STUDY OF THE OLIVE FRUITS INFESTATION BY *BACTROCERA OLEAE* IN THE AREA OF FEZ IN MOROCCO AND THEIR FERTILITY IN THE LABORATORY

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Abstract. In Morocco, the olive cultivation recognizes a serious problem due to its infestation by the olive fly *Bactrocera oleae*. The aim of this study was to evaluate the rate of this infestation in the area of Fez during the season 2002. We have found that this rate is of 86 % in some sites in this area and that the number of larvae per fruit can reach 8 in the different studied sites. However, this number is of 65 punctures per olive fruit in laboratory conditions. Moreover, the study of *B. oleae* fertility in cages shows that the stage of olive maturity has an influence on the fertility of *B. oleae* females: the olive fruits at an intermediate maturity stage shows the best sensitivity for *B. oleae* in comparison with those at an early or a late maturity stage. On the other hand, the study of *B. oleae* embryogenesis in laboratory shows that it is accomplished in 55 hours at 25°C with some differences with that of *D. melanogaster*.

Key words: *Bactrocera oleae,* olives, infestation, area of Fez, fertility.

Introduction

The olive tree has an important social and economical significance in the Mediterranean basin, which occupies 98% of the world's cultivated olive trees. The tree's number is about 800 millions and occupy a surface area of approximately 10 million hectares. They produce about 1.6 million metric tonnes per year of olive oil, in addition to 750,000 metric tonnes of table olives [1]. Nevertheless, the olive production and the oil quality are strongly influenced by different factors, mainly the infestation by the olive fly *Bactrocera oleae* [4].

B. oleae is one of the major pests of the olive trees all around the Mediterranean basin. Damage caused by the larvae, which feed on the olive fruits, is severe and economically important [3]. The damage on the olives production is of two kinds: (i) quantitative: the infested olives become blacks and fall prematurely, and (ii) qualitative: the presence of more than 10 % of infested olives leads to a degraded oil with a high degree of acidity, a low organoleptic quality and an unpleasant flavour. Moreover, with regard to the commercial standard, which tolerates a maximum of 2 % of infested olives, the commercial value of these olives decreases.

The most commonly used way to control *B. oleae* is by spreading organophosphate compounds mixed with attractants products to kill adults. Several alternative methods including the male sterilization technique [9, 19] and the use of pheromone baits [5, 8] or natural endoparasites [3] are also used to control this pest.

B. oleae could have three to five generations per year depending upon local

conditions. When the olive has overtaken the phenological phase of hard stone, the female, inserting its ovipositor in the olive's skin, lays eggs in this fruit. The adult female can lay 50 to 400 eggs, usually one egg by one olive fruit [13]. The fly lays eggs singly in the mesocarp of the olive fruits. After that, the eggs become a larvae which feeding in the mesocarp, undertake three different growing stages leading to a pupa. The larvae pupate in the fruit or in the soil. The olive fly can then pass the winter as a pupa in the soil or as an adult in or outside the olive grove [2, 4, 14, 16]. The adult's population decline to low levels in February or March, however new adults from over wintered pupa begin to emerge in March and April [13].

In this paper, we report the results of a study on the *B. oleae* female fertility and degree of infestation of the olive's cultivation in Morocco. The aim of this study was to estimate the premature crop loss through dropping caused by *B. oleae* infestation. In Morocco, the olive's cultivation is widespread and takes place in very different ecological surfaces like the Rif Mountains in the north and the Drâa and Souss valleys in the south. Although the noted dispersion of the culture, the olives plantations are especially concentrated in two principal areas: (i) The zone of Fez, Meknes, Taza and (ii) The zone of the interior plains, mainly the areas of Beni-Mellal, El Kalâa, El Haouz and Marrakech. Our study was accomplished within the zone Fez, Meknes and Taza, which is the first area in Morocco regarding the olives cultivation.

Materials and Methods

Sites of this study and laboratory rearing conditions of *B. oleae*

Seven sites were chosen in 300,000 hectares in the area of Fez (figure 1). The rate of infestation by *B. oleae* was estimated by the determination of the rapport between infested and non-infested olive fruits on different olive trees. The olives infested by *B. oleae* were collected in three different times: September 20th, October 07th and 30th. September 20th was chosen because it is within the month during which we started to easily observe the olive's fruits infestation by *B. oleae*. The two other dates (October 07th and 30th) were selected to allow us to follow the evolution of the infestation by the olive fly. The fruits were dissected and the pupa incubated at 25°C and 70 % of humidity until the adult's emergence. These flies were then put in cages at the same conditions of temperature and humidity and with a periodic cycle of light: dark incubation (16 h: 8 h). The diet trough, running parallel to water trough, is supplied with solid diet composed of yeast hydrolyzate, sucrose and fresh egg yolk at a ratio of 1:4:0.7. The *B. oleae* larvae were cultivated in an artificial food medium according to [7, 17, 18].

