

Ecological and Biological Studies on the Citrus Flower Moth, *Prays citri* (Millière) (Lepidoptera: Hyponomeutidae).

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ABSTRACT

The present investigation indicated that the citrus flower moth, *Prays citri* (Millière) showed that differences in their response to host plant species. However, *P. citri* population greatly preferred lime over navel orange and mandarin. Lime flowers received the highest numbers of *P. citri* throughout the whole period of investigation, while no infestation occurred on mandarin. Navel orange flowers were of intermediate rank for *P. citri* population. The obtained results illustrated that both larval and pupal populations exhibited one peak of seasonal abundance during 2015 and 2016 seasons. Distribution of *P. citri* population within host plants, based on the analysis of variance, was affected by directions. However, *P. citri* population was significantly higher at the south direction than the other cardinal directions (north, west and east) and center of the canopies. The present study showed that *P. citri* population exhibited positive response to the increase of mean temperature, while, it showed negative response to both relative humidity and wind speed. No significant differences were observed between the duration periods of all developmental stages which reared on different host (lime, navel orange and mandarin) flowers. Analysis of life table of *P. citri* population indicated that mortality of larval stage is the Key-stage mortality, which can induce population changes comparing to other specific stage mortalities.

INTRODUCTION

In Egypt, citrus is the most important fruit as far as its acreage, production and exportation potentials (El-Kassas, 1984). Citrus trees are mostly infested with many pests which affect their productivity such as flower moths and fruit flies. The citrus flower moth (CFM), *Prays citri* Millière (Lepidoptera: Hyponomeutidae), is regarded as a key pest of citrus in parts of the Mediterranean region (Toth *et al.*, 2009), including Portugal (Silva *et al.*, 2006), Sicily (Conti and Fisicaro, 2015) and Egypt (Abo-Sheaasha, 1994). Sweet lime, *Citrus aurantifolia* (Christm.) is the most susceptible species to the pest, followed by lemon, sweet orange, mandarin and grapefruit. On clementine, *P. citri* only caused damage at the time of flowering, and this was seldom of economic importance. Also the citrus flower moth attacks flowers of *Ligustrum lucidum* Ait, (Sinacori and Mineo, 1997).

CFM causes economic damage to different *Citrus* species, particularly to lemon flowers. The larva feeds both internally and externally on flowers, flower buds and small fruits (Carimi *et al.*, 2000 and EFSA, 2008). *P. citri* caused a loss of 30-40% of citron crop, during the year (Talhok, 1969, and Perez-Ibanez *et al.* 1973), in case of heavy infestation. The cumulative percentages reached 72.8-99.8. According to Mineo *et al.* (1980) infestation of flowers and fruits with *P. citri* reached over 50%, and flower infestation reached 100% in May and June.

For the success of management control program, it is essential to know several information concerning the biology and ecology of the pest (Abd El-Kareim, 1992). Temperature, relative humidity and wind speed are important environmental factors that affect the success of an insect to live in a given environment. The study of these parameters is interesting for insect of economic importance to obtain a useful orientation for good forecasting and prediction system of insect population (Dahi, 2003).

Therefore, the present studies aim to elucidate the following principal objectives:

- Host plant – *P. citri* interactions.
- Seasonal population trends of *P. citri* in lime orchard.
- Spatial distribution of *P. citri* in different directions of lime trees:

- Response of *P. citri* population to the changes in temperature, relative humidity and wind speed.
- Influence of host plant species on the biology of *P. citri*.
- Determine the key-stage mortality which may cause changes in the insect population.

MATERIALS AND METHODS

Estimation the seasonal abundance of *Prays citri*

1. Seasonal abundance of *P. citri* in response to host plant species

To evaluate the seasonal abundance of *P. citri* in response to each host plant (lime, navel orange and mandarin), four trees of each host plant homogenous in size and age were selected and marked for the present study. Samples were weekly collected at the beginning of the first flushes flowering period. Each sample consisted of 100 flowers / host plant (25 flowers / tree) which were picked up at random from the different cardinal directions (north, south, east and west) and the center of the tree (five flowers / direction). Sampling was started from mid. of February to the end of April during the first season (2015) and from the last week of February to the end of April during the second season (2016). Each sample of each direction was put in a plastic cup and transported to the laboratory for investigation. Numbers of larvae and pupae were counted and recorded.

