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Study of the flight dynamics of *Prays oleae* (Lepidoptera: Yponomeutidae) using sexual trapping in olive orchards of Essaouira region, Morocco

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Abstract
The population dynamics of the olive moth, Prays oleae (Bern) (Lep., Yponomeutidae) was monitored, using pheromone traps containing (Z)-7-tetradecenal, from 2009 to 2012 in olive groves of south-western Morocco. The carpophagous generation lasted significantly longer than the anthophagous generation.
The relationship between climatic conditions and pheromone trap captures of male P. oleae in olive groves, in Essaouira, showed a significant relationship between carpophagous and anthophagous flights. Although there was generally a linear relationship between cumulative males catch and olive tree phenology.
The interval between oviposition and emergence of adults could be interesting for the better understanding of moth monitoring.

Keywords: *Prays oleae*, population dynamics, olive tree phenology, Essaouira

1. Introduction

The olive moth, *Prays oleae*, belongs to the major olive pests in all olive-growing countries of the Mediterranean, the Black Sea, the Middle East and the Canary Islands [1, 2]. It is the most abundant pest in its area of distribution [3, 4]. The cycle of *Prays oleae* development stretches over three generations per year, each developed on a separate body of the olive tree. The first generation females lay the eggs on the flower buds, when hatched the larvae feed on the buds and flowers (anthophagous), the larval stages of the second generation live and bore into the kernel of olive fruit (carpophagous). The third generation larvae make mines in the olive leaves (phyllophagous). The damage caused by this insect can reduce the olive production by 49 to 63%, which equates to 8 - 11 kg per tree of modern cultivars [5, 6].
The anthophagous and carpophagous generations are difficult to manage with insecticides, because, after hatching, the larvae live into the buds, flowers and fruits. The phyllophagous generation occurs during, or after the harvest so it does not require an insecticides treatment [3, 6-8].
The use of attractive females *Prays oleae* to capture the males of this species during the different generations per year is demonstrated [9]. Because of permanent breeding difficulties of these females, this method for monitoring pest populations is not efficient. To attract males for mating the female moth emits sex pheromones.
Using pheromone-baited traps to control populations of *Prays oleae* have been effective, and the pest management system and implementation of control measures has been proposed [10, 11]. The identification and synthesis of the main component of the pheromone secreted by the females of the olive moth, (Z)7- tetradecenal or [(Z)-7-14: Ald] [12], in the extracts of the receptive females abdomen, caused a significant response in males, and opened prospects for the development of sexual entrapment in Mediterranean olive groves.
There is a proportional relationship between the catches and the estimated population of *Prays oleae*, hence the usefulness of this technique to define a catch threshold, necessary to trigger phytosanitary measures. Afterwards, several control methods, involving sexual attractants have been proposed.

The mating disruption method proceeds by disseminating, as constantly as possible, a sufficiently large amount of pheromone, which interferes with the natural pheromone emitted by the female of the species. The objective is to confuse the male's tracks and prevent coupling [13]. The use of sex pheromones was successful in tests of sexual confusion technique. First for the suppression and then for the control of populations of several Lepidopteran species [14-17].

In the case of *Prays oleae*, with a simple population estimation technique, the possibility of rapid risk assessment of the infestation is being much easy. The eggs are very small, and are lead under conditions that make its detection difficult. Therefore, its observation requires very careful review of a large number of samples, so the use of sexual trapping technique is more interesting. Mass trapping method of males has quickly shown its limits because of its very high cost. Authors faced the same problems with *Prays citri* monitoring [18].

In orchards where the emergence of the imago and reproductive activity of phyllophagous and anthophagous generations are observed, trapping histograms are perfectly adapted to these phenomena curves. Thus, sexual pheromone trap proves as a simple and effective way to monitor the activity of *Prays oleae* adult males during the olives campaign in Italy [19]; in France [38]; in Corfu [20] and in Tunisia [21].

Pheromone baited traps is a selective, effective and simple way to monitor the *Prays oleae* adult activity. This possibility of monitoring adult populations predicts risks and triggering phytosanitary interventions. This trapping system is effective for the adult stage population's limitation by mass trapping and the decrease their fertility by mating disruption.

The present study aims at demonstrating the influence of the continental gradient of the abiotic factors on the population dynamics of the olive moth generations, to contribute to the fight against this pest of major economic importance.