Fertility of *B. oleae* at the laboratory

120 flies (50 % females) were put in wooden cages (35 cm L x 20 cm H x 25 cm W) with 50 olives per cage. These olives were chosen to have the same stage of maturity (green olives, green-black olives or black olives) and without any *B. oleae* infestation. After 24 hours of incubation at 25°C and 70 % of humidity, the olives were exchanged with a new lot of 50 healthy ones and the number of punctures per olive was determined. The number of laying embryo per a female during its life is calculated by the mean of the embryo laying by all the females in the cage.

Statistical analysis

The significance of the differences between the different results obtained is estimated by the statistical test of the percentage comparison [15].

Descriptive study of *B. oleae* embryogenesis

B. oleae embryo were collected using beeswax/paraffin (1/9) domes,

moulded using a 25 ml corex tube previously soaked in detergent solution and dipped in melted beeswax/paraffin. Domes were filled with hydrophilic cotton, fixed on heated glass plates (six domes per 8 x 12 cm plate) and wetted with water to prevent embryo dehydratation. To analysis the embryo development, an alive embryo was deposited on a microscope lame and covered by Voltalef oil to avoid its drying. The development embryogenesis was then followed under microscope (Zeiss axioplan).

Result

Study of the olive fruit's infestation by *B. oleae* in the area of Fez

Our study consisted in the following throughout the period of olive's manufacture, the appearance of *B. oleae* and the evolution of the olive infestation by this fly during the season of 2002. For that, seven sites were randomly selected within the area of Fez (figure 1) and three days (September 20th, October 07th and 30th) were selected to estimate the rate of infestation and to harvest the olive fruits. The results obtained (figure 2) show that the degree of infestation varies from 11 to 86 % according to the studied site. We can classify the infested sites *into* two groups: (i) sites with high rate of infestation (Sefrou Rfayef, Ain Tafrouat, Ain Taoujdate, Ain El Hayej and Ain Aicha) and (ii) sites with weak rate of infestation (Sefrou Hmamouch and Karya Ba-Mohamed).

At the beginning of this study (on September 20th), more than 45 % of the fruits are infested in Sefrou Rfayef, Ain El Hayej and Ain Aicha while only 7 to 40 % are infested in Sefrou Hmamouch, Karya Ba-Mohamed, Ain Tafraout and Ain Taoujdate.

To follow the infestation evolution in these sites, 17 days afterwards (October 07th), the infestation rate was determined (figure 2). A significant increase of the infestation was observed in Sefrou Rfayef, Ain Aicha, Ain El Hayej, Ain Tafraout and Ain Taoujdate: the percentage of the infested fruits almost doubled in the first one, whereas this percentage increased only by 1/3 in the other sites. On the other hand, in Sefrou Hmamouch and Karya Ba-Mohamed, there is an increase of the rate of infestation but it's not significant.

