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Seasonal trends in *Ceratitis capitata* reproductive potential derived from live-caught females in Greece

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Abstract

Reproductive data of individual insects are extremely hard to collect under natural conditions, thus the study of research questions related to oviposition has not advanced. Patterns of oviposition are often inferred only indirectly, through monitoring of host infestation, whereas the influence of age structure and several other factors on oviposition remains unknown. Using a new approach, in this article, we live-trapped wild *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) females on the Greek island of Chios during two field seasons. For their remaining lifetime, these females were placed individually in small cages and their daily oviposition was monitored. Reproduction rates between cohorts from different collection dates were then compared. The results showed that in the different captive cohorts the average remaining lifetime and reproduction were highly variable within and between seasons. Multivariate regression analysis showed that the month of capture had a significant effect on captive life span, average daily reproduction, and patterns of egg laying. The effect of year was significant on reproduction, but not on captive life span. These differences between sampling periods probably reflect differences in the availability of hosts and other factors that vary during the season and affect age structure and reproduction. Using a non-parametric generalized additive model, we found a statistically significant correlation between the captive life span and the average daily reproduction. These findings and the experimental approach have several important implications.

Keywords

reproduction; age structure; captive cohort; natural population; field study; trapping; sampling; medfly; Diptera; Tephritidae; life span

Introduction

An understanding of reproduction in wild populations is recognized universally by insect biologists as fundamental to both basic and applied ecology. Theoretically, the reproductive rate of an insect reflects its physiological age (Leather, 1995) and the egg-laying pattern can be used as a proxy for its frailty and risk of death. Thus, a physiologically young insect has a low risk of death and is capable of laying many eggs, whereas a physiologically old individual experiences a higher risk of death and is usually much less fecund (Reznick, 1985; Roitberg, 1989; Rosenheim, 1999; Papaj, 2000). Despite this and many other research questions that may be addressed through knowledge of reproductive patterns, it is remarkable that the entomological literature contains no studies that investigate reproductive patterns in the wild. The reason for this is that ovipositing insects, especially those that fly, distribute their eggs at long distances in various locations and are therefore difficult logistically to monitor in the wild.

Insect traps are commonly used to draw a sample from wild populations and then use information gained from this sample to infer age structure and other population parameters that apply to the entire population (Southwood, 1978; Pedigo & Buntin, 1994; Müller et al., 2007; Carey et al., 2008). The datum used is the number of dead flies in the traps because flies are usually dead by the time traps are checked. Extrapolations are then made regarding the actual size of the population and a number of other parameters. However, several types of traps could be modified to capture live insects from which we may then derive valuable information on age and reproduction, thus avoiding the methodological constraints associated with free-ranging adults.

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a pest of major world-wide importance that flies several hundred meters or even kilometers during its lifetime (Meats & Smallridge, 2007) and distributes eggs in a large number of hosts (Liquido et al., 1991). Measuring oviposition of this fly in the wild is not possible and, therefore, most knowledge about oviposition has been derived indirectly through monitoring of host infestation levels (Liquido et al., 1991; Katsoyannos et al., 1998; Papadopoulos et al., 2001). However, the medfly can be easily trapped alive with the use of McPhail-type traps (McPhail, 1939) baited with a food-based synthetic attractant. In recent studies, the survival patterns of wild-caught individual insects of unknown age were used to estimate age structure in the populations from which these individuals were derived (Müller et al., 2004, 2007; Vaupel, 2009), or to investigate age-dependent bias in trapping systems (Kouloussis et al., 2009). In one particular study, this approach was brought to bear on the captive life spans of large numbers of medfly individuals trapped in citrus orchards in Greece (Carey et al., 2008). In this study, residual reproductive data were also collected from samples of live-caught individuals, but these data were not analyzed.

Dealing with these data, in the present work, we used the reproduction and life span of 1 014 wild-caught medfly females in Greece with the objective to assess within- and between-season patterns of reproduction. We tested the hypotheses that (1) the captive oviposition rates of individual females would differ over the season reflecting differences in reproductive rates and (2) oviposition would be associated with captive life span.

