Susceptibility of *Ceratitis capitata* Field and Laboratory Strains to Malathion and Spinosad in Tunisia

Meriem Msaad Guerfali[1,*], Heitham Hamden[1], Salma Fadhl[1], Wafa Djobbi[1], Lotfi Sillini[1], Wafa Marzouki[1] and Mohammed Ammar[2]

[1]*Laboratory of Biotechnology and Nuclear Technologies, LR16CNSTN01, National Centre of Nuclear Sciences and Technologies, Technopole Sidi Thabet, Tunis, Tunisia*
[2]*Institut National Agronomique de Tunisie, Université Carthage, Tunis-Mahrajène, Tunisie*

ABSTRACT

The Mediterranean fruit fly (medfly), *Ceratitis capitata,* is a major problem for fruit production in Tunisia. *Ceratitis* was for long time treated with malathion as main conventional control method. Malathion upon repeated and prolonged use may cause resistance. In an attempt to reduce the insecticide residues in fruit, the government is trying to introduce the application of spinosad that was also reported to cause resistance under extensive use. The aim of this work was to monitor the susceptibility to malathion after prolonged use in wild caught tunisian *Ceratitis* as well as to spinosad after a survey carried out with farmers and to give precise informations about treatment management state. Surveys and sampling of the wild strain of *Ceratitis capitata* were conducted in several regions to learn about the nature and number of treatments. The samples were subjected to ingestion toxicity bioassays of malathion and spinosad at different concentrations. Histopathological effects of spinosad were also studied and showed that for doses up to LC_{50}, annoying effects begin to appear in glial and neurosecretory cells. The LC_{50} for malathion was 75.61 ppm for the populations treated more than 6 times. Low levels of resistance are suspected and should be confirmed by molecular analyses for malathion. On the other hand, spinosad LC_{50} were 127.95 ppm and 22 ppm for field and laboratory population, respectively. Resistance phenomenon is suspected, a rotation of insecticides with different modes of action is desirable in insect resistance management programs.

INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is considered as one of the most destructive pests in the world (Liquido *et al*., 1990; Liquido *et al*., 2013). In Tunisia despite repeated malathion-bait spray applications, medfly is a major problem for fruit production although data on exact losses are not published. Conventional control of medfly in Tunisia is based on aerial and ground chemical treatment. Aerial treatments are exclusively made in the Cap bon peninsula at the north east of the country, the main fruit growing area especially for citrus, by the national company "SONAPROV", following the instructions of the Ministry of Agriculture. When the medfly populations tend upwards and their level reached the thresholds of 2-3 medflies/trap/day, the treatments were initiated totalising until 6 passages. However, the farmers either in the Cap bon area or in the other fruit growing areas are applying several

chemical treatments with very toxic organophosphates (OPs) (Allister Vale, 2007; Jira *et al*., 2012), especially Malathion + Lysatex (food attractant) and Dimethoate reaching 10-12 applications (personnal observations, Boulahia khedher *et al*. 2012). Since repeated use of the same class of pesticides to control a pest can cause undesirable changes in the gene pool of a pest leading to a form of artificial selection which is pesticide resistance and given the important medfly infestation and reported losses we may interpret it as an increased tolerance to malathion among tunisian populations of medfly. Indeed resistance to malathion in the field has been reported for a number of insects, including some dipteran pests (Hughes *et al*., 1984; Hemingway, 1985; Raghavendra *et al*., 1998; Hsu and Feng, 2000). For medfly, Koren *et al*. (1984) reported its potential in developing resistance to malathion after 18 generations of selection in the laboratory and it was until 2007 that a Spanish team, Magana and colleagues, identified a resistance of a Spanish field population to malathion (Magana *et al*., 2007). The resistance to malathion and other organophosphorous insecticides (OPs) could be caused by mutations on the target site of the acetylcholinesterase (Mutero *et al*., 1994; Oakeshott *et al*.,

2005), or the detoxification of the insecticide by metabolic enzymes (Ranson *et al*., 2002; Feyereisen, 2005). In the Spanish field-derived strain (W) a single point mutation Gly328Ala in the AChE gene (*Ccace*) is conferring some degree of resistance (Magana *et al*., 2007, 2008).

On the other hand, the main trade partner of Tunisia for agricultural products is Europe; this is why the Tunisian government tends to follow the European regulations that are the strictest regarding imported goods. For some years now, the Ministry of Agriculture has been trying to use spinosad in the Takelsa region (North-ouest of the Cap Bon area), which has been declared a biologically productive area and where the use of chemical pesticides was banned. He has also been trying for two or three campaigns to replace at least one malathion treatment with spinosad in the main fruit growing citrus area. The Spinosad is a natural compound obtained from the fermentation products of the soil actinomycete *Saccharopolyspora spinosa* with an insecticidal activity on the insect nervous system (Salgado, 1998; Salgado and Sparks, 2005). Spinosad is considered as a substitute to organophosphates that is efficiently active against tephritidae (Gazit *et al*., 2013).

