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Effects of different temperatures on the demography of pistachio green stink-bug, *Brachynema germari* Kolenati (Hemiptera: Pentatomidae), under laboratory conditions

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Efectos de diferentes temperaturas en la demografía del chinche verde del pistacho, *Brachynema germari* Kolenati (Hemiptera: Pentatomidae), en condiciones de laboratorio

RESUMEN. La chinche verde del pistacho, *Brachynema germari*, es una de las plagas más importantes del pistacho en Irán, que anualmente causa significativos daños a este estratégico cultivo. En este trabajo se investigaron los efectos de diferentes temperaturas en la demografía de *B. germari* en condiciones de laboratorio. Los experimentos se realizaron a cinco temperaturas constantes [20, 25, 27.5, 30 y 35 (± 1) °C], en cultivar *Ohadi*. Las tasas de mortalidad más bajas y más altas tanto en los huevos como en los estados ninfales se observaron a 25 °C y 35 °C, respectivamente. Los tiempos de desarrollo inmaduro más largos y más cortos se observaron a 20 °C (53,75 días) y 35 °C (18,42 días), respectivamente. Los parámetros de crecimiento de la población se vieron significativamente afectados por las temperaturas experimentales. La tasa reproductiva bruta (*GRR*) se redujo severamente a 35 °C, así como la tasa reproductiva neta (R_0) que también se redujo al aumentar la temperatura. La tasa intrínseca de aumento (r_m) fue de 0.05, 0.06, 0.06, 0.07 y 0.09 (día^{-1}) a 20, 25, 27.5, 30 y 35 °C, respectivamente. Además, la tasa de aumento finita más baja (λ) (1,05 día^{-1}) se observó a 20 °C, mientras que el tiempo medio de generación (*T*) y el tiempo de duplicación (*DT*) más largo y más corto se produjeron a 20 °C y 35 °C, respectivamente. Finalmente, los valores de los parámetros reproductivos más altos y más bajos, incluyendo la fecundidad diaria y total y la fertilidad, se obtuvieron a 20 °C y 35 °C. Los resultados de esta investigación podrían ser de utilidad para el monitoreo y el control eficiente de esta plaga.

PALABRAS CLAVE. Cultivar. Tabla de vida. Temperatura.

ABSTRACT. The pistachio green stink-bug, *Brachynema germari* is one of the most important pests of pistachio in Iran that annually causes significant damages to this strategic crop. In this research, the effects of different temperatures on the demography of *B. germari* were investigated under laboratory conditions. The experiments were conducted at five constant temperatures [20, 25, 27.5, 30 and 35 (±1) °C], on *Ohadi* cultivar. The lowest and the highest mortality rates in both the eggs and nymph stages were observed at 25 °C and 35 °C, respectively. The longest and shortest immature development times were observed at 20 °C (53.75 days) and 35 °C (18.42 days), respectively. The population growth parameters were significantly affected by the experimental temperatures. The gross reproductive rate (*GRR*) was severely reduced at 35 °C and the net reproductive rate (R_0) was also decreased by increasing temperature. The intrinsic rate of increase (r_m) was 0.05, 0.06, 0.06, 0.07 and 0.09 (day^{-1}) at 20, 25, 27.5, 30 and 35 °C, respectively. Moreover, the lowest finite rate of increase (λ) (1.05 day^{-1}) was observed at 20 °C, whereas the longest and shortest mean generation time (T) and doubling time (DT) occurred at 20 °C and 35 °C, respectively. Finally, the highest and lowest reproductive parameters values, including daily and total fecundity and fertility, were obtained at 20 °C and 35 °C. The results of this research could be useful in monitoring and efficient control of this pest.

KEYWORDS. Cultivar. Life table. Temperature.

INTRODUCTION

Pistachio (*Pistacia vera* L., Sapindales: Anacardiaceae) is a nut crop that ranks first among different exportable agricultural crops in Iran (Sheibani et al., 1996). The annual exportation of pistachio in this country is about 100,000 tons. The country's associated income ranks second after the oil revenue (Zare-Nazari, 2018). In pistachio orchards, there are many pests that feed on trees and cause serious damages (Hashemi-Rad, 1999). Among these, pentatomid bugs must be controlled in order to get a good crop (Hashemi-Rad, 1999). The pistachio green stink-bug *Brachynema germari* Kolenati (Hemiptera: Pentatomidae) is one of the most important pests of pistachio in Iran that was collected for the first time in summer of 1965 on orchards from Kerman province (Nyman et al., 1967). Both adults and nymphs of this bug feed on the fruits and cause significant direct damage in the form of epicarp lesions that can result on nut drop (Daane et al., 2005).

