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Life Cycle of *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae), an Invasive Exotic Pest in Hyrcanian Forests of Iran

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ABSTRACT

Box tree moth, *Cydalima perspectalis* (Walker, 1859), is one of the major destructive pests that feed on the leaves and shoots of various *Buxus* species. In the course of this survey, the life cycle of *C. perspectalis* was studied in laboratory and natural (Hyrcanian Forests) conditions. The laboratory experiments were carried out at temperature of 25±1ºC, 70±10% relative humidity and a photo phase of 16 light: 8 dark hours. The average duration of an egg, larva, pre-pupa, pupa, as well as female and male longevity were 5.09±0.04, 23.15±0.17, 1.04±0.02, 7.80±0.05, 15.31±0.73 and 12.92±0.71 days, respectively. As an important pest newly introduced in northern Iran, the Box tree moth completes two and partial third generations per year. The results of this study would be useful for improving pest management strategies.

Keywords: Box tree moth, *Buxus* species, Iranian fauna.

INTRODUCTION

Box tree moth, *Cydalima perspectalis* (Walker, 1859), was described as *Phakellura perspectalis* by Francis Walker in 1859. It is an economically important pest and originally distributed in the temperate zone ecosystems in East Asia (Inoue *et al.*, 1982). It was introduced in Europe in 2007, and distributed in at least 20 European countries (Krüger, 2008; Nacambo *et al.*, 2014). *Cydalima perspectalis*, as an invasive exotic pest, has been recently introduced in the Iranian fauna (Farahani *et al.*, 2016; Ahangaran, 2016). It seems that this pest will spread into new geographical areas in Hyrcanian Forest in northeastern parts to Golestan Province and neighboring countries.

Cydalima perspectalis is highly monophagous and specialized on the plant genus *Buxus* sp. (Wan *et al.*, 2014). The larvae of Box tree moth is the damaging stage of this pest. The voracious caterpillars feed large areas of leaves within webbing

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and cause complete defoliation of box trees. In this case, in the absence of leaves, larvae feed readily on bark tissue, which may result in dieback and even tree death (Leuthardt and Baur, 2013). Life cycle characteristics, including adult seasonal emergence, oviposition, post-embryonic development and photoperiodic induction of larval diapause were studied by Maruyama and Shinkaji (1987) and Maruyama (1992, 1993) in Japan. Leuthardt *et al.* (2010) studied the distribution, rate of spread, and biology of *C. perspectalis* in Switzerland. A research was conducted on oviposition preference and larval performance on the five most common European box-tree varieties by Leuthardt and Baur (2013). Nacambo *et al.* (2014) predicted the potential distribution by bioclimatic model. Wan *et al.* (2014) reviewed the biological characteristics, host plants, phenology, distribution, and control options of the box tree moth in Asia. Ostojić *et al.* (2015) investigated on morphology, biology, damage and control measures in Bosnia and Herzegovina. Duration of developmental stages at different temperatures and efficiency of natural enemies were studied in laboratory condition by Göttig (2017), who compared the results between Asian and German populations. *Cydalima perspectalis* is a polyvoltine lepidopteran pest and number of generations has been connected to the weather conditions of the area where it occurs (Wan *et al.*, 2014).

Cydalima perspectalis is one of the most important insects known to cause visible damages to forest and recently threated Iranian forests, where the knowledge on the pest biology is very limited. In the present work, developmental period, emergence of overwintered adults, mortality rate in immature stages, number of larval instars, adult flight, overwintering and number of generation in Iran are provided. The results of this study could help researchers develop better control of *C. perspectalis* in Iran. This study is useful in providing which life stage is suitable to apply control measures.

MATERIALS AND METHODS

Area of Study

The present study was carried out along Caspian (Hyrcanian) Forests, located in the Euro-Siberian Phytogeographical Region. Hyrcanian Forest is a humid zone in the north of Iran. The average annual rainfall varies from 2000 mm in the west to 530 mm in the east. Sampling locations included Mazandaran, Guilan, and Golestan Provinces (Figure 1). Table 1 shows the geographical characteristics and elevation above sea level for each location. The Hyrcanian climate is warm Mediterranean in the east and temperate and semi-temperate Mediterranean and occasionally temperate xeric in the central and western parts (Sagheb-Talebi *et al.*, 2004).

