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Review Article
Current Status and Future Prospects of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) Control in Morocco

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Abstract

The Mediterranean fruit fly (medfly), *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is the major fruit fly insect pest in Morocco. It causes significant annual loss in fruit production and quality. It also poses serious limitations to growers in terms of export into medfly-free areas like the USA, Russia, China and Japan. The objectives of this review are to present a synthesis of data related to medfly-control methods in Moroccan citrus orchards and highlight the key findings from previous studies, discuss current control methods and investigate the potential of innovative ongoing and environmentally-friendly approaches as possible new management tools. Medfly control in Morocco has been primarily based on organophosphate and pyrethroid sprays. Recently, the organic compound spinosad and mass-trapping have also become prominent tools for managing this pest. The use of the sterile insect technique (SIT) has been approved in many countries as an alternative to chemical treatments and a pilot project is currently being validated in Morocco. Provided successful outcomes for this project, SIT can potentially become the main control method against medfly in all citrus-growing areas in Morocco. These studies involved (i) The use of soil of *Argania spinosa* (Sapotaceae) that has been shown to contain pathogenic *Beauveria bassiana* and *Bacillus thuringiensis* isolates, (ii) The introduction of parasitoids like *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) and (iii) The use of a self-limiting genetic approach that can effectively suppress insect pest populations for improved public health and agricultural returns. This review highlights the diverse current and prospective methods used to control the medfly population in Morocco. These include the development of new biopesticides, biological control and the use of a self-limiting genetic approach. A combination of all or several of these techniques as part of an integrated medfly-management approach would provide the most holistic and effective strategy.

Key words: *Ceratitis capitata*, mediterranean fruit fly, Tephritidae, citrus, insect control

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The citrus-growing area in Morocco has gradually grown, reaching 125,000 ha in marketing year 2015/2016 with a total production of more than 2 million tons[1]. The Moroccan government has fixed an important production target that is estimated to reach 2.9 million tons by Hanson and Fardaoussi[2]. Further plans include spending allocations for 200,000 t of fresh citrus for juice production[3]. The main production areas in Morocco are Souss, Gharb, Moulouya, Tadla and Haouz[2-4]. Since the 1990s, the Moroccan citrus market has changed remarkably. Exports to the European Union have decreased from 70% to only 25-30%[5]. Depending on the marketing year, Russia currently absorbs 45-50% of all Moroccan exports[6]. Some of the constraints that growers need to address are the scarcity of water after several years of severe drought and marketing (due to strong competition in the international citrus market).

In addition to the above constraints, pest management is also a major challenge for citrus growers due to the high fruit-quality standards demanded by the market. Effective citrus pest management provides fruit with almost no pest presence or damage, while leaving minimal pesticide residues[7]. The key citrus pests that dictate which control strategies are used, include: The Mediterranean fruit fly [*Ceratitis capitata* Wiedemann (Diptera: Tephritidae)], California red scale [*Aonidiella aurantii* Maskell (Hemiptera: Diaspididae)], citrus red mite [*Panonychus citri* McGregor (Acari: Tetranychidae)] and the citrus leaf miner [*Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)][4,8]. Recently, the citrus brown mite [*Eutetranychus orientalis* Klein (Acari: Tetranychidae)] and the red spider mite [*Tetranychus urticae* Koch (Acari: Tetranychidae)] have also been reported to significantly affect citrus production[4].

Among the key citrus pests, the Mediterranean fruit fly (referred to as 'medfly') is certainly the most damaging, with both direct and indirect economic impacts[4,8-10]. Average fruit infestation can reach 10-20% or more, despite control efforts during years of high medfly prevalence or due to the incorrect application of treatments. Growers are required to continuously monitor medfly prevalence during the fruit-maturation period and potentially apply several insecticide sprays to control this insect pest. In addition, because medfly is a quarantine pest for some of the more important fruit-importing countries, such as Japan and USA, export of fruit to these countries requires cold treatment, which raises costs[8].