• Seasonal abundance of *P. citri* in response to weather factors

To study the role of main weather factors (i.e. temperature, relative humidity and wind speed) on the seasonal activity of *P. citri*, daily records of mean temperature along with relative humidity and wind speed were obtained from the Agro meteorological Station at El-Tahrir district, El-Beheira governorate during 2015 and 2016 seasons. The daily records of each weather factor were grouped into weekly means according to the sampling dates. Correlation and regression analysis were done between the weekly averages of each weather factor and the seasonal abundance of the insect population.

Influence of host species on some biological aspects of *P. citri*

Insect source

The citrus flower moths were obtained in the larval stage from flowers of lime trees located in El-Tahrir

district, Beheira governorate. *P. citri* larvae were confined in Petri-dishes with a perforated cover and provided with adequate amount of lime, navel orange or mandarin flowers. Inspection was conducted until pupation and kept until adult emergence. The sex of the emerged adults was determined on the basis of morphological differences between the terminal segments of the abdomen (Carimi et al, 2000), and pairs of a female and a male were kept separately in tubes for the experiments.

To estimate the influence of host plant species (lime, navel orange and mandarin) on the duration of each developmental stage of *P. citri*, females emerged from each host plant were released into a cylinder glass jars (each jar measured 10 cm in diameter and 25 cm in height) and kept until deposit eggs. These jars were provided with 10% sucrose solution for moths' feeding. Fine branches of each host plant contained a lot of flowers were introduced into the jar for oviposition.

Fifty newly deposited eggs were investigated daily until hatching. After egg hatching, twenty newly hatched larvae were separately confined in Petri-dishes with a perforated cover and provided with two fresh flowers (renewal daily) of lime, navel orange or mandarin for larval feeding until pupation. Inspection was conducted till adult emergence. This experiment was replicated three times. The durations of all developmental stages were estimated on the different citrus species infested with *P. citri* under laboratory conditions (27.0±1.8°C and 74.0±4.0% R.H.).

Determine the key-stage mortality of *P. citri*

The percentage of mortality during each stage were estimated to study the age stage mortality of *P. citri*, numbers of emerged adults (males and females) were released in the previously mentioned glass jar to have a laboratory culture. An initial population of *P. citri* was started as 50 newly deposited eggs on fine branches of lime contained a lot of flowers that were introduced into the jar for oviposition under laboratory conditions (27.0±1.8°C and 74.0±4.0 % R.H.). A test was replicated three times for each generation (50 newly deposited eggs/ replicate).

To estimate the stage-specific survival (Ix) life tables were constructed with the following columns:

Ix : The number of survivals at the beginning of age class x.

dx : The number of deaths during the age interval x.

k- value: Killing power acting on each developmental stage, which is the difference between the logarithm of the initial and final populations.

To assess the relative contribution of various stage-specific mortality to the total generation mortality, the following k- values were calculated:

k₁, k₂ and k₃: mortality of egg, larval and pupal stages, respectively.

K: Total generation mortality.

Key factor analysis: To determine the key-stage specific mortality the largest coefficient for the relation "k" on "K" (Podolar and Rogers, 1975).

RESULTS

Host plant preference to citrus flower moth, *P. citri*

Data presented in Figure (1) show the general mean of population density of *P. citri* as affected by host plant

species throughout two seasons (2015 and 2016) on lime, navel orange and mandarin trees.

As shown in Figure (1) lime flowers harboured the highest average number of *P. citri* throughout the whole period of investigation, while mandarin flowers did not invaded by *P. citri*, where the observed average numbers of the pest individuals was nil. Navel orange flowers were of intermediate rank for *P. citri* population. The general mean of *P. citri* population on lime flowers was significantly higher (5.07±4.16 individuals/ sample) than that of navel orange (0.28±0.28) and mandarin (0.0) during 2015 season. In the second season (2016), the same trend was observed, where flowers of lime, navel orange and mandarin were invaded by 5.05±3.54, 0.16±0.21 and 0.0 individuals/ sample, respectively.

