Does Geographic Region of Pomegranate Affect Life History of Carob Moth? A Case Study on Fruits Obtained From Three Different Climate Regions of Iran

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ABSTRACT

Many studies have been done so far on the reproductive biology of carob moth, *Ectomyelois ceratoniae*, considering different environmental conditions, however, climate regions' indirect effects cascaded up to the carob moth performance are not studied yet. A soil-pomegranate fruit cv. Malas-carob moth system was utilized based on three populations of pomegranate cv. Malas grown in three different climate regions of Iran (Aqda, Tarom-e-Oliya, and Saveh). Aqda region supported the highest nitrogen content for both soil and fruit; however, according to the two-sex life table, population growth parameters did not vary significantly in the carob moth reared on the fruits collected from these three climate regions. There was no correlation between soil nitrogen content and all population growth and biological parameters of the pest. Among all population growth and biological parameters, pupal period (r= -0.997, P= 0.047) and development time (r= -0.997, P= 0.051) showed inverse correlations with fruit nitrogen at 10% significance level. It was concluded that climate region indirect effect on the carob moth performance could not emanate under the pomegranate cultivar shade, however, this hypothesis should be tested in future.

Keywords: Age-stage two-sex life table; *Ectomyelois ceratoniae,* Fruit nitrogen, Plant quality, Soil nitrogen.

INTRODUCTION

Carob moth, *Ectomyelois ceratoniae* (Zeller) (Lep.: Pyralidae) is the most important pest of pomegranate in Iran and, in average, it damages more than 30% of pomegranates annually (Shakeri and Sadat Akhavi, 2003). Although few studies are available about genetic diversity in a plant species (e.g. different varieties of pomegranate), there are more documentations of genetic variations among plants' populations (e.g. different populations of a given variety of pomegranate from different climate regions) (Kinloch and Stonecypher, 1969). Moreover, spatial distribution of different organisms in different climate regions indicates different ecological services to different plant and animal species and certainly towards different climate regional populations of such species (Boyd and Benzhaf, 2007). These diverse ecological services, however, can lead to various resource allocation patterns among different plant and animal species. A good example for such diverse ecological patterns is the different generation numbers of a given insect species like carob moth in two different geographic

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regions: 4-5 generations in Iran vs. 3 generations in Iraq (Shakeri and Sadat Akhavi, 2003). For another instance, Millar and Shorey (1998) showed that carob moth could be controlled by a sex pheromone mimic in dates in the California while that lure was not effective against carob moth in Iran (Goldansaz *et al.,* 2008). This incapability of synthetic sex pheromone in a different geographic region (i.e. Iran) can be related to climate interference with chemical communications in the insect world or differences between two different host plant species (date vs. pomegranate). Also, to express the importance of considering climate region in carnivorous insects' ecology and behavior, we suggest the role of regional conditions in the success of different biocontrol agents released during many years in Iran: *Trichogramma* wasps were usually successful in regions in which relative humidity was higher than dry land regions (unpublished data).

Insects' dispersal, reproduction, development, and mortality are affected directly and indirectly by changes in their environmental conditions through many factors such as plant nutritional quality and resistance; however, these altered nutritional qualities are studied under "climate change" theme nowadays that is differ from geographical regions substantially (Nethere and Schopf, 2010). Many researches have been done so far on the effects of climate on insects' performance considering compatibility with environmental conditions as the driving force of behavioral variations among different populations of an insect species (Noldus and Potting, 1990). For instance, Hashemi *et al.* (2011) showed that carob moth damage was dependent on both geographical region and pomegranates variety under natural conditions. Moreover, there are many studies in which reproductive biology of this pest in different environmental conditions, such as different host plant species or different varieties of a given host plant, has been documented (Norouzi *et al.* 2008, Mortazavi *et al.* 2015), however, geographical regions' indirect

effects cascaded up via plant quality to the carob moth fitness are not studied yet. we selected a cultivar of pomegranate (Malas) from three different climate regions of Iran including Aqda, Saveh, and Tarom-e-Oliya. Then, we studied life history of the carob moth on these three different climatically different Malas fruits and potential correlations of the carob moth life history attributes with the fruit and climate region quality [measured through nitrogen analysis as a good quality index for plant and region (Mattson 1980; Brejda *et al.,* 2000)] were evaluated herein. To this end, the following hypotheses were tested in the current study: (1) Soil and plant nitrogen content is different among the three different climatic regions. Although this hypothesis has been frequently tested and proved by many researchers, however, We need this approval again to show that selection of experimental regions has been done correctly, (2) Fruit quality is different in various climatic regions, (3) Fruit nitrogen is positively correlated with soil nitrogen, (4) Life history parameters of the carob moth are different on the pomegranate fruits of Malas variety grown in three different regions, (5) Carob moth performance has positive correlation with both soil and fruit nitrogen.