Although natural, spinosad tolerance was reported for some fruit flies, such as for *Bactrocera olea* after the detection of an increased tolerance in areas where the insecticide has been more extensively used (Kakani *et al*., 2010). Likewise, for the Melon fly *Bactrocera cucurbitae* in Hawaii and Taiwan populations, where extensive treatments are applied (Hsu *et al*., 2012) and used even as a grain protector (Khan *et al*., 2018).

Knowing the important medfly infestation rates from which suffers the fruit production sector in Tunisia and the importance of the treatments frequency, our aim with this study is to monitor the susceptibility to malathion and spinosad in wild caught tunisian medflies after a survey carried out with farmers and to give precise informations about treatment management state.

MATERIALS AND METHODS

Ceratitis capitata *wild strain field sampling*

Medflies were obtained from periodically fruit sampling from different orchards (2009-2017) after investigation on chemical treatments (Table I). Sampling concerned 7 localities in the governorates of Tunis, Ariana and Bizerte (Fig. 1).

Laboratory strain (Vienna 8 strain)

The laboratory strain is a colony of the VIENNA 8 genetic sexing strain (GSS) maintained in the Tunisian Medfly rearing facility situated in the National Center of Nuclear Sciences and Technologies (CNSTN) since 2003 (Guerfali *et al*., 2007, 2011, 2018). Adult flies were fed with sugar: yeast (3:1) and water. The larval diet is based
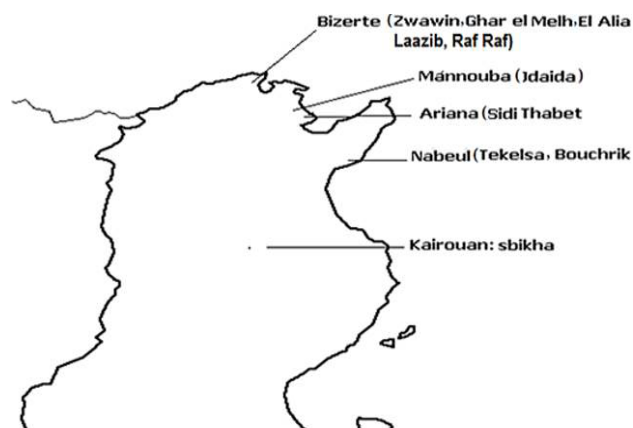

Fig. 1. Sampling localities of tunisian *Ceratitis capitata* populations.

Table I.- Year of sampling, host and spraying regimens of the fruit orchards where *C. capitata* infested fruits were collected.

Area	Population	Year	Fruit	Treatment
Bizerte /ElAlia	EABZ1209	2009	Oranges	4 /year Fyfanon 50EC
Bizerte/Laazib	LAB1210	2010	Oranges	6 /year Fyfanon 50EC
Bizerte/Zwawin	ZWB20910	2010	Peaches	Non-treated*
Bizerte/ Raf Raf	BIRA0911	2011	Figs	3to5/yearFyfanon50EC
Ariana/ Sidi Thabet1	STAR10810	2010	Figs	15/year Envidor
Ariana/ Sidi Thabet 2	STAR20611	2011	Peaches	3/year Fyfanon 50EC
Ben Arous/ Mornag M	MOBE0811	2011	Oranges	Non -treated*
Mannouba/ Jdaida 1	JDMA10911	2011	Figs	5/year Ultracid
Mannouba/ Jdaida 2	JDMA20611	2011	Oranges	Non-treated*
Nabeul/Takilsa	TKNA0411	2010	Pears	6 /year Fyfanon 50EC
Nabeul/Takilsa	TKNA0917	2017	Sour Orange	Success appat
Kairouan/ Sbikha	SBK0611	2011	Oranges	3/year Fyfanon 50EC

*Samples collected from non-treated parcels but their neighboring parcels were treated.

on the formulation originally suggested by Tanaka *et al*. (1969). It contains wheat bran as a bulking agent (28%), torula yeast as a protein source (7%), sugar as a phagostimulant and carbohydrate source (14%), and water (50%). All the experiments were carried out under laboratory conditions (23°C ±1°C and 60% RH). This strain is maintained without exposure to insecticides.

