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Assessment and forecast of damages caused by *Cydia pomonella* in apple orchards of Northern Africa (Algeria)

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Abstract

The codling moth, *Cydia pomonella* (L.), is a major pest in apple orchards worldwide. We carried out a two-year study in Northern Africa (Algeria), where the environmental conditions could affect codling moth phenology and host selection. Infestation rates in Golden Smoothie apple orchards (18% on average) were twice as high compared to Gala Brookfield and Granny Smith due to differences in the nutrient content and maturation phenology. A significantly higher proportion of fruits were attacked in the eastern and southern sides of the canopies, doubling those recorded at the centre, northern or western orientations. Infestation rates at the eastern sides of Golden Smoothie apple trees (average 32%) were especially remarkable. Adult flight phenology did not differ between years, and infestation rates were related to the number of adults caught in the middle of the first flight period (beginning of May). Hence, farmers could predict infestation rates in advance and start treatments against eggs and larvae to avoid further damages when necessary. In the two study years, adult moth numbers were already very high then, with peaks of more than 40 adults collected per trap weekly (four times the threshold recommended to start treatment). The application of insecticides in our study area does not start until the last days of April, but our results show that this is too late. Treatments targeting eggs and larvae should start no later than April 12th, when adult numbers are not so high yet and there are already eggs. Only doing this it will be possible to keep apple infestation rates below the acceptable threshold for commercialization in this region of Algeria (Sidi Bel Abbes).

Key words: apple cultivars, apple damage, codling moth, flight phenology, orchards.

Introduction

The codling moth *Cydia pomonella* (L.) (Lepidoptera Tortricidae) is a major pest in apple *Malus domestica* Borkh. (Rosales Rosaceae), pear *Pyrus communis* L. (Rosales Rosaceae) and walnut *Juglans regia* L. (Fagales Juglandaceae) orchards, being one of the most harmful pests in economic terms for fruit production worldwide (Riedl, 1983; Lacey and Unruh, 2005; Asser-Kaiser *et al*., 2007). Codling moth larvae bore through the mesocarp towards the seed chamber and severely reduce apple quality (Öztemiz *et al*., 2017). The fruit market demands high-quality fruits with no signs of damage by this pest. However, in spite of chemical control treatments, infestation rates often remain over 1%, which is an unacceptable level of damage according to the guidelines of apple production (Chen and Dorn, 2010; Pajač *et al*., 2011). To be effective, control management needs to be based on sound information on pest life cycles and infestation rates, which may change based on geography. In this article, we study both in the main apple production region of Algeria, an area for which these data remain scarce.

The codling moth is distributed worldwide after being introduced from its native European distribution range (Riedl 1983; Willet *et al.*, 2009). In Europe, the life-cycle differs with latitude, in the north of its distribution range it is univoltine, whereas in the south of the continent, it may have up to three generations per year (Ricci *et al*., 2009). In northern Africa, the number of generations may be as high as five, depending on the latitude and elevation (Muhammad *et al*., 2014; Guermah and Medjdoub-Bensaad, 2018). In turn, infestation rates may vary among host plants and among cultivars within the same plant species, as many hosts are fruit trees. In some trees (e.g., apple trees), certain cultivars have different sugar contents and maturation times that may make them more or less vulnerable to codling moth attacks (Lombarkia and Derridj, 2002; Brahim *et al*., 2013; Joshi *et al*., 2015).

The efficiency of any pest control management at a small (i.e. orchard) scale depends on its adaptation to the local phenology of codling moth populations (Judd *et al*., 2005; Mahzoum *et al*., 2017; Tomas *et al*., 2018). Additionally, whenever possible, it is important to have clues (e.g. adult counts in traps) that allow forecasting the fruit damages and schedule pest control treatments. Furthermore, it is necessary to record infestation rates in a standard way to make measurements comparable. In this regard, in Algeria, the scarcity of detailed information on codling moth phenology and/or the lack of standard sampling method for adults and infestation rates complicate pest control planning. The general objective of this study is to contribute to reduce this knowledge gap and provide useful information to guide stakeholder decision-making and management.