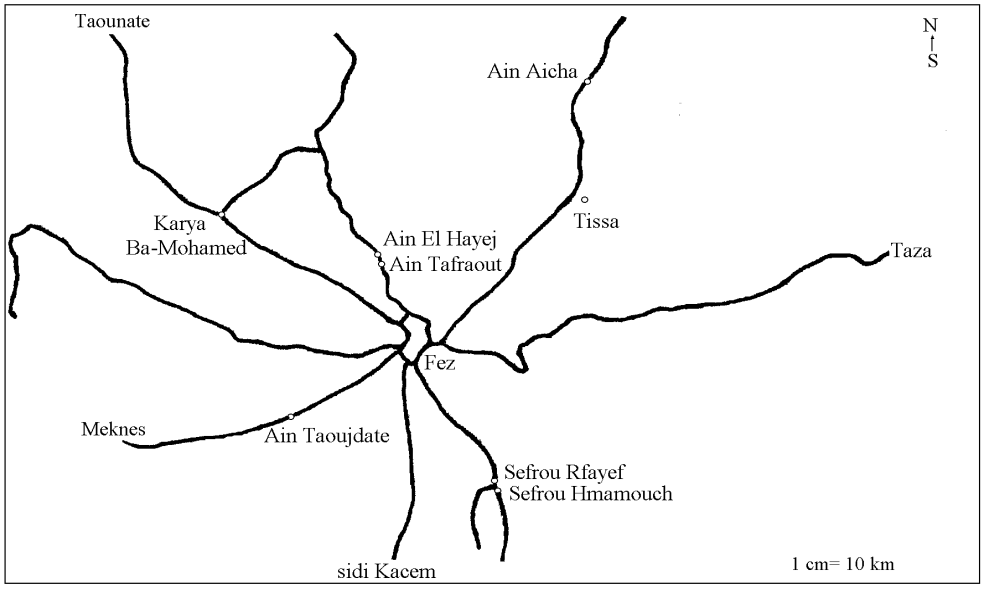

Figure 1. Distribution of the studied sites in the area of Fez. The seven selected sites are: Karya Ba-mohamed, Ain El Hayej, Ain Tafraout, Ain Aicha, Sefrou Rfayef, Sefrou Hmamouch and Ain Taoujdate.

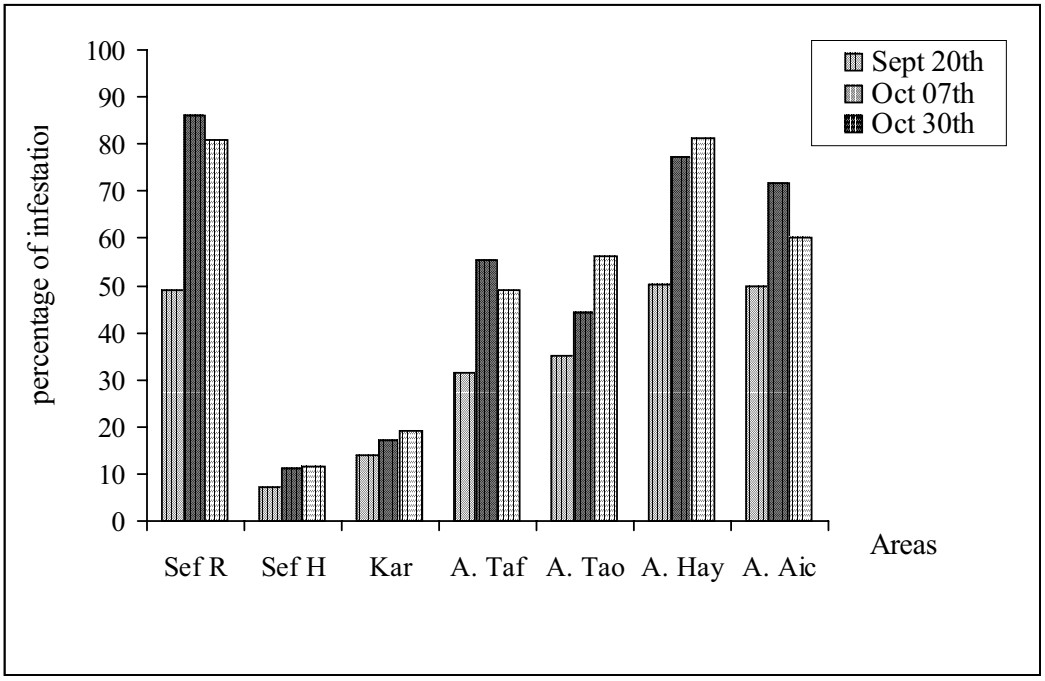

Figure 2. Percentage of the olive fruits infestation in the seven studied sites: Sef R: Sefrou Rfayef; Sef H: Sefrou Hmamouch; Kar: Karya Ba-Mohamed; A. Taf: Ain Tafraout; A. Tao: Ain Taoujdate; A. Hay: Ain El Hayej; A. Aic: Ain Aicha. This percentage was calculated on the field during three times: September 20th, October 07th and 30th 2002.

23 days later (October 30th), a third sampling time was carried out. The results (figure 2) show that except the sites of Ain Taoujdate and Ain El Hayej, where the rate of infestation varies significantly, no significant variation is observed in the other sites (figure 2).

Number of punctures per olive in the area of Fez

A batch of 100 infested olives were randomly harvested in each studied site and sampling time of this study. We have followed the number of punctures per olive during the three chosen times. Figure 3 summarizes the results corresponding to the mean of punctures observed for 100 pricked olives.

At the beginning of the study (September 20th), the number of punctures varies from 1 to 2 per olive; two sites, Sefrou Rfayef and Ain Tafraout, show the low rate of punctures per olive, 1 and 1.2, respectively, whereas the sites of Ain Aicha and Ain Taoujdate show the greatest rate of punctures per olive (2). The three other sites, Ain El Hayej, Karya Ba-Mohamed, and Sefrou Hmamouch show an intermediate rate of punctures per olive (1.5).