Malathion and Spinosad susceptibility feeding bioassays

For analyzing dose-response relationships of field and laboratory populations to malathion and spinosad at least 300 adults of medfly should be recovered from fruits collected across each infested field. Fruit samples were brought to the laboratory and placed in plastic trays. They were kept at an environmentally controlled rearing room, at a temperature of 26°C, until pupation occurred. Emerging adults from field-collected fruits (F_0) were used for testing their susceptibility to each pesticide. At 3-5 d of maturity emerged adults were kept in ventilated plastic dishes (89 mm diameter, 23 mm high) fed with sugar: yeast (3:1) and water. Ten to 15 pupae were contained per plastic dish. The insecticide was used mixed with diet (feeding bioassay), as in the field the exposure to malathion and spinosad is mainly by ingestion (Magana *et al*., 2007, 2008; Arrouri *et al*., 2015). The insecticide used is a commercial formulation approved for the control of *C. capitata* in Tunisia: Malathion (Fyfanon 50EC), an emulsifiable concentrate of malathion 50% (wt:vol) and Spinosad (Success Appat, Dow AgroSciences Co.). Both pesticides were diluted in water and mixed with the diet to obtain a range of concentrations. The day before the test the adults are kept without food. Three to 5 d after emergence of adults, the rearing diet was replaced by diet containing the appropriate concentration of insecticide. The prepared diet consisted of 0.9g of the mixture sugar: yeast (3:1) diluted with 100µl of the insecticide desired concentration. For Malathion concentrations were 6, 10, 30, 60, 80, 100 and 300 ppm and for Spinosad the concentrations were 10, 20, 30, 40, 70, 100, 150, 200, 240 ppm. The control consisted of diet mixed with water. The susceptibility of the laboratory population was also investigated by ingestion of the two tested pesticides. Mortality is recorded for each replication per dose 48 h after setting. We performed nine replications per dose.

Histopathological effect of spinosad on laboratory strain V8 of Ceratitis capitata

Laboratory adults were dissected immediately after the bioassay and treatment with spinosad. The chosen individuals were those exposed to the LC_{50} dose of 22ppm, 70 ppm, 100 ppm and 240 ppm. The brain of each treated individual was removed and a Bouin's fixative was used for a minimum of 24 h and a maximum of 48 h (Seroogy *et al*., 1988; Homberg *et al*., 1991). After fixation, brains were rinsed in phosphate buffered saline (PBS) five times, each time for 10 min at room temperature. The tissue was dehydrated through an ethanol series to toluen. Toluens were replaced with paraffin in increasing concentrations. Brains were placed in TissueTeck molds containing molten paraffin, mounted on TissueTek cassettes, and stored at -20°C for at least 24 h. Serial longitudinal sections at 7 microns were made by microtome (Redcamp 2030) and mounted on clean slides. Sections stained with Heidenhain's stain (Gabe, 1968) and prepared for observation and photomicroscopy (Olympus).

Data analyses

Mortality data were used to estimate the concentrations needed to cause 50% mortality (LC_{50}) by probit analysis using the computer program POLO-PC (LeOra Software, Berkeley, CA) (Russell *et al*., 1977) Which automatically corrected for control mortality by Abbott's transformation.

Table II.- Susceptibility to malathion and Spinosad of wild populations of *Ceratitis capitata*.

Population	Host	Treatment	Bioassay	N	Slope±SE	LC$_{50}$	Chi²	d.f
EABZ1209	Orange	Malathion	Malathion	240	1.736±0.18	46.39 (28.67-67.76)	143,76	43
LABZ0210	Orange	Malathion	Malathion	280	2.082±0.22	48.74 (36.66-62.07)	58,118	34
ZWBZ0910	Peaches	Non treated	Malathion	360	1.98±0.23	131.31 (96.12-200.15)	41.25	26
BIRA0911	Figs	Malathion	Malathion	381	2.19±0.24	32.17 (26.27_39.47)	13.92*	18
STAR0810	Figs	Envidor	Malathion	167	1.34±0.28	75.619 (33.61-138.18)	17.94*	13
STAR0611	Orange	Malathion	Malathion	216	1,793±0.54	29.51 (6.44-43.01)	10,71*	10
MOBE0811	Figs	Non treated	Malathion	382	1.66±0.16	28.72 (22.24-37.24)	29.74*	26
JDMA10911	Pears	Ultracid	Malathion	281	1.49±0.27	27.14 (15.46-43.37)	54.06*	18
JDMA20611	Oranges	Non treated	Malathion	234	1.88±0.34	18.98 (13.76-24.2)	18.10	13
TKNA0411	Pears	Malathion	Malathion	188	1.22±0.é"	42.88 (40.53-45.23)	15.87*	16
TKNA0917	Sour oranges	Success Appât	Success Appât	270	-0.25± 0.12	127.952 (99.75-99.75)	33.34	25
SBK0611	Oranges	Malathion	Malathion	336	2.037±0.38	15.08 (9.68-24.78)	20.62*	14

All data are presented as means ± SE. Differences between groups were tested using one-way analysis of variance (ANOVA) (Stat graphics Centurion XVI) using the data that had previously been checked for normality (Finney, 1971).

RESULTS

Susceptibility of field and laboratory populations to malathion

The survey carried out within the farmers showed that 25% do not apply pesticide against medfly. Nonetheless, 75% of them are applying four to five treatments per year as they stated. The generation of the laboratory strain Vienna 8 was highly susceptible with mean LC_{50} of 33.12 ppm. The susceptibility status of field populations to malathion is shown in Table II. For all the field populations the mean LC_{50} varied between 15.08 (SBKA0611) and 131.13 ppm (ZWBZ0910). Nonetheless, six of the sampled populations were more susceptible to malathion than the laboratory population (15.08 ppm (SBKA0611), 18.98 ppm (JDMA20611), 27.14 ppm, (JDMA10911), 28.72 ppm (MOBE0811), 29.51 ppm (STAR 0611), 32.17 ppm (BIRA0911), respectively).

