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Biological characteristics of Orthonama obstipata (Fabricius, 1794) (Lepidoptera: Geometridae), an emerging defoliator of mint (Mentha spicata L., 1753) in Morocco

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The looper caterpillar, *O. obstipata* is one of the defoliator species of cultivated mint in Morocco. Its identification and its biology constitute the preliminary step for the development of a program aiming at its integrated management. The larvae were collected from a plot of mint grown in a region of northern central Morocco, and reared in the laboratory. The identification of the species was based on comparing the male aedeagus and the female spermatheca to the reference slides. In this study, the elements of biology and morphological characteristics of the different ecophases of *O. obstipata* are clarified and discussed. The life cycle of the species, from egg to egg, is completed in 31.3 ± 2.16 days. The emergence occurs linearly in time after the 27^{th} and 28^{th} days of oviposition according the sex. The sex ratio (Male/Female) is 0.88:1. The fecundity of the females is positively correlated with their lifespan. Females have an average fecundity of 210 eggs and a fertility rate of 97%. Adults live on average 22.0 ± 6.27 days. The highest mortality rates occur in the last larval instar and pupa.

Keywords: *Orthonama obstipata*, Biology, Reproduction, *Mentha spicata*

INTRODUCTION

Migratory Geometrid pests are widely distributed around the world (Skou, 1986; Scoble et al., 1995; Scoble, 1999; Thibaudeau et al., 2013). The looper caterpillars are polyphagous. They feed on a wide variety of herbaceous and cultivated or spontaneous woody taxa (strawberry, cabbage, plum, green onion, Chinese bellflower, golden ragwort, etc.) (Choi, 2012; Thibaudeau et al., 2013). The larvae of some species are closely associated with herbaceous plants (Brehm and Fiedler 2005; Brehm et al., 2005; Axmacher et al., 2009). Some geometrid species cause damage to crops such as fodder crops (Ptychopoda herbariata) (Balachowsky, 1972) and apple trees (Chloroclystis rectangulata L.) (Balachowsky, 1966). In Morocco, some other species like Gymnoscelis pumilata have been reported on the flowers of Citrus (Balachowsky, 1966), Gymnoscelis pumilata Hubner on the cotton plant (Le Gall, 1965) and O. obstipata on cultivated spearmint (M. spicata L.) (Eddaya, 2015). Moroccan mint is cultivated on an area of about 3352 ha producing 98704 t with a yield of about 29 t/ha (FAO, 2018). The crop is attacked by various harmful biological agents (Eddaya et al., 2015) during its development. Among the pests associated with cultivated mint, O. obstipata is an emerging defoliator that is increasingly attracting the attention of producers.

O. obstipata is known for a remarkable sexual dimorphism. It is cosmopolitan, polyvoltine (Ford, 1972; Skou, 1986) and migratory (Soli, 1986; Pohl et al., 2010). O. obstipata was reported in several countries such as: the United States, southern Canada, Spain, France, India, China, South Korea, Malaysia, Turkey, Morocco, etc. (Albu and Metzler 2004; Schulze and Fiedler 2004; Ayberk, 2010; Bachelard and Fournier, 2010; Choi 2010; Pohl et al., 2010; Zamora-manzur et al., 2011; Eddaya, 2015). It frequents wasteland and gardens even in the heart of cities, meadows, agrosystems and urban parks (Schulze and Fiedler, 2004; Lim et al., 2009; Ayberk, 2010). O. obstipata accomplishes several generations from March to November (Skou, 1986). The development from egg to imago of O. obstipata lasts about a month under optimal conditions (Edelsten and Fletcher, 1961).

Some geometridae species have already been studied (King and Montesinos, 2017), e.g. Abraxas pantaria (Pernek et al., 2013), Ectropis sp (Prasad et al., 2013), Sangalopsis veliterna (Hernández et al., 2014) and Drymoea veliterna (Hernández et al., 2017). However, to our knowledge, the biology and morphology of O. obstipata have not been described in detail. Therefore, in an attempt to develop an integrated and sustainable management approach of O. obstipata, it is more appropriate to start by verifying its identity and specifying its elements of biology in Moroccan conditions. Thus, the aim of this work is to provide the morphological description and the biological characteristics of O. obstipata (viz. fecundity, fertility, sex ratio, longevity of adults and mortality rates of each stage) under laboratory conditions.