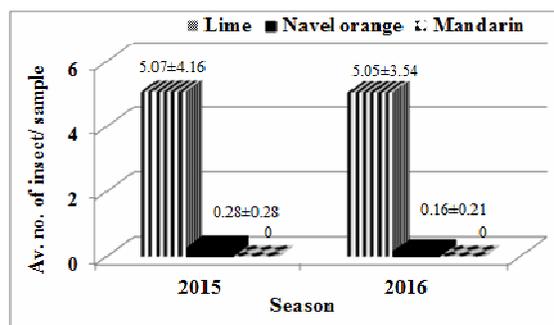


Figure 1. Average number of *Prays citri* individuals/sample on lime, navel orange and mandarin during 2015 (L.S.D. = 2.7, p=5%) and 2016 (L.S.D. = 2.06, p=5%) seasons.

Seasonal abundance of *P. citri* population in lime orchard

Flower samples were used to illustrate the seasonal abundance of the citrus flower moth, *P. citri* inhabiting lime orchard.

• Larval stage population

The average number of *P. citri* larvae / 5 flowers collected through weekly samples from lime flowers during 2015 and 2016 seasons are graphically illustrated in Figure (2). The first appearance of *P. citri* larvae was recorded during the ultimate week of February with few individuals, then larval population gradually increased to show the highest occurrence on April 10th, represented by 2.05 individuals / 5 flowers showing the highest peak. After that larval population gradually decreased toward the end of flowering period. In the second season (2016), larval population (Figure, 2) had similar trend. It was recorded from the first week of March to the last week of April. According to the abundance of *P. citri* individuals on lime trees, a peak was occurred on the 1st of April with an average of 1.9 individuals / 5 flowers.

b) Pupal stage population

As shown in Figure (2), the pupal population of *P. citri* started to appear on lime flowers with very low number (0.05 and 0.1 individuals / 5 flowers) on the 6th and 11th of March during the first and second seasons, respectively. Then, the mean number of pupae gradually increased to reach the highest abundance on the 17th and 29th of April, represented by 0.5 and 0.45 individuals / 5 flowers during 2015 and 2016, respectively.

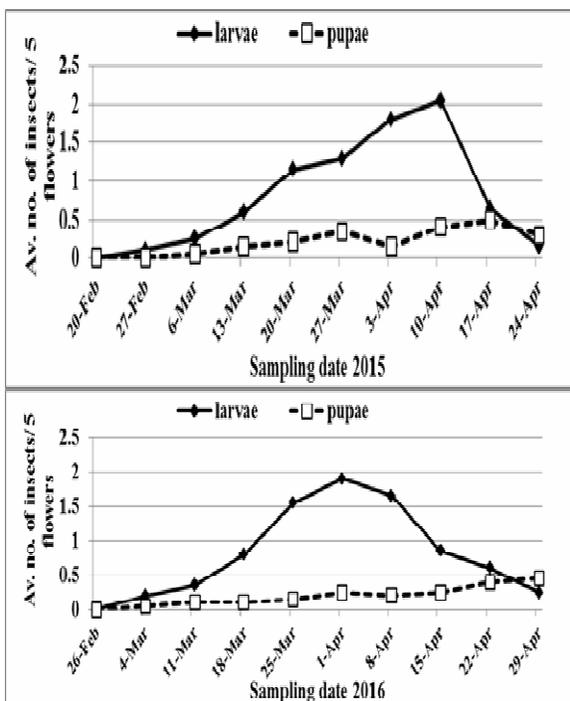


Figure 2. Seasonal abundance of *Prays citri* larval and pupal stages (number of larvae or pupae / 5 flowers) on lime flowers at El-Tahrir district, Beheira governorate during 2015 and 2016 flowering seasons.