Analysing the LC_{50} of field populations according to the frequency of treatments with malathion, we can notice a significant difference between the groups of treated populations (F=19.53, df1=3, df2=4, p<0.01). Figure 2 indicates the LC_{50} according to the number of treatments. Group 1: 0 treatment, Group 2: 2-3 treatments, Group 3: 4-6 treatments and Group 4: more than 6 treatments. The LC_{50} is significantly increasing with the number of treatments increasing. Group 4 was 75.61 ppm for the populations treated more than 6 times. This group is 2-fold more resistant than the susceptible laboratory strain (33.12 ppm).
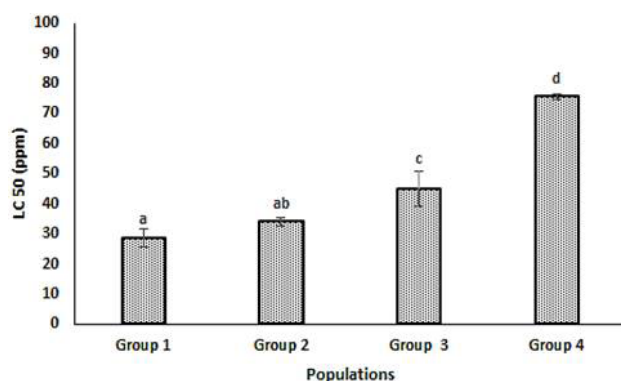

Fig. 2. LC_{50} of field populations according to the number of treatments. Group 1, 0 treatment; Group 2, 2-3 treatments; Group 3, 4-6 treatments; Group 4, more than 6 treatments.

Susceptibility of field and laboratory populations to spinosad

The susceptibility test for spinosad was carried out only for the field (Wild) population coming from Takelsa (TAK0917) because this area is treated with spinosad. The susceptibility of this population and laboratory strain of *C. capitata* to spinosad is significantly different (F=36.43, df1=4, df2=5, p<0.01). The LC_{50} of TAK0917 is about 127.95 ppm while in laboratory population the recorded LC_{50} is 22 ppm after 48 h. Laboratory population is highly susceptible to spinosad.
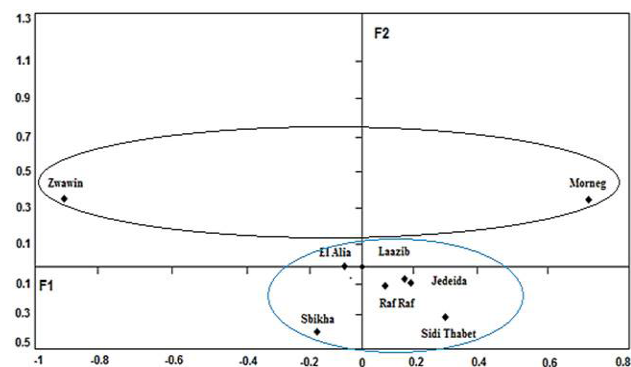

Fig. 3. Principle component analyses of LC_{50} in the different localities according to the frequency of treatments. The repartition is done on the plan Figure 1 and 2.

Histopathology

The brain of the untreated adults showed the presence of neurosecretory and glial cells with condensed chromatin within the *pars intercerebralis*. While in the treated adults, we noticed some cells' aspect change depending of the used dose. Indeed, for low doses such as 22 ppm no difference was observed from the untreated adults. However, we can notice a starting crystallisation within the cytoplasm (Fig. 4C). For the doses 50 and 100 ppm we observed chromophobe cells with condensed chromatin (Fig. 4D, E). Finally, at the dose 240 ppm which is the recommended dose for the field the glial cells are altered and cytoplasmic lesions are occurring for neurosecretory cells resulting in increased intercellular spaces.

DISCUSSION

Our survey that was conducted within the growers showed that the organization of the treatments is different from a grower to another. Nonetheless, the disparity of the medfly treatments demonstrated that 75% of them are treating 4-5 times a year which can be considred as a relatively high selection pressure. However, we noticed during the survey that the level of education of the farmers