2. Materials and Methods

2.1. Study Area

The choice of study sites is based on their latitudinal position from the ocean and along an increasing gradient of continental characteristics from the coast to the inner areas. We surveyed four sites (Ida ougard S1(31.437X,-9.714Y), Had dra, S2 (31.566X, -9.536Y), Korimate S3 (31.766X,-9.537Y) and Akrmoud S4 (31.448X,-9.278Y), distant from the coast 8 km, 17 km, 7 km and 49 km, respectively (linear distance) (Fig. 1). The climate of the region ranges from arid to semi-arid, characterized by erratic rainfall. The average rainfall in 38 years (1971- 2008) is of the order of 330 mm / year, a maximum of 633 mm was recorded during the campaign (1995/1996). The maximum rainfall period runs between November and February. The remaining months form a xeric period that lasts for about seven months. However, the proximity of the Atlantic Ocean relatively compensates the water deficit of the atmosphere. Oceanic influences are less influent from the coast to the interior, which leads to changes in climatic conditions. The continental features result in a temperature increase and a decrease in humidity.

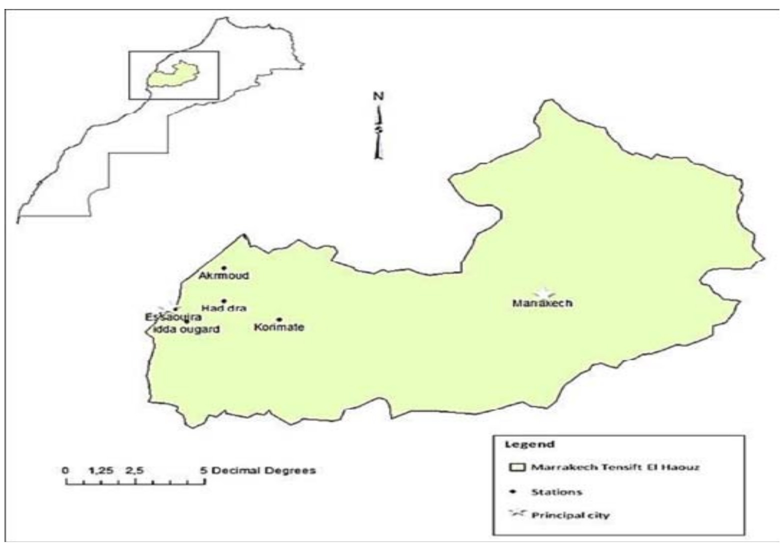

Fig 1: Location of the four Moroccan studied sites.

2.2. Sampling methods

2.2.1. Dynamic flights of *Prays oleae* adults during the olive crop

The researchers investigated the bioecology of the most important pests, performed at the Al Haouz region [22-24]. Investigations show that activity of moth adults start at the beginning of March (early spring). Therefore, the traps installation occurs on February or early March basing on these observations.

In the present study yellow Delta traps baited by 1 mg of the synthetic pheromone "Tetradecene Z7 Al 1" contained in a diffuser as polyethylene capsule was used, this type of pheromone traps were effective to obtain better results (Fig. 2).

Fig 2: Delta Trap with sticky adhesive plate, and diffusing pheromone capsule

The trap with a capsule placed at the center of the surface of the sticky plate covering its base. Three traps installed in each study area each trap hangs on a branch, at a height of 1.5 to 2 m from the ground and in the South-West exposition of the canopy. The traps are distant from each other about 70 m, to avoid mutual interference [25]. The pheromone capsules were changed after five weeks during spring and fall periods. Only four weeks during the summer when the temperatures become high and affect diffusers efficiency. The plates were glued at each levy and changed after 4 weeks, or immediately when congested or saturated due to a large number of captured males (Fig. 3).

The cumulative numbers of the moth caught in each trap after every week was counted, throughout the period for which a plate was in place. The number of adults captured per week and per generation can be a good indicator of population densities and an estimation tool for damage prevention.

Fig 3: Plate stuck with pheromone capsule with caught individual of *Prays oleae* (right).

2.2.2. Emergence and eggs laying of adults at different generations of *Prays oleae* on reproductive organs (flowers and fruits)

In general, the estimation of adults potential populations, based in sampling the latter preimaginal stages, which represent the stages of re-infestation (larvae of stages L3 and pupae). Population reduction factors affect these stages, such as parasitism, predation, mortality, whose importance was difficult to approach in the present study. Thus, the present study we can estimate the evolution of reproductive activity of *Prays oleae* on many samples of the host organs, bearing the oviposition involved in the study, flower buds and flowers for anthophagous generation, then fruits for the carpophagous generation.

Materialization of this activity based on the counted number of eggs in each sample. On each tree two branches, first flowering then fruiting, with uniform sizes, were taken from each cardinal direction of four trees per prospected station, meaning 32 branches per station. Because on the canopy, orientation and location significantly affect the distribution of eggs laying [26]. So 128 branches collected for each sample were examined under a dissecting microscope to identify and count eggs on the calyx, in the buttons, in the flowers in peduncle and in the pericarp of fruit.

