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Pheromone-mediated mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in Tunisian vineyards: Effect on insect population dynamics

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Abstract: The mating disruption technique using Checkmate® VMB-XL pheromone dispensers, in combination with imidacloprid-based insecticide treatments through drip irrigation, was tested against vine mealybug in Tunisian vineyards for the first time in the South Mediterranean area. The number of male vine mealybugs caught in traps of mating disruption (MD) plots was significantly lower than that of Non-MD plots, indicating that the application of Checkmate® VMB-XL significantly disrupted the male-female vine mealybug sexual communication. The cumulative effect of mating disruption on vine mealybug over time reached 120 days. Two months after Checkmate® VMB-XL application, the density of mealybug nymphs and adult females on grapevine basal leaves was significantly reduced in all MD plots. The obtained results provide evidence that the application of Checkmate® VMB-XL under Center-South Tunisian vineyard conditions proved to be very effective in confusing vine mealybug males and disrupting the mating process. The eventual future use of this technique in Integrated Pest Management programs in Tunisia is discussed herein.

Key words: vine mealybug; sexual behavior; mating disruption; population biology; biotechnological pest management; Mediterranean area

Introduction

Scale insects (Coccoidea), aphids (Aphidoidea), jumping plant lice (Psylloidea) and whiteflies (Aleyrodoidea) constitute the four superfamilies of the monophyletic suborder Sternorrhyncha within the order Hemiptera (Gullan & Martin 2003). Scale insects are major pests of cultivated crops worldwide (Sforza 2008). Economically important scale insects belong to the Families Diaspididae (armored scales), Coccidae (soft scales), and Pseudococcidae (mealybugs). A pseudococcid species, the vine mealybug (VMB) *Planococcus ficus* (Signoret, 1875), has been primarily located in Mediterranean vineyards and regarded as an introduced species on grapevine in South America and South Africa (Sforza et al. 2005). This species has been considered as key economic pest worldwide (Dalla Montà et al. 2001; Walton et al. 2009; Mansour et al. 2011a; Daane et al. 2012; Reineke & Thiéry 2016). In Tunisia, the VMB is considered the most economically important, devastating scale insect species (Mansour et al. 2017). This key mealybug species produce honeydew that supports the growth of the sooty mold fungus, affecting fruit marketable quality. Furthermore, *P. ficus* was shown to transmit Grapevine Viruses A and B (Tanne et al. 1989; Minafra & Haididi 1994; Bertin et al. 2010), and Grapevine Leafroll Associated Virus III (GLRaV-III) (Mahfoudhi et al. 2009; Bertin et al. 2010; Tsai et al. 2010). In Tunisian vineyards, *P. ficus* is capable of transmitting common grapevines viruses such as the GLRaV-III and other GLRaV (Mahfoudhi et al. 2009).

A challenging issue for researchers and grape growers has been to find the most suitable control tools of the VMB. As a consequence, several control strategies have been and are currently applied to reduce outbreaks of VMB populations and attempt to reduce severe plant damages and major economic losses.

In general, the most common control tactic adopted against mealybug pests is the application of insecticide treatments (Franco et al. 2009). Effective chemical control of the VMB can be achieved successfully by applying the systemic tetramic acid insecticide spirotetramat as foliar sprays. This lipid biosynthesis inhibitor showed excellent efficacy, long residual activity against this pest (Brück et al. 2009; Mansour et al. 2010a) and no adverse side effects towards main mealybug parasitoids (Mansour et al. 2011b). As such, the use of the systemic nicotinoid insecticide imidacloprid through drip irrigation (Daane et al. 2006; Mansour et al. 2010c) and the contact biopesticide Prev-Am® as foliar sprays (Mansour et al. 2010a) could also be promising in decreasing VMB densities in vineyards. Nevertheless, pest management based solely on the repetitive applications of insecticide treatments has proven to be neither sufficiently effective nor sustainable. This is mainly due to the fact that mealybugs often reside beneath the bark of their host vines or underground (Daane et al. 2006) and to the typical waxy body cover and the clumped spatial distribution pattern of these insects (Franco et al. 2009). In addition, this could also be induced by the occurrence of pesticide resistance in targeted mealybug pest due to repetitive use of ineffective insecticides (Flaherty et al. 1982; Franco et al. 2009) and adverse side effects of broad-spectrum insecticides towards VMB natural enemies (Walton & Pringle 1999; Mgocheki & Addison 2009; Mansour et al. 2011b, 2017). Consequently, these serious issues have dictated the need to finding and implementing environmentally-safe pest management methods as potential alternative to the use of broad-spectrum insecticides.