is of prime importance and related to their application of the treatments especially that some of them have difficulties to identify the medfly and the corresponding pesticide, although the pesticides are distributed by the government through the interprofessional group of fruits (GIF).
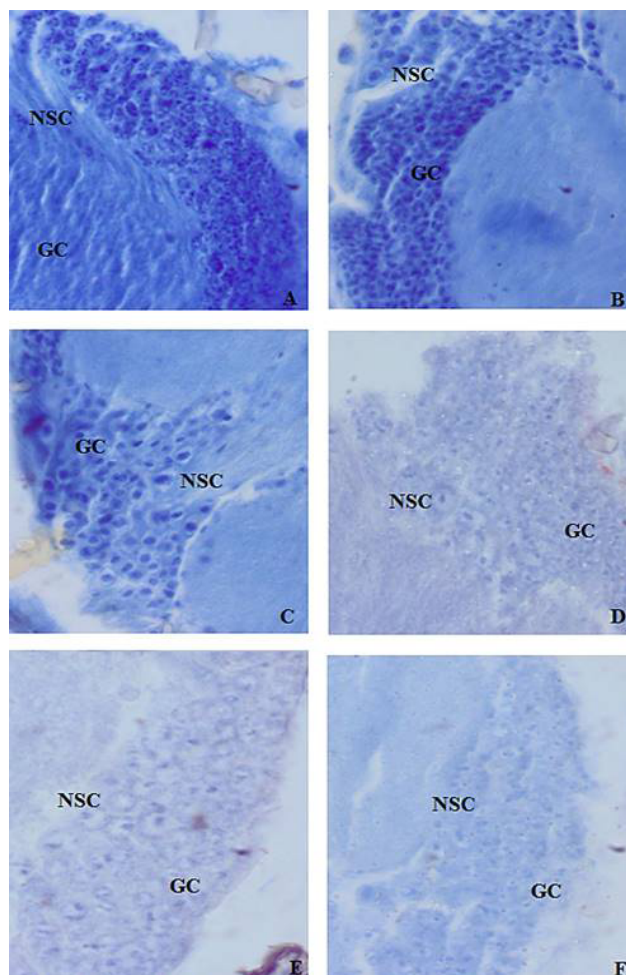

Fig. 4. Brain structure within *Ceratitis* adults treated with spinosad: A and B, controls; C, 22ppm; D, 50 ppm; E, 100 ppm; F, 240 ppm. Heidenhain's stain x1000. GC, glial cells; NSC, neurosecretory cells.

However, through this survey we tried to collect reliable information from the growers themselves because in previous field sampling operations, some of them stated that they treat 10 to 12 times a year as was observed by Khedher *et al*. (2012). We suspect that some of them feared the survey and didn't declare the exact number of the treatments. Interestingly the LC50 in our case is increasing accordingly to the frequency of treatments. However, the highest obtained LC50 was 75.61ppm for more than 6 treatments. Although in Spain, in the communidad of

Valencia 5 to 10 treatments/yr were applied and this was sufficient for the flies to develop a resistance with values of the LC 50 varying between 1000 and 3000 ppm (Magana *et al*., 2007). Nonetheless, low levels of resistance to malathion were also detected in field populations subjected to only one ground treatment per year or collected from fields that have not been treated, with values of the LC_{50} varying between 100 and 500 ppm suggesting that the resistant flies might have already dispersed from areas with high selection pressure to untreated areas. For this reason, we also analyzed adults coming from untreated fields. Moreover, the population coming from the locality of Zwawin (ZWBZ0910), where the farmers indicated after investigation that there is no treatment delivered, the value of LC_{50} is about 131.31ppm. This population has a significant low tolerance to the malathion compared to the laboratory strain (4-fold). Magana *et al*. (2007) considered that values of LC_{50} between 100 and 500ppm are considered to be low level of resistance.

These findings let us to suppose that in Tunisian medfly populations, low levels of resistance are already established, whereas we still need to investigate for high levels of resistance.

As the resistance to malathion and other organophosphorous insecticides (OPs) could be caused by mutations on the target site of the acetylcholinesterase (Mutero *et al*., 1994; Oakeshott *et al*., 2005), thus it is necessary to investigate whether these or other mutations are present in survivals with the highest LC50 values in order to obtain a coding sequence of the AChE gene for *C. capitata* (*Ccace*).

Magana *et al*. (2008), demonstrated that in the resistant strain (W) established from a field population collected in eastern Spain (Castellon) in 2004 and of which each generation was treated with malathion (about 3000ppm for 24–48 h), in order to maintain the selection pressure (50–70% of mortality); the resistance is multifactorial, with a single point mutation Gly328Ala in the AChE conferring some degree of resistance. This was also observed with other insects such as the 49R strain of the house fly, the Gly328Ala mutation is the only mutation observed, and it confers highest insensitivity to malaoxon (Walsh *et al*., 2001). Similarly, in a survey of *D. melanogaster* populations, three strains carrying only the Gly328Ala mutation were detected (Menozzi *et al*., 2004). In the case of the Tunisian strain, investigations should be carried at the molecular level to detect Single Nucleotide Polymorphism (SNP) with population presenting the highest LC50. It is worth mentioning that ElFekih *et al*. (2014) reported that they detected this mutation in Tunisian malathion treated populations (6 treatments/year) collected from only one area *i.e.* Bizerte. One out of the 27 collected flies was

heterozygous for the mutation. This work reinforces our hypothesis about the probable existence of resistance within the wild strain of the Tunisian medfly population.