We carried out a study in one of the major fruit production areas of northern Africa, the region of Sidi Bel Abbes (north-western Algeria). The codling moth *C. pomonella*, aphids *Aphis pomi* De Geer (Hemiptera Aphididae) and

Dysaphis plantaginea (Passerini) (Hemiptera Aphididae), the European red mite *Panonychus ulmi* (Koch) (Acari Tetranychidae) and scab *Venturia inaequalis* (Cooke) G. Wint. (Pleosporales Venturiaceae) are the most harmful pests and plant pathogen in these apple orchards. *C. pomonella* was by far the main moth species in our study area (accounting for more than 99% of the individuals trapped). Besides, we only collected 8 larvae that were identified thanks to DNA barcoding to the genus level as *Cadra* sp. (Tayeb Mahi *et al*., unpublished results).

For two years (2018 and 2019), we monitored apple infestation rates and the number of larvae and adults following the first flight period. We did so in orchards subjected to the same management but with three different cultivars of apples, Golden Smoothie, Granny Smith and Royal Gala. We specifically assessed: i) whether infestation rates differed between apple varieties, ii) whether canopy orientation had an effect on the proportion of apples attacked by moths, iii) whether adult flight phenology varied between the two study years, and iv) whether the number of larvae and infestation rates were related to the number of adults caught at each orchard.

Materials and methods

Study sites and experimental design

All experiments and observations were conducted in the municipality of Tenira, the largest apple-producing region of Sidi Bel Abbes (Northwestern Algeria) (35°11'38"N 0°38'29"W). Fieldwork was carried out from the last week of March to the last week of June in two different years (2018 and 2019). The first flight period of adult moths takes place at this time of the year, when climatic conditions are also favourable for apple production (figure 1).

The experimental design consisted of three replicates of each of the three apple cultivars: Golden Smoothie, Granny Smith and Royal Gala (total 9 orchards, table 1). These varieties were chosen because they were among the most common in the region. To focus on the effect of apple variety and minimize undesired confounding factors, all the orchards selected had similar characteristics: density 2800 trees/ha and tree heights from 2.5 to 3.5 m; orchard age, size and altitude differed very little as well (table 1). The maximum distance between study sites was 3 kilometres. Apple production did not differ too much either: it ranged from 40000 to 50000 kg per hectare at the orchards of the three varieties. In our study area, the orchards were regularly managed at fixed dates with conventional insecticides, namely, Chlorpyriphos ethyl (PYRYCAL 480 EC) on April 28th with doses of 1 L/ha, Lambda cyhalothrine (KARATE ZEON) twice (May 20th and 30th) with doses of 0.5 L/ha and, finally, on June 15th with Fenoxycab (INSEGAR) with doses of 0.5 kg/ha at the very end of the first generation.