Among eco-friendly pest control methods, the use of various pheromone-based tools to cope with VMB outbreaks on grapevine is currently the target of several research studies and has been of major concern in Tunisia and worldwide. In fact, mating disruption involves release of large quantities of synthetic sex pheromone to disrupt mate location, thus reducing the number of offspring produced in the next generation (Suckling et al. 2014). Pheromone-based monitoring systems have been developed and implemented against VMB populations (Millar et al. 2002; Walton et al. 2004; Zada et al. 2008; Franco et al. 2009; Mansour et al. 2009; Daane et al. 2012) after the identification and synthesis of the VMB sex pheromone by Hinkens et al. (2001). Additionally, VMB sex pheromone has been used as kairomone source for improving mealybugs' parasitoid host searching activity in vineyards (Franco et al. 2008, 2009; Mansour et al. 2010b) and it has been also applied in mating disruption programs, proving to be very effective in reducing mealybug densities on grapevine in the USA (California) and Italy (Daane et al. 2006; Walton et al. 2006; Cocco et al. 2014).

In the present study, we tested the effectiveness of mating disruption in decreasing *P. ficus* populations on grapevine in Center-South Tunisian vineyards. The main objectives were to assess: i) The effect of the application of mating disruption on VMB sexual communication (male behavioural response) and hence on VMB male flight activity in vineyards by using Checkmate® VMB-XL dispensers (Suterra LLC, Bend, OR-USA), which release a chemically formulated VMB sex pheromone component (lavandulyl senecioate), ii) the field longevity of Checkmate® VMB-XL dispenser under Tunisian (Center-South) vineyard conditions, and iii) the effectiveness of mating disruption in decreasing VMB life stages on grapevines.

Table 1. Climatic conditions near the experimental sites expressed by monthly means of different climatic parameters (climatic data were provided by the National Meteorological Institute of Tunisia – Station of Sidi Bouzid, the nearest meteorological station to the experimental vineyards).

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
MXT	17.6	19.1	18.5	26.1	29.3	33.3	37	38	35.3	32.3
MNT	5.7	6.7	7.4	10.3	13.7	17.7	20	20.8	20	17.7
TR	7.2	46.2	30.2	3.4	19.8	9.6	20.2	0.4	6	20.4
RH	62	62	68	56	58	50	52	50	56	57
WS	40.6	40	43.4	44.3	45.8	45	41.7	40.3	39.6	34.7

Explanations: MXT – Mean of maximum temperature (℃); MNT – Mean of minimum temperature (℃); TR – Total monthly rainfall (mm); RH – Mean of relative humidity (%); WS – Mean of maximum wind speed (km/h).

Material and methods

Trial location and growing conditions

The study was performed from April to October 2014 in three table-grape vineyards located in the region of Regueb (Center-South Tunisia). All of these vineyards have almost the same cultural practices and growing conditions: area between 0.7 and 1 ha each, drip irrigated, planted with 9-year-old grapevines (*Vitis vinifera* L.) cv. "Superior Seedless", cane-pruned and spaced 3.2 m between rows and 2.2 m within rows. The vineyard 1 (V1) has a sandy soil, whereas both the vineyards 2 (V2) and 3 (V3) have a clay soil. The grapevine fruit harvest in V1 is generally conducted about ten days earlier relative to the fruit harvest in either V2 or V3. The climatic conditions of the experimental sites are indicated in Table 1.

