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Assessment of control strategies against *Cydia pomonella* (L.) in Morocco

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Abstract: The codling moth, *Cydia pomonella* (L.), is the key pest of apple production worldwide. In Morocco, there is a sustainable presence of codling moth causing considerable damage in apple orchards despite frequent applications of broad spectrum insecticides. For 12 years, sexual trapping and chemical control were performed and the development of the codling moth population was analysed in an orchard which was in the region of Azrou. The efficacy of some insecticides (azinphos-methyl, chlorpyriphos-ethyl, diflubenzuron, thiacloprid, methoxyfenozide, spinosad, and deltamethrin) was also evaluated on neonate larvae and compared with a laboratory sensitive strain. This procedure was done to assess an eventual resistance in Moroccan populations. The action threshold was usually exceeded, leading to an intensive chemical control, with an average frequency of 9 to 13 days. The chemical control was done according to the action persistence time of the insecticides and the trap captures. However, those two parameters are compromised in Moroccan conditions because of the high summer temperatures which disrupt the action of insecticides and exacerbate populations. The pheromone traps may become ineffective and useless. Neonate larvae were resistant to five insecticides out of seven. Such results suggest the presence of a cross resistance in local strains. Overall, the insect resistance, the functioning of the sexual traps, and some insecticides properties (persistence action, pre-harvest interval) are the key factors that could explain the failure to control these moths under Moroccan conditions.

Key words: *Cydia pomonella*, insect resistance, insecticides control, sexual traps, toxicological bioassays

Introduction

Cydia pomonella (L.), is a serious pest of Rosaceae trees. This pest is native to southeastern Europe. It infests all production areas of apple, pear, quince, and walnut (Shel'Deshova 1967). Its wide distribution area, from cool temperate climates to the Mediterranean climate, results from its effective colonisation favored by human action and strengthened by its optional diapause (Steinberg *et al*. 1992). Codling moth occurs one to five generations (the latest generation is mostly partial) per year in different parts of the world. The number of generations depends mainly on altitude (Audemard 1992; 2003). In Morocco, studies, based both on a model of the degree days and captures of sexual traps, revealed that *C. pomonella* occurs in four generations. Two generations are complete, while the third and incidentally the fourth generation are partial because of the diapause of the insect (Hmimina and El Iraqui 2015). The non-fulfillment of those generations comes from the spatial-temporal equation, related mainly to photoperiod, temperature, and nutrition.

Growers fear this insect which downgrades their productions. The losses can reach 100% of the total production. Therefore, chemical control is intensively used to limit the extension of the codling moth but numerous cases of resistance have been observed (Thwaite *et al*. 1993; Reyes *et al*. 2007). In Morocco, chemical control is the main strategy used to protect orchards against codling moth (Hmimina 2007). Some conscientious producers introduced sexual traps, as a decision support tool, to get information on the accurate flight of the insect and to better manage the chemical control. However, the control of this insect seems to be failing. In this perspective, the present study, based on 12 years of capture data, aims to analyse the presence of the codling moth population and the reasons behind the failure of chemical control against this pest in the Moroccan conditions.

Materials and Methods

Sites

The study was conducted in an orchard in the region of Azrou (Tigrigra Valley) in Morocco (33°26'3 N – 5°13'16 O, 1,110 m altitude). This is a location of significant fruit production.

Sexual traps

Each year, from 2002 to 2013, pheromone traps were installed between early April and harvest time. Generally, one sexual trap was placed every 4 ha. If necessary, other

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traps are added proportionally to the orchard area. Traps were placed in the orchard at a distance of 50 m from each other and 10 m from the borders of the orchard. In our study, the number of traps decreased during some seasons because of tree grubbing which had been done as a result of fire blight disease. The number of traps increased in the later years because of the replanting of trees. The traps were checked at fixed days, three times per week. If the sum of these three catches exceeded the threshold of five males per week, it was possible to initiate chemical control. This threshold has been in effect in Morocco since the 90's.

The trap captures for the years studied were previously analysed, and permitted the delimitation of different generations in this orchard (Hmimina and EL Iraqui 2015). The percentage of the first generation in terms of captures, was calculated to highlight its proportion in the life-cycle of the codling moth.

Chemical control in the orchard

A wide range of synthetic insecticides was used in the orchard (Table 1).

Insect resistance

In order to assess the hypothesis of insect resistance, preliminary toxicological tests against some known and used insecticides, were tested on neonate larvae.

