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# Laying hens in apple orchards to reduce fruit damages caused by *Cydia pomonella*: myth or reality?

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## Abstract

*Codling moth (CM) is a major pest of apple, responsible for 90 % of insecticides' use in apple orchards in Provence. As an alternative way to manage it, some farmers put laying hens in their orchard. But their efficiency has yet never been scientifically tested.*

*We present experimental results on the effect of hens on CM carried out on a pilot farm in agroforestry (Provence, France), severely exposed to CM damages. The experimental device consisted in four apple tree rows covered by Alt'Carpo nets. Each row was divided into two sub-rows (H : Hens and C : Control). Two modalities for hens' presence were tested : presence of hens during the whole year and presence of hens during two months in winter. At the end of the season, fruit damages were evaluated by counting in each modality the total number of fruits presenting pest damages.*

*Results show contrasted effects of hens. We observed a significant decrease in pest damages in only one of the two plots with the 'Whole year' modality. On the opposite, in both plots of the 'Winter' modality, we observed a significant increase in fruit damages.*

*These results were supplemented with PCR analysis on hens' droppings, proving that hens were able to find and eat CM in the experimental plots. However, the impact of hens on codling moth during the winter seems to be too low to compensate a possible negative impact on the auxiliary fauna, which may explain our contrasted results.*

*So far, our study represents the first study bringing elements about a potential reduction of CM damages by hens in production orchards. Hence, integrating hens into orchards remains a limited pest management solution that may generate counter-effects if not managed properly. We conclude that hens are to be combined with other pest control techniques in a more multidimensional approach combining pest regulation, income diversification, fertilisation and weed management.*


**Keywords :** Hens pastured-orchards, field study, codling moth damages assessment, PCR

## Introduction

Codling moth (CM), *Cydia pomonella,* is a worldwide apple pest that causes a lot of damages in Provence, one of the main regions of apple production in France (Agreste, 2023). CM is responsible for around 90 % of insecticides' use in apple orchards in this region (Sauphanor *et al.*, 2009). In order to find alternative pest control techniques, some farmers introduced laying hens in their orchard (Solagro, 2016). Indeed, due to their natural behaviours and omnivorous diet (Horsted, Hermansen and Ranvig, 2007), hens are assumed to impact CM populations by different mechanisms depending on their developmental stages. During moth emergence period (spring and summer), larvae potentially migrate to the ground to seek for pupation sites (Balachowsky A., 1966) and would thus be vulnerable to hens' predation. When the photoperiod starts decreasing, CM larvae enter rather diapause than pupation in order to winter (Balachowsky A., 1966). They seek for diapausing sites, either in tree trunk crevices or in cavities close to the ground (straw, wood, weed) or direct in the ground (Balachowsky A., 1966). Hence, during autumn and winter phases, diapausing larvae would be durably exposed to predators located on the ground (Stairs, 1985), notably to hens and

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could also be directly damaged by hens scratching the ground. Furthermore, hens might also modify the local environment of diapausing larvae by removing vegetation, compacting the soil, and modifying soil composition due to manure deposition (Clark and Gage, 1997). CM could then for example be exposed to harsher temperature conditions or bird predation, endangering its winter survival (Glen and Milsom, 1978). Hence, for all those reasons, hens could be involved in the regulation of CM populations which could result in a diminution of fruit damages in the orchard. This idea pushes a greater number of farmers to adopt this practice, and feedbacks from farmers trials are always more numerous (Bosshardt *et al.*, 2022).

Though appealing, this hypothesis has never been scientifically confirmed. Very few studies explored this regulation potential over other pests (Bosshardt *et al.*, 2022) and all of them presented unclear conclusions (Clark and Gage, 1996; Hilaire *et al.*, 2001; Pedersen *et al.*, 2004; Guerin *et al.*, 2020). Based on these hypotheses, we designed an experimental study to examine the effect of hens' presence on fruit damages by CM. We explored two modalities for the presence of hens under apple trees : a *whole year* presence and a *winter* presence. The potential underlying mechanism of CM consumption was also assessed by using diagnostic PCR to detect the presence of CM DNA in hens' faeces collected during our field experiments.