Experimental design

All three vineyards were divided into two separate plots: Mating Disruption (MD) plot and Non-Mating Disruption (Non-MD) plot. Each plot in each vineyard was 50 m away from the other plot. In both V1 and V2, each plot was divided into three sub-plots, while each plot in V3 (smaller area relative to the two other vineyards) was divided into 2 sub-plots. The aim of dividing plots into subplots was to perform a more representative sampling from almost all areas (grapevines) within each plot. All MD plots were downwind of non-MD plots.

In MD plots of all three vineyards, Checkmate® VMB-XL membrane dispensers were applied manually by placing them as close as possible of the plant cross. This application was performed a few days after the beginning of the first VMB generation flight, exactly on April 5th at a rate of 620 pheromone dispensers / ha (1 dispenser / 16 m^2) and at a dose of 93 g a.i / ha, following a grid pattern. Later, on April 24th, insecticide treatments using imidacloprid were triggered through drip irrigation system at a dose of 2.5 L a.i. / ha in both Non-MD and MD plots within all three experimental vineyards. Imidacloprid is a systemic chloro-nicotinyl insecticide (Placke & Weber 1993), belonging to the pesticide group "Nicotinic acetylcholine receptor (nAChR) competitive modulators" (IRAC 2016). This insecticide induces the accumulation of the acetylcholine neurotransmitter in the central nervous system and results in the hyper-excitation, lethargy, paralysis and death of the target insect (Okazawa et al. 1998; IRAC 2016). Previous studies demonstrated that applying imidacloprid through drip irrigation system to control VMB in California and

Tunisian vineyards is more promising in reducing mealybug densities than applying it in a furrow-irrigated system (Daane et al. 2006; Mansour et al. 2010a, c). Indeed furrow-irrigated blocks have a more widespread root zone than the drip irrigation system, making delivery of imidacloprid to the entire root zone difficult, resulting in a more diluted application and poorer uptake of this insecticide (Daane et al. 2006).

In addition to the application of Checkmate® VMB-XL membrane dispensers, three delta traps baited with the vine mealybug sex pheromone were hung on the vine canopy (one trap / sub-plot), forming an imaginary triangle centered within each plot of both V1 and V2. In V3, two pheromone traps were placed in each of the two plots. Each trap was placed 40 m away from the other closest trap belonging to the same plot. Pheromone lures were changed every four weeks, while paper sticky delta traps were substituted only when they were dusty and/or damaged by mechanical devices. The aim of placing these traps in vineyards was to record the weekly number of male vine mealybugs caught /trap/ sub-plot.

Assessments and statistical analyses

In the laboratory, from April to October, all male vine mealybugs found on each trap were counted weekly using a binocular microscope (Leica® Model MS5). The cumulative effect of Checkmate® VMB-XL membrane dispensers on VMB males over time (disruption of sexual communication) was evaluated by calculating the percentage of catch inhibition of males in pheromone traps between MD and