Despite efforts made to control this pest, it is still considered an imminent threat to Moroccan citrus exports and consequently to the entire citrus industry in the country. In February, 2016, the Department of Agriculture's Animal and Plant Health Inspection Service had to temporarily prohibit the importation of tangerines, clementines, mandarins and sweet oranges produced in, packaged in, moved through or shipped from the Berkane region in Morocco because of medfly infestation[11].

This review aimed to summarize Morocco's diverse current and prospective methods used to control the medfly population and motivate for the implementation of an integrated medfly-management strategy that is based on environmentally-friendly methods, such as the development of new biopesticides, biological control or the use of a self-limiting genetic approach. The study objectives were to present a synthesis of data related to medfly-control methods in Moroccan citrus orchards, highlight the key findings from previous studies and provide an overview of current and prospective approaches that were used to manage this pest.

Medfly status in Morocco: The medfly is the major tephritid pest in Morocco and considered to be a serious threat to fruit production, diversification and export[12]. Nonetheless, this fly pest can be seen in traps year-round and its spatiotemporal occurrence and phenology depend on suitable environmental conditions, host plants and fruit availability[13-16]. According to the same authors, *Argania spinosa* from the Sapotaceae forest (7,000 ha) and *Opuntia ficus-indica* represent enormous potential reservoirs of medflies for nearby citrus orchards, particularly in the Souss valley (the main citrus-producing area in Morocco), which may negatively impact the eradication process in this area.

In addition to citrus, other traditional fruits, such as pears, apples, peaches, nectarines, plums and pomegranates are also affected by medflies, especially in Northern Morocco, but these crops are less widely grown commercially[17].

Control methods: Currently, pest management in Moroccan orchards is heavily reliant on chemical control. However, the implementation of ecological methods is slowly gaining momentum due to public concerns over chemical residues, medfly resistance to existing compounds and the withdrawal of several chemicals. Some eco-friendly approaches have shown efficacy in pilot studies and could be made ready for practical use to meet the new market requirements concerning fruit quality, a healthy environment, human health and good agricultural practices. Thus, alternative methods to chemical control such as mass trapping[18], cold treatment[19], the sterile insect technique (SIT)[20] and biopesticides[21-24] are under investigation. Other techniques, such as the sustained release of self-limiting medfly strains[25,26] are also being considered.

Chemical control: Chemical control is the most widely used practice in Morocco against medflies. Treatments are applied with a full-cover spray and a wide range of broad-spectrum pesticides, which are rarely extremely disruptive to beneficial insects. Alternatively, foliar spot treatments (1 row out of 3 or 4) using hydrolyzed protein baits comprises the other main chemical application methodology that is currently used[8]. The baits are based on the behavior and general biology of medflies. Data from several studies[27-29] have demonstrated that ingestion of proteins in the adult phase contribute to male medfly fitness by enhancing various aspects of male sexual behavior, such as participation in leks, pheromone production, the rate of copulation and the rate of sperm transfer. Protein is also necessary for egg production in females. Natural protein sources can include: Rotten fruit, bacteria and bird droppings. Bait sprays consist of an insecticide and a source of protein to attract adult medflies (Table 1). By combining both approaches in one formulation, the bait is attractive as a food source to both male and female medflies, which forage over the leaves for food.

Recently, the organically registered compound "Spinosad" has become the preferred chemical treatment, especially during the harvest period, because of its short sustainability in the environment and its pre-harvest interval of 1 day. Adult pest prevalence fruit and infestation define the commencement and frequency of chemical sprays. The monitoring threshold that indicates when spraying should commence is 3-5 adult males/(trap•day). However, in small farms, sprays are applied once every 8-10 days, in late summer and autumn for the early citrus varieties and at the end of winter to spring, for the late varieties, depending on the region[4]. However, to comply with Russian quarantine regulations of imported products, an agreement has been signed with Russia aiming to reinforce medfly control by growers interested in the Russian market and which has reduced the chemical treatment threshold to 0.5 females/(trap•day) or 1 male/(trap•day)[30].