Seasonal activity of *P. citri* in response to certain weather factors

Data in Tables (1 and 2) show the seasonal activity of *P. citri* in the lime orchard in response to mean of air temperature, relative humidity and wind speed at El-Tahrir district, Beheira governorate during spring flowering seasons of 2015 and 2016. In the first season (2015), *P. citri* population recorded the highest peak of 2.45 insects/ 5 flowers on the 10th of April at 18.4°C, 68.4% R.H. and 15.1 km/hr of wind speed. But, during the second season (2016), the highest peak (2.15 individuals/ 5 flowers) took place on the first of April at 16.4°C, 70.7%R.H. and 12.6 km/hr of wind speed (Table, 1). As shown in Table (2), the population of citrus flower moth exhibited significantly positive response to temperature ($r= 0.66$) during 2015 flowering season, while the correlation was insignificantly positive ($r= 0.15$) during the second season. On the other hand, the population of *P. citri* showed insignificantly negative correlation with the prevailing relative humidity during the two seasons of investigation. Finally, the correlation between population of CFM and wind speed was insignificantly positive ($r= 0.03$) during 2015 flowering season, but it was negatively insignificant ($r= -0.26$) during the second season of 2016.

To evaluate the common effect of the three tested environmental factors (mean temperature, relative humidity and wind speed) on *P. citri* population, multi-regression analysis was done. The combined effect of temperature, relative humidity and wind speed on the pest population were 49 % in the first season, and was low (represented by 25.5%) in the second season (Table, 2).

Table 1. Population density of *Prays citri* (Av. no. of larvae and pupae/5 flowers) in lime orchard in response to the changes of mean temperature, relative humidity and wind speed at El-Tahrir district, El-Beheira Governorate during 2015 and 2016 flowering season.

Sampling date	2015				2016				
	Av. no. of insect	Mean Temp.	Mean R.H.%	Wind speed(km/hr)	Sampling date	Av. no. of insects	Mean Temp.	Mean R.H.%	Wind speed (km/hr)
February 20	0	11.0	76.6	15.1	February 26	0	17.0	69.4	17.9
27	0.1	14.3	76.9	16.9	4	0.25	16.7	75.3	11.6
March 6	0.3	14.4	73.3	9.9	11	0.45	16.0	72.7	13.7
13	0.75	15.9	78.3	12.6	18	0.9	17.6	67.0	18.0
20	1.35	14.9	68.7	11.0	25	1.7	18.3	61.9	14.7
27	1.65	16.7	69.9	14.9	April 1	2.15	16.4	70.7	12.6
April 3	1.95	17.9	69.7	15.4	8	1.85	20.3	67.9	12.9
10	2.45	18.4	68.4	15.1	15	1.1	19.3	74.1	16.0
17	1.15	14.7	74.3	14.1	22	1	20.6	69.3	8.9
24	0.45	18.6	58.7	16.9	29	0.65	22.3	64.6	15.9

Table 2. Correlation and regression coefficients between *P. citri* population and weather factors (temperature, relative humidity and wind speed) in lime orchard during 2015 and 2016 seasons.

Year	Weather factor	Correlation and simple regression				Multi regression analysis		
		r	b	P	R ² %	b	P	E.V.%
2015	Temp.	0.66*	0.24	0.039	43.2	0.31	0.077	49
	R.H.	-0.32 ^{n.s.}	-0.05	0.375	9.9	0.04	0.556	
	Wind speed	0.03 ^{n.s.}	0.88	0.940	0.8	-0.05	0.651	
2016	Temp.	0.15 ^{n.s.}	0.05	0.674	2.3	-0.04	0.797	25.5
	R.H.	-0.33 ^{n.s.}	-0.06	0.346	11.1	-0.08	0.288	
	Wind speed	-0.26 ^{n.s.}	-0.07	0.460	6.9	-0.10	0.324	

Spatial distribution of *P. citri* in different directions of lime trees

Data illustrated in Figure (3) show the spatial distribution of *P. citri* in different directions (east, west, north, south and center) of lime trees during the two successive flowering seasons of 2015 and 2016. Distribution of *P. citri* population within the canopy of host plants, based on the analysis of variance, differently varied according to the cardinal directions and middle of the lime

trees. Significant difference of the population density of *P. citri* was observed among cardinal directions (north, west, south and east) and center, on lime trees. However, *P. citri* population was significantly higher at the south direction than the other cardinal directions (north, west and east) and center of the canopies (Figure, 3). As shown in Figure (3), *P. citri* population was the highest in south direction compared to the other directions during the first and second seasons with means of 1.27±0.27 and 1.50±0.43

individuals/5 flowers, respectively. On contrary, the lowest population of *P. citri* was recorded in north directions (0.77±0.41) during the first season; while, during the second season, the lowest population was recorded in west direction (0.75±0.33 individuals/5 flowers).