3. Results

3.1. Dynamic flights of *Prays oleae* adults during the olive crop

Curves of variations in average numbers of individuals caught in the three traps in each station per week (Fig. 4).

The first adult emergence occurred before buds receptivity and before the swelling of the buttons. Throughout all the study area, the numbers of adult males increase in early spring, due to emergence of adults of phyllophagous generation. The evolution curves of the average have the same gaits at all the stations. Differences in amplitudes and time shifts of about one to two weeks were remarked between locations. These variations show three phases of activity during which the numbers of adults reach peaks. Generally, the campaign period during which catches were low or null occurs around September and October.

The numbers of captured individuals for anthophagous and carpophagous generations were more important. During the carpophagous generation, the maxima of Captures were observed. More than 600 individuals per trap were caught at stations 2 and 3 per week. Moreover, the abundance of adult *Prays oleae* was very important in orchards studied during the 2010-2011 campaign than the others campaigns

Table 1: Average maximal values of the numbers of adults caught in the three traps per week at the four stations for each generation of *Prays oleae*.

Generation	Campaign	Station S 1	Station S2	Station S 3	Station S4
Phyllophagous	2009-2010	Late March 92	Late March 41	Mid March 89	Mid March 60
	2010-2011	Mid March 28	Mid March 22	Mid March 83	Late March 18
	2011-2012	End March 258	End March 115	Early March 258	Mid March 29
Anthophagous	2009-2010	Early May 104	Mid May 93	Late May 63	Early May 74
	2010-2011	Early June 388	Late May 505	Mid May 30	Mid May 418
	2011/2012	Early May 233	Early May 210	Early May 166	Mid May 85
Carpophagous	2009-2010	Mid November 43	Late October 16	Mid November 35	Mid November 18
	2010-2011	Early November 11	Early November 15	Late October 12	Late November 15
	2011-2012	Late October 165	Early October 90	Late September 133	Late October 75

The first adult's flights in the region occurred between late March and mid-April, according to the station and depending on the campaign due to the emergence of adults from overwintering larvae of phyllophagous generation. Towards the end of May and early June, a relatively large peak of individual numbers was stored in all the stations (Table 1). It remains constant, practically, through all the olive campaigns during the studied period corresponding to emergence of adults of anthophagous generation.

The anthophagous adults lay eggs around the fruit peduncle, when hatched; the larvae derived from dig tunnels in the direction of the fruits nuclei where the larval stages of carpophagous generation development occurred.

The third flights phase takes place at the onset of autumn. For this generation numbers of individuals were relatively low according to the numbers counted during the two previous emergences (Fig. 4).

The peaks of the anthophagous generation's captures were the most important despite the short-term of its course between the April and early June depending in stations. The amplitude of the temporal distribution of the carpophagous generation was marked by both two falls of the olives and the time interval between each other. The activity of adults is relatively low, but never nil. During the summer from July to early August, the quantities of fallen olives were more important. The autumn falls in September remain low, at all the stations. In this season, carpophagous generation's adult emergence showed a slight increase in its numbers. However, their observation stretches over a relatively extended period.

The maxima of captured adult numbers were very dissimilar from one to another campaign. During the 2009-2010 olive campaign, the captures reached average values of around 100 individuals per trap per week (Fig.5). These numbers increase during the 2010-2011 campaign and reach exceptional values between 380 and 505 individuals captured per trap per week in average (Fig. 6). The maximum registered during the 2011-2012 campaign fluctuate around 300-350 individuals in trap per week (Fig.7).