At the October 7th, a significant increase in the puncture's number per olive was observed in all the sites. This increase passes from the simple to the triple in Sefrou Rfayef and Ain Tafraout and from the simple to the double in Sefrou Hmamouch, Karya Ba-mohamed and Ain Aicha. Whereas, for the sites of Ain Taoujdate this increase is not significant. At the third sampling time (October 30th), the variation of the number of punctures per olive is not significant.

Figure 4 gives the percentage of olives pricked according to the number of punctures during the three sampling times of this study. We observes that the puncture's number of per fruit varies from 1 to 8 in the different sites and that the once pricked olives are the most abundant in these sites (25 to 35 %). The percentage

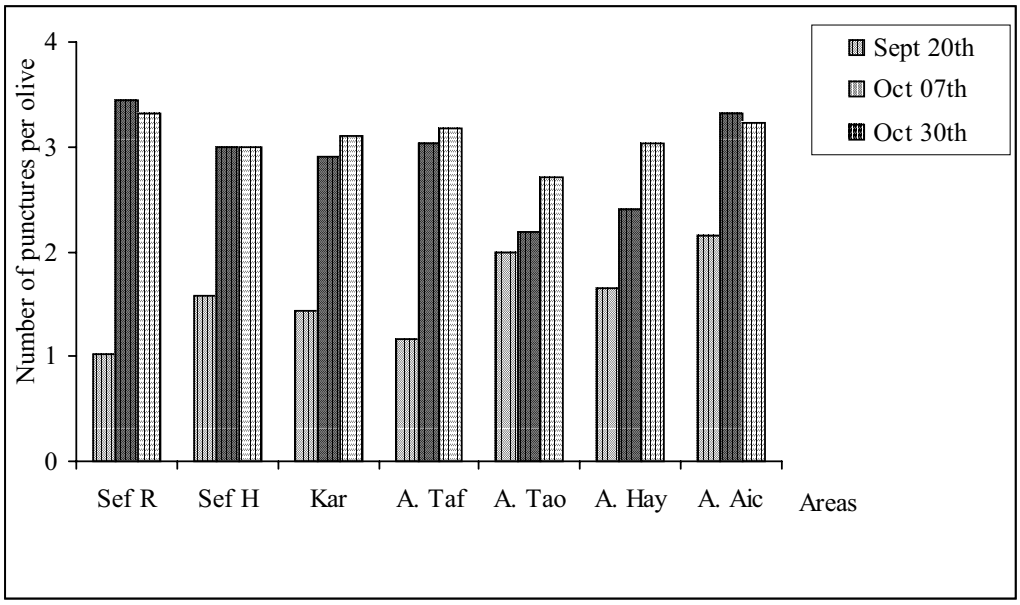

Figure 3. Means of the number of punctures per olive among 100 sampled olive fruits in the seven studied sites. These olives were harvested at three different times (September 20th, October 07th and 30th) in the seven studied sites.

of pricked olives decreases as the number of punctures per olive increases; for example the 8 times pricked olives accounts only for 1 to 3 %. We can note also that the distribution of the pricked olive's number according to the puncture's number is identical in all the studied sites.

Fertility of *B. oleae* at the laboratory

We have also studied the fertility of *B. oleae* in the laboratory. Figure 5 represents the number of punctures/day/olive, according to the female's ages. In the first week after adult eclosion, no puncture was observed in the olive fruits. After that, the flies started to prick the olives; the puncture's number of per olive varies with the fly age and the olive maturity. The greatest number of punctures observed on the same olive corresponds to 65 punctures per fruit. 20 days after the blossoming, the females reach the maximum of their fertility; a female can lay until 12 eggs per day. We observe that the end of female fertility corresponds to 40-45 days after the blossoming.

Figure 5 shows also that the stage of olive maturity has an influence on the fertility of *B. oleae* females. For the green olives, a female lays 168.6 eggs during its life, whereas for the green-black olives, the fertility is of 274.15 eggs by female (163 % compared to green olives). For the black olives, the fertility falls to 149.62 eggs/female (88 % compared to green olives). Furthermore, the maximum of fertility per day varies according to the maturity stage of the olives: 6.8, 11.6 and 7.8 punctures/day/female for green, green-black and black olives, respectively.