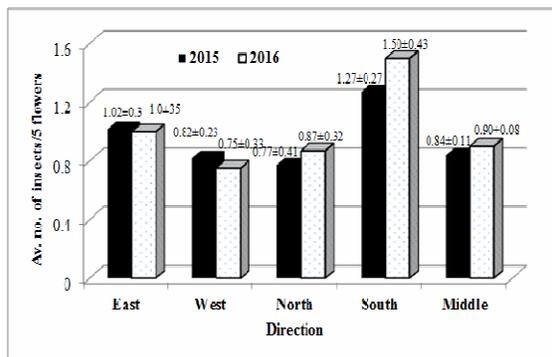


Figure 3. Population density of *Prays citri* in cardinal direction and center of lime trees at EL-Tahrir district, EL-Beheira governorate all over 2015 and 2016 flowering seasons (L.S.D. = 0.42 and 0.49 during 2015, and 2016).

Influence of different citrus species on some biological aspects of *P. citri*

The effect of different citrus flowers (lime, navel orange and mandarin) on the development of *P. citri* immature stages were studied under laboratory conditions (27.0±1.8°C and 74.0±4.0% R.H.). Data in Table (3) indicate that the duration of each developmental stage un affected by host plant species. The incubation period of *P. citri* eggs on all tested species was approximately the same recording 4.22±0.67, 4.33±0.71 and 4.56±0.73 days on lime, navel orange and mandarin flowers, respectively. The shortest duration of larval stage was 8.0±0.89 days for larvae reared on navel orange flowers followed by 8.13±0.64 and 8.33±0.82 days on lime and mandarin flowers. The pupal period insignificantly recorded 4.20±0.45, 4.33±0.52 and 4.60±0.55 days on navel orange, lime and mandarin flowers, respectively. Also, the total immature stages of *P. citri* insignificantly showed 16.68±2.23, 16.53±2.16 and 17.49±2.17 days on lime, navel orange and mandarin, respectively. The longest duration of *P. citri* immature stages was recorded when larvae were reared on mandarin flowers (Table, 3)

Table 3. Duration of the immature stages (in days) of the citrus flower moth, *P. citri* reared on lime, navel orange and mandarin flowers under laboratory conditions.

Stage	Lime	Navel orange	Mandarin	L.S.D
Egg	4.22±0.67	4.33±0.71	4.56±0.73	0.68 ^{ns}
Larva	8.13±0.64	8.0±0.89	8.33±0.82	0.94 ^{ns}
Pupa	4.33±0.52	4.20±0.45	4.60±0.55	0.69 ^{ns}
Total immature stages	16.68±2.23	16.53±2.16	17.49±2.17	1.75 ^{ns}

Table 4. Life table of the citrus flower moth, *P. citri* reared on lime flowers over three generations (G) under laboratory conditions.

Stage	Ix			dx	k-value				
	G ₁	G ₂	G ₃		G ₁	G ₂	G ₃		
Eggs	50	50	50	16	14	14	0.16	0.19	0.14
Larvae	34	32	36	18	17	17	0.33	0.39	0.28
Pupae	16	13	19	2	2	2	0.058	0.073	0.048
Newly emerged adults	14	11	17	-	-	-	-	-	-
Total (K)	-	-	-	-	-	-	0.548	0.653	0.468

Age-specific mortality of *P. citri*

Mortality among (k-value) larval stage was much higher than in other stages (Table, 4). The killing power (k-value) among they was 0.33, 0.39 and 0.28 for the 1st, 2nd, and 3rd generations, respectively. On the contrary, the pupal mortality was relatively low in the 1st, 2nd, and 3rd generations recording k-value of 0.058, 0.073 and 0.048, respectively.