Also, this pest is a vector of the pathogenic yeast *Nematospora coryli* Peglion (Saccharomycetales: Saccharomycetaceae) that concern the cork and turns the fruit somewhat bitter; thus, its quality, quantity and marketability decrease seriously (Ershad & Barkhordari, 1975).

Temperature is one of the most important abiotic factors that affects the life cycle of insects (Roy et al., 2003). Estimation of demographic parameters of an insect under different temperatures can reveal the relationship between it and growth potential of pests (Greenberg et al., 2005; Dong et al., 2007). Among the population growth parameters, the intrinsic rate of natural increase (r_m) is a useful indicator of the effects of temperature on the population growth, and can indicate the overall effects of this factor on insect's development, survival and reproductive characteristics (Southwood, 1978). The main purpose of this study was to investigate the effect of five different temperatures on the biological characteristics, life table parameters and reproductive features of *B. germari*, under laboratory conditions.

MATERIAL AND METHODS

Insect rearing

Adults of *B. germari* were collected from the pistachio orchards in Rafsanjan region located in southeastern Iran. The collected individuals were immediately transferred to the Pistachio Research Center and reared in a growth chamber set at 27.5 ± 1 °C, 65 ± 5% relative humidity (RH) and a photoperiod of 16:8 (L:D) h on *Ohadi* pistachio cultivar. The plant was replaced every two days. Nymphs were fed until the emergence of adults, and the emerged ones were used for egg-laying. One-day eggs were used for the experiments.

Demographic parameters study

The demographic parameters of *B. germari* were studied under 20, 25, 27.5, 30 and 35 ±1 °C in a growth chamber [65 ± 5% RH; 16:8 h (L:D)] on *Ohadi* pistachio cultivar. To determine the egg incubation period, a cluster of 200 one-day old eggs were selected and monitored every 12 hours and the number of hatched eggs were recorded. To determine the length of developmental period in the first and five instar nymphs, they were reared on the pistachio clusters of the cultivar. These clusters were collected from an unsprayed orchard in the pistachio research center in Rafsanjan (Kerman province, Iran). The nymphs were reared in transparent plastic containers of 20 × 20 × 30 cm on the pistachio clusters, which were replaced every two days. The nymphs were checked daily until adults' emergence and death events in each stage were recorded separately. After emergence, both male and female insects were kept in pairs for oviposition within a plastic container (7 × 17 × 10 cm) with fresh pistachio clusters. The containers were revised daily and the number of laid eggs and adult's longevity were recorded. Demographic parameters were obtained under above-mentioned temperatures by constructing a life table of the insect by the method described by Carey (1993). The estimated demographic parameters are detailed on Figure 1

Data analysis

Statistical analysis was carried out using SAS version 9.1 software (SAS Institute, 1998) and the means were compared by Tukey's test (P<0.05). Population growth parameters as net reproductive rate (R_0), intrinsic rate of natural increase (r_m), finite rate of increase (λ), generation time (T) and doubling time (DT) as well as reproductive parameters of *B. germari* were obtained following Birch (1948) and Carey (1993) equations under five constant temperatures. The longevity, growth, development, and reproductive period of the adults were analyzed with one-way ANOVA method. The variances and standard errors of the population growth and reproduction parameters were estimated by Jackknife technique (Meyer et al., 1986; Sokal & Rohlf, 1995).