Mass trapping: Mass trapping is an alternative and ecologically compatible insect-control method based on the

Table 1: Registered chemical compounds used to controlling medflies [*Ceratitis capitata* Wiedemann (Diptera: Tephritidae)] in Morocco[61]

Active ingredient	Commercial product	Concentration	Dose	Pre-harvest interval (days)
Deltamethrin	DECIS FLUXX	25 g L^{-1}	125 cc ha^{-1}	7
	DEXTRA 110	50 cc ha^{-1}	50 cc ha^{-1}	7
	SHARDELTA	28 g L^{-1}	100 cc ha^{-1}	7
	GRAFITI	25 g L^{-1}	125 cc ha^{-1}	7
	DELTAPLAN 25 EC	25 g L^{-1}	30 cc hL^{-1}	7
	DECIS EXPERT	100 g L^{-1}	50 cc ha^{-1}	7
	DELTA 2.5 EC	25 g L^{-1}	125 cc hL^{-1}	7
Malathion	KEMALAT 50 EC	500 g L^{-1}	200 cc hL^{-1}	7
	MALATHION 50	500 g L^{-1}	200 cc hL^{-1}	7
	POLATHION 50	500 g L^{-1}	200 cc hL^{-1}	7
	SIF MALATHION 50	500 g L^{-1}	200 cc hL^{-1}	7
	MALAPRON	500 g L^{-1}	200 cc hL^{-1}	7
	MALYPHOS 50	500 g L^{-1}	200 cc hL^{-1}	7
Lambda cyhalothrin	JACKPOT 50 G/L	50 g L^{-1}	100 cc ha^{-1}	7
	KARIS 10 CS	10%	50 cc hL^{-1}	7
	WARRIOR 10 CS	100 g L^{-1}	20 cc hL^{-1}	7
	KENDO	100 g L^{-1}	20 cc hL^{-1}	7
	SANTRI	50 g L^{-1}	100 cc ha^{-1}	7
	OSMOZE	50 g L^{-1}	100 cc ha^{-1}	7
	RAVANE 50	50 g L^{-1}	100 cc ha^{-1}	7
	REEVA 5 EC	50 g L^{-1}	100 cc ha^{-1}	7
	BILAD	50 g L^{-1}	100 cc ha^{-1}	7
	VAJRA	50 g L^{-1}	100 cc ha^{-1}	7
	KARATE	50 g L^{-1}	100 cc ha^{-1}	7
	TSUNAMI	5%	100 cc ha^{-1}	7
Azadirachtin	OIKOS	31.95%	80 cc hL^{-1}	3
Dimethoate	DANADIM PROGRESS	400 g L^{-1}	75 cc hL^{-1}	14
Spinosad	SUCCESS APAT	0.24 g L^{-1}	1 L ha^{-1}	1
Beauveria bassiana	NATURALIS-L	7.16%	2 L ha^{-1}	Not required
Fenthion	LEBAYCID 50 EC	500 g L^{-1}	0.3-0.5 L ha^{-1}	15
Lufenuron (in traps)	ADDRESS RBO3	3%	20 traps ha^{-1}	Not required
Cyantraniliprole	EXIREL TM	100 g L^{-1}	100 cc ha^{-1}	7

Table 2: Registered attractants and traps used to control medflies [*Ceratitis capitata* Wiedemann (Diptera: Tephritidae)] in Morocco[61]

Active ingredient	Commercial product	Concentration	Dose
Protein hydrolysate	BLOUZ	30%	1-1.5 L/200 L ha^{-1}
	PICK UP		
	CERA-TRAP	95%	100 traps ha^{-1}
Ammonium acetate	FLYCAP MASS	0.19%	40 traps ha^{-1}
1,5-Diaminopentane		43.49%	
Trimethylamine hydrochloride		4.10%	
1,4-Diaminobutane	MAGNET MED	0.10%	50 traps ha^{-1}
Ammonium acetate		14.81%	
Deltamethrin		0.03%	
Trimethylamine hydrochloride		6.28%	
1,4-Diaminobutane	CONETRAP CERATITIS	0.30%	50 traps ha^{-1}
Ammonium acetate		74.70%	
Lambda cyhalothrin		0.04 g/trap	
Trimethylamine hydrochloride		8.30%	
Alpha-cypermethrin	M3 FRUIT FLY BAIT STATION	0.01 g/trap	400 traps ha^{-1}
Plants extract		-	
Protein hydrolysate		5 g/trap	
Nitrogen	STARCE	8%	20 cc L^{-1} of water and 0.5 L of mixture/trap
Phosphorus pentoxide		16%	