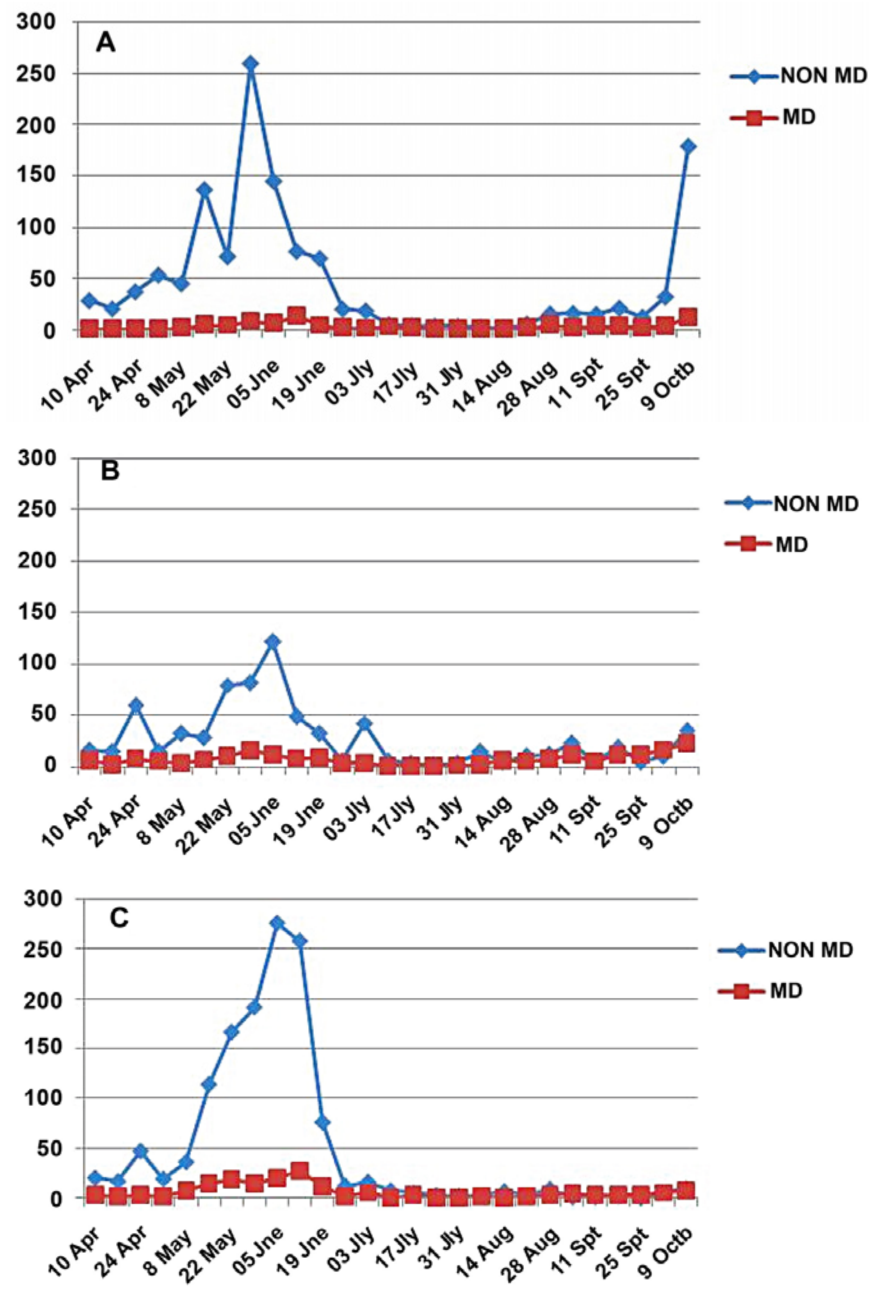

Fig. 1. Male flight activity in V1 (A), V2 (B) and V3 (C) expressed by mean number of male vine mealybugs / trap / week. MD – mating disruption plots; Non-MD – non-mating disruption plots.

Non-MD plots in each check date using the following formula (Abbott 1925):

% Catch inhibition (reduction) = $[(N_{Non-MD} - N_{MD} / N_{Non-MD}) \times 100]$

With: N_{MD} is the number of male vine mealybug trap catches in MD plots; N_{Non-MD} is the number of male vine mealybug trap catches in Non-MD plots.

Visual assessment of vine mealybug presence on leaves was carried out in all three vineyards at about two months after applying the mating disruption product. In both V1 and V2, a minimum number of 100 plants were checked per plot (treatment) for the presence of vine mealybug individuals. All mealybugs found on three basal leaves that are close to the main trunk of each selected plant were counted. Similarly, in V3, about 80 plants (240 leaves) were investigated per plot and all mealybugs found on leaves were counted.

