant transplants (*Solanum melonena* L. cultivar: classic) kept in the wooden cages maintained in the greenhouse at Faculty of Agriculture, Hebron University, Hebron, Palestine. To obtain eggplant transplants freshly infested with *B. tabaci*, healthy eggplant transplants (*S. melonena* L. cultivar: classic) were inserted in between the heavily infested plants that were kept in the wooden cages. After 48 hours, those freshly infested transplants were transferred to the Perspex cages and kept under ambient conditions in the laboratory to be used as a source of leaf discs freshly infested with eggs and larvae of *B. tabaci* used in bioassays**.** The predator bug, *O. laevigatus* obtained from Bio-Bee Company, Israel was provided in a package with two bottles; each containing 250 bugs. About 80% of the bugs were newly emerged adults and the rest were at 5^{th} nymphal instars mixed with Vermiculite carrier for ventilation.

2.2 Rearing Cages

Woody cages: Two woody cages (1m length x1m width x1m height) were constructed with woody arms and covered with 50 mesh screen from all sides. One cage was used to keep healthy transplants of eggplant; another cage was used to keep the culture of *B. tabaci* on eggplant plants. Perspex cages: Two perspex cages were made from transparent Perspex material (50 cm width x 70 cm depth x 50 cm height). To allow ventilation, a door of 50 mesh cloth (30 cm width x 40 cm high) was provided on the front of the cage. A ten cm diameter hole covered with 50 mesh net was provided in the rear side. The Perspex cages were placed on a metallic tray on laboratory bench with approximately 90 cm high under ambient conditions. Those cages were used to maintain the freshly infested eggplant transplants to be used as a daily resource for the infested leaf discs provided to the bioassays. Petri-dish cage: Each Petri-dish cage (5 cm diameter x 1.5 cm height) had hole of 2 cm diameter in the middle of the lid, which was covered by 50 mesh cloth to provide ventilation. These cages were used for rearing the predators on infested leaf discs in an incubator under the experimental conditions. Agar layer of 2 mm thick was used in Petri-dish cages as a source of nutrients as well as a source of moisture for the leaf-discs

2.3 Agar Medium

Fertilized Agar medium was prepared by adding Agar at rate of 15 g/liter to plant growth fertilizer (20N:20P:20K) diluted in a distilled water at a rate of 2 g/liter. The mixture was heated with a magnetic stirrer for 25 min on hot-plate for homogenizing and dissolving of Agar and then autoclaved for 40 minutes at temperature of 120°C under (1.4 bar) atmospheric pressure. After cooling to 45-50°C, a fungicide solution prepared by dissolving 0.3 g of Benlate ® (50% Benomyl) in 7 ml of ethanol 95% was then added to 3 ml of distilled water, was added to the Agar medium at rate of 1 ml/liter of fertilized Agar. The Benomyl was used to prevent the growth of mould on the agar layer.

2.4 Life Table Bioassay

Bioassays were carried out during fall 2009 in the laboratories of Hebron University, Palestine as part of a research project funded by the deanship of academic research in Hebron University. All tests were done in an incubator under standard conditions of 25±1ºC, 75±5% R.H and 16 L: 8 D photoperiod regime. The experiment was conducted for two successive generations. The 1st generation started on 10th September 2009 and included twenty replicates, each replication consisting of couple of female + male of newly emerged adults of *O. laevigatus* obtained from Bio-Bee Company. Those adults which were mass reared on *Ephestia* eggs during their developmental stages in the insectaries of the Bio-Bee company but, were fed on *B. tabaci* stages during their adult longevity in Hebron University laboratory under the standard conditions. The 2nd generation included 4 replicates obtained from the offspring of the 1st generation that were completely reared on *B. tabaci* stages in Hebron University laboratory under the standard conditions. To obtain adult *O. laevigatus* that were used for life table bioassay in the 2nd generation, 50 newly hatched nymphs were collected from Petri dish cages where the adult couples of the 1st generation reared. Each nymph was separately reared in a 5 cm Petri dish cage on heavy infestation of *B. tabaci* offered on eggplant leaf discs. These nymphs were daily transferred to a freshly prepared Petri dish cage by a fine paint brush while checking them under a binocular microscope (40X). Duration of development from egg to adult, mortality and survival of each bug, and the sex ratio of adults were assessed by daily checking of each nymphal stage.