But we should note that previous studies reported other point mutation in the AChE gene, such as the glycine-serine substitution (G488S) in *B. oleae* (Vontas *et al*., 2002) as well as the substitution (Q643R) found in *B. dorsalis* (Hsu and Feng, 2006). These mutations are also of great interest and should be investigated.

Spinosad was for long time considered as a good candidate and an alternative for the management of resistance problems. However, this is a misconception since it was demonstrated as for malathion, the appearance of relatively high levels of tolerance in areas where spinosad was routinely used (Kakani *et al*., 2010). The current level of tolerance in Tunisian field populations is satisfactory, 127 ppm is achieving 50% of mortality while the recommended dose in the field is 240 ppm. However, survivals from the bioassay doses that are up to the LC_{50} should be investigated and further analyzed. Anyway, our histopathology analyses on the laboratory strain have proved interesting and showed that for doses up to LC_{50}, annoying effects begin to appear in glial and neurosecretory cells. This is interesting since the glial cells are implicated in mediating and regulating behavior and physiology within adult insects (Jackson *et al*., 2014). They are involved in different important pathways such as metabolic homeostasis, lipid metabolism and long term memory, which make their damage critical even for the survivors.

Thus, we could not neglect the important role of the treatments management in the appearance of resistance. For instance in the Spanish population other operational factors may have contributed to the development of resistance such as the higher amounts of active ingredient used in the formulations, the expansion of extra early varieties of *citrus* extremely sensitive to the attack of medfly, and the switch from control measures based on malathion and fenthion to only malathion (Magana *et al*., 2007, 2008). In Tunisia the management of the treatment is differing between neighbour growers and different from a year to another. This kind of management may be the cause of the breaking of the selection pressure suffered by flies. The absence of resistance mutation does not mean that growers do not apply too many insecticides but that there is a random application. Malathion is used in Tunisia since 50 years, its use is banned from the European union since 2009, which is restrictive also for the fruits export, using spinosad as an alternative or a substitute will, with time, also conduce to tolerance.

An exclusive use of malathion and spinosad generate resistance. A cross-resistance phenomenon was also detected for laboratory strain of *B. dorsalis* between malathion and spinosad (Hsu and Feng, 2006). Thus a long term use of one pesticide or the other, even in rotation, for an integrated pest management program can be problematic. A rotation of insecticides with different modes of action and that does not show cross-resistance is desirable in insect resistance management programs.

CONCLUSION

A resistance phenomenon is suspected for malathion. This resistance can also take place with spinosad even if it is a biological pesticide. A rotation of insecticides with different modes of action is desirable in insect resistance management programs. Integrating biological methods to use pesticides in a sustainable way can help reduce the risk of resistance.

Statement of conflict of interest
The authors declare no conflict of interest.

REFERENCES

AllisterVale, J., 2007. Nerve agents: Why they are so toxic and can poisoning from these agents be treated? *Toxicology*, **240**: 141-142. https://doi.org/10.1016/j.tox.2007.06.023

Arouri, R., Le Goff, G., Hemden, H., Navarro-Llopis, V., M'saad, M., Castañera, P., Feyereisen, R., Hernández-Crespo, P. and Ortego, F., 2015. Resistance to lambda-cyhalothrin in Spanish field populations of *Ceratitis capita*ta and metabolic resistance mediated by P450 in a resistant strain. *Pest Manage. Sci*., **71**: 1281-1291. https://doi.org/10.1002/ps.3924

ElFekih, S., Shannon, M., Haran, J. and Vogler, A.P., 2014. Detection of the acetylcholinesterase insecticide resistance mutation (G328A) in natural populations of *Ceratitis capitata*. *J. econ. Ent*., **107**: 1965-1968. https://doi.org/10.1603/EC14166

Feyereisen, R., 2005. Insect cytochrome. In: *Comprehensive molecular insect science* (eds. L.I. Gilbert, K. Iatrou and S.S. Gill). Elsevier, Oxford, United Kingdom, pp. 1-77. https://doi.org/10.1016/B0-44-451924-6/00049-1

Finney, D.L., 1971. *Probit analysis*, 3rd ed. Cambridge University Press, Cambridge, UK, pp. 333.

Gabe, M., 1968. *Techniques histologiques*. Masson et Cie Editeurs, Paris, pp. 1113.