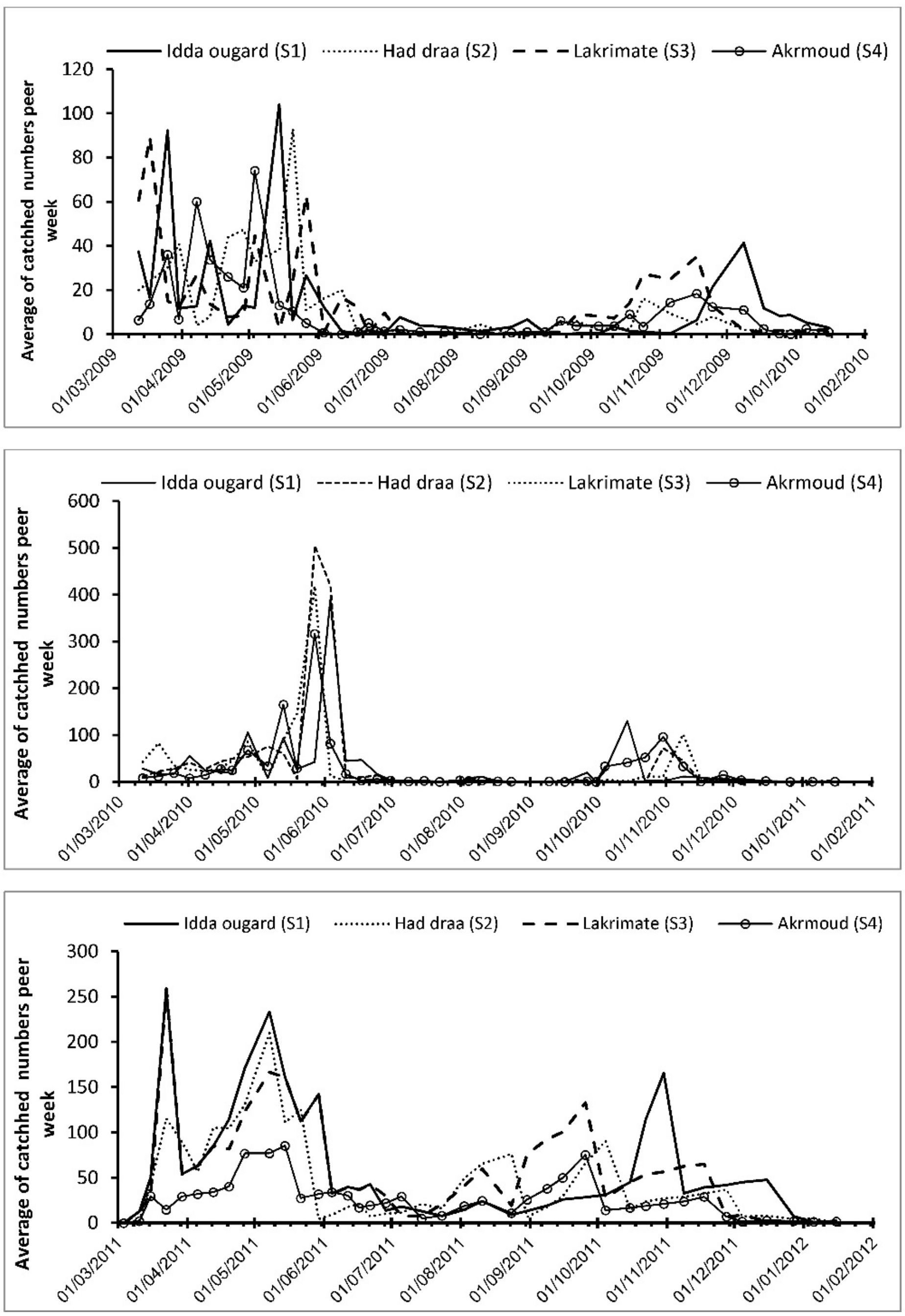

Fig 4: Evolution of the average numbers of adult males captured by the traps in each station for three successive campaigns, A: 2009-2010 campaign, B: 2010-2011 campaign and C: 2011-2012 campaign.