use of species-specific synthetic chemical lures, such as sex and aggregation pheromones or food/host attractants. Semiochemical-based, pest-management programs comprise three major approaches that are used as environmentally friendly control methods of insect pests: mass trapping, "attract and kill," and mating disruption[31]. Medfly mass trapping has been widely applied through the development of a synthetic food-based female attractant: A highly selective and effective combination of three chemical compounds developed for attracting female Mediterranean fruit flies[32]. It has proven to be highly effective in controlling *C. capitata* and its application in Mediterranean countries has increased notably as a consequence[33-36].

Several types of traps are registered for use in Morocco in mass trapping. A comprehensive list of these traps is shown in Table 2.

Different types of synthetic attractants, which have a long life span and are relatively easy to handle, have been developed to replace protein solutions. Examples of such food attractants are trimethylamine hydrochloride, ammonium acetate and 1,5-Diaminopentane (Putrescine), combined into one patch or in separate patches. These substances are highly female medfly-selective and have an action duration of at least 90 days[35].

In Moroccan areas, the "attract and kill technology," which involves using the M3 bait station (blue plastic trap impregnated with protein hydrolysate and alpha-cypermethrin) is very effective, resulting in zero spray applications in areas where M3 was set[37].

Generally, the use of mass trapping on its own or as part of an integrated medfly pest-management program, enables the reduction of chemical overuse. Consequently, mass trapping helps minimize environmental pollution, issues with chemical residues and pest proliferation, such as mites, scales, aphids and other secondary pests which cause damage to citrus production[36,38].

Cold treatment: Various citrus fruit-importing countries, such as the USA and Japan, request quarantine security protocols to avoid the risk of accidental introduction of insect pests and disease from imported fruit. The medfly belongs to a group of pests with the highest risk of introduction to pest-free (i.e., medfly-free) countries or regions[39].

Moroccan fruit growers (and growers in all countries in the Mediterranean basin where medflies occur) are therefore required to apply certain quarantine treatments prior to citrus export. In Morocco, this treatment is accomplished by fruit refrigeration at temperatures between 0-2°C for a period of 16 days. However, this method is costly and has many limitations, for instance the difficulty of reaching far away markets and the susceptibility of some varieties (such as clementines and tangerines) to low temperatures[40]. By following the strict standards and respecting market access regulations, Morocco's citrus exports to the U.S. market increased from 7,800 t in 2006/2007 to 42,011 t in the marketing year 2013/2014 (mostly clementine varieties). To counter the logistical constraint, Morocco exported citrus directly from Agadir to the U.S. for the first time in November, 2012. Previously, Morocco was forced to send these shipments via Europe for cold-treatment applications[2,41,42]. The direct shipment to the U.S. and the implementation of the U.S.-Morocco Free Trade Agreement were expected to raise Morocco's citrus exports to the U.S.

Likewise, China requires the treatment of commodities for a range of pests, including medflies that are indigenous to the regions from which the commodities originate. Moroccan citrus fruit that is destined for the Chinese market should be exposed to a minimum temperature of 1°C for a period of 16 days[43]. To penetrate other international markets, such as Japan (which is a promising destination for several Moroccan products) and other countries where medflies are considered quarantine pests, an agreement was signed between Maroc Citrus, ONSSA, IAV Hassan 2 and the SAOAS Company for the implementation of a cold-treatment experimental protocol delivered by Japanese authorities. The main objective of the study conducted by Anonymous[19] was to prove the effectiveness of cold treatment on infested fruit and fell within the overall strategic objective of eliminating the risk of medfly introduction to Japan[19].