Materials and methods

Trapping

Adult female medflies were trapped on the Greek island of Chios from August through November in 2003 and from July through November in 2004. We used the Multilure® trap (Better World Manufacturing, Fresno, CA, USA), a variation of the original McPhail trap

(McPhail, 1939), which is a standard tool for capturing numbers of *C. capitata* and other fruit flies (IAEA, 2003). The trap was baited with the two components, ammonium acetate and tri-methylamine, of the commercially available food-based synthetic attractant Biolure® (Suterra, Bend, OR, USA). No water or insecticide was added to the traps to maintain the captured flies alive. Thirty traps were distributed among 10 trapping locations (three traps per location) in two orchards located 1.3 km apart within the citrus-producing area of Campos, Chios Island, Greece (38°20′00″N, 26°08′00″E). Five 24-h collections were made on various dates in 2003 and 10 in 2004, resulting in a total of 350 and 664 females, respectively. The main cultivations in the area consist of mandarins (*Citrus reticulata* Blanco), sweet oranges [*Citrus cinensis* (L.) Osbeck], bitter oranges (*Citrus aurantium* L.), and lemons [*Citrus limon* (L.) Burm.f.] (Rutaceae), but there are also a few stone fruits, pome fruits, and figs. The climate of Chios has little diversity and is classified as Mediterranean. A detailed description of the orchards and the surrounding area as well as climatic data is given in Katsoyannos et al. (1998).

Measuring mortality and reproduction

The cohorts captured on the various dates were provided with adult food and water and were air-shipped alive for same-day arrival to the University of Thessaloniki, or to the University of Thessaly, Greece. For their remaining lifetime, these once-wild females were placed individually in 6 × 9.5 × 12-cm transparent plastic cages (inverted plastic cups of 0.4 l capacity), provided with water and adult food (4:1 ratio of sugar and yeast hydrolysate). The cages were kept in laboratory rooms at 25 ± 2 °C, 65 ± 5% r.h., and L14:D10 photoperiod. Each cage had a hemispheric 5-cm-diameter oviposition dome fitted to a similar-sized hole opened in the middle of a Petri dish lid that constituted the base of the cage. This surrogate fruit was constructed from a low-density polyethylene ball (1 mm thick) (Semadeni, Ostermundigen, Switzerland). To enable oviposition, the dome was punctured with an insect mounting pin with about 40 evenly spaced 1-mm-diameter holes. Oviposition was stimulated by 5 ml of juice from freshly squeezed oranges contained in a plastic cylindrical vial (2 cm high, 3 cm diameter) in the Petri dish base underneath the dome. The base of the Petri dish also contained a quantity of water to increase relative humidity. Orange juice volatiles and water vapors filled the space underneath the dome and also emanated outside it through the holes. The females inserted their ovipositor in the holes and laid eggs. Survival and the number of eggs laid by individual females were monitored daily. The juice and the water of the domes was renewed every 2nd day. Stress-related mortality (e.g., due to transport and transfer of the flies) was extremely low (<<1% during the first 48 h post-capture) (Carey et al., 2008).

Statistical analysis

The captive life span of wild-caught flies in the laboratory was measured in days since capture (denoted as day 0). To determine whether there was a time effect on captive life span and fecundity of wild-caught flies, we defined several indicator variables of 0–1 type, indicating the year and month of flies' capture. The indicator 'Year' is 0 for 2003 (baseline) and 1 for 2004. The indicators 'J', 'A', 'S', and 'O' are 1 for a fly being caught in July, August, September, and October, respectively, and 0 otherwise. The baseline was November where all month indicators were 0. At first a full model containing the year and month indicators along with multiplicative interaction terms between year and month was fitted (Life span = Intercept + Year + J + A + S + O + Year*J + Year*A + Year*S + Year*O), and two hypotheses were tested: no interactions between year and month, and no main month effect. Then a reduced model with significant terms and the corresponding lower-order terms only were selected by backward model selection. The same analysis was followed for reproduction.