MATERIALS AND METHODS

Collection and rearing larvae

One hundred O. obstipata larvae were collected from a plot of mint (M. spicata) located in the region of Meknes (GPS coordinates: 33 ° 53’44.6 “N; 5 ° 37’31.1” W, 549 m a.s.l.) in September 2017. They were brought to the laboratory. The larvae of different stages were reared in aerated plastic boxes 20 cm in diameter and 30 cm in height, at temperatures ranging from 25 to 31°C, with a relative humidity of 70% and a photoperiod of 13 h/11 h (Light/Darkness). The first two parameters were followed using a recording thermo-hygrometer (Ref. WK072). The caterpillars were fed with fresh spearmint leaves which were renewed every day after cleaning the boxes. The pupae, obtained from these larvae, were transferred daily and individually to other ventilated transparent plastic boxes 4 cm in diameter x 3 cm in height. When adults emerged, the two sexes were distinguished according to Schulze and Fiedler (2004) and Eddaya (2015). Then, they were placed together in aerated plastic boxes 11 cm in diameter x 7 cm in height with a sugar solution (10%). The confirmation of the species was based on the morphological genitalia in comparison with reference illustrations (Hausmann and Viidalepp, 2012; Lewis, 2013; Eddaya, 2015). The preparation of genitalia was done according to the modified method of Gates Clarke and Washington (1941). Some genitalia were colored by carmine (10% in distilled water) and some were not. They were placed in a few drops of water in watch glasses on a white or blue background to bring out some characteristics. The images were performed using the MOTIC digital stereo microscope “Model DM-143-FBGG” and prepared using Adobe photoshop Version 2018. The adults had fresh mint leaves as a support for oviposition. The leaves were renewed every two days (Chevin, 1995).

Biological parameters measured

Adult Longevity

The lifespan of each adult (i.e., the time interval between its emergence and its death) was expressed in days. It has been established in a sample of 32 males and 30 females that were followed from emergence to death. The average longevity was calculated according to the formula $D= \Sigma(fi*xi)/N$; with D: average longevity in days, fi: number of individuals dead at time xi, N: total number of individuals followed. Standard deviations were calculated using the grouped data method.

Fecundity and fertility

To estimate the fecundity of O. obstipata (i.e., the number of eggs laid per female), 33 new emerged pairs, at most 24-hour old, were used. Each pair was placed in a cylindrical plastic box 11 cm in diameter x 7 cm in height with a sugar solution (10% sucrose) and leafy stems of M. spicata as laying supports. The total number of eggs laid during the whole life of the females was recorded for each female. Thus, the relationship between the number of eggs laid/female and its longevity has been established. The eggs were isolated from the leaves under the stereomicroscope and glued onto white paper ribbons (80 g/m^2) of 1/10 cm using an aqueous solution of Arabic gum (10%). The counting of hatched eggs was performed using a stereomicroscope, then the fertility (= Number of eggs hatched / Number of eggs laid x 100) was calculated.

Larval and pupal development

Among the 240 eggs incubated, 60 neonate larvae, at most 2-hour old, were individually placed on a fresh mint leaf in a transparent plastic box (38 cm3) at temperatures ranging from 17 to 22°C, a relative humidity of 81-99.7% and a photoperiod of 10 h/14 h (Light/Dark). The fresh leaves of M. spicata were subsequently supplied to larvae regularly. The transformation from one larval instar to the next was monitored daily by the observation of exuvia and / or a rejected cephalic capsule. At the end of the larval development, the pupae were collected from the coiled mint leaves and placed individually in small transparent boxes to monitor the emergence of adults. The dimensions (length x width) of the eggs, larvae, cephalic capsules and pupae were measured using a MOTIC digital stereomicroscope “Model DM-143-FBGG”. The larvae and pupae were weighed using a “Precisa 125A” analytical balance. The duration of development of each stage was calculated according to the formula $D = \Sigma(fi * xi) / Ni$. With D: development time in days; fi = number of individuals belonging to stage i; xi = duration in days elapsed during stage i; Ni = total number of stage i). The standard deviations were calculated using the grouped data method.

To estimate the sex ratio (Male/Female), 221 pupae were distinguished according to the position of the genital orifices and their shapes. Then, they were placed individually in transparent plastic boxes (38 cm3) under the same larval rearing conditions. The emergence dates of adults were also recorded by sex.

Mortality of the pre-imaginal stages

To estimate mortality at each stage, 150 eggs were followed from oviposition to adult emergence. Dead individuals were counted in each stage of development. The percentage of mortality for each pre-imaginary stage was calculated by dividing the number of deaths recorded at this stage over the total number of individuals followed x 100.