Gross reproductive rate (*GRR*):

$$GRR = \sum_{x=\alpha}^{\beta} m_x$$

Net reproductive rate (R_0):

$$R_0 = \sum_{x=\alpha}^{\beta} l_x m_x$$

Intrinsic rate of natural increase (r_m):

$$\sum_{x=\alpha}^{\beta} e^{-rx} l_x m_x = 1$$

Finite rate of increase (λ):

$$\lambda = e^r$$

Mean Generation time (*T*):

$$T = \frac{lnR_0}{r}$$

Doubling time (*DT*):

$$\mathrm{DT} = \frac{ln2}{r}$$

Fig. 1. Demographic parameters estimated in this study, *sensu* Carey (1993). L_x: number of individuals life at time *x* (age specific survival); m_x: number of female offspring *per* female at time *x* (age specific fertility); *x*: age interval in days; *r*: *per capita* rate of population change; *e*: natural base = 2.71878 (constant), α: age of first laying; β: age of last laying

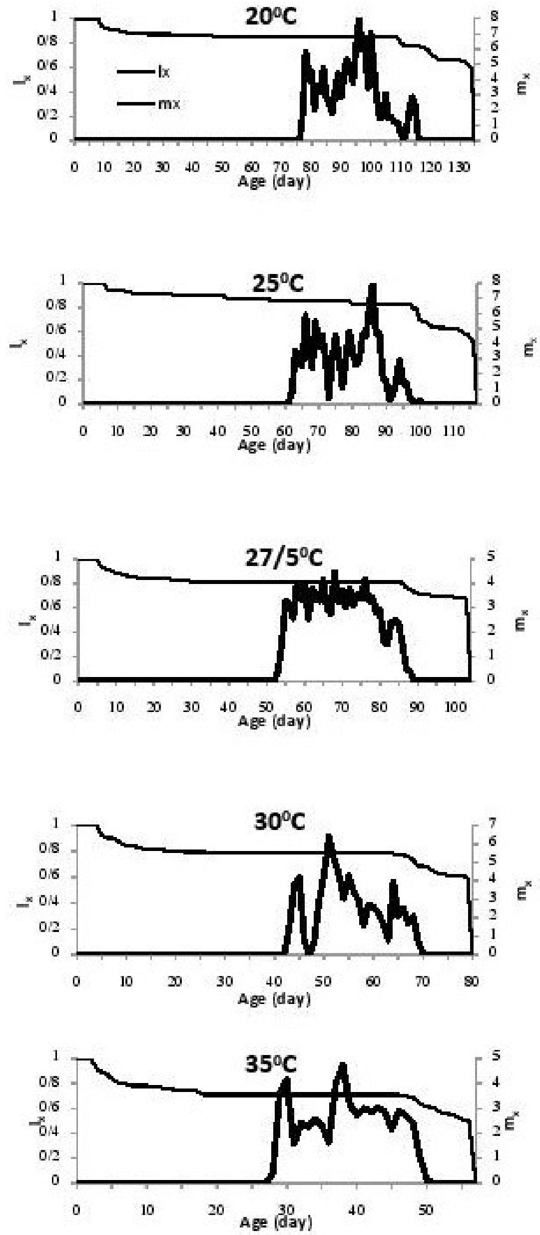

Fig. 2. Age-specific fecundity (m_x) and age-specific survival rate (l_x) of *Brachynema germari* under different temperatures.

RESULTS

Mortality rate

The mortality rate of eggs and nymph stages of *B. germari* under the studied temperatures are given in Table I. In both stages, the number of normal mortalities was separated from the abnormal mortalities (*i.e.*: non-inoculated eggs and dead nymphs by impact). The lowest mortality rate of the eggs (4.63%) was observed at 25 °C and with increasing temperature the mortality rate also increased, reaching 8.94% at 35 °C. Also, the mortality rates of the nymph stages under five different temperatures show that the highest value (23.68%) occurred at 35 °C, whereas the lowest (10.30%) being at 25 °C. The age-specific survival rates are shown in Figure 2. The total life cycle (egg to egg) reared at 20, 25, 27.5, 30 and 35 °C was 135, 117, 104, 80 and 57 days, respectively.

Developmental time and longevity

The developmental time of *B. germari* under the studied temperatures is given in Table II. Both the mean incubation period of eggs and the mean developmental time of nymph stages were the highest at 20 °C, and the length of these stages decreased with increasing temperature, until reaching the lowest values at 35 °C. The longest and the shortest total development times (egg to adult) also occurred at 20 °C and 35 °C, respectively.