Insecticides tested

The effect of organophosphates azinphos-methyl and chlorpyrifos-ethyl, diacylhydrazin methoxyfenozide, neonicotinoid thiacloprid, pyrethroid deltamethrin, spinosoid spinosad, and benzoylurea diflubenzuron were studied (Table 2). Fresh dilutions of formulated insecticides were prepared in distilled water for the bioassays on neonates.

Neonate larvae

Larvae of Moroccan strains were collected from injured apples of the orchard studied and were reared on an artificial diet (Stonefly Industries Ltd) until becoming adults. Once adults, 10 males and 10 females were placed in bottles to obtain the eggs of the codling moths. Those plastic bottles, containing eggs, were cut in pieces and were placed in boxes at 23°C, until the eggs hatched. Then, newly hatched larvae (0–4 h old) were individually placed in the microplate wells.

Toxicological bioassays

The microplate wells were filled with 150 μl of artificial diet (Stonefly Industries Ltd). Then 6 μl of each insecticide concentration was put on the surface of the diet. In the controls, distilled water replaced the insecticide (Reyes and Sauphanor 2008). Neonate larvae were put individually in the wells (on the surface of the diet) and mortality was registered after 5 days. A larva was considered dead when it did not respond to being probed with dissecting forceps. Missing larvae were subtracted from the initial number.

The discriminating dose of azinphos-methyl, chlorpyrifos-ethyl, and methoxyfenozid were defined according to the study by Reyes *et al*. (2015); whereas the dose of spinosad was determined based on Reyes and Sauphanor (2008). On the other hand, the discriminating doses of thiacloprid and deltamethrin were obtained from the Laboratory of INRA Avignon (personal communication). The Sv strain was kept by mass rearing on an artificial diet as described by Guennelon *et al*. (1981). The use of this strain as a reference population was suitable for our tests because previous studies have shown a similarity to the susceptible strain response of Spain, France, and Italy (Rodriguez *et al*. 2011).

Statistical analysis

Using SPSS software, the annual captures per trap for each year were subjected to analysis of variance (ANOVA) to figure out the differences between traps and years. Years were classified in terms of treatments and means of catches, using T-student-Newman-Keuls. Pearson's correlation was carried out to assess the correlation between number of treatments and catches. Mortalities to diagnostic concentrations in the field populations were compared to those of the Sv strain by using a Chi-square test after using Abbot's correction (Abott 1925).

Results

Orchard captures

Over all the years studied, the catches per trap per week were always higher than the action threshold. The captures per trap per week varied between 15.89 and 45.85 (Table 3). When considering the comparison between traps, the ANOVA statistical model revealed that traps homogeneously intercepted insects despite the location of the traps ($F = 0.059$, $p = 0.98$).

Analysis by ANOVA revealed a very significant effect of year ($F = 5.18$, $p < 0.001$) on captures. Three subsets were distinguished. The first group was composed of the years 2007, 2004, and 2008. The second group consisted of the remaining years (except 2011 which registered the minimum of captures).

When considering the catches of the first generation (from 1 April to 20 June), the results showed that this period represented about 44% of the captures. The percentage of captures in the first generation reached 60% of the total captures (2006, 2011 and 2013). The years 2005, 2007, and 2008 recorded fewer captures during the first generation than others.

Chemical control effect

The chemical control was applied at regular intervals of 9 to 13 days. As supported by the correlation analysis, the number of treatments was not correlated to total catches

Table 1. Characteristics of insecticides against *Cydia pomonella* and their frequency of use from 2002 to 2013 in Azrou orcard