Field Studies

Monitoring was done consistently over 2 years (2017 and 2018) to validate the biology. Seasonal life cycle of *C. perspectalis*, developmental stages, and moth flight activity were recorded based on monitoring data from field observations. The pheromone and light traps were used for monitoring adult emergence.

Laboratory Studies

The mass rearing itself originated from individuals collected in Cheshmeh-Bolbol Box Reservoir (Golestan Province). One hundred eggs of *C. perspectalis* were used to obtain data (one cohort in four groups with 25 individuals). Our experiments were conducted in a controlled environment (25±1°C, 70±10% Relative Humidity (RH) and 16 hours photo phase), with daily observations (70 days). Each egg mass was placed individually in a 10 cm-diameter Petri dish, where it remained until the eggs hatched. Mortality rate in the egg stage was

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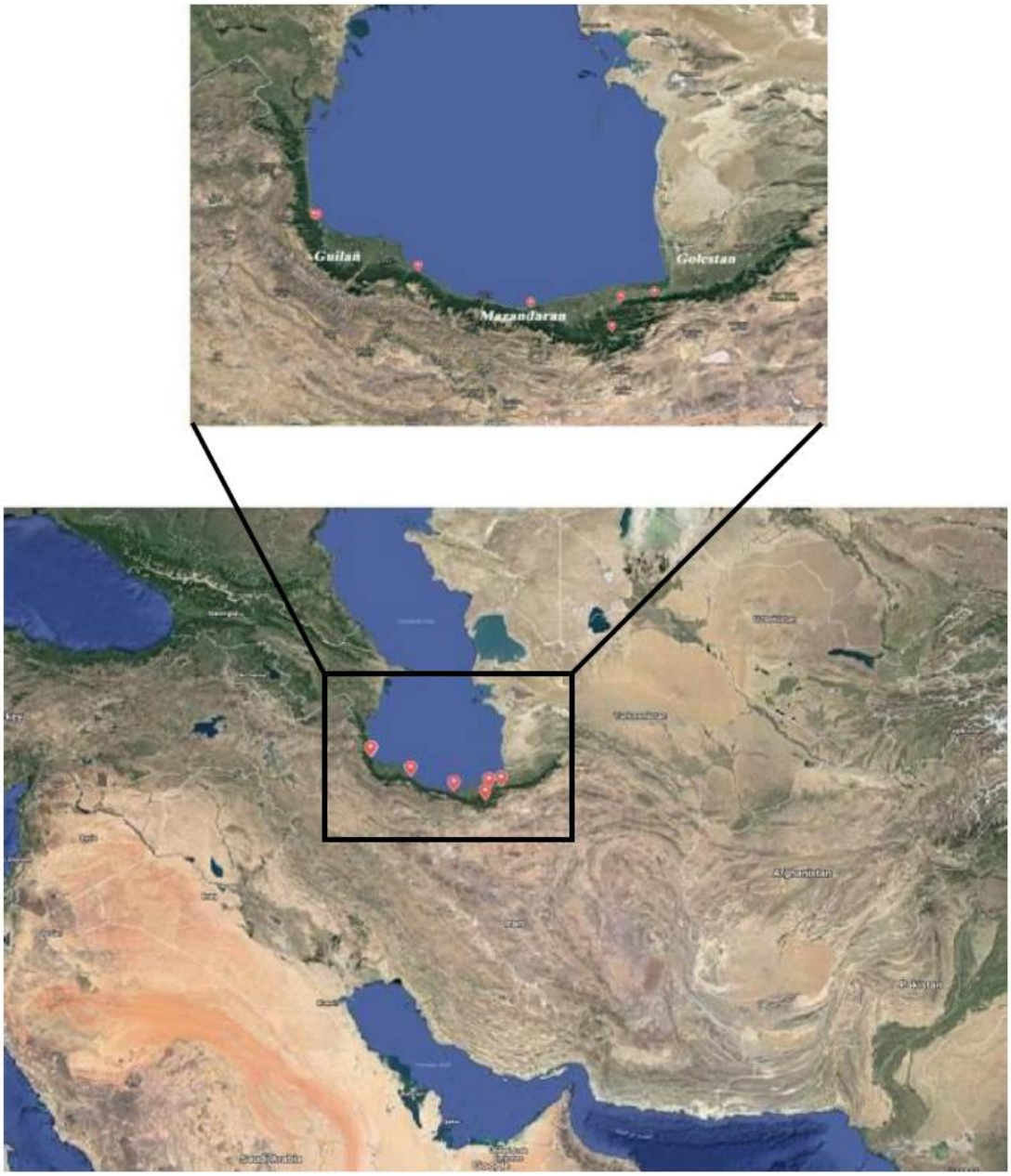