Sterile insect technique: This technique was developed during the 1930s and 1940s in different countries[44]. The key researchers were A.S. Serebrovskii at Moscow State University, F.L. Vanderplank at a Tsetse Field Research Station in rural Tanganyika (now Tanzania) and E.F. Knipling of the United States Department of Agriculture[45].

Due to the increase in customer demand for organic products and the continuous restrictions imposed on the use of insecticides, SIT has become increasingly viewed as a suitable technique as part of an area-wide integrated pest-management (AW-IPM) program for some agricultural pests[46]. Currently, SIT is included in several medfly AW-IPM programs as the major control strategy in relation to the type of area i.e., as a suppression strategy in Israel, Jordan, Madeira, South Africa, Spain and Tunisia as a containment measure in Argentina, Chile and Mexico and as a prevention strategy in California and Florida (USA)[46].

Since 2005, an agreement to establish an area-wide SIT program against the Mediterranean fruit fly has been approved by the Ministry of Agriculture of Morocco and the Citrus Producers, with the main aim of establishing low-prevalence zones in the Agadir region[4,8]. A pilot project was conducted to suppress medflies in approximately 5,000 ha in the Souss Valley using an AW-IPM approach with a SIT component. Each week, 8 million sterile male flies were released that were initially imported from Madeira in Portugal (2008-2011) and later from the mass-rearing facility in Valencia, Spain (2013 to present)[4]. In 2014, the Ministry of Agriculture in Morocco (in partnership with Maroc Citrus) decided to construct a medfly mass-rearing facility and a sterile male-release center, with technical assistance from FAO/IAEA. The constructions were designed to be large enough to process sufficient sterile males to cover the entire Souss Valley (40,000 ha of citrus-producing areas plus the surrounding areas). The mass-rearing, holding and release facility is expected to be capable of producing 130 million sterile males/week in its first phase and eventually 200 million sterile males/week[4,20]. However, efficacy field assessments are still in progress, prior to expansion to include all the Souss Valley[4]. The ambitious aim is that the SIT program will be applied nation-wide to cover all Moroccan citrus orchards at a later stage[20].

According to ALBRA, following 3 years of release and despite favorable climatic conditions for medflies between 2010 and 2011, the level of female medflies in the test zone decreased during 2010 and 2011 in comparison with the levels in 2009. A reduction of chemical sprays occurred according to surveys performed in 2010 that aimed to quantify the impact of SIT. The reduction rate during the 2008/2009 and 2009/2010 seasons in the test area ranged from 25-75%[20]. The completion of the SIT rearing and release facilities will ensure highly cost-effective levels of sterile medfly pupal production[20].

New potential control methods

Sustained release of self-limiting medflies: To increase the effectiveness and reduce the overall costs of SIT-like programs, research was conducted that showed that engineered sterile insects present a potential improvement to current SIT methods[47]. SIT is a mating-based technique whereby released males compete with their wild counterparts for successful mating with wild females. The use of irradiation to sterilize the insects causes a dramatic loss of competitive mating ability compared to wild-type insects. Irradiated insects are less competitive and have reduced life spans. The combination of these two factors leads to an estimated reduction in fitness by 4-10-fold for medflies[48,49]. The self-limiting technology is a novel alternative to SIT, which aims to address some of the limitations of current SIT methodologies, namely the expensive filter colony required for the stability of temperature-sensitive lethal mutation medfly strains, the use of irradiation and the ineffectiveness of fluorescent powders[25,26,50-52].

Genetic sexing for sterile male-only release can be several times more effective than releases involving mixed-sex populations[53]. The self-limiting approach provides effective genetic sexing and removes the need for the expensive and performance-reducing process of sterilization using ionizing radiation. Fluorescent marking allows for easy discrimination between wild and transgenic insects in the laboratory or field, removing the need for the use of fluorescent powders, which can be hazardous to employees and cannot always be scored for reliably, especially in damaged specimens[54].