The Reproductive cycle of *B. oleae*

During our study on *B. oleae*, we have followed the development cycle of this pest under the laboratory conditions (figure 7). After coupling (figure 7A), the female pricks the olive fruit with an ovipositor located at the end of its abdomen (figure 7B) laying one embryo under the olive skin. Two days after, the embryo becomes a larva, which evolves in 3 stages by nourishing from the olive pulp (figure 7C). At the end of the larvae development (stage 3), the larvae digs a hole on the olive surface and starts its transformation to a pupa at the interior of the fruit. At the end of this metamorphosis,

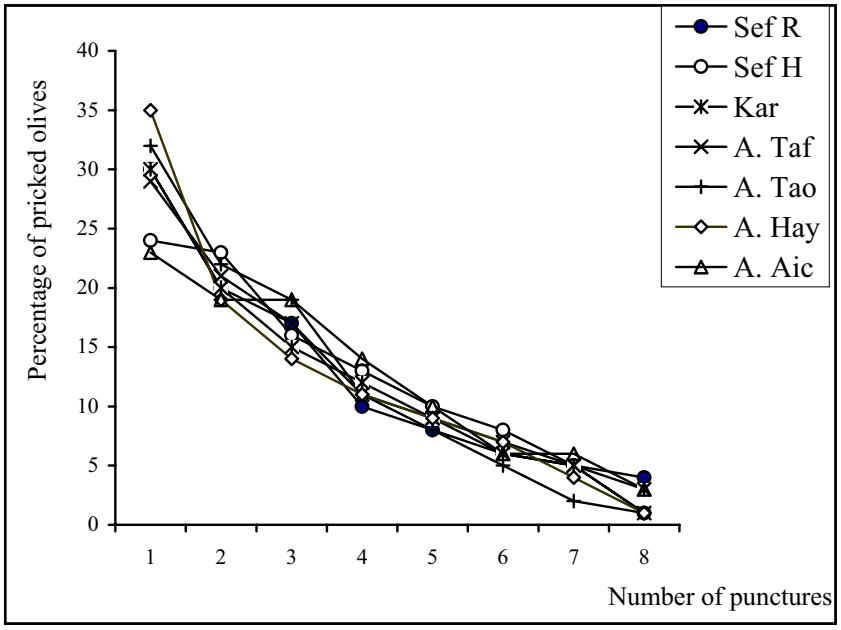

Figure 4. The percentage of the pricked olives according to the number of punctures per olive (Means of the percentages obtained in the three times of this study in the seven studied sites.

the adult emerges from the pupa (figures 7E and F) and leaves the fruit by the hole prepared by the larvae (figure 7D).

Descriptive study of *B. oleae* embryogenesis

The different stages of *B. oleae* embryogenesis were determined. After the first week in the cages (coupling period), the females present full ovaries and start to lay eggs in the olive fruits. Figure 6, shows the ovary dissection of a 10 days old female; each ovary is composed of overdoes. A *B. oleae* alive embryo, collected using beeswax/paraffin (1/9) domes, was deposited on a microscope lame and its development followed under microscope (figure 8). The antero-posterior polarity can be identified by the micropyle system position. *B. oleae* embryogenesis, which extends since the laying of the embryo until to the blossoming of the larvae, is accomplished in 55 hours at 25°C (figure 8). During the early stage (1-4 h), the cytoplasm is distributed in a uniform way as a whole of the embryo. Around the fifth hour of development, the vitellin membrane separates from the cytoplasm of the zygote (figure 8; arrow on 5-6 h). From 7 hours of development, we observe the appearance of a clear contour along the embryo periphery, which widens until the 12th hour (figure 8, 7-9 h and data not shown). The beginning of the elongation of the germinal band starts around 18 hours of the development (figure 8, arrow in 18 hours). The first segmentary marks appear in the 21st hour and become increasingly clear until the 26th hour (figure 8, 26 h). The blossoming of the *B. oleae* larvae takes place at the 55th hour of development (figure 8, 55 h).

Discussion

Study of the olive fruit infestation by *B. oleae* in the area of Fez

The results of this study show that the infestation of the olive fruits by *B. oleae* was important (until 86 %) during the olive-growing season 2002 in the area of Fez. Furthermore, this infestation rate varied between the seven studied sites within this area. This rate was important in the majority of the studied sites with the Hmamouch and Karya. In these sites, we