Gazit, Y., Gavriel, S., Akiva, R. and Timar, D., 2013. Toxicity of baited spinosad formulations to *Ceratitis capitata*: From the laboratory to the

application. *Ent. Exp. Appl.*, **147**: 120-125. https://doi.org/10.1111/eea.12051

Guerfali, M.M., Raies, A., Ben Salah, H., Loussaief, F. and Caceres, C., 2007. Pilot Mediterranean fruit fly *Ceratitis capitata* rearing facility in Tunisia: Constraints and prospects. In: *Area-wide control of insect pests: From research to field implementation* (eds. M.J.B. Vreysen, A.S. Robinson and J. Hendrichs). Springer: Dordrecht, The Netherlands, pp. 535-543. https://doi.org/10.1007/978-1-4020-6059-5_50

Guerfali, M.M., Parker, A., Fadhl, S., Hemdane, H., Raïes, A. and Chevrier, C., 2011. Fitness and reproductive potential of irradiated mass-reared Mediterranean fruit fly males *Ceratitis capitata* (Diptera: Tephritidae): Lowering radiation doses. *Fla Entomol.*, **94**: 1042-1050. https://doi.org/10.1653/024.094.0443

Guerfali, M.M., Djobbi, W., Charaabi, K., Hamden, H., Fadhl, S., Marzouki, W., Dhaouedi, F. and Chevrier, C., 2018. Evaluation of *Providencia rettgeri* pathogenicity against laboratory Mediterranean fruit fly strain (*Ceratitis capitata*). *PLoS One*, **13**: e0196343. https://doi.org/10.1371/journal.pone.0196343

Hemingway, J., 1985. Malathion carboxylesterase enzymes in *Anopheles arabiensis* from Sudan. *Pest. Biochem. Physiol.*, **23**: 309-313. https://doi.org/10.1016/0048-3575(85)90091-4

Homberg, U., Davis, N.T. and Hildebrane, J.G., 1991. Peptide-immunocytochemistry of neurosecretory cells in the brain and retrocerebral complex of the sphinx moth *Manduca sexta*. *J. comp. Neurol.,* **303**: 35-52. https://doi.org/10.1002/cne.903030105

Hsu, J.C. and Feng, H.T., 2000. Insecticide susceptibility of the Oriental fruit fly (*Bactrocera dorsalis* (Hendel)) (Diptera: Tephritidae) in Taiwan. *Chinese J. Ent.,* **20**: 109-118.

Hsu, J.C. and Feng, H.T., 2006. Development of resistance to spinosad in Oriental fruit fly (Diptera: Tephritidae) in laboratory selection and cross-resistance. *J. econ. Ent.,* **99**: 931-936. https://doi.org/10.1093/jee/99.3.931

Hsu, J.C., Haymer, D.S., Chou, M.Y., Feng, H.T., Chen, H.H., Huang Y.B. and Mau, RF., 2012. Monitoring resistance to spinosad in the melon fly (*Bactrocera cucurbitae*) in Hawaii and Taiwan. *Sci. World J.*, **2012**: 750576. https://doi.org/10.1100/2012/750576

Hughes, P.B., Green, P.E. and Reichmann, K.G., 1984. Specific resistance to malathion in laboratory and field populations of the Australian sheep blow fly, *Lucilia cuprina* (Diptera: Calliphoridae). *J. econ.*

Ent., **77**: 1400-1404. https://doi.org/10.1093/jee/77.6.1400

Jackson, M.D., Shapiro, M. and Shepard, B.M., 2014. Effects of spinosad and neem on the efficacy of a nucleopolyhedro virus on Pickleworm larvae. *J. Agric. Urban Ent.*, **30**: 28-37. https://doi.org/10.3954/JAUE13-10.1

Jira, D., Janousek, S., Pikula, J., Vitula, F. and Kejlova, K., 2012. Toxicity hazard of organophosphate insecticide malathion identified by *in vitro* methods. *Neuro Endocrinol. Lett.*, **33**(Suppl. 3): 53-59.

Kakani, E.G., Zygouridis, N.E., Tsoumani, K.T., Seraphides, N., Zalom, F.G. and Mathiopoulos, K.D., 2010. Spinosad resistance development in wild olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) populations in California. *Pest Manage. Sci.*, **66**: 447-453. https://doi.org/10.1002/ps.1921

Khan, H.A.A., Akram, W., Lee, S., Manzoor, S., Ayub, S.R., Rehman, K.U., Ali, S.W., Chattha, M.B. and Maqsood, S., 2018. Monitoring susceptibility to spinosad in three major product-stored insect species from Punjab. *Pakistan. J. Zool.,* **50**: 1355-1360.

Kheder, S.B., Trabelsi, I. and Aouadi, N., 2012. From chemicals to IPM against the Mediterranean fruit fly *Ceratitis capitata*. In: *Integrated pest management and pest control current and future tactics* (eds. L. Marcelo, M.L. Larramendy and S. Soloneski). InTech Open, pp. 301-320.

Koren, B., Yawetz, A. and Pery, AS., 1984. Biochemical properties of characterizing the development of tolerance to malathion in *Ceratitis capitata* Wiedmann (Diptera: Tephritidae). *J. econ. Ent.,* **77**: 864-867. https://doi.org/10.1093/jee/77.4.864

Liquido, N.J., Cunningham, L.A. and Nakagawa, S., 1990. Host plants of Mediterranean fruit fly (Diptera: Tephritidae) on the Island of Hawaii (1949-1985). *J. econ. Ent.*, **83**: 1863-1878. https://doi.org/10.1093/jee/83.5.1863

Liquido, N.J., McQuate, G.T. and Suiter, K.A., 2013. *Medhost: An encyclopedic bibliography of the host plants of the Mediterranean fruit fly,* Ceratitis capitata *(Wiedemann)*, version 1.1. United States Department of Agriculture, Center for Plant Health Science and Technology, Raleigh, N.C.