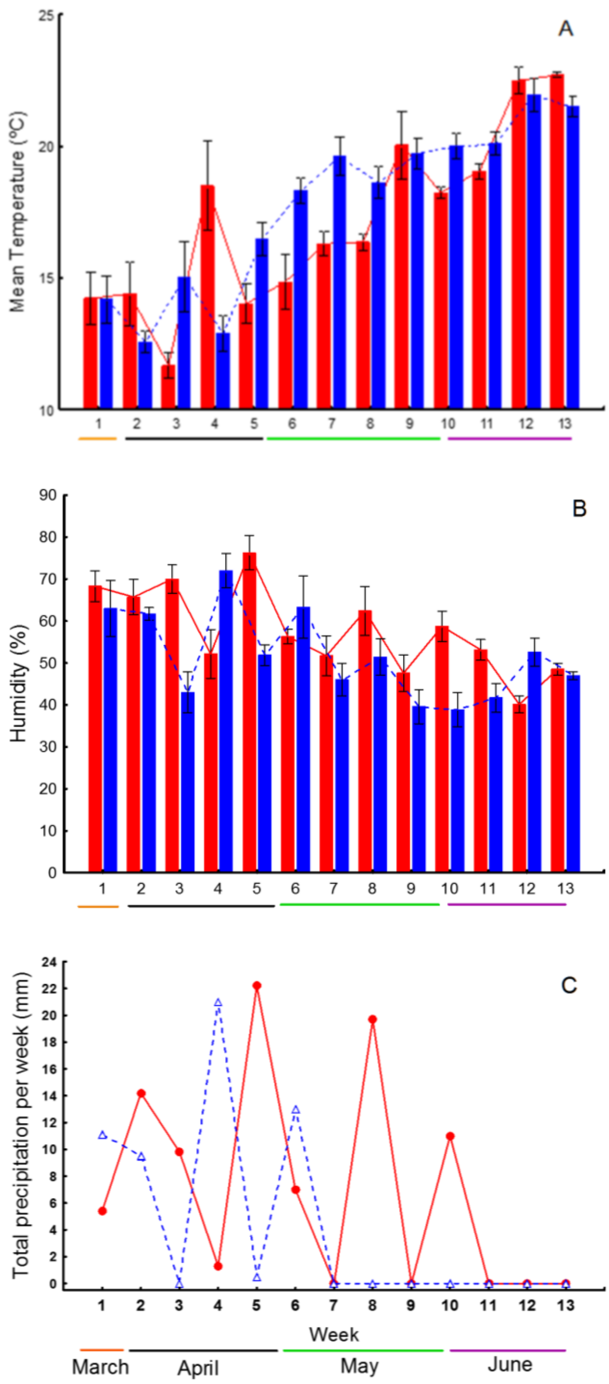

Figure 1. Mean temperatures (mean ± SE) (**1A**), air humidity (mean ± SE) (**1B**) and accumulated rainfall (mm) (**1C**). Data for 2018 (continuous red line, red bars) and 2019 (dashed blue line, blue bars) during the 13 weeks of the study period are shown. Week one starts in March 28th. Months are indicated with lines of different colours.

Fruit damage

We recorded damage on apple fruits by the first generation of codling moth larvae at the end of June, when adult first flight had finished. Following Ricci *et al.* (2009), we calculated infestation rates on 1000 fruits per orchard: at each orchard, 20 trees and 50 fruits per tree were taken randomly. To analyse the effect of canopy orientation, at each tree we collected 10 fruits per orientation (north, south, east, and west) and 10 more at the middle of the canopy. All these apple fruits were inspected in the field to see whether they had been attacked by *C. pomonella*

Table 1. Characteristics of the experimental orchards.

Orchard	Cultivar	Area (ha)	Elevation/m	Latitude/N	Longitude/W	Age (years)
1	Golden Smoothie	1.25	551	35°08'24"	0°40'42"	18
2	Golden Smoothie	2.25	551	35°08'10"	0°40'53"	18
3	Golden Smoothie	2.5	555	35°08'34"	0°42'02"	18
4	Gala Brookfield	2	560	35°08'31"	0°41'53"	14
5	Gala Brookfield	2	561	35°08'19"	0°41'54"	14
6	Gala Brookfield	2	659	35°01'15"	0°30'39"	18
7	Granny Smith	1.5	554	35°08'16"	0°41'00"	14
8	Granny Smith	1.5	699	35°00'26"	0°35'23"	14
9	Granny Smith	2	689	34°59'57"	0°35'29"	14

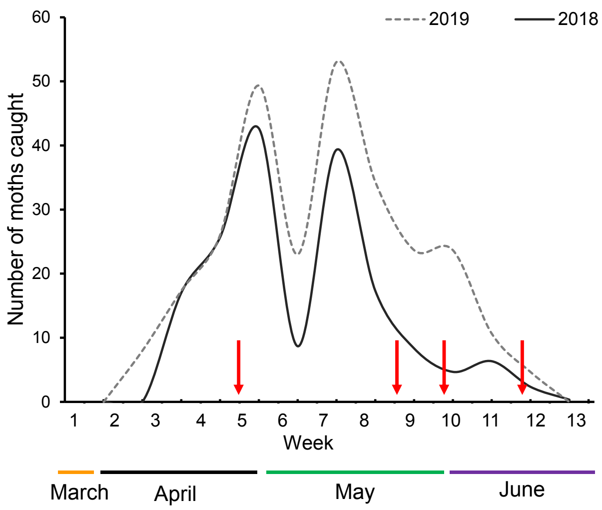

Figure 2. Flight phenology of *C. pomonella* adults (87% males) during the first flight periods of 2018 (continuous line) and 2019 (dashed line). The 13 study weeks and the corresponding months are shown. Week one starts on March 28th. The red arrows show the dates in which the insecticide treatments were applied.

or not. Infestation by *C. pomonella* can be visually checked easily, as attacked apple fruits show a dark scar (entrance hole with larval excrements inside).