As shown in (Table, 4) the total mortality from eggs to adult stage was considerably varied from a generation to another. Analysis of factors contributing to age stage-specific mortality indicated that the determining key-stage specific mortality on the basis of regression coefficient of k-values was k₂ (Figure, 4). Regression analysis illustrated that the regression of k₂ values on K showed the highest slope (b= 0.59) for larvae, while the slope values of k₁ and k₃ on K were (0.27 and 0.14). So, k₂ appeared to be the key-stage mortality which could induce population changes of *P. citri*.

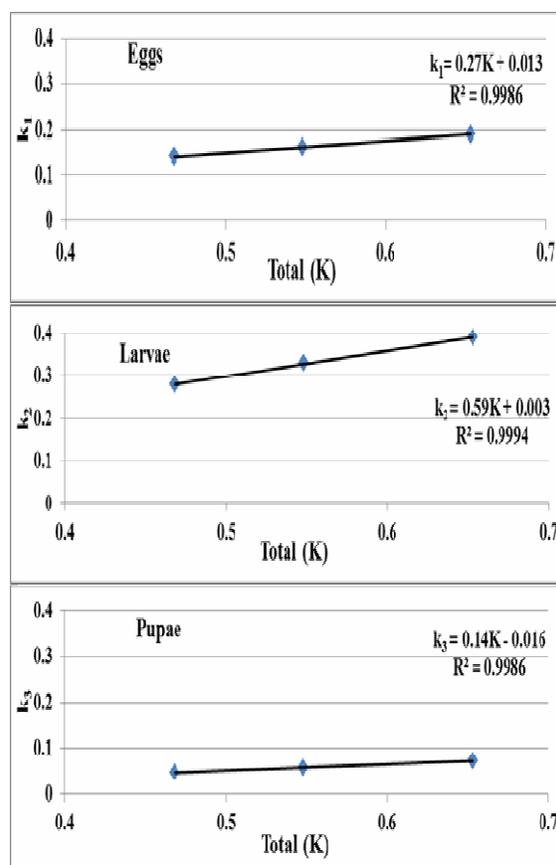


Figure 4. The k-values for the different citrus flower moth mortalities plotted against the total generation mortalities.

DISCUSSION

1. Seasonal activity of *P. citri* in lime orchards

The present results revealed that *P. citri* populations exhibited the highest abundance on lime in comparison with navel orange and mandarin trees. Also, Ibrahim and Shahateh (1984) reported that sweet lime (*Citrus aurantifolia*) was the species most susceptible to *P. citri*, followed by lemon, sweet orange, mandarin and grapefruit in order of decreasing susceptibility. According to Surin (1992) and Abo-Sheaasha (1994), lime and navel orange flowers are the most favorable hosts for *P. citri* larvae, while, mandarin is the least favorable host. El-Sayed *et al.* (1994) demonstrated that the volatile odors from different citrus flowers may activate (e.g., limonene and citronellol) or inhibit (e.g., nerol, terpineol, beta -caryophyllene, methyl lantheranlate and geraniol) females to lay eggs.

P. citri population showed a single peak of activity on lemon flowers at the end of main flowering period (the first half of April) after a gradual increase from the late of February. These results are in agreement with those obtained by Buchelos *et al.* (1963) in Greece and Mineo *et al.* (1980 and 1991) in addition to Sinacori and Mineo (1997) in Sicily who found that *P. citri* activity showed an increase at the end of main flowering period of lemon (at the late of May in Greece and at May-June in Sicily). By using sex pheromone traps in citrus orchards, Garrido *et al.* (1984) stated that *P. citri* were found throughout the year in eastern Spain exhibiting two peaks; in May-July and October-November. They added that the largest population was occurred when the trees carried the maximum number of flower buds. While, in Lebanon, Talhouk (1969) reported that *P. citri* was most severe in November and February. In Portugal, *P. citri* exhibited its highest peaks of activity during July and October (Mendonca *et al.*, 1997).