Magana, C., Hernandez-Crespo, P., Brun-Barale, A., Couso Ferrer, F., Bride, J.M., Castanera, P., Feyreisen, R. and Ortego, F., 2008. Mechanisms of resistance to malathion in the medfly *Ceratitis capitata*. *Insect Biochem. mol. Biol.*, **38**: 756-762. https://doi.org/10.1016/j.ibmb.2008.05.001

Magana, C., Hernandez-Crespo, P., Ortego, F. and Castanera, P., 2007. Resistance to malathion in field populations of *Ceratitis capitata*. *J. econ. Ent*., **100**: 1836-1843. https://doi.org/10.1093/jee/100.6.1836

Menozzi, P., An-Shi, M., Lougarre, A., Hua-Tang, Z. and Fournier, D., 2004. Mutations of acetylcholinesterase which confer insecticide resistance in *Drosophila melanogaster* populations. *BMC Evol. Biol.,* **4**: 4. https://doi.org/10.1186/1471-2148-4-4

Mutero, A., Pralavorio, M., Bride, J. and Fournier, D., 1994. Resistance associated pont mutations in insecticie-insensitive acetylcholinesterase. *Proc. natl. Acad. Sci. U.S.A*., **91**: 5922-5926. https://doi.org/10.1073/pnas.91.13.5922

Oakeshott, J.G., Claudianos, C., Campbell, P.M., New comb, R. and Russell, R.J., 2005. Biochemical genetics and genomics of insect esterases. In: *Comprehensive molecular insect science* (eds. L.I. Gilbert, K. Iatrou and S.S. Gill). Elsevier, Oxford, United Kingdom, pp. 309-381. https://doi.org/10.1016/B0-44-451924-6/00073-9

Raghavendra, K., Subbaraos, S.K., Pillai, M.M.K. and Sharma, P.V., 1998. Biochemical mechanisms of malathion resistance in Indian *Anopheles culicifacies* (Diptera: Culicidae) sibling species A, B, and C: Microplate assays and synergistic studies. *Annls. entomol. Soc. Am.,* **91**: 834-839. https://doi.org/10.1093/aesa/91.6.834

Ranson, H., Claudianos, C., Ortelli, F., Abgrall, C., Hemingway, J., Sharakhova, M.V., Unger, M.F., Collins, F.H. and Feyereisen, R., 2002. Evolution of supergene families associated with insecticide resistance. *Science*, **298**: 179-181. https://doi.org/10.1126/science.1076781

Russell, R.M., Robertson, J.L. and Savin, N.E., 1977. POLO: A new computer program for probit analysis. *Bull. entomol. Soc. Am*., **23**: 209-213. https://doi.org/10.1093/besa/23.3.209

Salgado, V.L. and Sparks, T.C., 2005. The spinosyns: Chemistry, biochemistry, mode of action, and resistance. In: *Comprehensive molecular insect science* (eds. L.I. Gilbert, K, Latrou and S.S. Gill). Elsevier, Oxford, pp. 137-173. https://doi.org/10.1016/B0-44-451924-6/00078-8

Salgado, V.L., 1998. Studies on the mode of action of spinosad: Insect symptoms and physiological correlates. *Pestic. Biochem. Physiol.,* **60**: 91-102. https://doi.org/10.1006/pest.1998.2332

Seroogy, K., Tsuruo, Y., Walsh, J., Fahrenkrug, J., Emson, P.C. and Goldstein, M., 1988. Further analysis of presence of peptides in dopamine neurons. Cholecystokini, peptide histodine-isoleucine/vasoactive intestinal polypeptide and substance P in rat upramammillary region and mesencephalon. *Exp. Brain Res.,* **72**: 523–534. https://doi.org/10.1007/BF00250598

Tanaka, N., Steiner, L.F, Ohinata, K. and Okamoto, R., 1969. Low-cost larval rearing medium for mass production of oriental and Mediterranean fruit fly. *J. econ. Ent*., **62**: 970–971. https://doi.org/10.1093/jee/62.4.967

Vontas, J.G., Hejazi, M.J., Hawkes, N.J., Cosmidis, N. and Lo, M., 2002. Organophosphate insensitive acetylcholinesterase, in the olive fruit fly *Bactrocera oleae*. *Insect mol. Biol.,* **11**: 329-336. https://doi.org/10.1046/j.1365-2583.2002.00343.x

Walsh, S.B., Dolden, T.A., Moores, G.D., Kristensen, M., Lewis, T., Devonshire, A.L. and Williamson, M.S., 2001. Identification and characterization of mutations in housefly (*Musca domestica*) acetylcholinesterase involved in insecticide resistance. *Biochem. J*., **359**: 175-181. https://doi.org/10.1042/bj3590175