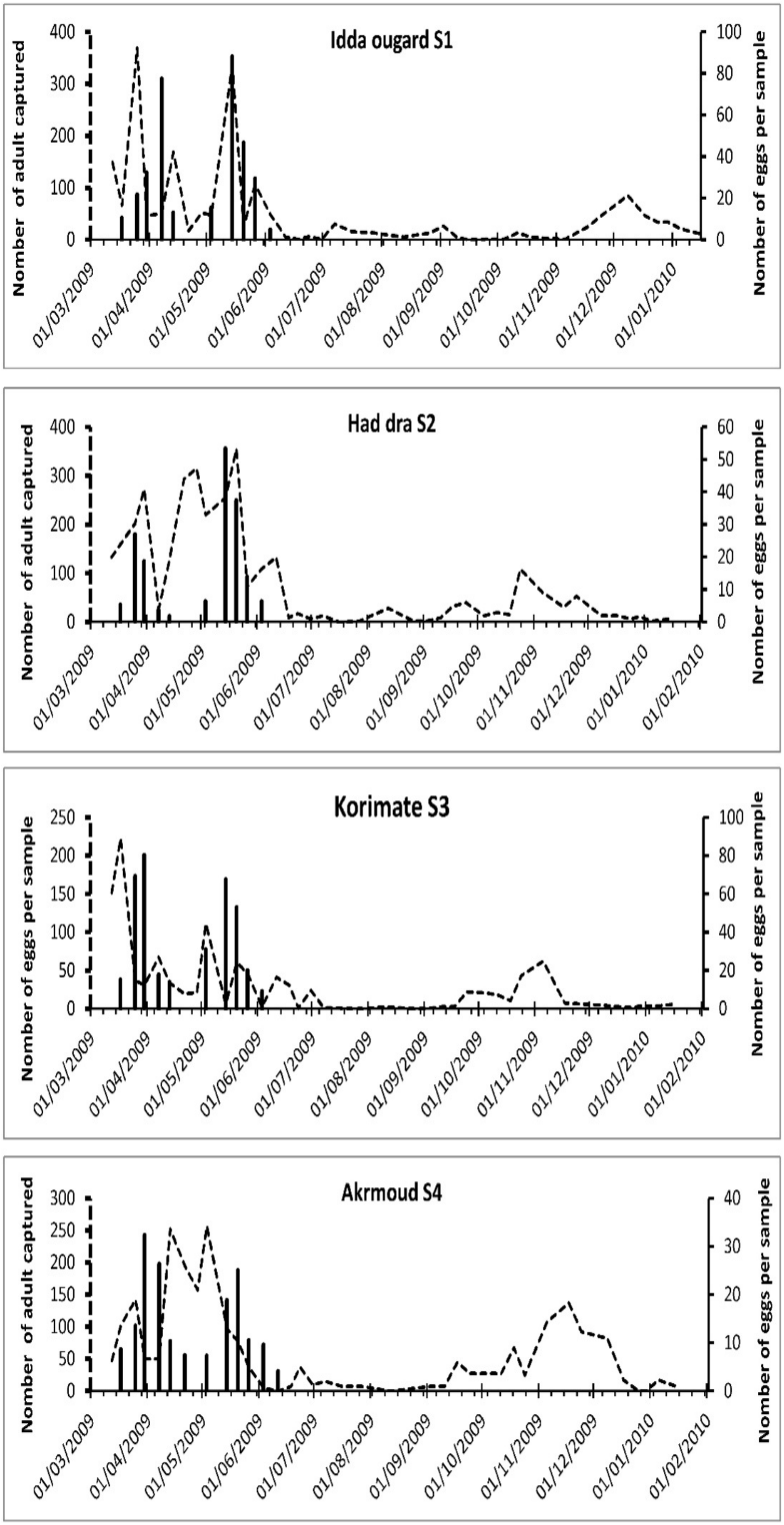

Fig 5: Evolution of the number of captured males and eggs laid by the females of *P. Pleae* stations in the study during the olive crop, 2009-2010.