Figure 1. Map of Iran and geographical characteristics of the collecting sites in Hyrcanian Forests, northern Iran, where biology of *Cydalima perspectalis* has been recorded.

Table 1. Geographical characteristics of sampling locations in Hyrcanian Forests.

Province	Locality	Latitude and longitude	Altitude (m)
Guilan	Gissoom Jungle	37° 38′ N, 49° 01′ E	55
	Asalem, Pirharat	37° 40′ N, 48° 57′ E	119
	Roodsar, Chaijan	37° 01′ N, 50° 27′ E	9
Mazandaran	Neka, Abelo	36° 38′ N, 53° 20′ E	72
	Noor, Sisangan	36° 34′ N, 51° 47′ E	19
Golestan	Bandar-e-Gaz, Cheshmeh-**B**olbol	36° 41′ N, 53° 54′ E	45

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derived from direct observations of dead eggs in the egg-batches. After hatching, larvae were separately placed in 500 mL plastic cups and fed with fresh leaf of *B. sempervirens* subsp. *hyrcana*. Fresh leaves were provided daily in the morning. Larval instars were determined by the presence of newly molted exuviae and head capsules. The pre-pupal period was recorded when a larva stopped feeding and decreased in size. Pre-pupae were individually kept in plastic containers (3 cm in diameter and 5 cm high) for pupation. After emerging of adults, moths were kept individually in transparent plastic container with small cotton wick soaked in 10% honey solution. The adult moths are alike in general appearance, except for the abdominal tip of the male ends inside the tuft of scales, while in females, the tip of the abdomen is without any side tufts and has a defined hole. Developmental stages were checked daily and developmental periods and mortality of eggs, larvae and pupae were recorded. The experiment continued until the death of all individual members of each cohort. Age-specific survival rates (lx) and life expectancy (ex) were calculated, but we were not able to evaluate the reproduction and demographic parameters, because the adult moths did not mate in laboratory condition.

RESULTS

Area of Study

Annual life cycle of Box tree moth in some of Hyrcanian Forests Sites can be seen in Figure 2.