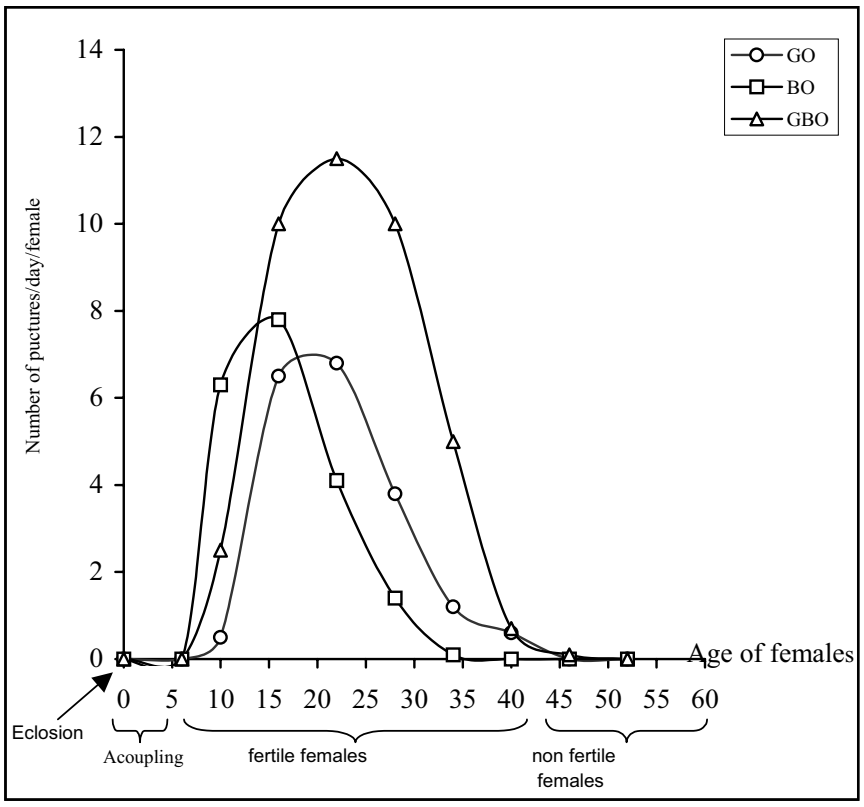

Figure 5. Number of punctures/day/female according to the age of the female for the various olive types. GO: green olives. BO: black olives. GBO: green-black olives.

can note that the rate of infestation was weak and didn't increase significantly in the time. The treatment with insecticides could be responsible of this situation. This is confirmed by the fact that the site of Sefrou Rfayef witch is located near the Sefrou Hmamouch site had a great rate of infestation.

In the second sampling time (October 7th) of this study, we note a significant increase of the infestation rate in the sites with high level of infestation in contrast with those less infested (Sefrou Hmamouch and Karya Ba Mohammed). These results confirm those of the first point witch suggested that the two later sites were treated by insecticides.

In the third point (October 30th), a stability of the infestation rate is noted in the majority of the sites with the exception of Ain Taoujtate and Ain Aicha. These results can be explained by several things: (i) the fall of the infested fruits after being damaged by the larvae (ii) the important rainfall which fell between October 7th and 30th involving a reduction of the fly population; (iii) When the infestation percentage is very high (e.g. 80 % for Sefrou Rfayef), the flies tend to prick the olive fruits which are already infested and thus the same fruit is pricked several times.

Number of punctures per olive

The adult female can lay 50 to 400 eggs in its life, usually one egg by one olive fruit [13]. In this study we have found that the fruits could be pricked more than one once (until 8 punctures per olive) and that the number of puncture was variable between the various studied sites. It is interesting to note that in the first point of this study, the most infested site, Sefrou Rfayef, shows the lowest rate of punctures per olive, whereas the two least infested sites (Sefrou Hmamouch and Karya Ba-Mohamed) show the intermediate rate of punctures per olive (figure 3). The high rate of punctures per olive this season can

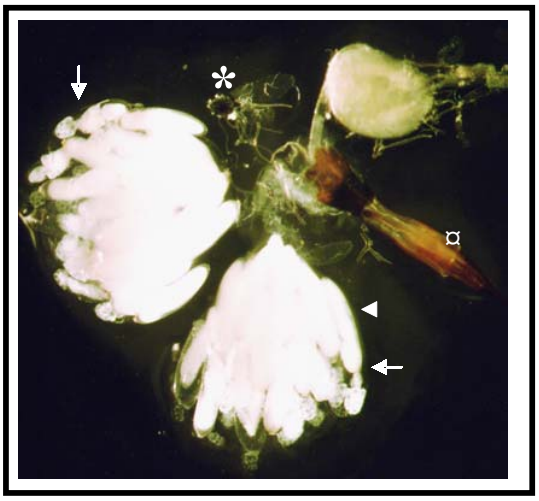

Figure 6. The female ovary of a 10 day old *B. oleae*. Each ovary is made of 20 ovarioles composed of ovary rooms at various stages of maturation (Arrows), and of mature oocytes (head of arrow). The female uses the sperm preserved in the spermthacae (star) to fertilize the ovocytes. The embryo is laid within olives, with the assistance of the ovipositor (¤).