Infestation rates were calculated as the number of infested fruits divided by 50 (the total number of fruits evaluated per tree). Rates were assessed in the last week of June, when adult first flight is over (figure 2) and most of the larvae of the first generation are finishing their development. By that date, almost all the infested apples are still in the canopy and we rarely saw any on the floor. In the case of the study trees, in very few could we see one or two fallen apple fruits. This did not bias or underestimate infestation rates, as all trees produced at least 150 apples. Manual fruit thinning (including the removal of infested apples) started in the first week of July, and hence did not affect our assessment of infestation rates.

Codling moth flight phenology

Codling moth males and females were monitored at each orchard with one green cardboard delta trap (two sides of 15 cm and a base of 20 cm) placed at one tree in the middle of the field. In all of them the distance from the border was at least 25 m. The trap was hung in the outside part of the canopy at a height of 1.5 m above the ground on the last week of March. The traps were baited with red rubber septa with 3 mg of codlemone (commercial sex pheromone, PH-227-1 RR, RUSSEL IPM) (to attract males) and 2 ml of a pear ester (kairomone - (E, Z)-2,4-decadienoate) (synthesized at our chemistry laboratory to attract both males and females). These were deposited on the stuck plates of the traps, as recommended by Knight and Light (2005). The traps were checked weekly and all moths were counted, sexed and then removed. Pheromone and synthesized pear ester were renewed monthly and weekly respectively, and sticky inserts were changed if necessary. To take into account the potential effect that temperature could have on adult flight, we analysed mean daily temperatures, air humidity and rainfall recorded at the meteorological station of Sidi Bel Abbes (35°10'26"N 0°35'43"W), 20 km from the study area.

Number of larvae in the first generation

We assessed the number of larvae corresponding to the first generation using cardboard strip traps attached to the tree trunk. Larvae crawling down the trunk after finishing their development stopped when they arrived at the traps, within which they pupated. At each inspection, the number of larvae in the traps could be easily recorded. The traps were set at the beginning of May, in the middle of the first flight period (figure 2), when the first signs of infestation are usually observed in the developing apples. The final inspection of the traps was carried out on June 25th, once the first flight period was over (figure 2) and the larvae of the first generation had finished their development. After this, the cardboards were removed and replaced.

Statistical analysis

We analysed the effect of the apple variety on infestation rates using a generalized linear mixed model (GLMM). Infestation rate was the dependent variable (binomial distribution, number of apples infested vs non-infested), whereas the apple variety and the year were the independent factors. In a second GLMM we assessed whether canopy orientation (independent factor) had any effect on infestation rates after controlling for the year and apple variety. We also used a GLMM to test whether the variety and the year (independent factors) had any effect on the number of larvae collected at each tree (Poisson distributed dependent variable). Finally, we performed a linear mixed model LMM to assess the relationship between the number of adults trapped at each orchard (independent variable) and the mean infestation rates (dependent variable). The same analyses were performed to examine the relationship between the number of adults per orchard and the mean number of larvae per tree.

In all GLMMs, the tree and the orchard identities were included as random factors, with the former nested in the latter. In LMMs, we only used one random factor (orchard identity), as the dependent variables were averaged per orchard. In GLMMs, we used Logit-link functions when the dependent variable was binomial and logarithmic for Poisson. The significance of the dependent variables was tested using the likelihood ratio test (LRT). Pairwise posthoc comparisons between the different levels of the significant independent factors were assessed using Tukey´s tests. All the GLMMs were performed with the statistical analysis programme R Core Team (2016) using the following libraries: multcomp (Hothorn *et al*., 2008), lme4 (Bates *et al*., 2015) and ggplot2 (Wickham, 2016). The LMMs were carried out with the STATISTICA 7.0 software package (Statsoft. Ltd, Sweden).