Statistical data analysis

The data have been synthesized in graph or table forms. The comparisons of the measured parameters (adult longevity, fecundity, fertility, development times of the pre-imaginal stages, dimensions of the stages or of the cephalic capsules) were made using Student t-test at 5% or by the one-way analysis of variance followed of the Tukey test at 5%. In the case of adults, survival curves were established and compared using the log rank test according to Kaplan-Meier (1958). Regressive models linking fecundity to longevity of each female or to the growth of pre-imaginal stages over time have been established. The sex ratio or the percentages of deaths recorded at each stage were compared using chi-square test at 5%. The software tools used were those of Excel version 2013 and Statistica version 7.

RESULTS

Adults

The patterns on the fore wings of both sexes are very different. Males are yellowish brown with a darker transverse area between the base of the wing and the midline. A small black discoid spot is located in the center of the forewing. The transverse lines on the two wings are generally dark. The coloration of female wings is much darker and the black discoid spot on the front wings is surrounded by white. The transverse bands of the wings are very dark in color and alternate with light colored lines. The antennae of adult males are bi-pectinate, whereas those of females are threadlike.

Male genitalia:

It is small and strongly sclerotized. The uncus is glabrous and has an orifice in the form of a beveled slit (Figure 1a). The aedeagus is long and narrow (Figure 1b). The cornuti has fine hairs (Figure 1c and d). The valva consists in a round V-shaped basal part with a crenellated upper edge and a sub-rectangular distal part carrying long bristles (Figure 1c-f). The sclerotic costa is ear-shaped and carries fine small distal spines (Figure 1a, d and f). It is inserted at the border of the seed coat and the vinculum at one end and at the valva on the other end (Figure 1e). The saccus is in a form of Greek upsilon (Figure 1f).

Female genitalia: The posterior apophyses are longer than the anterior ones (Figure 2a). The ovipositor does not have a longitudinal crest on the ventral side. Its lobes bearing short hairs have the basodorsal parts covered with a small sclerified membrane (Figure 2b). The antero- and post-vaginal lamellae are weakly sclerosed (Figure 2c). The posterior antrum is elongated and well sclerosed. The ductus bursae is short, sclerotic and as long as it is wide. It has a longitudinal dorsal line and two others on both sides. The corpus bursae is globose and membranous (Figure 2d).

Longevity

Among a sample composed of 32 males and 30 females, the males live from 5 to 34 days and the females from 12 to 34 days. Nevertheless, the individual variability is rather high: the coefficient of variation is around 30% for females and 41% for males. Considering the average longevity of the two sexes, there is no significant statistical difference between both sexes. The survival probability of females is higher at the beginning of the adult stage, unlike that of males which increases rather towards the end of the stage (Figure 3).

Fecundity and fertility

During their lifetime, the females of O. obstipata lay from 94 to 391 eggs / female (Figure 4), which corresponds to an average of 210.3 ± 79.84 eggs/female and a high individual variability (coefficient of variation = 38.0%). There is also a significant statistical difference between the fecundity of females followed ($t = 15.13$; $dl = 32$; $P < 0.0001$) (Table 1). In fact, the fecundity of the females monitored depends directly on their longevity; indeed, it is positively correlated with their lifespan (Figure 5). Moreover, not all laid eggs reach the term of their embryonic development. The number of hatched eggs varies between 92 and 381 depending on the female considered (Figure 4), with an average of 204 ± 76.4 eggs / female, i.e., 97.2% of eggs laid by each female hatch. The statistical comparison between the fertilities observed in the females tested allowed concluding that the percentage of eggs hatched vary among individuals ($t = 15.34$; $dl = 32$; $P < 0.0001$), or $t = 150.9$ in %), with a high individual variability (Table 1).

Pre-imaginal development

Eggs

The egg is more or less oval in shape. It measures 0.55 ± 0.03 mm long and 0.39 ± 0.03 mm wide (N = 60). The chorion is decorated with juxtaposed hexagonal rings. The eggs are laid individually or in a group around the edges of the leaves, preferably on the lower side where they can be easily detached. They are whitish in color during lay. They turn, a little later, to pale yellow then to dark yellow during incubation if they have been fertilized. The mean duration of embryonic development is 4.16 ± 0.36 days (N = 60). Unfertilized eggs are easily recognizable as their color does not change. They shrivel a few days after being laid.

Caterpillars

The caterpillar goes through four successive larval instars before their transformation into a pupa. The passage from one stage to the next one is marked by the rejection of the exuvia with the cephalic capsule. The weight and size of the larvae increase with their development (Table 2).

First instar

After hatching, the neonate larva is pale green in color with three pairs of legs and five pairs of white prolegs. Subsequently, it turns bright orange on both sides (dorsal and ventral). Its length varies from about 1.37 mm after hatching to an average of 4.12 ± 0.36 mm at the end of the stage. The cephalic capsule is brown with 0.249 ± 0.005 mm of width. The average duration of this instar is 5.00 ± 0.84 days which constitute the longest instar compared to the other ones (Table 2).