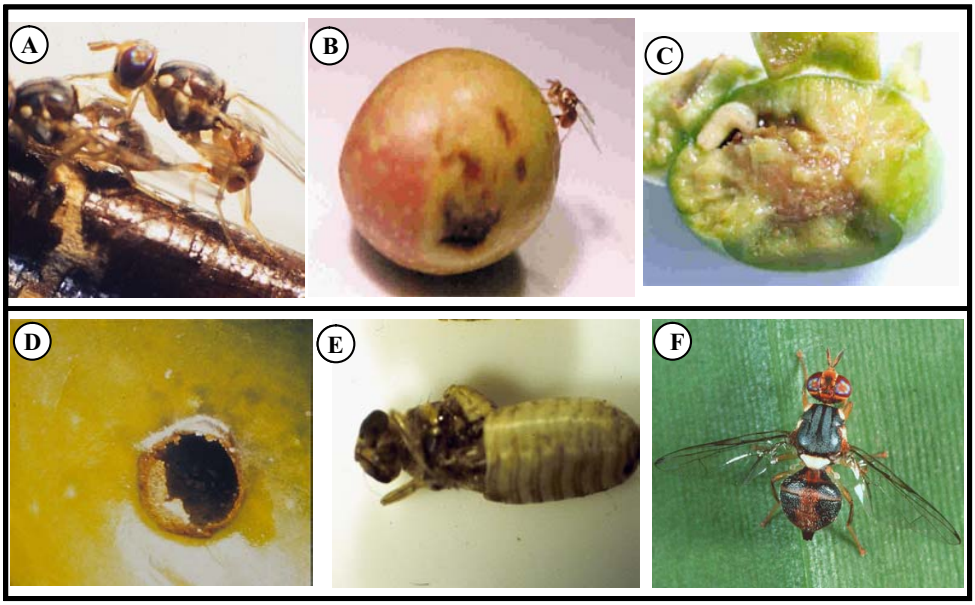

Figure 7. Reproductive cycle of the olive fly, *B. oleae* (A-f). (A) Adult coupling; (B) an olive fruit pricked by a fertilized female; (C) a dissected olive showing a larvae nourishing the olive pulp, (D) a hole formed by the larvae on the olive surface, (E) an adult emerged from the pupa, (F) a female of the olive fly.

be explained by the low rate of the olive production and the strong infestation during the year 2002 [10].

Fertility of *B. oleae* at the laboratory

This study showed that we could have until 8 punctures per olive in nature. However, this number can reach 65 punctures per olive in laboratory conditions; the small space of the cage and the high number of the fly might be responsible of this result.

Furthermore, the stage of the olive fruits maturity has an influence on the fertility of *B. oleae* females. The maximum of fertility per day varies according to the maturity stage of the olives: 7.8, 11.6 and 6.5 punctures/day/female for green, green-black and black olives, respectively. These results show that the olive fruits with intermediate maturity (green-black olives) are more receiving for the females than the other types of olives. These results can be explained by the fact that the green- black olives have a skin less resistant to the fly than the green olives. On the other hand, the black olives are less receptive than the