Results

Fruit damage

Apple infestation rates differed across years (df = 1, LRT = 22.30, P < 0.001) and apple varieties (df = 2, LRT = 7.98, P = 0.01, figure 3). The interaction between the two factors was not significant (df = 2, LRT = 3.30, P = 0.19), as in both years, infestation rates were higher in Golden Smoothie apples, followed by Granny Smith and Gala Brookfield apples (figure 3). Posthoc tests showed that there were pairwise significant differences between Golden Smoothie and Gala Brookfield apples, and marginally significant differences with Granny Smith ones. On the other hand, infestation rates did not differ significantly between Gala Brookfield and Granny Smith apples (table 2).

Infestation rates differed significantly among canopy orientations (df = 4, LRT = 805.74, P < 0.001, figure 4). All posthoc pairwise comparisons were significant except between centre and west (table 3). The effect did not differ across years (interaction between factors not significant (df = 4, LRT = 0.63, P = 0.95), but it was affected by the apple cultivar (df = 8, LRT = 38.97, P < 0.001). In all cases, the highest infestation rates were recorded in the east, followed by the south, centre-west, and north; however, the magnitude of the differences changed between cultivars (e.g., the differences between east and south were higher in Golden Smoothie apples than in Granny Smith apples, figure 4).

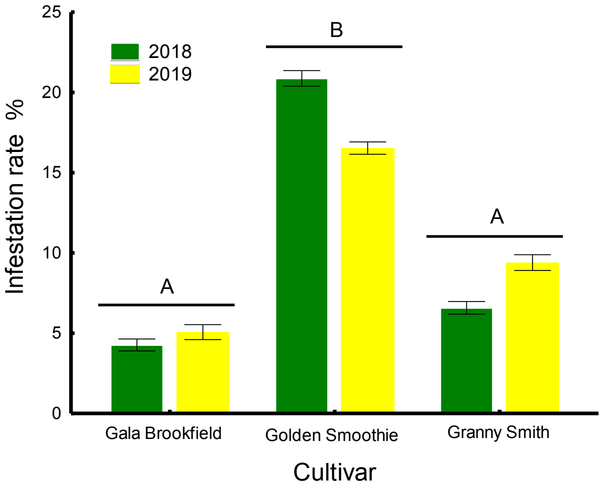

Figure 3. Apple infestation rates (mean ± SE) in the three different apple cultivars and years. Different letters on the bars indicate statistically significant differences between groups.

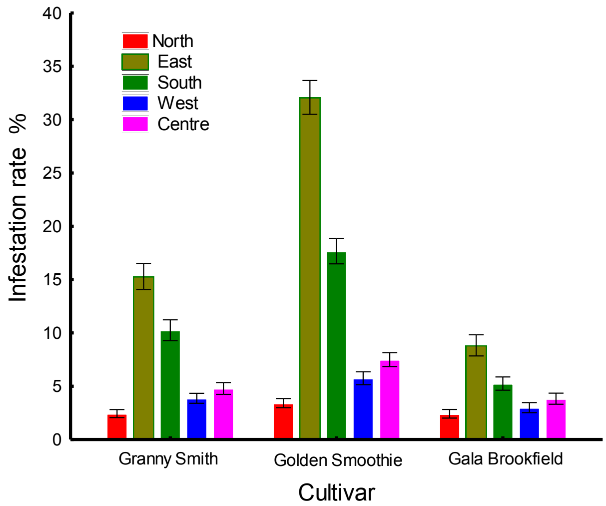

Figure 4. Apple infestation rates (mean ± SE) in relation to canopy orientation.

Codling moth flight phenology, larvae numbers and damage forecast

In 2018 and 2019, there were two major peaks of adult captures followed by a much smaller one (figure 2), this pattern was observed for the three apple varieties. Temperature increased during the first adult flight period in both years, but the average of daily mean temperatures

Table 2. Results of the pairwise comparisons (Tukey tests) of infestation rates between apple varieties.