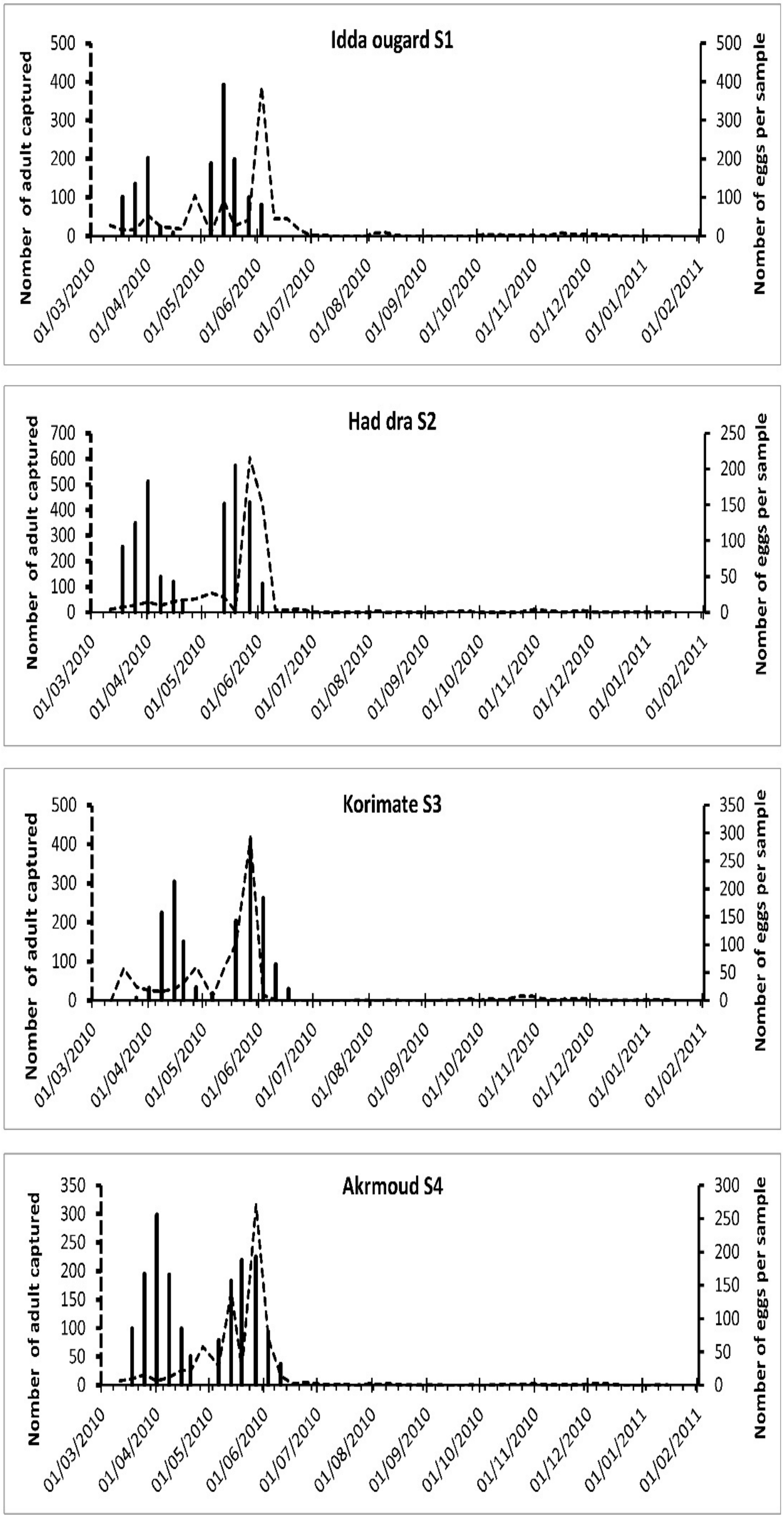

Fig 6: Evolution of the number of captured males and eggs deposited by *P. oleae* females in the study stations during the 2010-2011 olive crop