The longitudinal egg-laying trajectories of the flies in the captive cohorts were represented with functional principal component analysis (FPCA) (Yao et al., 2005). Functional principal component analysis was used to identify important components of variation and to summarize the egg-laying trajectories in terms of the principal component scores, which could then be entered into multivariate regression analysis to detect monthly and yearly effects. To avoid possible bias on the right tail part due to the decreasing number of surviving flies during captive aging, only the measurements obtained within 123 post-capture days were used to perform FPCA. With this truncation on the longitudinal trajectories, it is guaranteed that there are at least 10 flies alive at each age. Besides, FPCA was also employed to fit mean trajectory of egg laying in each month with monthly data obtained at those captive ages with at least five live flies at each measured time point. These fitted monthly mean trajectories of egg laying provide a primary analysis of the yearly or monthly effects through a visual comparison.

The association between the captive life span of the females and the average daily reproduction was explored using a non-parametric approach by fitting generalized additive models and incorporating smoothing techniques. Such an approach has the advantages of exploring the relation between captive life span and average daily reproduction without imposing any parametric model assumption. A confidence band is also available for further statistical inference.

Results

Life span

Data pertaining to captive life span have been included in an earlier publication by Carey et al. (2008). Here, we give only a summary and a re-analysis of them in combination with egg production. In accordance with Carey et al. (2008), measures of post-capture longevity for groups of individuals of unknown ages that were trapped during the 2003 and 2004 field seasons were highly variable both within and between seasons. The average time-to-death for the 350 and 664 females captured during 2003 and 2004, respectively, was 47.7 and 40.7 days (Table 1). The average captive life span of flies caught in each month and year is shown in Table 2 along with the median and standard error of life span. In our analysis, the P-values of the full model were smaller than 0.001 for both null hypotheses, suggesting that the month of capture has a significant effect on the captive life span and the size of this effect depends on year. The fitted result of the reduced model in Table 3 suggests that the yearly effect is insignificant whereas the month effects of July, September, and October are highly significant. Moreover, the effect of October is different in the 2 years.

Average daily reproduction

Net reproduction averaged 147.6 eggs per female for the females trapped in 2003 and 126.1 eggs per female for the females trapped in 2004 (Table 1). Of the captured medflies, 73.7% laid eggs in 2003 and 63.4% in 2004. For the females that oviposited, the average number of days before the first egg was laid was 20.3 days in 2003, 18.4 days in 2004, and 19.1 days for both years combined. The percentages of days without egg laying in individual post-captured life span of flies ranged from 24.4 to 99.4% with yearly means of percentages being 74.4 and 74.1% for 2003 and 2004, respectively. Captured medflies laid over 10 eggs on only 11.0% of all post-captured life-days in 2003 and 10.6% in 2004.

An event-history chart (Carey et al., 1998a) of reproduction for all captured flies is given in Figure 1. The leftmost region shows an irregularity of initiation of egg laying with a few females laying some eggs shortly after capture but most not laying for at least 2 weeks and many not until nearly 4 weeks. The mottled pattern reflects the irregularity of both the

timing and intensity of egg laying throughout the egg-laying period. The cessation of egg laying was time-to-death dependent for the majority of flies, although with some major exceptions. For example, the 3–7 days width of the light gray band shows the post-reproductive period prior to death. There was a large fraction of females not laying eggs. Most eggs were laid at later ages. Long-lived females laid the most eggs and laid them at older ages as well. Pronounced post-reproductive periods were observed in cohorts with high reproduction. Few females laid eggs beyond 100 days.

Average daily counts of egg laying were used to explore time effects. Summary statistics of average daily reproduction are shown in Table 2. Comparing the mean and median in each cell in Table 2, the distributions of average daily reproduction are seen to be skewed to the right. Moreover, the patterns of change between months differ between 2003 and 2004. In 2003, median average daily reproduction is highest in females captured in August and then slightly decreases for the following 3 months. In contrast to the situation in 2003, median average daily reproduction has an oscillating pattern in 2004.