Overwintering: Our results showed the

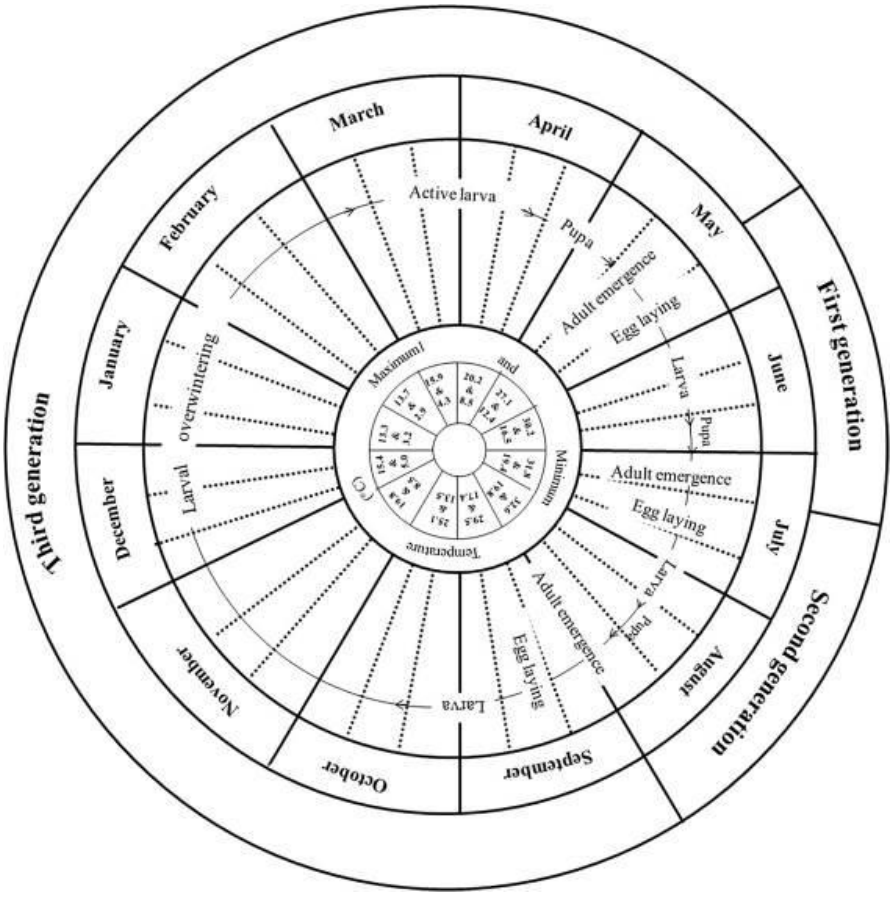

Figure 2. Annual life cycle of *Cydalima perspectalis* in sampling locations of Hyrcanian Forests.

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Box tree moth overwinters as second, third, and particularly fourth instar larvae, in hibernacula of silk webbing.

Overwintering larvae appeared from their nest, became active in mid-March, and transformed into pupae in the last ten days of April to early May. Pupa was found in a sheltered place between injured leaves. Pheromone traps were used to detect the presence of male adults. The first flight of moth occurred in early to mid-May (overwintering generation). According to monitoring data, the first flight peak and egg laying occurred in mid-late May. Box tree moth has two distinct color forms, normal white and brown forms.

First Generation: The adult moths were active at night, fed on nectar and searched for a specific host (e.i, *Buxus* sp.) for egg laying. The adult moths laid eggs/egg-clusters on the underside of leaves of terminal shoots. Eggs were laid singly or in clusters of 2 to 19 eggs per mass and a tiny larva was visible developing inside. A freshly laid egg was opaque and pearly white. The eggs became yellowish and their contents were more noticeable as they aged, and at the time of hatching, they were black. Neonate larvae were less mobile and started feeding within few hours after their emergence. Also, larvae tended to aggregate until they reached 3rd developmental stage. Caterpillars moulted five times to complete post eclosion development، and thus had six distinct instars. The larvae were taken approximately 38-40 days to develop, reaching up to 40 mm long in the sixth instar. The first generation of adults began to fly in early July and their flight peak occurred in mid to late-July.

Second Generation: In the first generation, many adults emerged and, as a result, population density increased remarkably during the second generation. Eggs/egg-clusters were most abundant in late July. Overlapping of the development time of immature stages of *C. perspectalis* was observed and exact record for all stages was somehow difficult. Our observations indicated that pupae and adults were formed in mid- and late August, respectively.

Third Generation: Egg of the third generation was observed from the first ten days of September to mid-September. Development rate and overall abundance decreased during the autumn and winter months. An overwintering larva spun a cocoon between box leaves in autumn. Larvae entered diapause in the 3rd or 4th instar in early October.