The longevity of adult females was significantly affected by the temperatures (F=421.52, df=346, P<0.0001). In addition, there were significant differences among the studied temperatures on the longevity of adult males (F=141.41, df=346, P<0.0001). The longest and the shortest male and female adult longevity were observed at 20 °C and 35 °C, respectively (Table II). According to the results, the longevity of adult males was lower than that of the adult females under the experimental temperatures. The longest pre-oviposition period was observed at 20 °C, with significant differences with the other temperatures (F=2909.29, df=346, P<0.0001). The oviposition period also showed significant differences among different temperatures (F=524.74, df=346, P<0.0001). The post-oviposition period was significantly different at 20 and 25 °C compared to the other temperatures (F=47.04, df=346, P<0.0001). The longest and the shortest pre-oviposition, oviposition and post-oviposition periods were observed at 20 and 35 °C, respectively.

The age-specific fecundity of *B. germari* under five experimental temperatures is presented in Figure 2. The first oviposition was observed on day 78, 63, 54, 44 and 29 at 20, 25, 27.5, 30 and 35 °C, respectively.

Population growth parameters

The population growth parameters of *B. germari* under the studied temperatures are given in Table III. The highest gross reproductive rate was observed at 20 °C, and showed significant differences with the other temperatures (F=2737, df=346, P<0.0001). The highest net reproductive rate (R_0) was 66.53 at 25 °C, and showed significant differences with the others (F=1659, df=346, P<0.0001). The intrinsic rates of natural increase (r_m) were 0.05, 0.06, 0.06, 0.07 and 0.09 (day^{-1}) at 20, 25, 27.5, 30 and 35 °C, respectively. Also, there were significant differences in the finite rate of increase (λ) among five temperatures (F=1076, df=346, P<0.0001), being highest at 35 °C and lowest at 20 °C. The highest values of mean generation time (*T*) and doubling time (*DT*) were also observed at 20 °C, and these parameters decreased by increasing the temperature from 20 °C to 35 °C (F=2.68, df=346, P<0.0001; F=1155, df=346, P<0.0001).

Reproductive parameters

The reproductive parameters are presented in Table IV. The gross fecundity rate showed significant differences under different temperatures (F=2636, df=346, P<0.0001). The highest and the lowest gross fecundity rates were observed at 20 °C and 35 °C (273.33 and 124.96 eggs *per* female), respectively. Also, the net fecundity and net fertility rates were significantly different among the five temperatures (F=1495, df=346, P<0.0001; F=1371, df=346, P<0.0001). The highest and the lowest gross fertility rates (251.64 and 119.49 eggs *per* female, respectively) were observed at 20 °C and 35 °C. The daily fecundity (number of eggs laid *per* female *per* day) was the highest at 35 °C, and it was significantly different from the other temperatures (F=3087, df =346, P<0.0001). The highest daily fertility (number of fertile eggs laid *per* female *per* day) was observed at 35 °C, and was different from the other temperatures (F=4030, df=346, P<0.0001).

DISCUSSION

The suitable temperature for pistachio growth is 25 °C to 35 °C, and under this environmental condition some of its main pests are active and can cause important damage (Hokmabadi, 2011). Our results provided useful information about the growth, survival and reproduction of *B. germari* feeding on pistachio at five constant temperatures under laboratory conditions. These results can help us to understand the ecology and biology of this pest. In addition, estimation of life table parameters can forecast the relationship between temperature and growth potential (Greenberg et al., 2005; Dong et al., 2007). We found that temperature affects the development time of immature stages of *B. germari*, agreeing with other researchers that have reported similar results (Bommireddy et al., 2004; Zamani et al., 2006; Liu & Meng, 2007; Da Silva & Daane, 2014; Basirat et al., 2016). In this research, the development time of immature stages of *B. germari* decreased with increasing temperature from 20 °C to 35 °C, and the shortest growth period was observed at 35 °C. In contrast, the highest mortality rate and the lowest survival rate in the immature stages were observed at this temperature, indicating that the survival rate decrease at higher temperatures. These results are also in agreement with findings previously reported (Bommireddy et al., 2004; Da Silva & Daane, 2014; Basirat et al., 2016). Temperature had a significant effect on the longevity of males and females of this pest, and this feature decreased with increasing temperature from 20 °C to 35 °C, similar to that reported by Bommireddy et al. (2004). The reproduction parameters were also significantly affected by the different temperatures. The highest reproduction parameters were observed at 20 °C. A similar result has been reported by Mehrnejad (1998) on *Agonoscena pistaciae* Burkhhardt & Lauterer (Homoptera: Sternorrhyncha).