A significant interaction between year and month factors and a significant monthly effect were detected by the full model ($P < 0.001$ for both tests). Although the main effect of October was not significant, it was kept in the model due to its significant interaction with year. The final model in Table 4 implies that the average daily reproduction of females trapped in August and October is significantly different from that of females trapped in November in 2004 and the difference only happens to be significant between August and November in 2003. Moreover, the overall yearly effect is also significant.

Longitudinal trajectories of egg laying

The estimated mean trajectories of egg laying for flies caught in each month and year are shown in time order in Figure 2. The estimated means are obtained from FPCA, using only those captive ages with at least five remaining flies at each measured time point. Mean reproduction peaks between 20 and 50 post-capture days. Post-peak shapes are variable. In 2003, the highest peak of the mean curves occurs in August, whereas for 2004 it is highest in November. Mean peak locations also differ between these 2 years.

Differences were formally investigated by conducting a principal component regression to test for time effects using the first four principal components, which explain 86.7% of the total variation, based on truncated data with at least 10 live flies at each measured time point. The result in Table 5 indicates a significant yearly effect between 2003 and 2004, and significant monthly effects of July, August, and October. This supports the finding that the patterns of egg laying varies between flies captured in different months. Moreover, as the interaction terms are significant, the pattern of monthly changes of egg laying is different in each year.

Relationship between life span and reproduction

A scatter plot, along with Pearson correlation, of captive life span against average daily reproduction of the 1 014 caught flies can be found in Figure 3. The Pearson correlation coefficient is 0.22 ($P < 0.00001$) indicating a positive correlation between the captive life span and average daily reproduction of a caught fly.

The association between the captive life span of the females and the average daily reproduction was explored further by fitting a generalized additive model with the effect of the average daily reproduction on the captive life span represented by a smoothing function without a specified form. The fitting procedure was performed by the software R (Wood, 2006). The estimated mean function along with its 95% confidence band is shown in Figure

3. Although the average daily reproduction is found to be significantly associated with the life span of a caught fly, their relation as shown in Figure 3 is non-linear and complicated.

Discussion

In this study, we used a novel method for estimating reproduction in wild-caught medfly females, which circumvents the constraints associated with measuring oviposition in free-ranging flying insects. Our approach was based on analysis of reproduction data gathered from 1 014 wild individual flies that were live-caught on various collection dates in Greece and subsequently monitored through death in the laboratory. The results indicate that substantial within- and between-season changes occur in reproduction in wild medfly populations. Oviposition was high in females caught at the beginning of the season and with few exceptions (e.g., in November 2004) it decreased in females caught toward the end of the season. In addition, a significant correlation was found between the captive life span and average daily reproduction of a caught fly that provides a statistical foundation for analyzing and interpreting the data derived from captured flies.

The observed reproductive trends may be interpreted in terms of variation in aging or age structure. In addition, a strong seasonal element may be involved and different predominant hosts in each period probably play a strong role. Wild *C. capitata* populations on Chios Island tend to be older under a shortage of suitable hosts and younger when good hosts are abundant (Carey et al., 2008). Medflies are the ultimate generalist using over 200 hosts for their larvae and there is a large body of data showing that larval host has considerable effects on life span, reproduction, and other fitness components (Krainacker et al., 1987). Thus, the presented differences in reproduction could be interpreted as evidence of temporal variation in host plants causing variation in fitness/condition affecting longevity. In the orchards where we made the collections, there are a few apricots that mature during June, whereas bitter oranges mature during winter and from mid-June through mid-July. Sweet oranges of local varieties mature during winter, whereas Valencia oranges mature from late-July through late-August. A few existing fig trees mature from early- to mid-August through October, mandarins from late autumn through the winter, and lemons during the winter as well as during the summer.