## Material and methods

Plots

An on-farm trial was set up on an organic agroforestry market gardening pilot farm located in Avignon (South-East of France), severely exposed to CM damages (up to 100% damages for some apple varieties), and willing to test alternative pest management solutions. The objective of this study was to test the interest of such a practice on a real production farm. The experimentation was carried out between July 2021 and October 2022. Due to farm's configuration, trials were not conducted on a whole orchard but on four apple rows planted in 2015-2016. Each row (from 9 to 12 trees) corresponds to one apple variety and was separated into two sub-rows, each associated to one of the two main modalities : (H) with hens, (C+) control without hens (Table 1 and Figure 1). To avoid migration effects of CM and due to the high CM pressure, both modalities were protected separately using netting systems (Alt'Carpo). As the objective was to study the local impact of hens on fruit damages, we chose to apply an *incomplete* exclusion system including the soil : nets were thus left hanging to the ground in a row-by-row system (Chouinard *et al.*, 2017). We also chose to add a third modality to evaluate CM pressure outside the net by excluding from the netting system two trees located at each extremity of *whole year* rows. Nets were closed in spring, at the end of the flowering period and reopen after harvest, both dates depending on varieties. Winter pruning and harvest were the only interventions realized on the rows during this period (no treatment, no apple thinning, no fertilization).

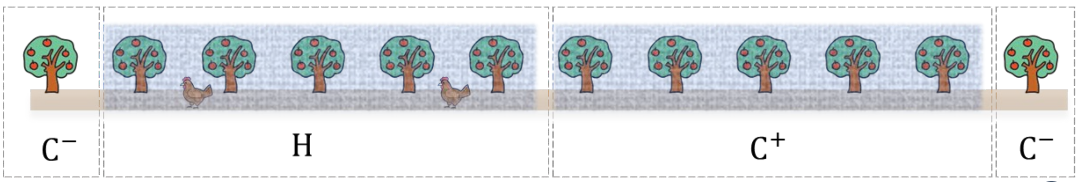


Figure 1: Apple rows' spatial organisation of modalities for the whole year trial (row ① or ② of Table 1). Hatched areas represent Alt'Carpo nets, set up during spring/summer periods. Letters refer to modalities: C- (no net, no hens), C+ (net, no hens) and H (net, hens)

## Hens

For each (H) sub-row in the *whole year* trial, two hens (resulting in a density of 243 hens/ha in row ① and 192 hens/ha in row ②) were introduced from 27th July 2021 to 27th October 2022 (Table 1). For the *winter* trial, two and four hens (resulting in 548 hens/ha in row ③ and 727 hens/ha in row ④) were introduced from 13th January 2022 to 1st March 2022. Farmers chose standard “brown hens” known to be performant layers and widespread among farms. Hens were aged around two, and originated either from private individuals or from another organic farm. Hens were thus used to explore the environment, as they had been raised outdoors, and even for some, in a fig orchard. Electric fences surrounding hens’ plots were used to avoid predation. Farmers implemented a limited feed restriction (between 90 and 110 g/hens/day instead of 130g/hens/day) in order to encourage foraging behaviour (Horsted and Hermansen, 2007).

Table 1: Summary of trial characteristics for each row. C- : Negative control (no net, no hens), C+ : Positive control (net, no hens), H : Modality with hens

| Row number | Cultivar | Modalities | | Net | Hens | Number of trees |
|---|---|---|---|---|---|---|
| ① | Canada Reinette | Whole year (07/21-10/22) | $C^-$ | - | - | 2 |
| | | | $C^+$ | + | - | 5 |
| | | | H | + | + (243 hens/ha) | 5 |
| ② | Rouge provençale d’hiver | | $C^-$ | - | - | 2 |
| | | | $C^+$ | + | - | 4 |
| | | | H | + | + (192 hens/ha) | 5 |
| ③ | Garance | Winter (01/21-03/22) | $C^+$ | + | - | 5 |
| | | | H | + | + (548 hens/ha) | 4 |
| ④ | Goldrush | | $C^+$ | + | - | 6 |
| | | | H | + | + (727 hens/ha) | 6 |

## Monitoring of fruit damages and statistical analysis

Damaged fruits were counted at two moments in the year : at the end of the first CM generation[2] (28th and 30th June 2022) and at harvest (between 7th September and 27th September 2022, depending on varieties). All fruits present on trees or on the ground were visually examined and counted. Fruits were classified into three categories : damaged by CM, undamaged by CM, unknown in case of doubts. Results were obtained individually for each tree and grouped by sub-row. To eliminate difference in term of total fruit number, we calculated a percentage of damaged fruits per sub-row. Results for the hens’ modalities (H) were then compared to their control (C+) using a Chi2 test.