Laboratory Studies

The larvae were rather aggressive, occasionally carnivorous, and could even cannibalize each other. Therefore, mass rearing was individually performed from the 4th to 6th instar larval. The 1st to 3rd instar larvae consumed the inferior epidermis layer and the mesophyll of the leaf; but the 4th and 6th instar tended to burrow holes in leaves and remained in thick epidermis and veins. The life cycle of *Cydalima perspectalis* in laboratory condition is presented in Table 2. Egg incubation period was obtained as 5.09±0.04 days. Six larval instars passed until the pupation. The minimum and maximum duration of larval stage were 20 and 31 days. The results indicated that pupal period was 7.80±0.05 days. The longevity of females adult was longer in comparison with the males (Table 2). In the laboratory conditions, total life span from egg to adult emergence was completed in 50.20±0.54 days (ranging from 39 to 62 days).

The results on survival rate (lx) and the life expectancy (ex) of *C. perspectalis* are summarized in Figure 3. In the present work, we obtained survival rate and the life expectancy of one-day-old larvae were estimated as 0.91 and 40.75 days, respectively. The age-specific survival rate at the time of adult emergence was 0.81 and the life expectancy (*ex*) of one-day-old adults of *C. perspectalis* was estimated as 16.12 days. Further, the mortality rate was comparatively high at the early ages.

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Table 2. Mean±SE duration of immature stage, adult longevity and life span of *Cydalima perspectalis* on *Buxus sempervirens* subsp. *hyrcana* in laboratory condition (25°C).

Different life stage/period (day)	n (n0= 100)	Mean±SE	Range (Minimum-Maximum)
Incubation period	91	5.08±0.03	4-6
1st Instar larval period	87	3.01±0.03	2-5
2nd Instar larval period	85	3.13±0.06	2-7
3rd Instar larval period	84	2.88±0.04	2-4
4th Instar larval period	82	2.99±0.04	2-5
5th Instar larval period	81	3.11±0.05	3-6
6th Instar larval period	81	6.95±0.12	5-12
Pre-pupal period	81	1.04±0.02	1-2
Total larval period	81	23.15±0.17	20-31
Pupal period	81	7.80±0.05	6-8
Developmental time	81	36.04±0.16	33-44
Female longevity	42	15.31±0.73	5-27
Male longevity	39	12.92 ± 0.71	4-25
Life span	81	50.20 ± 0.54	39-62

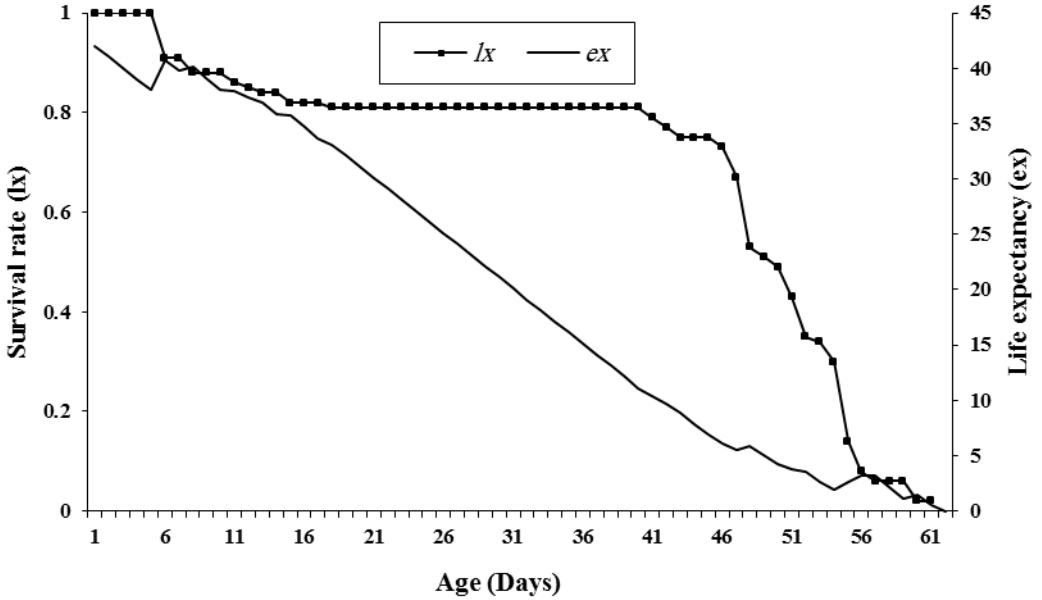

Figure 3. Age specific survival rate (*lx*) and life expectancy (*ex*) of *Cydalima perspectalis* on *Buxus sempervirens* subsp. *hyrcana* in laboratory condition.