MATERIALS AND METHODS

Sufficient amount of pomegranate and soil samples were obtained from three climatically different regions of Aqda, Saveh, and Tarom-e-Oliya and were used in experiments. Soil samples were obtained from 20 cm depth of the soil near trunks of nearly 10 trees in orchards in which no record of fertilizers was available. These soil samples were mixed and used in the next analyses. Before elemental analysis of the soil samples, all organic materials were removed carefully from the soils. Aqda is a dry desert in central Iran (32° 26' 42" N, 53° 37' 46" E) with less than 60 mm annual rain at 1,256 m above mean sea level (amsl), Saveh (34°53'50"N, 50°08'59"E) is steppe

dry land with about 230 mm annual rain at 1,245 m amsl and Tarom-e-Oliya (36˚ 44' 01" N, 49˚ 14' 47" E) is a lowland region with more than 700 mm annual rain and at 296 m amsl (for more information see https://fa.climate-data.org/). To evaluate the nitrogen content of pomegranate fruits and soil samples, we dried a small piece of fruit peel plus 3-4 perforated arils of each plant cultivar and soil samples at 55˚C for 24 and 12 hours, respectively, to get the dry weights. Then, the nitrogen content of these dried ground fruit and soil samples was estimated by the Kjeldahl method (Karla, 1998). Nitrogen values for either soil or plant samples had three replications.

In order to obtain the synchronized eggs of *E. ceratoniae*, nearly 50 infested pomegranate fruits, each containing about 10 overwintering larvae, were collected from an orchard located in Alborz province of Iran during autumn of 2016. Totally, 120 reproduced eggs of these feral carob moths were used in life table study as forty eggs per experimental cohort on each fruit treatment under laboratory conditions (29±2˚C, 75±5% RH, and 16/8 D/L). A cohort life table was constructed based on unlimited food supply (a small piece of fruit peel plus 3-4 arils of Malas fruit collected from each climatic region), where natural enemies were excluded from the experiment. To measure reproduction, pairs of newly emerged *E. ceratoniae* (male and female) reared on each fruit were placed in ventilated plastic cylindrical cages (20 cm diameter and 15 cm high) containing the same host-plant fruits (a small piece of soft textile). For each cage, replacement textiles were provided ad lib, until all the females had died. The number of eggs laid was recorded daily and used as a measure of fecundity.

Statistical Analysis

Data of nitrogen contents in both fruit and soil were analyzed using multivariate general linear model and means were compared using *LSD* procedure at 5% significance level (Proc GLM, SAS, 2003). Correlations among different attributes were done using Spearman and Pearson moment coefficient at 5% significance level. The life history data including males, females, and immature individuals that died before the adult stage and the intrinsic rate of natural increase (r, calculated by using the bisection method and Euler-Lotka equation), mean generation Time (T), finite rate of increase (λ), and net Reproduction rate (R_0) (Table 1) were analyzed according to the age-stage, two-sex life table using the TWOSEX-MSChart (Chi, 2016). The age-specific survivorship (l_x) including both male and

Table 1. Population growth parameters and their formula used in two-sex life table analysis proposed by Chi (2016).

Population growth parameters	Formulae
Net reproductive rate	$$R_0 = \sum_{x=0}^{\omega} \sum_{j=1}^{m} s_{xj} f_{xj}$$
Intrinsic rate of natural increase	$$\sum_{x=0}^{\omega} l_x m_x e^{-r(x+1)} = 1$$
Finite rate of population increase	$$\lambda = e^{r}$$
Mean generation time	$$T = \frac{Ln R_0}{r}$$

female and the age-specific fecundity (m_x) were calculated according to Chi and Liu (1985) as below:

$$l_x = \sum_{j=1}^{\beta} s_{xj} \tag{1}$$

$$m_x = \frac{\sum_{j=1}^{\beta} s_{xj} f_{xj}}{\sum_{j=1}^{\beta} s_{xj}} \tag{2}$$

The variability of life table parameters was estimated in bootstrap procedure (Iteration= 100,000) and the bootstrap values on three different fruits were compared using the paired bootstrap test (P< 0.05).

RESULTS

Soil nitrogen contents were different among the climatic regions ($F_{2,6}$= 242.00, P< 0.0001). The highest and lowest soil nitrogen contents were observed in Aqda and Saveh regions, respectively (Figure 1-a). Moreover, fruit nitrogen contents varied among climate regions ($F_{2,6}$= 307.94, P< 0.0001) and the highest and lowest values of the fruit nitrogen were measured in Aqda and Saveh, respectively (Figure 1-b). The age-specific survivorship (l_x) curves of the pest on fruits of three different regions showed a similar pattern with high mortality