Besides hosts, differences in reproduction could also result from effects on fly physiology and behavior by such factors as temperature. Katsoyannos et al. (1998) provide temperature data for the area in relation to the phenology of the medfly. Also, these differences could be due to environmental (e.g., unknown effects on pupae or young adults in the wild), nutritional (e.g., acquisition of certain amounts or kinds of symbiotic microbials), or other attributes that living in the wild in different times over the season has conferred upon females. Much further research is required to address all these factors.

In our study, most females did not lay eggs for at least 2 weeks after capture and a number of them did not lay eggs for 4 weeks. The initial stress associated with introducing the flies to the laboratory and having them lay eggs in artificial substrates probably accounts for this long adjustment period. In fact, this delay in the initiation of egg laying is much longer than would be found in the field. Following the same methodology for collecting the flies as in our study and keeping the flies with the same type of food, Katsoyannos et al. (1999) showed that mated wild females caged with natural hosts (pears, *Pyrus communis* L.) started oviposition an average of 1.9 days after capture. The delay in our study may be because the artificial substrates we used lack the physical and chemical stimuli present in natural host fruits, which influence fruit discovery and acceptance for oviposition by the females (Nakagawa et al., 1978; Papaj et al., 1989; Light et al., 1992; Katsoyannos et al., 1997). Furthermore, previous research has revealed that medflies require a certain degree of

experience with artificial substrates to accept them for oviposition (Cooley et al., 1986; Papaj & Prokopy, 1988; Prokopy et al., 1990).

Another possible explanation for the delayed oviposition of the live-caught females may be nutrient restriction. Indeed, the reproductive response in the present study was similar to that observed in experiments in which females were given access to a rich source of nutrients after a long period in which they were provided only carbohydrates (Carey et al., 1998b, 2002). In these cases, a lag period was observed that undoubtedly corresponded to the time required for egg development. Although flies partially compensated for egg laying by increasing egg production, they never caught up to full capacity. The oviposition patterns were also similar to results of studies in which reproduction is hindered via host deprivation (Carey et al., 1986). It is therefore likely that the delayed start of captive individuals was because they were unable to acquire a sufficient quantity of nutrients in the wild. In addition, recent findings showed that the food-based Multilure trap we used to collect the flies is susceptible to an age-dependent bias (higher probability of capturing younger flies), as well as a bias stemming from the feeding history of the flies (flies provided only with carbohydrates are captured at a higher rate compared with those given access to yeast hydrolysate) (Kouloussis et al., 2009). Based on these findings, we may speculate that the sample of the collected flies contained a larger proportion of young and/or hungry flies compared with the actual abundance in the wild. Once introduced to the laboratory, captive flies were provided and consumed a rich source of nutrients, which led to ovarian development, egg production, and subsequent oviposition.

Age structure and reproductive potential are fundamentally important aspects of population dynamics in insects. This is particularly true in insects of agricultural and medical importance, where accurate estimation of populations forms the basis of population studies, management of pests, and prediction of the epidemiology of diseases (Styer et al., 2007; Kouloussis et al., 2009). Besides age and hosts, oviposition is also influenced by other factors that need to be addressed in natural settings, including mating status (Benz, 1969; Levinson et al., 1990; Fox, 1993; Jang, 1995), social context (Prokopy & Duan, 1998), diet (Wheeler, 1996; Carey et al., 2005), and an array of other factors that have mainly to do with the physiological state of females (Miller & Strickler, 1984; Browne, 1993). All these should be taken into consideration when trying to find the association between life span and reproduction.

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Figure 1.

Event-history graphs for survival and reproduction in the wild-caught cohort of 1 014 female medflies. Each horizontal line represents the life course of an individual fly, the length of which is proportional to its life span. Segments within each life line are coded to depict egg-laying levels at each age: light gray, 0 eggs; dark gray, 1–20 eggs; black, >20 eggs.

Figure 2.
Mean trajectories of egg laying of medflies caught in each month and year of consideration.

Figure 3.

Scatter plot of medflies captive life span against average daily reproduction along with Pearson correlation coefficient and fitted curves from the generalized additive models with identity link function. Open circles are the observed data and broken lines the 95% confidence bands. Captive life span refers to the period individuals lived after being captured and introduced to the laboratory.