Numerical data linked to male VMB trap catches were log transformed [log(x)] to meet the assumptions of homoscedasticity. Then, the effect of mating disruption on male vine mealybugs was analyzed by comparing male catches using a linear mixed model with repeated measures. Treatments were considered as fixed affects, whereas vineyards (each containing MD and Non-MD plots) as random effects. In the analysis, vineyards were treated as replicates. Numbers of VMB nymphs and adult females on basal leaves were normalized with a log(x+1) transformation and compared using a one-way ANOVA to reveal whether mating disruption significantly reduced or not VMB densities on grapevine leaves. All statistical analyses were performed at 95% level of significance using the software IBM SPSS Statistics for Windows version 20.00 (IBM Corp. 2011).

Table 2. Mean numbers of male vine mealybugs caught per trap in Non-MD and MD plots in each check date within the three experimental vineyards.

Date	Mean number ± SE of males caught in all Non-MD plots	Mean number ± SE of males caught in all MD plots
10 April	21.22 ± 3.8	3.27 ± 1.64
17 April	17.16 ± 1.67	1.22 ± 0.61
24 April	48 ± 6.65	3.77 ± 2.24
1 May	28.88 ± 12.29	2.38 ± 1.69
8 May	37.66 ± 3.84	4.22 ± 1.92
15 May	92.66 ± 32.76	8.61 ± 3.04
22 May	105.83 ± 30.43	11.11 ± 4.34
29 May	177.16 ± 51.69	12.44 ± 2.25
5 June	180.33 ± 48.29	12.6 ± 4.31
11 June	127.77 ± 65.58	16.22 ± 5.57
19 June	59.22 ± 13.58	8.22 ± 2.43
26 June	13 ± 3.78	2.11 ± 0.48
3 July	25.66 ± 8.52	3.16 ± 1.3
10 July	6.38 ± 0.73	1.44 ± 0.44
17 July	4.11 ± 0.48	2 ± 0.57
24 July	2.88 ± 0.48	0.61 ± 0.2
31 July	2.88 ± 0.44	0.72 ± 0.3
7 August	6.38 ± 3.8	1.27 ± 0.49
14 August	4.05 ± 1.61	2.27 ± 1.86
21 August	6.33 ± 2.14	2.72 ± 1.3
28 August	11.99 ± 1.83	5.05 ± 1.16
4 September	13.16 ± 6.36	6.16 ± 2.8
11 September	7.16 ± 4.12	3.83 ± 0.83
18 September	13.83 ± 5.96	5.83 ± 2.83
25 September	5.66 ± 3.48	5.83 ± 3.11
2 October	15.83 ± 8.18	8.33 ± 4.14
9 October	74 ± 52.49	14.16 ± 4.6

Results

Pheromone trap catches

The statistical analysis revealed a highly significant effect ($F_{157,1} = 63.076$; $P < 0.001$) of mating disruption application on modifying the vine mealybug sexual communication: VMB males were not capable of finding the right source emitting sex pheromones, which significantly reduced pheromone catches. Indeed, the overall mean weekly number of males caught in MD plots in all vineyards, equal to 5.54, was significantly lower than the mean number of males caught in Non-MD plots, which was equal to 41.08. From early April to late July, overall mean numbers of male vine mealybugs caught per week in traps of MD plots were numerically lower than the numbers recorded in traps of Non-MD plots in almost all check dates. Conversely, since early August, numbers of VMB males caught in MD plots were numerically equal to numbers of VMB males caught in Non-MD plots (Table 2; Fig. 1). Under the study conditions, the cumulative effect (satisfactory trap catch reduction) of mating disruption over time against male vine mealybugs reached 120 days (Table 3). The highest percentages of trap catch reduction (> 80%) were recorded during April, May and June. A slight reduction in catch inhibition % was reported during July, August, September and October (Table 3).