occurring during first and last ten days (Figure 2). Fecundity was valued at nearly 10 last days of the female moths' life span (Figure 2). Also, age-stage Survival rates (S_x) of the carob moth for all stages were similar on fruits of these three regions (Figure 3). Age-stage life expectancy of different stages of the carob moth followed the same pattern on fruits of the three regions (Figure 4). Age-stage reproductive Value (V_x) is expanded at the last 15 days of the life span in favor of female reproduction (Figure 5). Incubation period, 1st, 2nd, and 3rd larval instar durations were significantly different among individuals fed with fruits of the climatic regions. Also, the 4th larval instar time was different among individuals fed with fruits of the climate regions, but the 5th instar and pupal period lasted equally for the individuals fed with fruits of all three regions. However, development time of the moths varied among the regions. Female adult longevity and Adult PreoviPosition Period (APOP) (shortest and longest time were 3 and 7 days for Aqda, 2 and 4 days for Saveh, and 0 and 7 days for Tarom) of the pest were not different on the climatically different fruits but male adult longevity as well as male adult life span were significantly different among the different fruits. However, female life span and Total PreoviPosition Period (TPOP) were different among individuals who were reared on the climatically different fruits. Oviposition
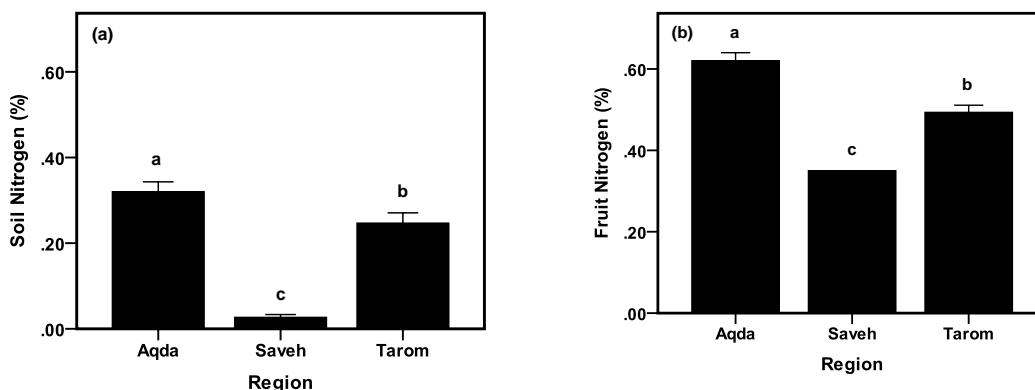

Figure 1. Percent nitrogen of soil (a) and fruit of Malas variety (b) in orchards of the three climatic regions, Aqda, Saveh, and Tarom-e-Oliya based on *Kejeldahl* method. Different letters on each error bar show significant difference among treatments based on *LSD* test at 5% significance level.
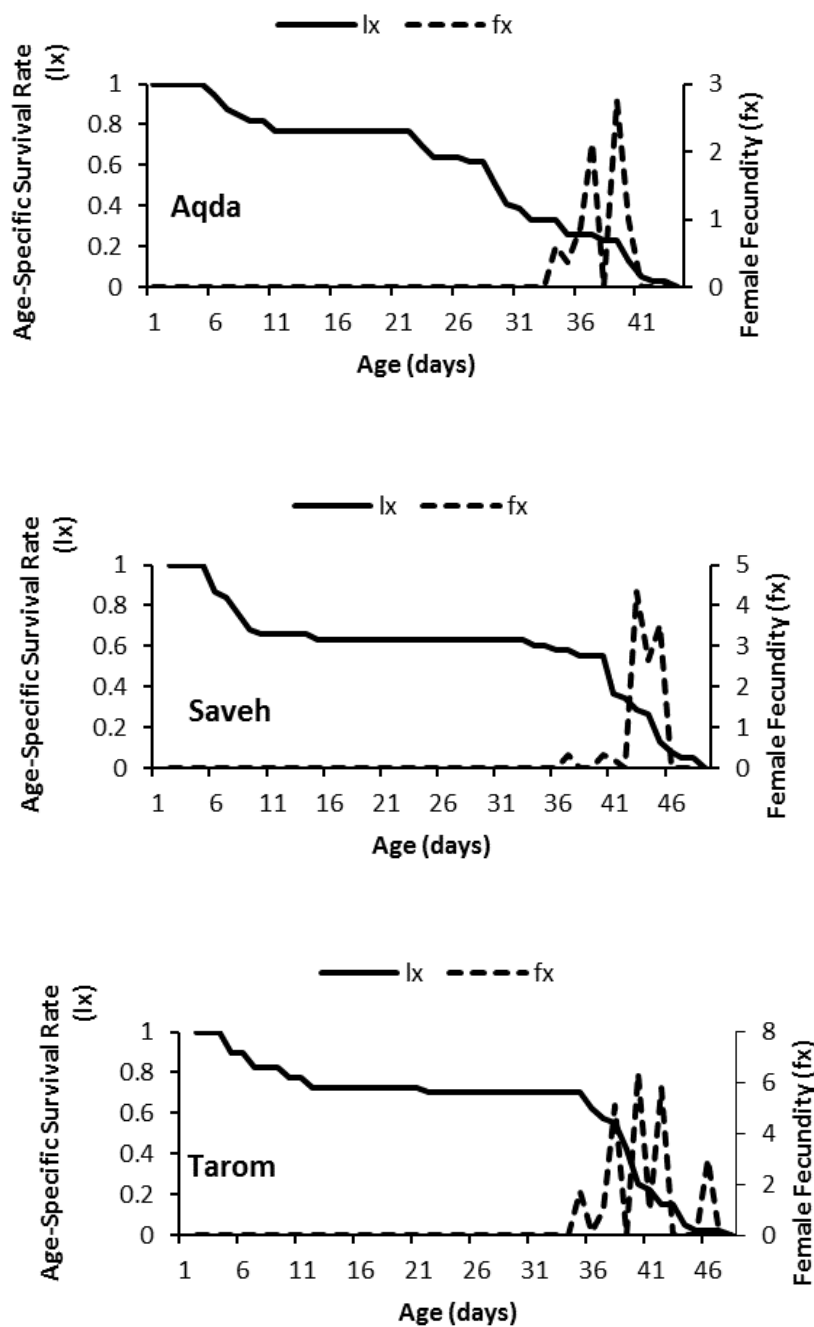
Figure 2. Age-specific survival rate (l_x) and female fecundity (f_x) of the carob moth on fruits of Malas variety of pomegranate grown in three different climatic regions, Aqda, Saveh, and Tarom-e-Oliya under laboratory conditions.