Biological control: Despite the specificity of each region in terms of the crop-pest interactions, almost all citrus-producing areas have the following key pests; armored scales, mealy bugs, thrips, mites and fruit flies[23]. Most, if not all insect or mite pests are susceptible to attack by entomopathogens and therefore citrus pests should be seen as potential targets and investigated as such[23]. Among the natural enemies of the medfly, parasitoids of the Braconidae family are the most frequently used in biological control. Several hymenopteran parasitoids such as the egg-pupal parasitoid *Fopius arisanus* (Sonan) and the larval-pupal parasitoids *Fopius vandenboschi* (Fullaway), *Diachasmimorpha longicaudata* (Ashmead), *Diachasmimorpha tryoni* (Cameron) and *Psyttalia incisi* (Silvestri), have resulted in significant suppression of the medfly population[55-58].

Several trials were performed in Spain to assess the ability of *D. longicaudata* (Ashmead) to control medflies. The results showed that the parasitoid could disperse and parasitize medfly larvae under Mediterranean climatic conditions[59]. This parasitoid is a good candidate for testing in Morocco especially as the only medfly parasitoid present in Morocco. *Psyttalia concolor*, showed parasitism rates for reducing medfly populations that were below an economically acceptable threshold[8,15]. Thus, a project aiming to integrate *D. longicaudata* in a biological control program is underway. This parasitoid was imported from Spain in 2016 and is currently being reared under restricted conditions, prior its release in the field[60].

Use of biopesticides: Although medflies may be controlled by Moroccan growers through pesticide use, awareness of their negative effects is mounting. Consequently, biopesticides like Spinosad, Azadirachtin and *Beauveria bassiana* have been recently registered for use in Morocco[61]. These active ingredients are considered to have low environmental persistence and limited side effects to non-target organisms. Other natural products can be extracted from Moroccan soils. The use of *Bacillus thuringiensis* (Berliner) (Bt) as a biopesticide is actually one of the best chemical alternatives for controlling insect agricultural pests, as it is environmentally friendly but also economically sustainable[62]. Previous data suggested that the soil from the Argan region may be a suitable target for future and wider screening programs, in an effort to identify strains bearing toxins or combinations of them, to develop novel and more efficient Bt-based formulates[63]. Previously, it was reported that most autochthonous isolates of *Beauveria bassiana* isolated from *Argania spinosa* endemic to Moroccan forests were virulent (86.44%) to medfly pupae[22,64]. Several of these Moroccan *Beauveria bassiana* isolates might be considered as highly entomopathogenic and may serve as a source of potential biological control agents against medflies. In addition, 12 preselected actinobacteria, isolated from various Moroccan habitats were screened and were shown to have important insecticidal activities against medfly larvae, pupae and adults[24].

This review highlights the current and future prospects of Mediterranean fruit fly control methods used in Morocco, with the objective to inspire specialists in other countries to also control this key citrus pest and also serves as a roadmap for researchers to broaden their research focus, especially on lesser-known techniques such as biological control or the use of self-limiting approaches against medflies. The main limitation of this study is the current lack of references related to the medfly situation in Morocco. Hence, it would be interesting to integrate the solutions and experiences of other countries in the control of medflies in order to develop a global approach to manage this worldwide pest. Further studies on medfly population dynamics, natural enemies, feeding behavior, life history and population genetic structures are also crucial for the success of any program.

CONCLUSION

The current and prospective methods that can be used to control the medfly population in Morocco include, in addition of chemical control and mass trapping, the development of new bio pesticides, biological control or the use of a self-limiting genetic approach. A combination of all or several of these techniques as part of an integrated medfly-management approach can provide a holistic and effective strategy, with a limited negative impact on the environment.

SIGNIFICANCE STATEMENT

This review highlights Morocco's current and prospective control methods that can be used to implement an integrated medfly-management strategy, with the objective to inspire specialists in other countries to adopt or further develop more environmentally-friendly medflycontrol approaches.

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