## PCR study

A method using diagnostic polymerase chain reaction (PCR) to detect CM DNA in hens’ faeces was performed to study whether hens in field conditions were able to predate CM. We collected hens’ faeces for both modalities at different periods of the year : in autumn

[2] The right period was determined by using local model predictions available on https://www.bsv-paca.fr/

2021 and in summer 2022 for the *whole year* group, and in winter 2022 for the *winter* group. Fresh faeces were collected individually in sterile collecting tubes and frozen at -20°C. DNA was extracted using DNeasy® mericon Food Kit (Qiagen) and purified using OneStep™ PCR Inhibitor Removal Kits (Zymo) (Mangan, Pejchar and Werner, 2017). Singleplex PCR was performed using C. pomonella primers developed by a partner laboratory (PSH, INRAE Avignon)(Boreau de Roincé, 2012) and amplified samples were analysed using sequencing (ABI DNA sequencer).

## Results

<u>Fruit damages</u>

Results show contrasted effects of hens. In the *whole year* trial, only one row (①) shows a significant decrease in fruit damages in the modality with hens compared to its control C+ (Figure 2). This row corresponds to the most damaged variety of the trial. For the other apple variety of the trial (②), no significant difference was observed between H and C+ modality but impact on C+ modality was already marginal. In both cases, unnetted controls ($C^-$) confirmed the elevated CM pressure in the environment, as well as the significant effect of nets on damage reduction.

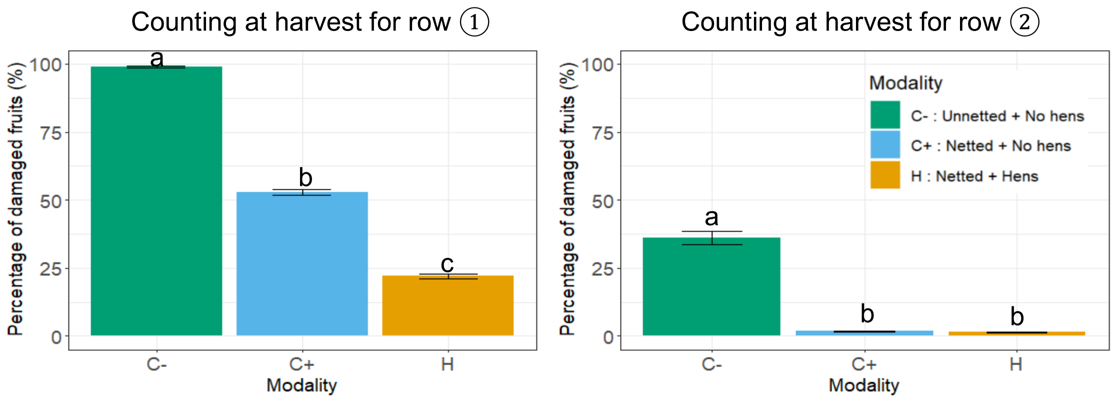


Figure 2: Fruit damages at harvest for rows ① and ② of the whole year trial. Error bars represent standard errors. Percentages with different letters are significantly different at P ≤0.05.

Surprisingly, opposite effects were obtained for the *winter* trial. For both varieties, results show a significant increase in fruit damages in the hen-modalities compared to their respective control (C+) (Figure 3).