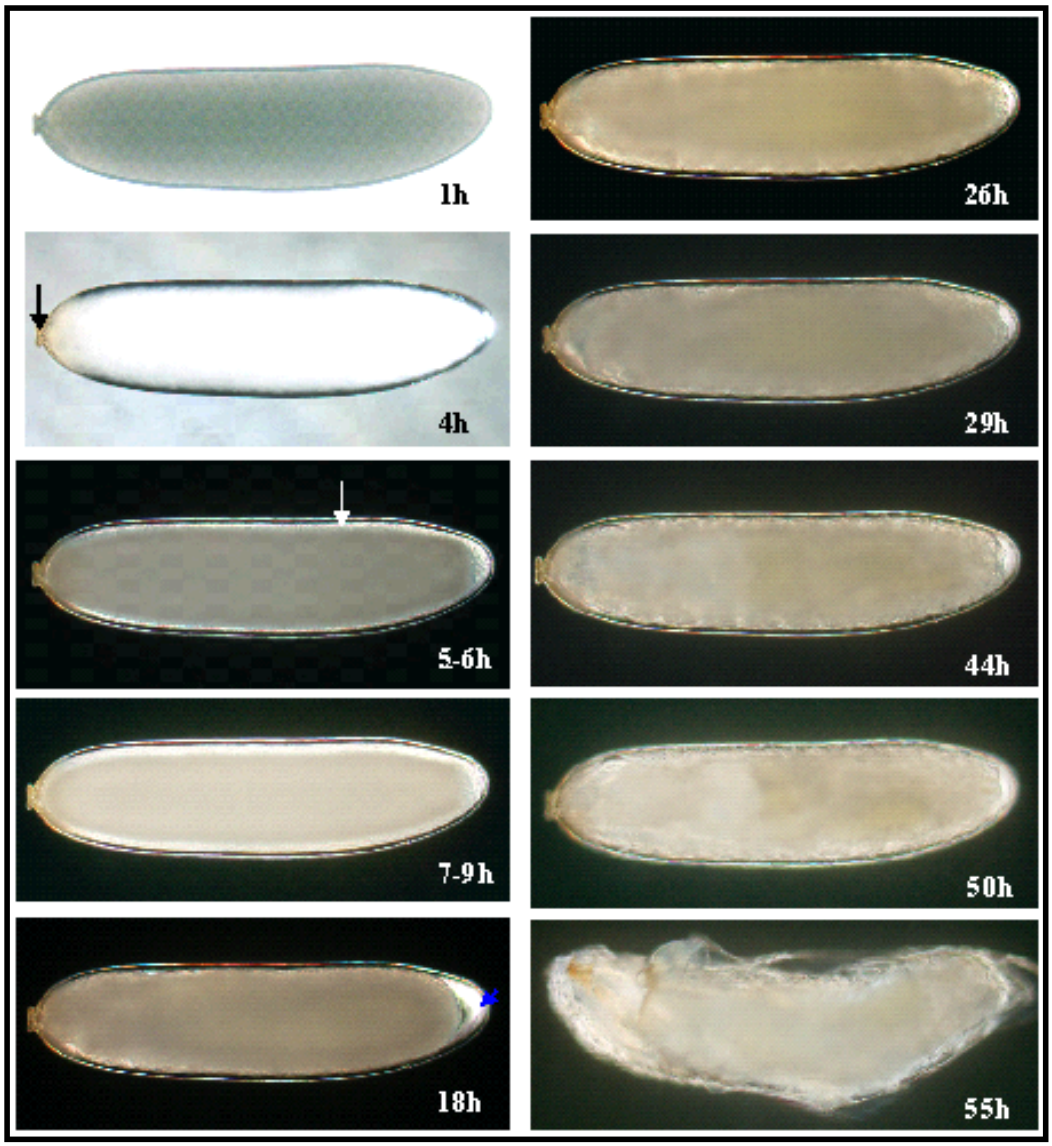

Figure 8. Development of *B. oleae* embryo: All the photographs were taken on the same embryo. The cellularisation starts around the 7th hour of development. The embryonic development requires 55 hours at 25°C. Arrow in 4 h: micropyle system. Arrow in 5-6 h: Vitellin membrane separates from the cytoplasm of the zygote. Arrow in 18 h: The beginning of the elongation of the germinal band. 55 h: blossoming of the larvae.

black-green olives because they are rich in polyphenol. These results are in agreement with those of Orphanidis and Soultanopoulos [11], which demonstrates that *B. oleae* is a monophage species, which can produce in means 2 to 5 generations per year according to the precocity or the lateness of the maturation of the fruits.

Descriptive study of *B. oleae* embryogenesis

B oleae embryogenesis, which extends since the laying of the embryo until to the blossoming of the larvae, is accomplished in 55 hours at 25°C. This study shows that the embryogenesis development present some differences

between *B. oleae* and *D. melanogaster*. The development duration is 24 h and 55 h at 25°C in *D. melanogaster* and *B. oleae*, respectively. Moreover, one hour of embryonic development in the *D. melanogaster* corresponds to the stage 1 and the major part of stage 2; this stage is recognized by the zygotic separation of the cytoplasm of the membrane vitellin [6, 12]. This characteristic is observed in *B. oleae* after the 5th hour of the development. From 7 hours of development, we observe the appearance of a clear contour along the periphery of the embryo, which widens until the 12th hour. In the *Drosophila*, the appearance of this cytoplasm band marks the beginning of the stage syncytial blastoderm (stage 4).

In summary, our results demonstrates the damage extent of *B. oleae* on the olive fruits in the area of Fez and emphasizes the need to adopt a suitable strategy to fight against this pest.

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