Summary of life expectancy and reproduction in medflies of unknown age trapped during the 2003–2004 field seasons in Chios Island, Greece

Table 1

	Survival/longevity (no. days)		Reproduction (no. eggs)							
	n	e _x	Max	GRR	NRR	ADR	0 eggs (%)	>10 eggs (%)	% laying	T
2003	350	47.68	152	365.0	147.6	2.8	78.4	11.0	73.7	33.3
2004	664	40.73	126	322.6	126.1	2.5	77.7	10.6	63.4	26.7
Both years	1014	43.13	152	350.2	133.5	2.6	78.0	10.8	67.0	29.0

e_x, life expectancy; Max, maximum lifespan; GRR, gross reproductive rate; NRR, net reproductive rate; ADR, average daily reproduction; '0 eggs' and '>10 eggs', percentage of all post-capture life-days with 0 or >10 eggs laid, respectively; '% laying', percentage of medflies that laid at least one egg; T, mean captive age of reproduction.

Table 2

Mean and median of medflies captive life span and daily reproduction in each month by year

	n	<u>Survival/longevity (no. days)</u>		<u>Reproduction (no. eggs)</u>	
		Mean $\pm$ SE	Median	Mean $\pm$ SE	Median
2003					
August	109	38.3 $\pm$ 19.1	33.0	4.8 $\pm$ 5.2	3.1
September	42	53.0 $\pm$ 23.9	47.5	2.5 $\pm$ 3.8	0.5
October	99	59.4 $\pm$ 32.1	51.0	1.8 $\pm$ 3.3	0.4
November	100	44.2 $\pm$ 32.0	36.5	1.7 $\pm$ 3.0	0.2
2004					
July	113	51.9 $\pm$ 27.7	47.0	3.9 $\pm$ 4.6	1.6
August	63	40.8 $\pm$ 17.7	40.0	2.5 $\pm$ 3.8	0.4
September	100	47.8 $\pm$ 20.7	44.5	3.3 $\pm$ 4.0	1.9
October	288	33.9 $\pm$ 26.6	30.0	1.2 $\pm$ 2.7	0.0
November	100	40.8 $\pm$ 18.6	38.5	3.5 $\pm$ 4.5	1.5

Table 3

Fitted result of the reduced model for medfly life span

Coefficient/factor	Estimate $\pm$ SE	t-Value	Pr(> t)
Intercept	41.611 $\pm$ 1.663	25.028	<0.001
Year	-1.505 $\pm$ 2.311	-0.651	0.52
July	11.814 $\pm$ 3.025	3.906	<0.001
September	8.801 $\pm$ 2.583	3.407	<0.001
October	17.743 $\pm$ 3.049	5.818	<0.001
Year*October	-24.001 $\pm$ 3.758	-6.387	<0.001

Table 4

Fitted result of the reduced model for medfly reproduction

Coefficient/factor	Estimate $\pm$ SE	t-Value	Pr(> t)
Intercept	1.944 $\pm$ 0.317	6.132	<0.001
Year	1.654 $\pm$ 0.382	4.327	<0.001
August	2.822 $\pm$ 0.481	5.867	<0.001
October	-0.094 $\pm$ 0.495	-0.190	0.85
Year*August	-3.876 $\pm$ 0.709	-5.464	<0.001
Year*October	-2.260 $\pm$ 0.583	-3.878	<0.001

The main effect of October is kept in the model due to the significance of the Year*October interaction term.

Table 5

Analysis of the longitudinal trajectories of medfly egg laying: multivariate regression analysis of four principal component scores from functional principal component analysis

Coefficient/factor	Pillai (d.f. = 1)	F _{4,1004}	Pr(> F)
Year	0.012	3.049	<0.05
July	0.016	4.036	<0.01
August	0.0480	12.662	<0.001
October	0.0133	3.396	<0.01
Year*August	0.030	7.782	<0.001
Year*October	0.011	2.856	<0.05