Chemical group	Active ingredient	Common noun	PA [days]	PHI	Year of use [frequency]
Anthranilic diamides	chlorantraniliprole	Coragen	12–14	14	2013(1)
Avermectin	abamectin	Tinamex	21–42	10	2009(1)
Avermectin + Benzoylurea	emamectin benzoate + lufénuron	Denim	14	28	2013(1)
Benzoylphenylurea	flufenoxuron	Cascade	15–21	45	2013(1), 2004(1)
Carbamates	methomyl	Lannate	7	21	2004(1), 2009(1), 2010 (2), 2013(1)
Diacylhydrazin	methoxyfenozide	Runner	21	14	2013(1), 2012(1)
Neonicotinoids	thiacloprid	Calypso	15	14	2003(2), 2013(2)
Organophosphates	azinphos-methyl	Azinkothion	15	30	2009(3)
	phosmet	Imidan	10–15	28	2013(1), 2010(2), 2007 (2)
	fenthion	Lebaycid	60	15	2013(1), 2011(1), 2006 (2), 2005(1)
	malathion	Malathion	8	7	2013(2), 2012(1), 2011(1), 2009(1), 2008(1), 2007(1), 2006(2), 2005(2)
	methidathion	Ultracide	15–21	30	2002(3), 2003(2), 2005(2), 2007(2), 2008(2), 2011(3)
	phosalone	Zolone PM	15–21	21	2003(2), 2008(1),
	chlorpyriphos-ethyl	Dursban	10–15	30	2004(3), 2005(1), 2006(2), 2008(3), 2009(2), 2010(2), 2011(1), 2012(2)
	dimethoate	Promethion	15–21	30	2007(2)
	parathion-methyl	Folidol	20	14	2002(1), 2003(1), 2004 (1)
Oxadiazines	indoxacarbe	Avaunt	14	7	2012(3), 2011(1), 2006 (1)
Pyrethroids	beta-cypermethrin	Akito	15–20	7	2013(2)
	cypermethrin	Arrivo	15–20	14	2011(1), 2010(2), 2008 (1), 2002(1)
	deltamethrin	Decis	21–28	7	2002(1), 2003(1), 2004 (2), 2005(2), 2006(1), 2008(1), 2011(2), 2012 (1), 2013(1)
	lambda-cyhalothrine	Karate	21–28	14	2005(1), 2006(1), 2007 (1),
	cypermethrin	Nurelle	15–20	14	2009(2)
	bifenthrine	Talstar	15–20	25	2002(2), 2003(1), 2004 (3), 2005(2), 2006(1), 2007(2), 2008(2), 2009(1), 2010(1), 2011(2), 2012(3)

PA – persistence of action; PHI – pre-harvest interval

Table 2. Diagnostic concentrations used for the detection of insecticide resistance on neonate larvae of the susceptible laboratory strain (Sv) and the field population of *Cydia pomonella* from Azrou (Morocco)

Active ingredient	Formulation	Concentration [mg · l^{-1}]	Supplier
Azinphos-methyl	Azinkothion 25%	45	SAOAS
Chlorpyrifos-ethyl	Dursban4 (480 g · l^{-1})	2,600	Dow Agrosciences
Methoxyfenozide	Runner (240 g · l^{-1})	260	Dow Agrosciences
Thiacloprid	Calypso (480 g · l^{-1})	30	Bayer
Deltamethrin	Decis fluxx (25 g · l^{-1})	1.6	Bayer
Spinosad	Tracer (480 g · l^{-1})	50	Dow Agrosciences
Diflubenzuron	Dimilin (480 g · l^{-1})	10,000	Chemtura

Table 3. Average catches per week from 2002 to 2013 of *Cydia pomonella* and percentage of captures in the first generation (G1)

Years	Period of trapping	Traps	% of catches (G1)	Catches/trap/week
2002 ab	12/05 to 02/09	4	54.73	17.99
2003 ab	01/05 to 04/09	4	30.00	29.82
2004 bc	19/05 to 08/09	3	38.89	31.57
2005 ab	07/05 to 08/09	3	18.87	29.05
2006 ab	29/04 to 08/09	3	61.34	21.63
2007 c	18/05 to 09/09	3	24.10	45.85
2008 bc	04/05 to 09/09	3	29.11	35.56
2009 ab	14/04 to 02/09	3	50.37	17.64
2010 ab	23/04 to 04/09	2	59.17	23.24
2011 a	22/04 to 05/09	2	63.19	15.89
2012 ab	03/05 to 07/09	3	29.62	25.20
2013 ab	23/04 to 04/09	4	60.25	22.47
The mean	–	–	–	44.24

The small letters a, b, c refer to the three groups of years generated from t-Student Newman Keuls analysis

Table 4. Number and frequency of chemical treatments in Azrou orchard from 2002 to 2013

Years	Catches/trap	Number of treatments	Average frequency of treatments
2002	293.0	8	12.67
2003	542.0	9	12.70
2004	509.0	11	10.27
2005	519.3	12	11.36
2006	411.3	10	13.30
2007	746.6	10	11.50
2008	656.0	11	11.73
2009	358.0	11	12.91
2010	447.0	9	13.50
2011	307.0	12	11.42
2012	506.3	13	10.38
2013	434.0	14	9.64