period and total fecundity were not different among the moths that were fed with fruits of the three regions (Table 2). Moreover, population growth parameters of the carob moth were not significantly different among individuals fed with the fruits of the three regions (Table 3).

There was high correlation between soil and fruit nitrogen in the three regions

Table 2. Biological life history parameters (mean±SE) of the carob moth on the fruits of Malas variety of pomegranate grown in three different climatic regions, Aqda, Saveh, and Tarom-e-Oliya under laboratory conditions.

Biological parameters[a]	Climate regions		
	Aqda	Saveh	Tarom-e-Oliya
APOP (Days)	4.11± 0.48[ns]	3.00±0.33[ns]	3.22±0.60[ns]
Oviposition period (Days)	1.67±0.24[ns]	1.75±0.25[ns]	1.56±0.24[ns]
TPOP (Days)	35.11±0.54[b]	40.12±0.93[a]	37.11±1.18[b]
Incubation period (Days)	4.11±0.09[a]	3.62±0.1[b]	3.31±0.10[c]
1st instar duration (Days)	2.97±0.14[a]	2.58±0.11[b]	1.65±0.14[c]
2nd instar duration (Days)	2.67± 0.15[c]	3.08±0.11[b]	4.62±0.24[a]
3rd instar duration (Days)	4.53±0.17[b]	2.83±0.1[c]	5.59±0.28[a]
4th instar duration (Days)	2.33±0.15[c]	10.38±0.19[a]	4.14± 0.27[b]
5th instar duration (Days)	6.76±0.31[ns]	6.58±0.29[ns]	6.82±0.36[ns]
Pupal duration (Days)	6.62±0.26[ns]	6.9±0.22[ns]	6.65±0.16[ns]
Development time (Days)	30.62±0.65[c]	35.86±0.59[a]	32.35±0.56[b]
Male longevity (Days)	4.00±0.73[b]	5.11±0.59[ab]	5.69±0.33[a]
Female longevity (Days)	7.70±0.78[ns]	6.42± 0.43[ns]	6.92±0.49[ns]
Male life span (Days)	34.33±2.03[c]	40.00±0.83[a]	36.85±0.60[b]
Female life span (Days)	38.50±0.78[c]	43.00±0.73[a]	40.46±0.68[b]
Total fecundity (Eggs/Female)	5.90±1.62[ns]	6.92±3.11[ns]	12.77±6.82[ns]