Each replication (consisted of couple of newly emerged adults of *O. laevigatus*) was reared in 5cm diameter Petri dish cage and offered eggplant leaf disc heavily infested with *B. tabaci* eggs and larvae. The predators were provided with infested leaf-discs placed underside upwards on 2mm-thick Agar medium. A filter paper was used as a layer between the leaf-disc and the Agar medium enabling the free movement of the insects and decreasing the possibility of their sticking to the Agar. Each couple was transferred to freshly prepared cages every day and the previous leaf discs from each cage were kept in the incubator under the standardized conditions during incubation period of eggs and then observed for egg hatching. A solution of 0.5 ml/liter of Merpan® (50% Captan) was sprayed on the leaf discs to prevent the growth of mould on the leaf surface.

The following parameters of *O. laevigatus* adults were observed for each replication:

1. The survival of adult female at 24 h intervals.
2. Daily observation was done on the presence of eggs of *O. laevigatus* in leaf discs where females reared.
3. Fertility of *O. laevigatus* females reared in each replication by daily observation and counting the number of nymphs hatched from cages where the *O. laevigatus* females were reared. Due to the difficulty of counting the eggs laid per female, the fecundity was considered as the number of newly hatched nymphs from eggs oviposited per every female and referred to as fertility

Each couple reared in a 5 cm diameter Petri dish was followed individually till the death of all individual members of the cohort. The surviving adults were maintained and monitored individually to collect necessary data for constructing the life tables. The age of each female (***x***), the probability that a new individual is alive at age ***x*** (***Lx***) and the number of female offspring produced by a female with attributed ***x*** (***mx***) were also recorded. Life-table analyses for the study were undertaken using QBASIC computer program (Jervis and Copland, 1996) (see Appendix 1). In addition to the intrinsic rate of increase (***rm***), the other main fertility life table parameters including net reproductive rate, generation time, doubling time, and finite rate of increase were also calculated.

3. Results and Discussion

Life-table analyses were undertaken using QBASIC computer program (Jervis & Copland, 1996) (see Appendixes 2 & 3). These analyses were done on the basis of a cohort female proportion of 40%, survival rate to adults of 20% and average duration of development from egg to adult of 17.5 days (Table 1). In addition, the daily fertility of *O. laevigatus* was recorded by counting the nymphs which emerged from the eggs laid in each day of the oviposition period. Results of the present study described the life table characteristics of *O. laevigatus* that fed on eggs and larvae of *B. tabaci* offered on eggplant leaf-discs for two generations under laboratory conditions of 25ºC, 75% r.h. and 16:8 L:D. Results show that, the life table parameters of *O. laevigatus* of the 2nd generation that fed throughout its lifetime on *B. tabaci* were approximately similar to that of the 1st generation which mass reared during developmental stages on *Ephestia* eggs (in Bio-Bee Company) but fed during adult longevity on *B. tabaci* (in Hebron University Laboratory) (Table 2). Even though, *Ephestia* eggs are considered the most preferred food that currently used for mass rearing of predatory bugs in company insectaries (Bonte & De Clercq, 2008). Thus, *B. tabaci* proved to be with higher nutritional values for feeding *O. laevigatus* and therefore, *O. laevigatus* might be a promising bio-control agent for suppressing populations of *B. tabaci* infesting eggplant under greenhouse conditions similar to the standard conditions of this study.