The total number of male vine mealybugs caught in traps in all Non-MD plots was 8,035, while only 1,045 males were caught in traps of all MD plots (Table 4). Overall, the highest season-long percentage of catch inhibition (94.29%) was recorded in V1, whereas this percentage was lower in both V2 (75.58%) and V3 (86.83%) (Table 4).

Visual assessment of VMB density on grapevines

Statistical analyses revealed a highly significant effect ($F_{198,1} = 14.97$; $P < 0.001$) of mating disruption on densities of VMB nymphs and adult females on grapevines, two months after applying Checkmate® VMB-XL dispenser. In fact, the numbers of vine mealybugs on grapevines were significantly reduced in all MD plots as compared to Non-MD plots. A total number of 3,611 vine mealybugs were found on leaves in all MD plots, while in Non-MD plots, a total number of 5,364 vine mealybugs were observed (Table 5). Among MD plots, the highest number of VMB nymphs and adults females (2,582) was found on grapevines in V2, compared to the same plot of either V1 or V3 where less than 700 mealybugs were recorded (Table 5).

Discussion

Over the last two decades, semiochemical-based (biotechnological) pest control tools have received total and unconditional support from scientific community due to their effectiveness on arthropod pests and safety to-

Table 3. Percentages of male vine mealybug catch inhibition during the whole study period within the three experimental vineyards.

Date	Total number of males caught in all Non-MD plots	Total number of males caught in all MD plots	Catch inhibition (%)
10 April	171	26	84.79
17 April	138	9	93.47
24 April	385	31	91.94
1 May	241	20	91.70
8 May	303	30	90.09
15 May	720	63	91.25
22 May	786	81	89.69
29 May	1403	98	93.01
5 June	1347	93	93.09
11 June	893	119	86.67
19 June	457	62	86.43
26 June	105	17	83.80
3 Jul	215	23	89.30
10 Jul	50	12	76
17 Jul	32	15	53.12
24 Jul	23	5	78.26
31 July	24	6	75
7 August	55	10	81.81
14 August	30	20	33.33
21 August	54	23	57.40
28 August	99	42	57.57
4 September	55	28	49.09
11 September	28	17	39.28
18 September	50	21	58
25 September	22	33	N/A
2 October	63	47	25.39
9 October	266	73	72.55

Table 4. Overall percentage of trap catch inhibition by mating disruption product in each of the three vineyards, based on total number of male vine mealybugs caught in all pheromone traps used.

Vineyard	Non-MD plots	MD plots	% catch inhibition
V1	3329	190	94.29
V2	2093	511	75.58
V3	2613	344	86.83
Total (V1+V2+V3)	8035	1045	86.99

wards ecosystem components including non-target auxiliary fauna, plants, surrounding environment, and humans. A key benefit of pheromone-based programs is that they are highly selective: only the primary target insect species responds to the pheromone, and non-target effects on biological control agents within a field are not observed (Welter et al. 2005). Pheromones have been exploited by researchers in an attempt to manipulate insect behavior for pest management issues. In this sense, a keen interest in assessing and applying mating disruption technique against various pests, such as moths (Lepidoptera) and mealybugs (Hemiptera), has long been noted. Until recently, mating disruption technique has been investigated for more than 120 species of Lepidoptera (Suckling et al. 2014). Interestingly, mating disruption seems to be more advantageous in mealybugs than in moths because mealybug females are sessile and cannot migrate from one area to another as moths do (Franco et al. 2009). Currently, mating disruption is the most suitable control strategy against VMB infestations in organic viticulture, especially when integrated with rational practices such as pruning, nitrogen fertilization, and irrigation that could reduce the grapevine vigor and pest populations (Cocco et al. 2014, 2015).

Table 5. Total numbers of vine mealybugs (nymphs and adult females) found on basal leaves in all Non-MD and MD plots within the three experimental vineyards.