[a] Adult pre-oviposition period was abbreviated as APOP and Total pre-oviposition period counted from birth was abbreviated as TPOP. Significant level at $p < 0.05$.
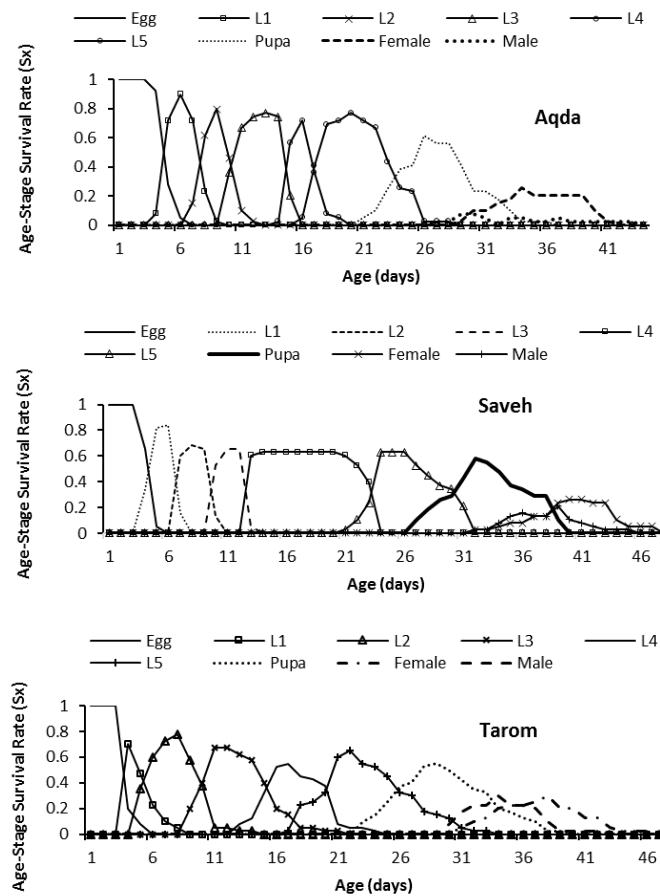

Figure 3. Age-stage Survival rate (S_x) of the carob moth on fruits of Malas variety of pomegranate grown in three different climatic regions, Aqda, Saveh, and Tarom-e-Oliya under laboratory conditions.

Table 3. Population growth life history parameters of the carob moth on the fruits of Malas variety of pomegranate grown in three different climatic regions, Aqda, Saveh, and Tarom-e-Oliya under laboratory conditions.

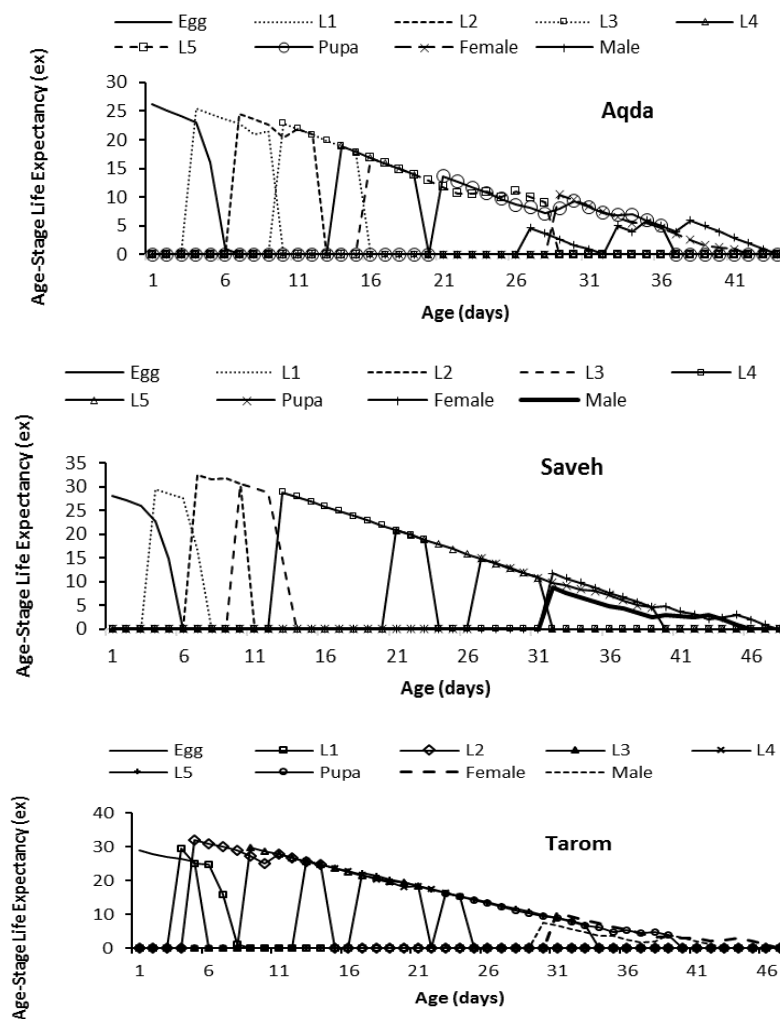| Population growth parameters	Climate regions		
	Aqda	Saveh	Tarom-e-Oliya
Net Reproduction rate (R_0) (offspring)	$1.51 \pm 0.57^{n.s}$	$2.18 \pm 1.07^{n.s}$	$4.15 \pm 2.33^{n.s}$
Intrinsic rate of increase (r) (d^{-1})	$0.011 \pm 0.01^{n.s}$	$0.018 \pm 0.01^{n.s}$	$0.037 \pm 0.02^{n.s}$
Mean generation Time *(T)* (Days)	$37.41 \pm 4.25^{n.s}$	$42.38 \pm 3.71^{n.s}$	$38.19 \pm 2.25^{n.s}$
Finite rate of increase (λ) (d^{-1})	$1.01 \pm 0.01^{n.s}$	$1.02 \pm 0.01^{n.s}$	$1.04 \pm 0.02^{n.s}$

Significant level at p<0.05.