From the obtained results, it can be concluded that the highest activity of *P. citri* was recorded when mean temperature was 18.4 and 16.4°C (during the first and second seasons, respectively). These results are supported by Burgio *et al.* (1974) who found that the maximum number of laid eggs was obtained at 20°C. According to statistical analysis of the present study, mean temperature and relative humidity exhibited relatively low effects on *P. citri* population. While, Mineo *et al.* (1980) reported that climatic and cultural factors had considerable effects on *P. citri* activity in Sicily. The variations between the present results and others may be attributed to the variations of climatic and/or cultural factors; however, Mineo *et al.* (1980) added that the activity of *P. citri* varied so much according to time of year and locality. With respect to wind speed, there was insignificantly positive and negative correlation between *P. citri* and the changes of mean wind speed during the first and second years of study. According to Jonason *et al.* (2014) flight activity of micro moths is markedly affected by wind speed with no flight occurring above at wind speeds greater than 10km/hr. Wind speeds above 8km/hr reduce the moth flight activity.

Significant difference of the population density of *P. citri* was observed among cardinal directions (north, west, south and east) and center, of lime trees. However, *P. citri* population was significantly higher at the south

direction than the other cardinal directions (north, west and east) and center of the canopies. The causes of aggregation in these species might be due to their inherent active aggregative behavioral response such as in a situation where the presence of one individual attracts the others, and also due to some heterogeneity of the environment such as microclimate and preferred part of the plant (Southwood 1978 and Wratten & Fry 1980).

2. Biological aspects of *P. citri*

The present results indicate that incubation period of *P. citri* eggs were approximately similar on all tested host plants. These results are confirmed with those obtained by Ibrahim and Shahateh (1984), Surin (1992) and Abo-Sheaasha (1994) who reported that incubation period of this pest ranged between 2–9 days. The duration of all developmental stages slightly varied according to the host plant, where lime and navel orange flowers are the most favorable hosts for development of *P. citri* larvae. While, mandarin is the least favorable host. The duration of immature stages lasted 16.90 days; these results are in agreement with those obtained by Abo-Sheaasha (1994) who found that life cycle of *P. citri* lasted a period from 12 to 51 days according to the time of year. While, Carimi *et al.* (2000) mentioned that the life cycle of this pest on callus lasted about 21 days.

According to the present study, the durations of larval and pupal stages ranged between (8.0 and 8.33 days) and (4.2 and 4.6 days), respectively. Also, Ibrahim & Shahateh (1984) and Abo-Sheaasha (1994) mentioned that the durations of larval and pupal stages ranged between 6–25 and 3–12 days, respectively; while, Surin (1992) mentioned that these stages lasted between 22–30 and 5–7 days, respectively under laboratory conditions.

Analysis of life table of *P. citri* population indicated that mortality of larval stage is the Key-stage mortality, which can induce population changes comparing to the other specific stage mortalities. While, Abo-Sheaasha (1994) found that larval mortality percentages ranged between 28.4 and 46.9%. The variations between the present results and the others may be attributed to the variations of experimental conditions and/or host plant species/varieties.

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دراسات إيكولوجية وبيولوجية علي فراشة أزهار الموالح

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تشير تلك الدراسة إلى وجود اختلافات في إستجابة فراشة أزهار الموالح لنوع العائل النباتي. فقد أظهرت الحشرة تفضيلاً بدرجة واضحة لأزهار الليمون مقارنة بأزهار البرتقال أبو صرة أو اليوسفي، حيث تواجدهت بأعداد عالية على أزهار الليمون طوال فترة الدراسة في حين لم تسجل الحشرة على أزهار اليوسفي، بينما تواجدهت بأعداد قليلة على أزهار البرتقال أبو صرة. وهذا وقد أوضحت النتائج وجود ذروة واحدة لتعداد كلا من يرقات و عذارى الحشرة خلال موسم ٢٠١٥، ٢٠١٦. كما أثبتت الدراسة تفضيل الحشرة للجانب الغربي للشجرة مقارنة بالجانب الرئيسية الأخرى. كذلك أبدت أعداد الحشرة إستجابة موجبة مع درجات الحرارة وسالبة مع كلاً من الرطوبة النسبية وسرعة الرياح. هذا وقد تبين من الدراسات البيولوجية وجود اختلافات غير معنوية بين فترات حياة الأطوار غير الكاملة عند تربيتها على أزهار كلاً من الليمون، البرتقال أبو صرة و اليوسفي تحت الظروف المعملية، كما أثبتت نتائج جداول الحياة الخاصة بالعمر ان الموت في طور اليرقة يعتبر العامل المحدد لتعداد فراشة أزهار الموالح.