Second instar

The caterpillar has purple and green dorsal bands interspersed with longitudinal stripes. It has an average length of 7.58 ± 0.90 mm and an average cephalic capsule width of 0.446 ± 0.018 mm. The development time is 2.89 ± 0.41 days on average (Table 2).

Third instar

The individuals of this stage are green or brown and slightly translucent. They measure on average 12.05 ± 1.81 mm long and weigh 6.575 ± 1.990 mg at the end of the stage. The cephalic capsule is 0.725 ± 0.033 mm wide on average. The development time is around 2.51 ± 0.72 days (Table 2).

Fourth instar

The caterpillars of this stage are variable in color. Their length is 18.75±0.58 mm, their weight is 37.8 ± 6.70 mg and their development lasts 4.46 ± 0.90 days (Table 2). Before pupating, the caterpillar weaves silk threads and turns pale green.

Pupa

The pupa is a green at the beginning of the stage and turns brown towards the end of the pupation with light brown transverse bands in the abdominal region. The cremaster has four curved thorns, 2 long and 2 short. Females are characterized by the presence of the genital opening in the 8th abdominal segment, whereas the genital opening of males is located in the 9th segment and presents a slight swelling. On the basis of a sample of 31 individuals, the pupae measure on average 7.21 ± 0.49 mm long, 2.502 ± 0.163 mm wide and their weight is 20.0 ± 0.005 mg. Their development lasts about 13 days (Table 2).

In this work, the development of the pre-imaginal stages of O. obstipata, fed on M. spicta leaves, lasts an average of a month; their duration varies according to the stage considered and the strong variation within the same stage (Figure 6). It is statistically different ($F_{(5, 12)} = 40.9$; P =

0.0000004). Caterpillars of the second and third instars develop more quickly than the others. The average duration of development in the other larval instars varies from 4 to 5 days, whereas pupae take longer to complete their development (Table 2). Furthermore, the growth of all the pre-imaginal stages of this moth fits a linear time model (Table 2).

Emergence of adults and sex ratio

The adult starts to emerge 27 days after the oviposition for females and 28 days for males. For both sexes, the emergence occurs linearly over time spanning 10 days for females and 5 days for males. Statistically, both males and females have the same average duration of emergence (Figure 7). It is worth noting that, under our conditions, O. obstipata completes a generation (from egg to egg) in 31.3 ± 2.16 days.

The sex ratio of the species (Male/Female) is 0.88:1 (N=221 adults); meaning that there are more females than males in the studied population.

Mortality before emergence

In a sample of 150 eggs monitored, approximately 77% of them reach the stage of reproductive adults; the remaining 23% succumb before (Figure 8). Among the dead individuals, 1.33% of eggs die before hatching and 9.33% die at the larval stage, whereas 10% and 2% die at the pupal and imago stages before spreading their wings, respectively (Figure 8).

The survivors of O. obstipata accomplish their life cycle in different stages (Figure 9). Under our conditions, 41% of the life cycle takes place in the adult stage and 24% occurs in the pupal stage. The durations of the other ecophases represent 35% (egg=8%; caterpillars=27%) of the ontogenesis of the species (Figure 9).

DISCUSSION

The development of an integrated pest management program against Orthonama obstipata involves the identification of this pest and the acquisition of accurate knowledge of its biology. Although O. obstipata has been reported in Morocco (Eddaya, 2015), its biology is little known there. To set-up an integrated management approach, it is fundamental to study the species under local conditions (e.g., Norris et al., 2003).

O. obstipata is known by sexual dimorphism, strong individual variability of adult longevity and polygyny. After their emergence, males and females have the same average longevity. Adults are active at night, like other Geometrids (Scoble, 1992). The male genitalia is characterized by very hairy valvae differently from that illustrated and presented by Schulze and Fiedler (2004). This would be due to the removal of hair during the preparation of these organs. The costa has two connection points, one with the valva and the other at the border of the seed coat and the vinculum. According to Schulze and Fiedler (2004), the costa presents only one point of connection at the level of the vinculum. This difference could be due to the mounting of the genitalia between blade and coverslip. In our case, the costa was placed in a watch glass with a few drops of water. The genitalia of adults studied in this work resemble those of Nycterosea obstipata (Geometridae) synonymous with O. obstipata (Lewis, 2013).