Figure 4. Age-stage life expectancy (e_x) of the carob moth on fruits of Malas variety of pomegranate grown in three different climatic regions, Aqda, Saveh, and Tarom-e-Oliya under laboratory conditions

(Spearman rho= 1, P< 0.0001). There was no correlation between soil nitrogen and all population growth and all biological parameters; however, pupal period (r= -0.946, P= 0.210) and development time (r= -0.984, P= 0.113) were high and close to significance. Among larval instars, the 4th and 5th larval instars showed inverse (r= -1,
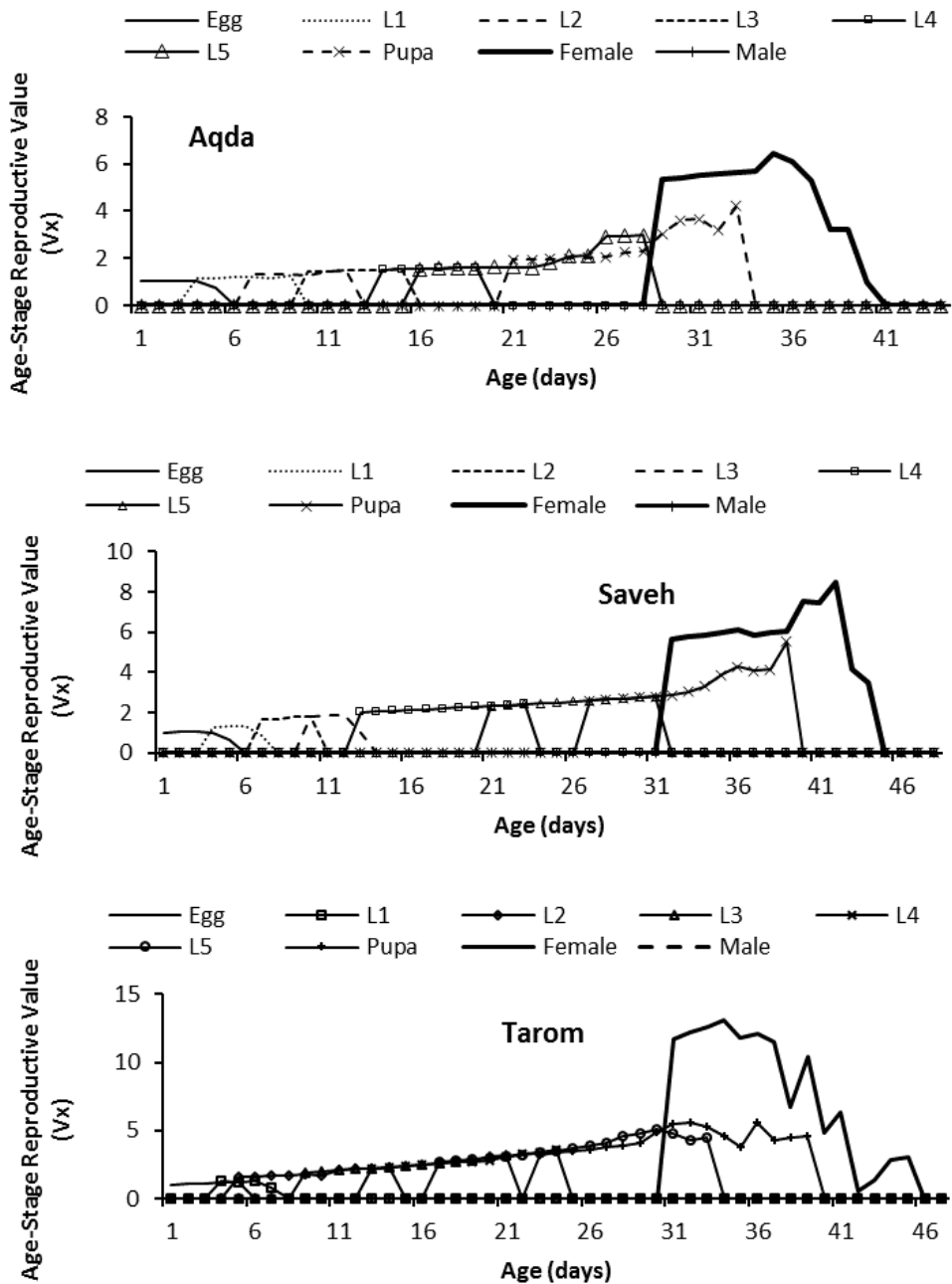
Figure 5. Age-stage reproductive Value (V_x) of the carob moth on fruits of Malas variety of pomegranate grown in three different climatic regions, Aqda, Saveh, and Tarom-e-Oliya under laboratory conditions.