Table 5. Toxicity of seven insecticides to neonate larvae of one susceptible laboratory strain (Sv) and field population of *Cydia pomonella* from Azrou orchard

Active ingredient	Populations	n	Abbot's corrected mortality for neonate larvae [%]
Azinphos-methyl	Sv	96	100
	Azrou	187	15.40***
Chlorpyrifos-ethyl	Sv	192	100
	Azrou	96	100
Methoxyfenozide	Sv	96	100
	Azrou	161	87.18***
Thiacloprid	Sv	96	100
	Azrou	96	61.70***
Deltamethrin	Sv	96	100
	Azrou	279	74.49***
Spinosad	Sv	96	100
	Azrou	104	95.99
Diflubenzuron	Sv	96	100
	Azrou	114	2.06***

Mortality followed by asterisks was statistically significant at ***$p < 0.001$ according to the Chi-square test per traps (r = 0.017). However, based on the Student's t-test, three groups of years were distinguished: the year 2002 with the lowest treatments (8), the years from 2003 to 2011 with an average of 10 treatments (SE = 1.13), and finally the years 2012 and 2013 receiving 13 and 14 treatments respectively (Table 4).

Toxicological bioassays

Diflubenzuron showed less than a 3% corrected mortality in the field population. Azinphos-methyl also showed a high percentage of larval survival and recorded 15.4% of corrected mortality. A reduced sensitivity of the field population was noted for thiacloprid and deltamethrin. The field population also had a small but significant resistance to methoxyfenozide (Table 5). Only two insecticides, chlorpyrifos-ethyl and spinosad, were highly effective against neonate larvae.

Discussion

The present study was performed to explain the failure to control the codling moth in a Moroccan orchard. The weekly catches per trap were always higher than the action threshold, confirming the permanent risk of this insect over years. In Morocco, populations of codling moth are very abundant compared to other countries. For instance, the Durance valley orchards in France recorded an average of 73 and 55 moth catches in the first generation during 2006 and 2007, respectively (Ricci 2009). On the other hand, the Azrou orchards in Morocco recorded 765 and 560 moths during the first generation of 2006 and 2007, respectively. The Moroccan recordings were about 10 times that of the moth population in France (Hmimina and El Iraqui 2015).

Similarly to previous studies (Setyobudi 1989; Hmimina and El Iraqui 2015), the importance of the first generation in terms of captures (44%) was confirmed, in comparison with the subsequent ones. An effective control management against the first generation can greatly reduce the development of the following generations during a growing season, thus reducing the pest population abundance (Miletic *et al*. 2011). In fact, during the period of the first generation, it is possible to clearly differentiate growth stages, as opposed to the later generations where all growth stages are present at the same time. This situation makes the control of the codling moth much more difficult during later generations (Miletic *et al*. 2011).

Chemical control involved frequent, intensive spraying with insecticides for 9 to 13 days, along with the use of sexual traps data (action threshold). In the south of France, 8 to 15 treatments were needed to control *C. pomonella* despite the use of traps. This intensity of treatments led to the appearance of several cases of resistance (Boivin and Sauphanor 2007). In Washington State, USA, the use of pheromone traps was associated with the degree-day model. Trapped moths are accumulated from biofix to 250 degree-days and treatments are sprayed based on the action threshold fixed at 5 moths per week (Brunner *et al*. 1993). In our case, the action threshold is usually exceeded. As long as the catches are higher than this threshold, chemical treatments are always justified and producers rely less on trapping data. In fact, the frequency of the treatments is based on the persistence action of the insecticides. The decrease in the catches only allows the treatments to be spaced out or for the treatments to be stopped. This means the role of pheromone traps, as a support tool, is limited, except for the initiation of the first treatment targeting the first generation.