The infestation of M. spicata plots occurs by females of O. obstipata laying their eggs on the leaves of the plant from which they are easily detached, as in the case of Drymoea veliterna (Hernández et al., 2017). The average number of eggs laid by O. obstipata in the laboratory is comparable to the one of D. veliterna. Similarly with Bupalus piniarius (Brandt, 1947), the fecundity of O. obstipata is positively correlated with the longevity of females. Like other geometrid species (Coaker, 1959; Reed, 1965; Brader-Breukel, 1970; Heard et al., 2010; Hernández et al., 2017), the fertility of O.

obstipata is characterized by a high individual variability. This issue could be explained intrinsically by the number of mating, the amount of sperm received by females to fertilize the oocyte stock and the nutrients provided by males during mating (Mollema, 2004). Indeed, in Tuta absoluta (Meyrick), the fertility is 79.6% of the eggs laid by females mated only once and 98.1% in the case of those mated several times (Kaouthar et al., 2010). The variability of fertility can also be influenced by the photoperiod (Way et al., 1951) and the thermal conditions in which the female stayed before mating (Bonnemaison, 1961). Furthermore, according to Sharma and Chaudhary (1988), increasing temperature contributes to higher rate of egg hatching. The duration of incubation is around 4 days in O. obstipata, as for Abraxas pantaria (Geometridae), whose embryonic development lasts 4-5 days at 22°C, 60% relative humidity and a photoperiod of 18/6h (Pernek et al., 2013). By contrast, in some other species, like Drymoea veliterna, the eggs hatch in 10-12 days at 23.5 ± 2.0°C of temperature and 60% relative humidity (Hernández et al., 2017). In this connection, a relationship between temperature and incubation time of eggs has been established in Helicopverpa armigera (Noctuidae) (Hmimina, 1986).

O. obstipata has four larval instars during its life cycle, like Abraxas pantaria (Pernek et al., 2013) and Timandra griseata (Chevin, 1995), whereas in some Geometrid species, the larvae pass through five instars (Prasad et al., 2013; Hernández et al., 2017). The different larval instars of O. obstipata showed chromatic polymorphism as observed by Eddaya (2015). Moreover, according to Pernek et al., (2013), the chromatic variation is a common phenomenon in Geomtridae. Pupation lasts on average 13 days in O. Obstipata, whereas in Drymoea veliterna (Druce, 1885) (Geometridae), it lasts about 19 days (Hernández et al., 2017). The discrepancy in the durations of embryonic, larval and nymphal development could be due to temperature variations (Chevin, 1995) and host plant (Pinault et al., 2007; Razafimanantsoa et al., 2013). In pupae, the location of the genital opening with its swelling makes it possible to distinguish males from females. The sex ratio of O. obstipata is 0.88: 1 (Male/Female). It leans in favor of females, as for some Geometrid species, such as Sangalopsis veliterna and Drymoea veliterna with sex ratios of 2: 3 (Male/Female) and 1: 2 (Male/Female) respectively (Hernandez et al., 2014; Hernandez et al., 2017), contrary to Abraxas pantaria whose sex ratio is 1.5: 1 (Male/Female) (Pernek et al., 2013). When there is an excess of females in a population, polygyny can compensate for the male deficit.

In our study, all stages of O. obstipata are affected by mortality, but the highest mortality rates are observed in the pupa and the last larval instar. These mortality rates are relatively low compared to those recorded in Iridopsis ephyraria (Lepidoptera: Geometridae) for which the pupal mortality reaches 94% and the percentage of late instar survivors is too weak (15.3±3.4%) in comparison with that of early instars (71.4±5.0%) (Pinault et al., 2007). The variation in larval mortality rates differs according to the instars and the host plant (Pinault et al.,2007; Razafimanantsoa et al., 2013).

O. obstipata is a multivoltine species. On average its life cycle from egg to reproductive adult is completed in one month. Our results are similar to the study of Edelsten and Fletcher (1961) who found that the development from egg to imago takes place in one month under optimal conditions. In other species, like Abraxas pantaria and Drymoea veliterna, the time interval separating the oviposition from the emergence of the imago is long (Pernek et al., 2013; Hernández et al., 2017). This may be due to the genotype of species and the variability of climatic and food factors. Moreover, the hatching of eggs and the development of different ecophases depend on the number of accumulated degree-days of temperature (Prasad et al., 2013).

CONCLUSION

Considering its polyvoltinism and its high biotic potential, O. obstipata is a very serious pest of mint in Morocco. This work constitutes an essential step to elaborate an integrated management program for O. obstipata associated with the culture of M. spicata in Moroccan conditions. Field studies on population dynamics with key mortality factors are underway. In addition, the use of appropriate biopesticides and biological control by conservation are considered in order to avoid the inherent disadvantages of synthetic pesticides.

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