Percentage of viability of *C. perspectalis* is shown in Figure 4. The number that survived from 100 eggs to adult emergence was 81. Egg hatchability was recorded as 91±1% in laboratory condition. Accordingly, there was 9±1% mortality in the egg stage, whereas mortality rate during larval period was 8±1%. The results on the number of individuals survived during developmental periods did not reveal any mortality during a pupal stage.

DISCUSSION

The field works showed that *Cydalima perspectalis* was distributed from the west to the east of Hyrcanian Forests. Box tree moth has been reported on *Euonymus japonicas*,

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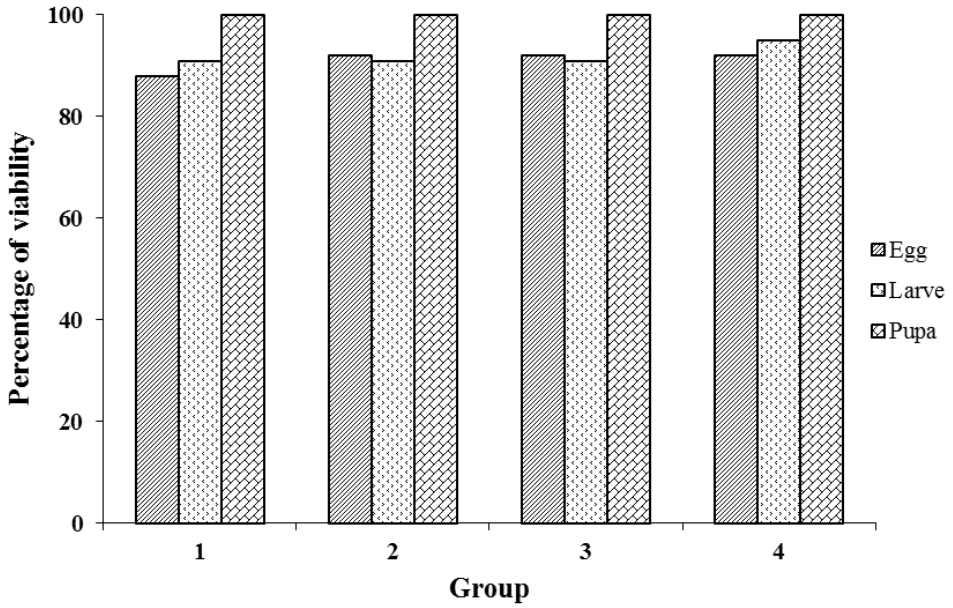

Figure 4. Percentage of viability of *Cydalima perspectalis* immature stage on *Buxus sempervirens* subsp. *hyrcana* in laboratory condition (the number of 100 eggs in four groups with 25 individuals).

E. alatus, *Ilex purpurea* and different *Buxus* species in the area of origin (Wan *et al.*, 2014), but there are no reports of these plant species being attacked in Iran. In Europe, it has also been reported on *Buxus* sp. (Hizal *et al.*, 2012; Bella, 2013; Plantwise Knowledge Bank, 2015).

As incidental occurrence, Box trees in some sites at higher altitudes were not damaged (as, Mazandaran Province, Yakhkesh, besides of Senbi River, 36° 35′ N, 53° 44′ E, 910 m) and in some sites, non-infested trees were observed among infested trees (Guilan Province, Parehsar, Lomir, toward Pisesovan Jungle, 37° 33′ N, 48° 46′ E, 1000 m). The timing of adult emergence and development time depended upon altitude and latitude and environmental condition. These results corroborate findings of a great deal of previous works (López and Eizaguirre, 2019). Males emerge before females to achieve maximal reproductive success (Wiklund and Fagerström, 1977). This finding confirms that development time and number of generation are associated with temperature of the environment. The number of generations produced in a year is mainly dependent upon the climate conditions. *Cydalima perspectalis* has three generations annually in Hyrcanian Forests (two complete generations and a partial third; all third generation larvae overwintering), while it completes 3-5 generations per year in the area of origin (Niu *et al.*, 2008; Wang *et al.*, 2008; She and Feng, 2006; Sun *et al.*, 2009) and 2 generations per year occur in Europe (Nacambo *et al.*, 2014).