Furthermore, the mean calculated life table parameters of *O. laevigatus* for the two generations were as follow: gross reproductive rate of 17.8 insect/ female/ lifespan, net reproductive rate of 2.6 female/ female/ generation, intrinsic rate of increase of 0.038 insects/ female/ day, a generation time of 25.2 days, and doubling time of 18.2 days (Table 2). It's important to note that no literature was found containing the life table parameters *O. laevigatus* when fed on related whiteflies, and the current study might be the first research that recorded the life table parameters of *O. laevigatus* when used as natural enemy against *B. tabaci* infestation on eggplant plantation. However, previous studies (Hamdan, 1997; 2006) reported records of the life table parameters of a mired predatory bug (*Macrolophus caliginosus*) when fed on a related whitefly (*Trialurodes vaporariurum*) offered on eggplant leaf discs under similar laboratory conditions. Meanwhile, *M. caliginosus* is currently recommended as a biocontrol agent against the greenhouse whitefly *T. vaporariurum* in most European and Mediterranean countries (Lucas, & Alomar, 2002; Bonato, *et al*., 2006; Rasdi *et al*., 2009).

Therefore, comparing the life table parameters of *O. laevigatus* preying on *B. tabaci* offered on eggplant leaf discs in the present study and that of *M. caliginosus* preying on the greenhouse whiteflies, *T. vaporariurum* offered on eggplant leaf discs (Hamdan, 1997; Hamdan, 2006), showed that the intrinsic rate of increase of *O. laevigatus* (0.038) was three times higher than that of *M. caliginosus* (0.009), the gross reproductive rate of *O. laevigatus* (18) was ten times higher than that of *M. caliginosus* (1.6), and the doubling time of *O. laevigatus* (18 days) is one fourth that of *M. caliginosus* (72 days). In conclusion, results of the present study suggest that the *B. tabaci* infestation on eggplant is a satisfactory diet for establishing and maintenance of *O. laevigatus* which proved to have the potential to be used as predator against *B. tabaci*, and this predator would probably be able to overcome populations of *B. tabaci* in greenhouses under the conditions similar to those used in this study.

Table 1: Duration of Development, Survival to Adult and Sex Ratio of *O. Laevigatus* Nymphs Fed on *B. Tabaci* Infestation Offered on Eggplant Leaf Discs

	Duration of Development Egg-Adult Mean ± sdv	**Nymph Survival To Adult Stage (%)**	**Sex Ratio (Female %)**
Value	17.5 ± 0.53days	20%	40%

Table 2: Life-Table Parameters of *O. Laevigatus* Fed on *B. Tabaci* Stages Offered on Eggplant Leaf Discs

LIFE TABLE PARAMETER		**1ST GENERATION**	**2ND GENERATION**	**MEAN ± SDV**
gross reproductive rate	***GRR***	21.53	14.06	17.795±5.28
net reproductive rate	***Ro***	2.75	2.469	2.6095±0.198
capacity for increase	***Rc***	0.0375	0.0370	0.0373±0.000345
intrinsic rate of increase	***Rm***	0.0386	0.0375	0.0381±0.000778
cohort generation time	***Tc***	26.9715	26.4186	26.6951±0.3909
generation time	***T***	26.1909	24.1343	25.1626±1.454
finite capacity for increase	***λ***	1.0394	1.0382	1.0388±0.000849
doubling time	***DT***	17.95	18.50	18.225±0.3889