P= 0.0001) and positive (r= 0.966, P= 0.166) correlations with soil nitrogen, respectively. Among population growth and biological parameters, pupal period (r= -0.997, P= 0.047) and development time (r= -0.997, P= 0.051) showed inverse correlations with fruit

nitrogen at 10% significance level. Also, among larval instars, the 4th and 5th instars showed inverse (r= -0.967, P= 0.164) and positive (r= 1, P= 0.002) correlations with the fruit nitrogen, respectively; although the

correlation of the 4th instar was close to significance.

DISCUSSION

Results of soil nitrogen content in the experimental regions showed that selection of these regions based on soil quality had been done correctly. Moreover, fruit nitrogen and its positive correlation with soil nitrogen showed that the climatic regions had significant effects on the host plant quality of the carob moth (based on the second and third hypotheses). It must be noted that the first hypothesis was proved by many researchers (e.g. Brejda *et al.,* 2000), but we tested it again to conclude the current data more precisely. Most studies done on the plant–insect interactions have focused on the role of secondary metabolites in insects' performance (either herbivore or carnivore) (Harvey, 2005; Ode, 2006) while nutritional elements such as nitrogen that are known to have important effects on the insect performance are studied insufficiently (Soufbaf *et al.,* 2012). Moreover, different levels of synthetic nitrogenous fertilizers were applied by researchers to change the plant nutrient quality (e.g. Aqueel and Leather, 2011), but in such studies precise isolation of plant quality on insect fitness is nearly impossible (Soufbaf *et al.,* 2012). Our fourth hypothesis was not proved and carob moth showed similar potential of population growth on all fruits examined. Towards fifth hypothesis and as an important result, there was no relationship between soil quality (as an index for climate region) and insect performance in terms of population growth potential. More importantly, biological parameters including larval period, pupal period, and development time (during which an insect feeds directly on plants and, so, is expected to be affected more by plant quality) decreased after either soil or fruit nitrogen increased. Positive effect of nitrogen on insect longevity is suggested by many researchers (Coley *et al.,* 2006; Soufbaf *et al.,* 2012); however, biomass

growth rate (measured by Relative Growth Rate, RGR) is usually shown to be lower on the plants with lower amount of nitrogen (Loader and Damman, 1991; Lou and Baldwin, 2004). Johnson (2008) found an inverse correlation between leaf nitrogen and intrinsic rate of population increase in an herbivore insect, while many researchers reported such correlations as positive (Aqueel and Leather, 2011; Winter and Rostâs, 2010). Lack of correlation between climate region and population growth potential could be ignored at this step and leave it up to more findings about validation of currents methods of population ecology. For instance, we see that Jacknife algorithm is now deniable even after many years of application (Huang and Chi, 2012). But, we link this similarity in population growth parameters of the pest on the three types of pomegranate to the "host plant variety", which is the same. Logically, we accepted and concluded that host plant variety could be more reliable than climate region in predicting insect fitness characteristics in predicting insect fitness correlates than climate region. However, many researchers have proved the climate region effects on the insect species performance and they believe that behavioral variations among different populations is related to their compatibility with environmental conditions (Noldus and Potting, 1990). For instance, Hashemi *et al.* (2011) showed that carob moth damage is dependent on both geographical region and pomegranates variety. After testing five different geographical regions (but not climate regions) and three different pomegranate varieties, these researchers found that Natanz region and Torsh variety suffered more damage than Najafabad region and Malas variety. Interestingly, there were inverse and positive correlations between the 4th and 5th larval instar durations with nitrogen (of either soil or fruit), respectively. As a first result, nitrogen could decrease the 4th larval instar duration but increase larval 5th instar duration. In other words, carob moth passed the 4th larval instar fast, but in the 5th larval instar, the

moth allocated more time to obtain resources. Although these results are contradicting in terms of larval growth, due to the same pattern seen on both soil and plant nitrogen, it should be studied with more details in future to determine the mechanism(s) of the phenomenon. Agrawal (2004) suggested plant nitrogen as a good index for predicting insect herbivores population dynamics and, as shown here, our data in part is confirming this claim.

Survival trend showed the same pattern in all three climate regions. The survival can be represented as a combined type I and III survival curves. High susceptibility of immature stages, however, causes some mortality in these stages and, so, the survival curve has some decreasing fluctuations in this period. However, through laboratory experiments of life history of different insects, almost the same pattern has been observed so far for different insect and mite species. For instance, the survival pattern of *Aphis gossypii* (Tazerouni *et al.* 2016), *Plutella xylostella* (Nikooei *et al.* 2015), and *Tetranychus urticae* (Maleknia *et al.* 2016) are very similar, especially at immature stages. In age-stage survival curves, the survival curve of egg is exactly of type II, while for other stages, due to graduate entrance of individuals to a given stage, survival value changes from zero to a maximum value and then it declines along individuals' mortality towards zero again.