The overwintering larvae tends to weave silk web between damaged leaves (Nacambo *et al.*, 2014), which seems very important for their thermoregulation during winter. Sometimes, a number of active larvae were observed in the mid-January due to warm winter days. These larvae could not survive the following low temperatures.

Mortality of immature stages –eggs and initial larvae– was observed due to high temperature (above 38 °C) in warm locations such as Golestan Province. Thus in August, rate of larval development reduced larval final mass, which may also be the

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reason for reduced female fecundity for the next season in warm areas.

We were able to determine that *C. perspectalis* has six larval instars and that those instars can be identified using five different head capsule size, whereas German population had 7 larval instars at 25°C (Göttig, 2012). In the present study, developmental parameters of *C. perspectalis* were evaluated in the laboratory. The duration of each instar was also obtained from our data. Our findings confirm that 1-5 larval instars had similar developmental times (3 days approximately), while the sixth-instar larvae presented the longest average interval with 7 days at 25°C. The development of larvae and pupae lasted 23.15±0.17 and 7.80±0.05 days, respectively. Maruyama and Shinkaji (1987) reported a longer duration of the larval and pupal stages (24.9±2.89 and 10.0±0.36 days). Zhou *et al.* (2005) determined pupal period duration as 8.8±0.32 days at 26.4±0.68°C. Göttig (2012) showed that in German population of *C. perspectalis* average time from egg incubation to adult emergence were 36±10.8 days at 25 °C, which is in agreement with our result (36.04±0.16 days).

Two natural enemies have been identified and reported from Iran. The first one, i.e. *Compsilura concinnata* (Meigen) (Diptera: Tachinidae), previously reported from Japan (Shima, 1973; Wan *et al.*, 2014), was reported from Golestan Province (Farahani *et al.*, 2018). The second one, fungal mycelium of *Beauvaria bassiana* Vuillemin, was observed on larval stage of *C. perspectalis* by Zamani *et al.* (2017). Both parasitoids are reported in literature as polyphagous. In the area of origin, known parasites and predators include families of Tachinidae, Braconidae, Chalcididae, Encyrtidae, Ichneumonidae and Aeolothripidae (Wan *et al.*, 2014). Most probably, some other species of natural enemies may be present, which needs further studies.

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چرخه زندگی *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae)، آفت خارجی مهاجم در جنگل‌های هیرکانی ایران

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چکیده

شب‌پره شمشاد، *Cydalima perspectalis* (Walker, 1859)، یکی از آفات مخربی است که از برگ‌ها و شاخه‌های گونه‌های مختلف *Buxus* تغذیه می‌کند. در طی این بررسی، چرخه زندگی *C. perspectalis* در شرایط آزمایشگاهی و طبیعی (جنگل‌های هیرکانی) مورد مطالعه قرار گرفت. بررسی‌های آزمایشگاهی در دمای ۱±۲۵ درجه سلسیوس، رطوبت نسبی ۱۰±۷۰ درصد و دوره نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی انجام شد. میانگین طول مدت دوره رشد تخم، لارو، پیش‌شفیره، شفیره و همچنین طول دوره زندگی حشرات ماده و نر به ترتیب ۰/۰۴±۵/۰۹، ۰/۱۷±۲۳/۱۵، ۰/۰۲±۱/۰۴، ۰/۰۵±۷/۸۰، ۰/۷۳±۱۵/۳۱ و ۰/۷۱±۱۲/۹۲ روز بود. شب‌پره شمشاد به عنوان یک آفت مهم که به تازگی به شمال ایران وارد شده است، دارای دو نسل کامل و یک نسل زمستانگذران در سال می‌باشد. نتایج این مطالعه برای بهبود استراتژی‌های مدیریت آفات مفید خواهد بود.

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