Comparison	Estimate ± SE	Z	P
Golden Smoothie - Gala Brookfield	1.25 ± 0.35	3.52	0.001
Granny Smith - Gala Brookfield	0.51 ± 0.35	1.43	0.32
Golden Smoothie - Granny Smith	−0.74 ± 0.35	−2.09	0.09

Table 3. Results of the pairwise comparisons (Tukey tests) of infestation rates between canopy orientations.

Comparison	Estimate ± SE	Z	P
East-Centre	1.51 ± 0.08	17.37	0.001
North-Centre	−0.71 ± 0.12	−5.66	0.001
South-Centre	0.82 ± 0.09	8.96	0.001
West-Centre	−0.26 ± 0.11	−2.38	0.11
North-East	−2.22 ± 0.11	−20.01	0.001
South-East	−0.68 ± 0.07	−9.68	0.001
West-East	−1.77±0.09	−18.75	0.001
South-North	1.53 ± 0.11	13.37	0.001
West-North	0.44 ± 0.13	3.40	0.005
West-South	−1.09 ± 0.09	−10.98	0.001

Table 4. Results of the pairwise comparisons (Tukey tests) of the number of larvae between apple cultivars.

Comparison	Estimate ± SE	Z	P
Golden Smoothie - Gala Brookfield	0.96 ± 0.42	2.29	0.05
Granny Smith - Gala Brookfield	0.02 ± 0.42	0.06	0.99
Granny Smith - Golden Smoothie	−0.93 ± 0.42	−2.22	0.06

did not differ between 2018 and 2019 ($F_{1, 166}$ = 0.91, P = 0.34, figure 1A). Mean air humidity was higher in 2018 ($F_{1, 162}$ = 9.23, P < 0.01), especially in the last 5 weeks of the study period (figure 1B). In 2019, no rainfall was recorded in those weeks (figure 1C), although the average daily rainfall across the whole study period did not differ significantly between years ($F_{1, 166}$ = 1.31, P = 0.25).

There were significant differences between years in the number of larvae trapped (df = 1, LRT = 5.94, P = 0.01). However, the effect of the year depended on the variety (df = 2, LRT = 42.59, P < 0.001). In Golden Smoothie apples, the number of larvae collected was significantly higher in 2018 than in 2019, whereas in the other two varieties, it was similar in both years. The pairwise posthoc tests showed that the pairwise comparisons between Golden Smoothie apples and the other two were very close to statistical significance (table 4).

The number of adults trapped was 1012 in 2018 and 821 in 2019. In both years males represented an 87% of the total sample, with almost no differences (1%) among varieties. The average number of larvae per tree was positively related to the number of adults trapped at the end of the first flight period ($F_{1, 14}$ = 4344, P < 0.001, figure 5, model R^2 = 0.83). Mean infestation rates were strongly related to the number of adult moths as well ($F_{1, 14}$ = 274, P < 0.001, figure 5, model R^2 = 0.67). The mean number of larvae per tree and infestation rates were also related to the number of adults collected at the middle of the first flight period ($F_{1, 14}$ = 2800, P < 0.001, model R^2 = 0.48) and ($F_{1, 14}$ = 176, P < 0.01, model R^2 = 0.36), respectively. The effect of the year or the interaction between the covariates and the factor were not significant in any model (P > 0.30 in all cases).