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5. Appendixes

5.1 Appendix 1: Qbasic program to calculate life-table parameters (Jervis and Copland 1996)

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10 REM program to calculate life table parameters (MJWC 1996)
15 CLS
20 DIM x(100), k(100), m(100):            REM dimension arrays (n<100)
30 READ n
35 FOR h = 1 TO n: READ x(h), k(h), m(h): NEXT h: REM  read data
40 PRINT "calculating data"
50 ct = 0: mx = 0: ro = 0: j = 1:         REM set variables
60 REM ---------------------------------- cumulative calculations
70 FOR h = 1 TO n
80 ct = ct + x(h) * k(h) * m(h): mx = mx + m(h): ro = ro + k(h) * m(h)
90 NEXT h
100 rc = LOG(ro) / (ct / ro): rm = rc:            REM calculate rc
110 crm = 0: FOR h = 1 TO n
115 crm = crm + k(h) * m(h) * EXP(-x(h) * rm): NEXT h
120 REM ---------------------------------- iterative substitution for rm
130 IF ABS(1 - crm) < .00001 THEN GOTO 180: REM accurate to 4 dec points
140 IF crm > 1 THEN rm = rm + ABS(1 - crm) / x(1)
150 IF crm < 1 THEN rm = rm - ABS(1 - crm) / x(1)
160 PRINT j: j = j + 1: GOTO 110
170 REM ---------------------------------- display results
180 PRINT "gross reproductive rate      (GRR)    ="; mx
190 PRINT "net reproductive rate        (Ro)     ="; ro
200 PRINT "capacity for increase        (rc)     ="; rc
210 PRINT "intrinsic rate of increase   (rm)     ="; rm
220 PRINT "cohort generation time       (Tc)     ="; ct / ro
230 PRINT "generation time              (T)      ="; LOG(ro) / rm
240 PRINT "finite capacity for increase (lambda) ="; EXP(rm)
250 PRINT "doubling time                (DT)     ="; LOG(2) / rm
260 END
270 REM
280 REM ---------------------------------- data
290 DATA 31:        REM number of days data
300 DATA 17.5, .6,   0:: REM x (day), lx (survival), mx (fecundity) female offspring
310 DATA 18.5, .6,   2.325: REM
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5.2 Appendix 2: Life-table data for 1st generation of *O. laevigatus* reared on *B. tabaci* infestation on eggplant leaf-discs at 25ºC, 16:8 L: D and 75% r.h.

290 DATA 27: REM number of days data
300 DATA 18.5, .2, 0: REM x (day), lx (survival), mx (fecundity) female offspring
310 DATA 19.5, .2, 1.3: REM
320 DATA 12.5, .2, 1.42
330 DATA 21.5, .2, 1.44
340 DATA 22.5, .2, 1.1
350 DATA 23.5, .18, 1.31
360 DATA 24.5, .18, 0.73
370 DATA 25.5, .15, 0.77
380 DATA 26.5, .12, 1.00
390 DATA 27.5, .12, 0.67
400 DATA 28.5, .12, 0.70
410 DATA 29.5, .12, 0.63
420 DATA 30.9, .12 0.93
430 DATA 31.5, .10, 0.64
440 DATA 32.5, .10, 0.80
450 DATA 33.5, .10, 0.88
460 DATA 34.5, .10, 0.72
470 DATA 35.5, .10, 0.72
480 DATA 36.5, .09, 0.75
490 DATA 37.5, .09, 0.80
500 DATA 38.5, .08, 0.65
510 DATA 39.5, .07, 0.80
520 DATA 40.5, .05, 0.80
530 DATA 41.5, .05, 0.56
540 DATA 42.5, .04, 0.48
550 DATA 43.5, .04, 0.40
560 DATA 44.5, .03, 0.53

5.3 Appendix 3: Life-table data for 2nd generation of *O. laevigatus* reared on *B. tabaci* infestation on eggplant leaf-discs at 25ºC, 16:8 L: D and 75% r.h.

290 DATA 17: REM number of days data
300 DATA 18.5, .2, 0: REM x (day), lx (survival), mx (fecundity) female offspring
310 DATA 19.5, .2, 1.9: REM
320 DATA 12.5, .2, 1.4
330 DATA 21.5, .2, 0.9
340 DATA 22.5, .2, 1.2
350 DATA 23.5, .2, 1.1
360 DATA 24.5, .2, 0.7
370 DATA 25.5, .15, 1.33
380 DATA 26.5, .15, 1.00
390 DATA 27.5, .15, 1.2
400 DATA 28.5, .15, 0.67
410 DATA 29.5, .15, 0.40
420 DATA 30.9, .15 1.20
430 DATA 31.5, .15, 0.53
440 DATA 32.5, .15, 0.267
450 DATA 33.5, .15, 0.267
460 DATA 34.5, .15, 0.0