The intrinsic rate of natural increase (r_m) is the most informative demographic parameter that denotes pest's increasing capacity (Andrewartha & Birch, 1954; Wang et al., 2000). This rate has been considered as the most complete parameter for comparing population growth under various environmental conditions (Aldyhim & Khalil, 1993; Tsai & Liu, 1998; Wang & Tsai, 2001; Kuo et al., 2006). In the present research, the highest and the lowest r_m values were observed under 35 °C and 20 °C, respectively. Mehrnejad (1998) investigated the $r_m$of *A. pistaciae* under 20 °C to 35 °C and obtained the lowest value at 20 °C, which is in agreement with our results. However, this researcher determined the highest r_m of *A. pistaciae* at 30 °C (vs. 35 °C in our study). The longest and shortest mean generation time of *B. germari* also occurred at 20 °C and 35 °C, respectively. Mehrnejad (1998) obtained the mean generation time of *A. pistaciae* at 20 °C and 35 °C and the lowest value of this parameter was observed at 20 °C, similar to our results. It also have been documented that the mean generation time decreases with increasing temperature in the range of 20 °C to 30 °C (Mehrnejad, 1998; Basiratet al., 2016). The highest rmand R0 were observed under 35 °C and 20 °C, respectively. This could imply the highest population growth under this temperature range.

Our results showed that the temperature has significative impact on growth, survival and reproductive rate of pistachio green stink-bug, playing an important role in phenology regulation, seasonal abundance and population growth of this pest. Although insects in nature are not always exposed to constant temperatures, our studies under controlled conditions provided useful information about dynamics of B. germari that could be used in forecasting populations growth and evaluate integrated pest management (IPM) programs.

Stage	20.0 °C	25.0 °C	27.5 °C	30.0 °C	35.0 °C
Egg	5.64	4.63	5.85	6.91	8.94
	(n=195)	(n=194)	(n=188)	(n=188)	(n=190)
Nymph 1st instar	4.54	2.77	5.35	7.36	1.68
	(n=176)	(n=180)	(n=168)	(n=163)	(n=119)
Nymph 2nd instar	2.43	1.15	3.92	4.13	5.59
	(n=164)	(n=173)	(n=153)	(n=145)	(n=143)
Nymph 3rd instar	0.62	1.18	2.08	2.20	3.05
	(n=159)	(n=169)	(n=144)	(n=136)	(n=135)
Nymph 4th instar	0.63	0.60	1.43	1.52	2.41
	(n=157)	(n=166)	(n=139)	(n=131)	(n=124)
Nymph 5th instar	0.64	0.60	1.48	1.57	1.68
	(n=155)	(n=164)	(n=135)	(n=127)	(n=119)
Egg to Adult	13.33	10.3	17.55	20.21	23.68
	(n=195)	(n=194)	(n=188)	(n=188)	(n=190)

Table I. Immature mortality rate (%) of *Brachynema germari* under five constant temperatures.

Stage	20.0 °C	25.0 °C	27.5 °C	30.0 °C	35.0 °C
Egg	9.01 ± 0.04 [a]	7.02 ± 0.03 [b]	6.03 ± 0.04 [c]	5.01 ± 0.03 [d]	3.03 ± 0.03 [e]
Nymph	44.75 ± 0.84 [a]	36.33 ± 0.54 [b]	30.13 ± 0.63 [c]	24.65 ± 0.65 [d]	15.42 ± 0.47 [e]
Egg-to-adult	53.75 ± 0.84 [a]	43.34 ± 0.54 [b]	36.13 ± 0.63 [c]	29.66 ± 0.66 [d]	18.42 ± 0.47 [e]
Male longevity	61.07 ± 1.37 [a]	53.48 ± 1.02 [b]	54.78 ± 0.99 [b]	35.75 ± 0.88 [c]	31.31 ± 0.75 [d]
Female longevity	81.02 ± 1.04 [a]	73.58 ± 0.79 [b]	67.65 ± 0.82 [c]	50.10 ± 0.72 [d]	38.45 ± 0.57 [e]
Pre-oviposition period	24.52 ± 0.10 [a]	21.37 ± 0.07 [b]	19.35 ± 0.12 [c]	14.43 ± 0.10 [d]	10.63 ± 0.09 [e]
Oviposition period	34.90 ± 0.38 [a]	30.95 ± 0.37 [b]	29.97 ± 0.16 [c]	22.36 ± 0.16 [d]	18.26 ± 0.12 [e]
Post-oviposition period	21.59 ± 0.90 [a]	21.26 ± 0.64 [a]	18.32 ± 0.82 [b]	13.30 ± 0.67 [c]	9.55 ± 0.55 [d]