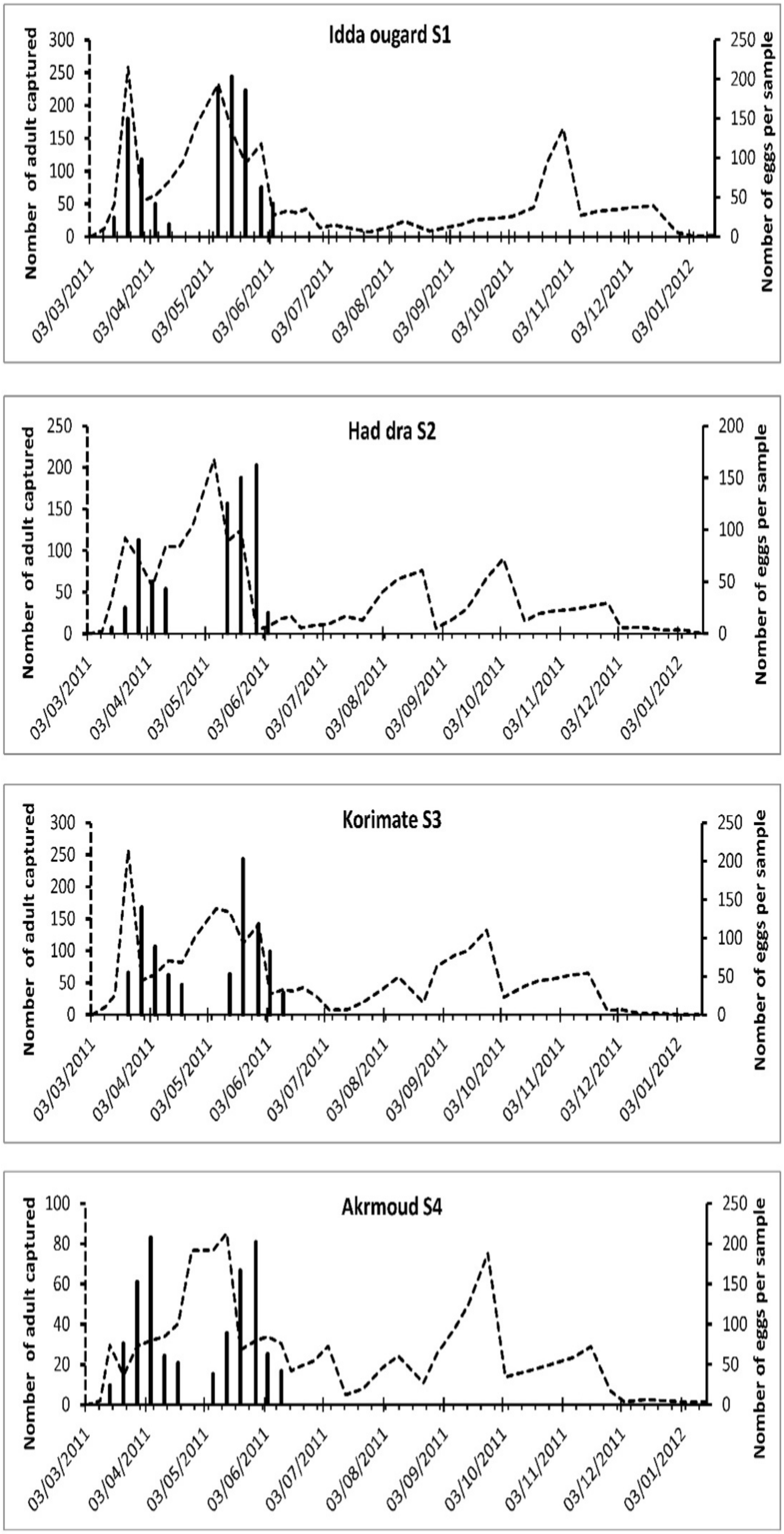

Fig 7: Evolution of the number of captured males and eggs laid by the females of *P. oleae* stations in the study from 2011 to 2012 during the olive crop

3.2. Emergence and eggs laying of adults at different generations of *Prays oleae* on reproductive organs (flowers and fruits)

To facilitate comparisons between the two phenomena, the evolution of individual's moth emerging numbers and changes in numbers of eggs laid by the females after their emergence, on the same graph was presented.

The emergence of adults of phyllophagous generation started in late-February and early-March, and their amplitude increases gradually. The oviposition (eggs laying) of females start at the second decade or the third one of March, when the olive fruits were at the phenological stage D (swelling flower). The advent of these two events occurs earlier compared to those observed for the same generation at the Haouz region of Marrakesh [24]. However, oviposition always extended after one week at the station S3.

Oviposition takes place after emergence and the first flights of the adults, which determines a period of pre-oviposition, lasts between 5-12 days, depending on the geographical situations and olive campaigns. These variations are ascribed to weather conditions and the sequence of phenological stages of the host.

Adult of anthophagous generation emerged at the beginning of May, which coincides with the stage of flowering (F) to early stage of falling petals (G). In fact, the flights of adults were completed in late April or early May. Populations of eggs laid by the females this generation have higher densities, compared to those of carpophagous and phyllophagous ones.

Adult emergence of carpophagous generation happens during the fall period of the olive campaign. The numbers of male captured were lower than the other generations despite their extension over a significant period. However, it was the shorter duration generation; adult's emergence ranges for 30 to 40 days the stations S1, S2, and S4 does not exceed 30 days. At the station S3, flights extend over 40 to 50 days.

Flights of the adult from the carpophagous generation overlaps a relatively longer period compared to the consecutive generations in the region, over two months, from early September to mid-November. At the station S1, the period extends until December.

As the traps were in place catches remains, but the numbers of individuals get very low until the emergence of adults of phyllophagous generation from overwintering larvae in early spring.

4. Discussion

4.1. Dynamics of adults flights Prays oleae during the olive crop

The phenological stage of the olive tree corresponding to the appearance of flower buds and flowers triggered oviposition of phyllophagous generation. The flight of emerging adults explains the increasing numbers of captured males. Eggs observing dates are closely related to the spatial lags in phenology hots, so it changes according to stations. These two latter elements are both controlled by climatic factors [3, 29, 32, 36, 37]. In coastal stations, the development cycle of the olive tree was anticipated, following the early onset of favorable weather conditions [3, 29, 32]. The favorable climatic conditions and the availability of the host organs, buds and flowers explain the increasing densities of different development stages during summer at late July and during August [24, 38]. The activity resumes at September, but the numbers of fruit falling by the emergence of the adults at the autumn remain low compared to olives that fall during summer at all the studied stations [24, 29].

In autumn adult emergence of the carpophagous generation manifested by a slight increase in captures numbers, however, their observation spread over a relatively extended time. The numbers caught in this phase during the 2009-2010 campaign are lowest than consecutive seasons. The low production recorded during the 2008-2009 campaign can explain this situation [41].

The males of carpophagous generation captured at all stations are still very low, compared to other generations. Previous studies carried out in different regions of Morocco shows similar results [24, 27]. The encysted larvae in many vascular bundles of summer fruits and falls of small-infested fruit cause removal of a large part of the larvae. Their emergence takes place over a short period.

The anthophagous and carpophagous generations cause major damages. By reducing fruit set, the larvae cause premature falling olives. The damage fluctuates each year in correlation with the fluctuating climatic conditions [28]. Economic losses are a combined function of populations of larvae and the olive yield in a given year [28, 29].