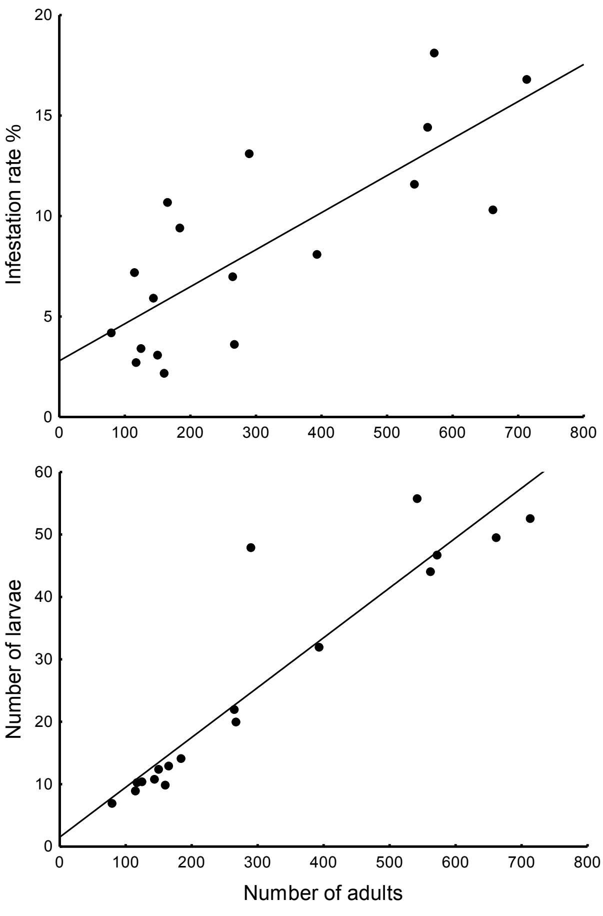

Figure 5. Relationship between the number of adults (87% males) collected at each orchard with apple infestation rates (y = 0.018x + 2.798) and with the mean number of larvae collected per tree (y = 0.079x + 1.515).

Discussion and conclusions

Fruit damage

Previous reports have shown differences in susceptibility to *C. pomonella* attacks among cultivars (Audemard *et al*., 1979; Stoeckli *et al*., 2009). We also found it in our study area: infestation rates in Golden Smoothie were twice as high compared to Gala Brookfield and Granny Smith. Some authors have proposed that codling moth females would prefer Golden Smoothie cultivar because of its sugar and polyphenol richness and its large crops (Lombarkia and Derridj, 2002; Brahim *et al*., 2013). In our study area there were not significant differences among varieties in fruit production, but the sugar content could differ.

In addition, the semiochemicals could also play a role in host selection. Light *et al*. (2001) and Coracini *et al*. (2004) demonstrated that the volatiles produced by green apples could provoke olfactory responses in *C. pomonella* and stimulate egg laying. Some sesquiterpenes, (E,E)-α-Farnesene among them, are present in apples (Murray *et al*., 1964) and have been shown to both attract *C. pomonella* adults and stimulate egg laying (Sutherland and Hutchins, 1972; Light *et al*., 2001; Coracini *et al*., 2004). In our study the pear ester was not very effective for trapping females, what could suggest that they are not too much attracted by these compounds. However, we must be cautious before making this statement, as the methodology used (combination of pear ester and pheromones in rubber septa) could have reduced its efficiency. In addition, attractiveness may change geographically and be higher in other *C. pomonella* populations (Preti *et al.*, 2021).

The phenology of fruit maturation may also explain infestation rates variability. Joshi *et al*., (2015) reported that females preferred to oviposit on later maturing Golden Delicious cultivars than in the early maturing Gala ones. Accordingly, Sutherland *et a*l., (1977) found that codling moths preferred Golden Delicious, but only during the first generation, and Mahzoum *et al*., (2017) confirmed the same in Morocco. Early in the season the late maturing Golden apples are comparatively richer in sugars and polyphenols than other varieties, what attracts egg-laying females (Yan *et al*., 1999). Thus, if our study shows higher infestation rates in Golden Smoothie, this could differ later in the season (second and third flight periods). However, since most damage is due to the first generation larvae, our results will largely resemble the final infestation rates in each variety.

In the three study cultivars, the eastern and southern sides of the trees suffered stronger attacks than the rest. The highest rates were recorded in the east, especially for the Golden Smoothie. Interestingly, infestation followed the same decreasing trend in the three varieties, and was always lowest in the north. Factors such as temperature, light and humidity may explain these results. Stoeckli *et al.,* (2008) and Mahzoum *et al*., (2017) reported that, during the first generation (G1), infestation rates were lower in the northern (coolest) side of the canopy. Arthropods are ectothermal organisms (Kührt *et al*., 2006), and in April-May adults might avoid northern orientations. Moreover, adverse whether conditions increase mortality of newly hatched larvae searching for fruits (Geier, 1963, Jackson, 1979), what could also reduce infestation rates in the cooler northern side of the canopies. These differences among canopy orientations could however change along the year, and disappear during the second and the third generations as temperatures increase. Whatever the case may be, these results have important methodological implications, and stress that it is mandatory to sample all sides of the canopy and its centre. This method should be implemented in Algeria to obtain unbiased and comparable data on infestation rates (as already recommended by Blomefield, (1997) for South Africa, MacLellan, (1962) for Australia and Meni Mahzoum *et al*., (2017) for Morocco).