Moreover, age specific life expectancy showed the same pattern in all three climate regions such that after some fluctuations in the first 11 days, a smooth decrease was observed towards natural deaths in adulthood. Following the story about survival curves described above, life expectancy trends are also similar among different insect and mite species [see life expectancy curves in the papers of Tazerouni *et al.* (2016), Nikooei *et al.* (2015), and Maleknia *et al.* (2016)]. Again, a plausible reason for this pattern could be some mortalities through immature stages that are usually due to unsuitable conditions for the insects under laboratory conditions

and natural enemy pressure in the natural conditions. Also, age-stage life expectancy showed the same pattern in three climate regions and following the discussion about the survival curves, except for the egg stage, other stages started from zero and, after a maximum value, a general decrease was observed. Reproductive value of the female stage had the biggest under-curve area that is expected. Further, reproductive value of those individuals who would become female is also combined to this area. However, male reproductive value was zero throughout as excepted. Norouzi *et al.* (2008) studied life table of the carob moth on four different host plant species under laboratory conditions and suggested that the pest had the highest value of the intrinsic rate of natural increase on the pomegranate as 0.107 ♀/♀/day. This value is about three times of the value obtained in our study on the Malas variety of Tarom-e-Oliya region and ten times of the Malas variety of Saveh and Aqda regions. Further, Mortazavi *et al.* (2015) studied the effects of some different diets on the life table parameters of the carob moth and found 0.09 ♀/♀/day and 1.096 d^{-1} values of intrinsic rate of natural increase and finite rate of increase of the pest on the pomegranate. The result of these researchers for the intrinsic rate of natural increase is nearly 3-9 time of our estimations of the *r*. One of probable reasons for these high variations among our data and others is that we got eggs for the cohort from the wild moths without further rearing under fixed environmental conditions. Clearly, this procedure does not allow insects to adapt to new environment and, therefore, the number of offspring declines. But, other differences about the insect strain and environmental conditions are also determinant in this variation, either in laboratory or field. The survival and life expectancy in the current study was similar to Norouzi *et al.* (2008) and Mortazavi *et al.* (2015).

In conclusion, we suggested that climate region indirect effect on the carob moth performance could not be critical and we hypothesized that the same performance of

the pest in different regions could be related to the plant variety chosen, which was the same in the three sites, and should be tested in future studies. Further, we found that population growth parameters, which are calculated based on the daily number of laid eggs, have less biological sense than biological parameters such as development time, larval period, and incubation period. This hypothesis was well documented through current study in correlations of the insects' growth parameters with the soil-plant quality. Following this observation, it can be suggested that, to study host plant effect on the biology of a given insect, using artificial population growth parameters should be avoided or population growth of the insects' wild parents should be reconsidered instead.

ACKNOWLEDGEMNTS

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آیا ناحیه جغرافیایی درخت انار بر تاریخچه زیستی کرم گلوگاه انار اثر دارد؟ یک مطالعه موردی روی میوه های انار به دست آمده از سه ناحیه اقلیمی مختلف ایران

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چکیده

تاکنون مطالعات زیادی در زمینه بیولوژی تولید مثلی کرم گلوگاه انار، *Ectomyelois ceratoniae* با در نظر گرفتن شرایط محیطی مختلف انجام شده است ولی به هر حال اثرات غیر مستقیم ناحیه جغرافیایی گیاه انار بر کارایی کرم گلوگاه انار تا به حال مورد مطالعه قرار نگرفته است.

یک سیستم خاک –میوه انار رقم ملس – کرم گلوگاه انار رقم ملس متعلق به سه جمعیت مختلف انار رقم ملس متعلق به سه منطقه جغرافیایی مختلف ایران (عقدا، طارم علیا و ساوه) مورد بررسی قرار گرفت. منطقه عقدا بیشترین مقدار نیتروژن را برای خاک و میوه نشان داد؛ با این حال، بر اساس جدول زندگی دو جنسی، پارامترهای رشد جمعیت بین مناطق تفاوت معنی دار نشان ندادند. همچنین همبستگی بین نیتروژن خاک با پارامترهای زیستی و رشد جمعیت آفت مشاهده نشد. در میان کلیه پارامترهای مورد آزمون، طول دوره شفیرگی (r=-0.997 و $P=$ 0.047) و طول دوره نشو و نما (r=-0.997 و $P=0.051$) همبستگی معکوس با نیتروژن میوه و در سطح احتمال ۱۰ درصد نشان دادند. در مجموع، چنین استنباط می شود که اثر غیر مستقیم ناحیه جغرافیایی بر کارایی کرم گلوگاه انار حداقل در آزمایشگاه در سایه رقم یکسان انتخاب شده، نتوانسته است اثر خود را نشان دهد که البته این فرضیه می بایست در آینده مورد آزمون قرار گیرد.