4.2. Emergence and oviposition of *Prays oleae* adult on reproductive organs

Around the beginning of March or shortly before, we observed the first's individuals of adult males and their numbers increased in order to achieve maximum during different periods in the same month, in different periods depending on the station. These situational variations are the result of changes in local climate conditions characteristic of each station [3, 24, 34].

The evolution of emergences in the station S3 occur with a slight lag of at least a week, compared to the coastal stations S1, S2 and S4. Climatic factors in S3, characterized by low humidity and temperatures relatively high, cause a delay vegetative cycle of the olive among 2 to 4 weeks depending on the campaign, and consequently reduced the development of the larval stages of the pest [39].

Females appeared before the buds are fully receptive to phenological stages B and C [30]. The first observed eggs marked the reproductive activity in varying dates depending on conditions in the biotope [31]. Egg laying behind the anthophagous generation occurred after time of more or less important period, called pre-oviposition phase. Then, the females deposited eggs on the calyx of flower buds and flowers. [24, 38].

During 2010 - 2011, the olive crop striking phases of the insect activities and phenological stages of the olive tree were anticipated in time. Indeed, an outstanding wave of flowering occurred at late January and early February, which lead to fruit maturation stage to the month of July. The year 2010 was more rainy and humid; so the production was exceptional in the region this situation may explain the earlier set of development stages for the insect and the host [41].

The highest densities of adult male population were captured by the traps at all the stations during the anthophagous generation. Average densities have reached their maximum during the 2010-2011 campaign. The captured were 418, 500 and 388 individuals per trap, respectively in the stations S1, S2 and S4. Duration of this generation is the shortest compared to other generations. These observations are attributed in part, to favorable spring conditions and the high density of buds and flowers in early flowering. Previous studies reported similar results [21, 38]. Densities of deposited eggs peaked in the phenological stage of corolla differentiation and the early flowering stage. Then gradually decreases then disappears in mid-April at the stations S1, S2, and S4.

The weather conditions, the characteristics of the host plant and the influence of natural antagonists, conditioned the dynamics of the moth populations [3, 29, 32]. The bio-ecology of Lepidoptera and phenology of the olive tree are highly correlated with temperature oscillations, which presents intra and inter-annual [29, 33-37]. Development, fertility, and mortality rate, in this case, are associated with a dependent non-linear function of the temperature, or triggered delayed according to specific environmental conditions.

The period of oviposition for the olive moth, *Prays oleae,* is determined by the availability of nutritional resources associated with the phenology of the olive tree. The linkage of the olive moth reproductive cycle with the host plant vegetative cycle is crucial for the pest's survival and insect efficiency [33-35, 37, 40]. The lay of eggs, at the anthophagous generation, occurs at the onset of the first receptive fruit, usually on the southern sector of the tree, where the phenological development is earlier [31]. The term of eggs laying is sensibly identical for all generations. Eggs deposition occurred in 15-20 consecutive days at the beginning of the infestation 15 to 18 days after the first captures.

Sexual trapping was a simple way to detect and monitor flights from *P. oleae*. This technique would be especially useful as it sets a capture threshold for phytosanitary interventions and determines whether there is a proportional relationship between captures and the densities of existing populations. Other elements may be involved in the decision on the date, intensity and type of intervention [25, 36].

The results of the sampling carried out during the three years, trapping with sexual pheromone used to attempt estimation of intervention thresholds. These estimations are the only tools of appreciation densities and prevention of the development of adults populations the pest.

Catches traps are important, in all stations, for the phyllophagous and anthophagous generations during the three campaigns of study. However, the numbers of adult trapped have levels significantly low during the carpophagous generation. Among the causes of this situation, the high density of females that emits significant amounts of natural pheromones, that come into interference and competition with synthetic pheromones spread from traps, which greatly reduces their effectiveness. The ability to capture a significant proportion of the male population could be because the females of *P. oleae* mate from the first nights of leaving and then lose their appeal after mating [23, 24].

The length of eggs laying is sensibly identical for all generations. The deposited eggs are grouped in 15-20 consecutive days at the beginning of the infestation. Concerning carpophagous generation after 15-18 days first captures the majority of the eggs hatched, then begins the fall of infested fruit that host young larvae and is still low.

5. Conclusion

The intensity and the course of several generations of *Prays oleae* are variable depending on the conditions of local mesoclimates and host phenology. Coastal areas with mild temperatures and important humidity show high densities of adult populations. In inland areas, warmer and less humid climate, the emergences were late and densities were lower. The gap between adult emergence and early oviposition followed this trend and varies according to the continental gradient whose determination is a way to control populations *Prays oleae.*

The importance, of the numbers of individuals caught per trap per week, shows that trapping is an effective means of regulating the population of the butterfly *Prays oleae*, despite its high cost.

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