Our study has also demonstrated some inconsistency in the use of insecticides because the number of treatments is not related to the captures recorded. Thus, two hypotheses were investigated: the first one concerns the choice of insecticides and their spray timing and/or the second one concerns the eventual development of insect resistance in Moroccan orchards. To clear up the first hypothesis, the program of sprays as analysed. The grower followed an intensive use of some organophosphates such as (methidathion, chlorpyrifos-ethyl, and malathion) and the pyrethroids (deltamethrin and bifenthrine) (Table 1). Methidathion and chlorpyrifos-ethyl were mainly used early in the season. They represent 23% of the 130 treatments listed. Methidathion and chlorpyrifos-ethyl have in common a good action persistence time (10 to 20 days) and 30 days as pre-harvest interval. The second generation of the codling moth was covered by several insecticides belonging to numerous chemical groups (organophosphates, pyrethroids, benzoylureas, neonicotinoids, carbamates, oxadiazines, Avermectine) with more than 10 days as the action persistence time. Before harvest, malathion and deltamethrin (having 7 days as pre-harvest interval) were usually applied at the end of the season; starting from August. The application at this time generally corresponds to the third or fourth generation. These insecticides are similar to the insecticides ordinarily authorised worldwide. However, the choice and the frequency of sprays depend especially on the pre-harvest interval and the action persistence time. This latter parameter is never known precisely, especially in Moroccan conditions where hot summer temperatures (often ≥ 35°C) could quickly deteriorate products.

For the second hypothesis, the response of larvae originating from Azrou, to discriminating doses of the different insecticides used in toxicological tests, is unexpected and suggests the presence of a cross resistance in Moroccan strain. In literature, the inefficacy of insecticides is due to their excessive use. Paradoxically, this was not the case of the two insecticides (azinphos-methyl and diflubenzuron) tested in our study. Diflubenzuron has never been used in the orchard. As for azinphos-methyl, marketed under multiple formulations and once considered to be one of the remarkable chemicals used in the 1970s, there are few occasions when it has been applied in the last ten years in the orchard. However, both insecticides showed the highest percentage of larvae survival (98% and 84% respectively for diflubenzuron and azinphos methyl). The same situation was noticed in USA for diflubenzuron (Moffitt *et al*. 1988). Moffitt *et al*. (1988) explained the resistance to diflubenzuron by repeated treatments with azinphos-methyl, which was not true in our case. In California, resistance to several classes of insecticides was correlated to azinphos-methyl resistance in codling moth.

In laboratory tests and field populations, cross resistance was positively correlated with azinphos-methyl and two of organophosphates (diazinon, phosmet), a carbamate (carbaryl), a chlorinated hydrocarbon (DDT), and two pyrethroids (esfenvalerate and fenpropathrin) (Dunley and Welter 2000). These findings could explain the resistance detected through toxicological tests.

Unlike diflubenzuron and azinphos-methyl, deltamethrin was often sprayed in the orchard especially before harvest. In France, in the early 1990s, resistance to both deltamethrin and diflubenzuron was explained by common mechanisms based on mixed function oxidases activity (Sauphanor *et al*. 1998; Sauphanor *et al*. 2000). Thiacloprid and methoxyfenozide, recently introduced in our orchard, also showed low mortality in the Azrou strain. Reyes *et al*. (2007) showed the clear role of multiple function oxydases (MFO) and esterase (EST) in the resistance to thiacloprid and the involvement of these enzymes in the resistance to spinosad. However, the latter product has shown 100% efficacy in the Moroccan strain. For methoxyfenozide, although the survival rate is low in relation to azinphos-methyl, the result is surprising because of the absence of this insecticide in both orchards. The same situation was described in Michigan, USA, despite its unusual use against codling moth. It was usually used to control *Choristoneura rosaceana* (Harris) and the treatment periods against this pest did not coincide with the life stages of *C. pomonella* (Mota-Sanchez *et al*. 2008).

In conclusion, the abundance of codling moths cannot be explained by a single factor. Several factors may interfere and affect pest population abundance. The control failure is mainly related to: the inadequate use of the sexual trap data by farmers, insecticides properties, and insect resistance. The use of sexual traps is not optimised and should be associated with the degree day's model. Information about life stages are required to better control the different generations of *C. pomonella*, especially the first one which is spread out over time and more populated than others. The persistence action and pre-harvest interval are not sufficient to choose insecticides related to the high temperatures dominant in Morocco. Finally, repeated and intensive treatments have not been without consequences. The toxicological tests have noted the eventual presence of a cross resistance in Morocco; a situation that could endanger the health of our farms. In perspective, enzymatic and molecular analyses should be done to confirm and understand the mechanisms involved in this resistance. Moreover, environmentally-friendly and more sustainable (such as biological control) alternative control methods should be adopted in Morocco. This is necessary in order to establish a strong integrated pest management strategy against this pest.

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