Table II. Immature developmental time, adult longevity and reproduction period values (days) of *Brachynema germari* under five constant temperatures. Mean of values that followed by the same letter in each row are not significantly different (Tukey test, P<0.05).

Parameter	20.0 °C (n=76)	25.0 °C (n=80)	27.5 °C (n=70)	30.0 °C (n=65)	35.0 °C (n=60)
Gross reproductive rate (*GRR*)	131.48 ± 0.01 [a]	112.46 ± 0.02 [b]	102.85 ± 0.01 [c]	72.50 ± 0.01 [d]	58.56 ± 0.01 [e]
Net reproductive rate (R_0)	63.75 ± 0.04 [b]	66.53 ± 0.04 [a]	49.92 ± 0.03 [c]	34.21 ± 0.02 [d]	28.43 ± 0.02 [e]
Intrinsic rate of increase (r_m)	0.05 ± 8.26 [e]	0.06 ± 9.75 [d]	0.06 ± 1.24 [c]	0.07 ± 1.68 [b]	0.09 ± 2.95 [a]
Finite rate of increase (λ)	1.05 ± 8.64 [e]	1.06 ± 1.03 [d]	1.06 ± 1.31 [c]	1.07 ± 1.79 [b]	1.09 ± 3.23 [a]
Mean generation time (*T*)	107.61 ± 0.004 [a]	87.88 ± 0.003 [b]	78.40 ± 0.01 [c]	64.77 ± 0.004 [d]	45.28 ± 0.004 [e]
Doubling time (*DT*)	15.26 ± 0.002 [a]	12.53 ± 0.002 [b]	11.87 ± 0.002 [c]	10.49 ± 0.002 [d]	7.65 ± 0.002 [e]

Table III. Population growth parameters of *Brachynema germari* under five constant temperatures. Mean of values that followed by the same letter in each row are not significantly different (Tukey test, P<0.05).

Parameter	20.0 °C (n=76)	25.0 °C (n=80)	27.5 °C (n=70)	30.0 °C (n=65)	35.0 °C (n=60)
Gross fecundity rate	273.33 ± 0.02 [a]	218.33 ± 0.05 [b]	200.17 ± 0.01 [c]	152.83 ± 0.02 [d]	124.96 ± 0.03 [e]
Gross fertility rate	251.64 ± 0.02 [a]	201.19 ± 0.04 [b]	187.46 ± 0.01 [c]	144.65 ± 0.02 [d]	119.49 ± 0.03 [e]
Net fecundity rate	132.28 ± 0.09 [a]	129.12 ± 0.09 [b]	97.10 ± 0.07 [c]	71.98 ± 0.06 [d]	60.51 ± 0.06 [e]
Net fertility rate	121.78 ± 0.08 [a]	118.98 ± 0.08 [b]	90.93 ± 0.07 [c]	68.13 ± 0.05 [d]	57.86 ± 0.05 [e]
Egg/female/day	1.55 ± 0.0006 [c]	1.58 ± 0.001 [b]	1.54 ± 0.001 [d]	1.51 ± 0.001 [e]	1.85 ± 0.001 [a]
Fertile egg/female/day	1.42 ± 0.0006 [d]	1.45 ± 0.001 [b]	1.44 ± 0.001 [c]	1.42 ± 0.001 [d]	1.76 ± 0.001 [a]

Table IV. Production parameters of *Brachynema germari* under five constant temperatures. Mean of values that followed by the same letter in each row are not significantly different (Tukey test, P<0.05).

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