Vineyard	Non-MD plots	MD plots
V1	885	361
V2	2808	2582
V3	1698	668
Total (V1+V2+V3)	5364	3611

Following this direction, we tested in the present study a pheromone-based mating disruption tactic against VMB in Center-South Tunisian table-grape vineyards. Our study clearly revealed a highly significant effect of a single application of the mating disruption dispenser Checkmate® VMB-XL on male vine mealybugs' natural flight behavior. Similar results were obtained in Sardinian (Italy) vineyards using the same pheromone dispensers (Cocco et al. 2014). Besides, in California vineyards, the use of a sprayable microencapsulated formulation of the sex pheromone, applied three to four times in combination with a single application (mid-June) of the insect growth regulator pesticide buprofezin, also generated great success in disrupting VMB mating process (Daane et al. 2006; Walton et al. 2006). Indeed, in our study, the total vine mealybugs caught in Non-MD plots was almost eight times greater than number of males found in traps of MD plots. In fact, wingless adult females are located, through the pheromone plume that they emit, by winged adult males for ensuring mating process. The inability of VMB males to find pheromone traps in MD plots indicates that these males are not able to find females. As a consequence, this significantly decreased season-long trap catches of VMB males in MD plots during the whole study period (Fig. 1). Disrupting mate location will result in the reduction of the number of insect offspring produced in the next generation (Suckling et al. 2014), thus reducing grapevine fruit damages by preventing the development of damaging VMB life stages: nymphs and adult females.

The cumulative effect of mating disruption on VMB over time reached 120 days after a single application (Table 3). This result implies that membrane dispensers used worked well even at the high temperatures recorded during the period June-October (Table 1), characterizing the Center-South region of Tunisia where the present study was carried out. However, it should be taken into account that prolonged high temperatures may shorten field longevity of the mating disruption dispensers. On the other hand, as indicated in Table 1, the mean of maximum wind speed during the whole experimental period was stable and relatively low, not exceeding 46 km/h, and thereby it did not affect our study, avoiding moving pheromone cues away from the MD plots. In the present study, the highest percentages of VMB male catch inhibition, exceeding 80%, were recorded from the beginning of the experiment (10 April) until early July, decreasing afterwards until early October (Table 3). Two possible major factors that could explain the decrease in number of male catches are both the higher temperatures reported in all vineyards during July and August (Table 1) and the increased VMB parasitoid activity. In Coachella Valley vineyards (California), the dramatic decline in VMB density reported in the summer is perhaps due to increased mortality due to high temperatures (Anonymous 2003). As such, in California vineyards, the parasitoid *Anagyrus pseudococci* (Girault, 1915) (Hymenoptera: Encyrtidae) became more effective toward the end of the growing season, thereby reducing the pest population in August and September (Daane et al. 2004; Walton et al. 2006). As compared to our results, mating disruption trials in Sardinian vineyards induced similar season-long percentages of trap catch reductions, ranging from 85 to 100% in the peak dates (Cocco et al., 2014). However, Cocco et al. (2014) demonstrated that pheromone dispensers were effective for a longer time, as they did not observe a reduction in catch inhibition until October.