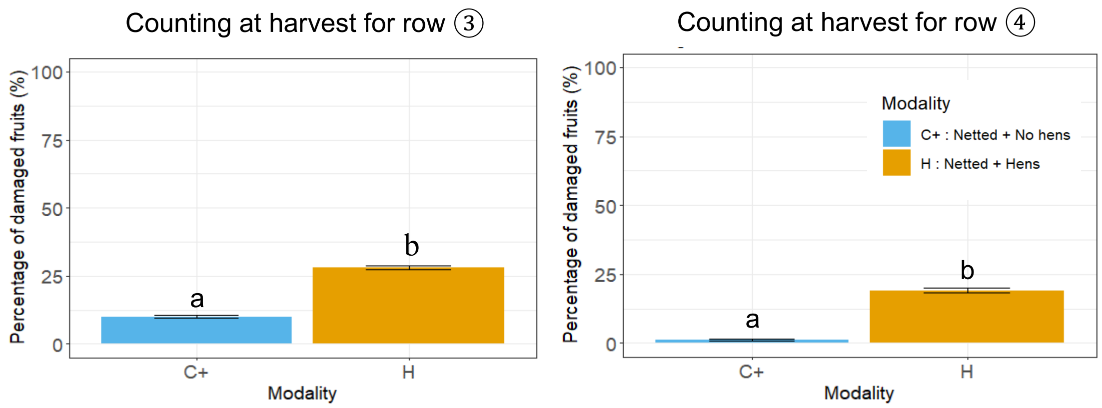


Figure 3 : Fruit damages at harvest for rows ③ and ④ of the winter trial. Error bars represent standard errors. Percentages with different letters are significantly different at P ≤0.05.

For all rows, previous counts (2020-2021) confirmed that no difference of damages percentages prior to the experimental setup was observed between sub-rows of the same apple variety.

PCR

Over 97 on-field faeces samples, 5 showed a significant positive signal for CM DNA presence. Positive results were obtained only for the autumn 2021 and for the winter 2022. Although limited, they represent the first proof that hens present under apple trees are able to find and consume codling moth. Moreover, positive samples were obtained for different weeks, confirming that hens more or less regularly consumed CM.

Table 2 : PCR results obtained over 97 individual faeces samples

| **Whole year** | | **Winter** |
|---|---|---|
| Autumn 2021 | Summer 2022 | Winter 2022 |
| 3 positives over 80 | 0 positive over 6 | 2 positives over 11 |

Preliminary tests in controlled conditions also showed that this method hardly detected the consumption of one single CM larva. This limited sensitivity can thus result in an underestimation of DNA presence, all the more that hens probably consume small amounts of CM at one time. This might also explain the small number of positives sample that we obtained.

## Discussion

Though limited, this first experimental study allows to draw first tendencies and hypotheses about hens' regulation potential on CM.

PCR results represent the first proof that hens are able to find and consume CM in real production plots during autumnal and wintering phases. A complementary study, using samples collected during spring and summer, would be useful to determine whether hens also predate CM larvae during the emergence period.

Nevertheless, this limited consumption did not necessarily result in pest regulation at plot scale. In the case of high CM pressure, an extended presence of hens during a whole year seems to have favoured a reduction of fruit damages. However, this effect could not be observed with marginal damages. For the second trial, contrary to our primary hypothesis, we observed a countereffect of hens' presence, resulting in a damage increase compared to the control. To date, the most probable hypothesis is that hens could have also directly or indirectly impacted other natural predators of CM and disturbed food webs, though pieces of evidence at our disposal remain sparse (Clark and Gage, 1997). Hence, a limited effect of hens on CM in winter might not be sufficient to compensate this countereffect, aggravated by a high density of hens during a critical period for vegetation and insect growth. On the contrary, for the whole year trial, hens' effect during the spring/summer period might have compensated this potential disservice. Other alternative hypothesis (experimental defects, initial differences of inoculum, difference in the total number of apples between modalities resulting in a density-effect of damages etc.) were also examined but were assumed to be less probable. Complementary approaches would yet be necessary to confirm those findings and deepen our comprehension of the mechanisms involved.

Integration of hens in orchards requires to be carefully handled to avoid potential countereffects. Moreover, hens only represent a limited pest management solution, that has to be combined with other pest control techniques. Though, benefits provided by hens have to be examined using a multidimensional approach. Indeed, farmers interested in this association also expect other services : weed management, fertilisation effects and income diversification.


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