Codling moth flight phenology, larvae numbers and damage forecast

The meteorological conditions during the study were frequently favourable for the codling moth flight (dry weather with temperatures over 15ºC, see Geier 1963). Average temperatures did not differ significantly between years, but there were some differences at the beginning and the end. The first flight started one week earlier in 2019, as temperatures were slightly higher in late March and higher temperatures advance adult emergence (Damos *et al.*, 2018). In both years it ended in the second fortnight of June (June 21st), but the number of adults collected in the last four weeks was lower in 2018. In that year there was a higher humidity and rainfall in late May-June, and adverse weather conditions hampers adult moth movement (Geier, 1963). In other regions of Algeria, the first flight begins at a similar date (Guermah and Medjdoub-Bensaad, 2016; 2018) and may finish earlier or later depending on the weather conditions and altitude, as both affect temperature and hence C. *pomonella* development speed (Brahim *et al.*, 2013; Iraqui and Hmimna, 2016).

Other environmental factors, such as the effects of crop management and orchard practices, may also influence codling moth biological parameters, and their role should not be underestimated (Joshi *et al*., 2016). Our study orchards were treated with insecticide from the middle of the first flight (late April) period onwards, what may have effects on both adults and larvae. *C. pomonella* populations are larger in abandoned orchards (absence of chemical insecticide programs) than in commercial (sprayed) ones (Joshi *et al.*, 2016). Moreover, insecticide may affect not only insect numbers, but also *C. pomonella* phenology at different stages of the life cycle (Boivin *et al.*, 2003, Lue, 2005; Joshi *et al.*, 2016). Boivin *et al*. (2003) showed that the fixation of alleles that confer resistance against pesticides could have pleiotropic effects and delay adult emergence. However, the emergence dates in our study sites were very similar (beginning of April) to those recorded at Algerian organic orchards (not treated with insecticides) (Belkadi and Hamli, 1998; Guermah and Medjdoub-Bensaad, 2016). This lack of differences may be because our data correspond to first generation adults, and the side effects of insecticide resistance on phenology (if occurred) would be more likely from the second generation onwards (Boivin *et al.*, 2003).

In spite of the slight differences in the onset of adult flight, the peaks of moth abundance were coincident between years and allowed anticipating larval population size and fruit damage. The number of adults collected during the whole first flight period was strongly correlated with the number of larvae and infestation rates. The moderate differences between orchards in apple production have probably favoured such a strong correlation. As the number of apples available for ovipositioning does not differ too much among orchards, infestation rates will mostly depend on the number of adult moths at each site. Thus, our data show that using a single adult trap per apple orchard is enough to assess those key variables for pest management. Moreover, we found that these data can predict in advance, as the number of adults trapped at the middle of the first flight period (first week of May) was already very high and significantly related to the final number of larvae and infestation rates.

Summarizing, our results show that infestation rates were higher in Golden Smoothie, and stress that unbiased estimations of apple infestation rates need to be balanced among canopy orientations. Adult flight phenology did not change too much between years, and the number of adults trapped in the middle of the first flight period (early May) predicted final infestation rates. Current control management in northern Algeria starts at the end of April, but our data show that is too late according to *C. pomonella* flight phenology. By that time, the accumulated number of adults collected per trap since the beginning of the season was over 200, with weekly peaks of more than 40, largely higher than the 5-10 recommended to start treatments (Charmillot and Höhn, 2004). In the second week of April the number of adults trapped was in both years over 10, and the adults emerged at the beginning of the month would have already laid eggs (Geier, 1963). In the Sidi Bel Abbes area (Algeria), insecticide treatments against eggs and larvae should start no later than April 12th to reduce apple infestation rates below the acceptable threshold for commercialization.

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