The results related to the influence of mating disruption on male VMB movements and sexual behavior were further confirmed statistically by a highly significant effect of treatment (mating disruption) on the VMB density on grapevines. However, more VMB nymphs and adults females were found on leaves of MD plot in V2, as compared to the MD plot in either V1 or V3 (Table 5). This could be explained by the fact that the insecticide applied was not sufficiently effective in decreasing VMB densities on highly infested vines within the MD plot of V2. Indeed, in a detailed manner of presenting results, more than 600 VMB nymphs and adult females were found on only three basal leaves of a plant in the MD plot of V2, and also more than 300 VMB nymphs and adult females were found on each three basal leaves of two other plants belonging to the same MD plot of V2. By contrast, in either V1 or V3, the number of VMB nymphs and adult females found on each plant in MD plots did not exceed 50 individuals. Hence, this is a strong indication that V2 had a higher VMB infestation compared to either V1 or V3. In such a situation, the action of mating disruption solely would not prove to be sufficient; thereby a more effective insecticide should have been applied in combination with mating disruption. In the case of the VMB, mating disruption proved to be more successful at low insect population densities (Suckling et al. 2014). In previous preliminary field trials in highly infested vineyards, a major mating disruption effect was observed in the second or third year of application. Conversely, under low pest pressure, significant pest disruption can be observed from the first year of applying mating disruption. In this context, Sharon et al. (2016) showed that, at high VMB population levels, mating disruption can be effective if applied for consecutive years. Indeed, at low initial VMB infestation levels, mating disruption induced a shutdown of pheromone traps and significant reduction in infested vines. While, at high initial VMB infestation levels, mating disruption generated a gradual reduction in infested vines, with a trap shutdown after the second year of applying pheromone dispensers (Sharon et al. 2016). Mating disruption does not kill mealybugs already infesting plants but rather prevents the formation of the next generation, so that high mealybug population density influence the effectiveness of mating disruption (Daane et al., 2006). As such, regarding moth pests, mating disruption has better performance at low or moderate population densities (Cardé & Minks 1995).

The overall results of the present study drawn earlier clearly show that a single application of 625 Checkmate® pheromone dispensers loaded with 150 mg of a.i. was adequate to disrupt VMB mating process in Center-South Tunisian (South Mediterranean) table-grape vineyards.

The same density of CheckMate® dispensers provided similar results under North Mediterranean climatic conditions (Cocco et al. 2014). In California vineyards, the optimal pheromone membrane dispenser density should be around 175 dispensers per acre (437 dispensers / ha) with a load rate of 37.5 g of a.i. per acre (93.75 g of a.i. / ha) to achieve sufficient control against the VMB (Langone et al. 2014).

Mating disruption is cost prohibitive in the case of high VMB populations, hence applying an insecticide as combination is strongly recommended for better performance. In our study the insecticide imidacloprid applied through drip irrigation did not show promising performance in reducing VMB densities, especially on highly infested vines belonging to V2 as indicated earlier. This insecticide could thereby be replaced by a more effective pesticide active ingredient for a better season-long control of VMB populations. In this regard, spirotetramat (Movento®), has been considered among the most effective insecticide used for VMB control (Brück et al. 2009; Mansour et al. 2010a) with no adverse side effects on main mealybug parasitoids (Mansour et al. 2011b). This systemic insecticide could thus be used in combination with mating disruption as a more promising option for reducing high VMB populations to non damaging levels. Refining our knowledge about biological and behavioral characteristics of the target pest would help to decide the best timing for applying mating disruption product in vineyards.

Future field trials will focus on evaluating the effect of mating disruption on reducing next generations's offspring and grapevine fruit damages and studying the eventual side effects of this biotechnological control technique on the behaviour of the most effective VMB parasitoid *Anagyrus* sp. near *pseudococci* (Triapitsyn et al. 2007; Mansour et al. 2012; Suma et al. 2012a, b). In California vineyards, it was shown that there was a significant reduction in crop damages when mating disruption was applied against vine mealybug (Daane et al. 2006) and that this technique did not negatively affect parasitism levels of vine mealybug by *A. pseudococci* since the percentage of parasitism by this parasitoid was higher in vineyards with mating disruption (Walton et al. 2006). In this context, testing whether the combination "mating disruption using Checkmate® VMB-XL membrane dispenser + release of the encyrtid parasitoid *Anagyrus* sp. near *pseudococci* + a well timed spirotetramat-based insecticide application" would provide better results in decreasing VMB populations in Tunisian vineyards might be a future potential avenue of research and could help in adopting suitable pest management decision-making process.

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