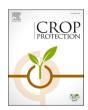
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# Influence of weather conditions on *Lobesia botrana* (Lepidoptera: Tortricidae) mating disruption dispensers' emission rates and efficacy

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#### ABSTRACT

Passive dispensers are the most widely used dispensers to control the European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), by mating disruption (MD). Although their efficacy is well-proven, efforts are needed to reduce the use of pheromone without compromising efficacy and to allow lower MD costs. For this purpose, two different widely employed dispenser types in Europe against this pest (Ampoule and Rope dispensers) were evaluated in the field to verify their performance (emission rates and efficacy) in relation to weather conditions. Their release profiles were studied by extracting and quantifying the residual pheromone load over time by gas chromatography. Dispensers' performance in relation to weather conditions was then assessed by correlating the daily emission rates with the mean daily values of several of these variables. Although both dispenser types were efficient in controlling *L. botrana* populations and reducing fruit damage, their pheromone content and their release rates differed at the end of the crop cycle. The Ampoule dispensers emitted lower amounts of pheromone throughout the study period, whereas the Rope dispensers' emissions were weather-dependent, with higher emission rates at higher mean temperatures and wind speeds. Our results showed that the current commercial MD dispensers could be improved by adjusting their release rates to better reflect actual pheromone requirements.

# 1. Introduction

The European grapevine moth (EGVM), *Lobesia botrana* (Denis & Shiffermüller), (Lepidoptera: Tortricidae), is a key pest of grapes in most wine-growing areas worldwide, especially in the Mediterranean region (Ioriatti et al., 2011; Bournier, 1977). The losses caused by this pest occur after oviposition in berry clusters where larvae feed, which facilitates the colonization of pathogenic fungi like *Botrytis cinerea* and *Aspergillus* spp. at mid-season, and leads to rotting at harvest (Cozzi et al., 2006; Fermaud and Le Menn, 1989).

This pest has been traditionally controlled by several applications of insect growth regulators or organophosphate insecticides per year (Lucchi and Benelli, 2018). Given the widely known effects of these treatments on non-target organisms and the environment, many efforts have been made to identify and develop new alternatives. Semi-ochemicals are well-known examples of alternatives used for both monitoring and direct control purposes (El-Ghany and Nesreen, 2019).

Today, mating disruption (MD) is successfully employed for pest control, mainly in the orders Lepidoptera and Hemiptera (Daane et al., 2020; Lucchi et al., 2019; Evenden, 2016; Miller and Gut, 2015; Vacas et al., 2015; Witzgall et al., 2010; Carde, 1990). MD offers many advantages over insecticides, such as specificity, lack of toxic residue on fruit and no negative effects on human health (Lucchi and Benelli, 2018; Witzgall et al., 2010). This control method reduces environmental impacts by improving pest control tools within integrated pest management programs and organic farming (Miller and Gut, 2015; Welter et al., 2005).

The availability of effective dispensers is critical for the success of pheromone-based pest control strategies, and a wide variety of dispensing technologies is available to apply MD against lepidopteran pests, including microencapsulated sprayable formulations (Il'Ichev et al., 2006), female-equivalent dispensers (Stelinski et al., 2008), automatic aerosol devices (Benelli et al., 2019), and hand-applied passive dispensers. Among the different alternatives, passive dispensers are the most used in MD programs (Evenden, 2016; Miller and Gut 2015).

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They release the pheromone contained in, or impregnated on, their material, namely rubber, biodegradable or plastic polymers and are deployed at rates above 250 units/ha (Daane et al., 2020; Lucchi et al., 2018b; Evenden, 2016; Miller and Gut, 2015).

EGVM mating disruption development was possible after the isolation and identification of the main component of its sex pheromone, (*E*, *Z*)-7,9-dodecadienyl acetate Roelofs et al., 1973. Although other types of dispensing technologies are employed, the use of passive dispensers is the most widespread to control EGVM (Ioriatti et al., 2011). Currently, there are several marketed passive dispensers for EGVM, and they usually provide effective control by reducing pest populations below the economic injury level (Ioriatti et al., 2004, 2011; Akyol and Aslan, 2010; Vassiliou, 2009).

The lifespan of MD dispensers should be designed to provide constant amounts of airborne pheromone to inhibit or delay mating throughout the crop cycle, especially during periods of moth activity, independently of weather conditions (Tomaszewska et al., 2005; Bradley et al., 1995; Van der Kraan and Ebbers, 1990). In this way, an ideal dispenser must meet the following requirements (Ogawa 1997; Hofmeyr and Burger, 1995): (1) come close to zero-order release kinetics and provide a constant emission rate, without substantial fluctuations throughout its life span; (2) temperature-independent emissions to avoid emission peaks corresponding to the highest temperatures; (3) moderate wind-dependent emissions to avoid pheromone waste under strong wind conditions; (4) a long life span to protect crops during its most susceptible period or the whole pest cycle without resorting to replacements.

Pheromone diffusion and its volatilization capacity, together with the characteristics of the dispensers, determine emission rates (Femenia, 2010; Hofmeyr and Burger, 1995). Pheromone volatilization is affected mainly by vapor pressure, which interacts with weather conditions. Higher environmental temperature leads to higher vapor pressure and vaporization (Femenia, 2011; Hofmeyr and Burger, 1995). In this way, temperature has been demonstrated to be the main parameter affecting the release rates of dispensers compared to other weather parameters. For example, Femenia (2011) carried out studies with experimental mesoporous pheromone dispensers designed for EGVM based on the technology of inorganic molecular sieves. She found differences between the emission rates of dispensers kept under constant laboratory conditions (30 °C) and those of dispensers deployed under field conditions, the latter displaying a higher mean emission level. Van der Kraan and Ebbers (1990) described a 2-2.5-fold increase of the emission of low-density and microporous polythene tubes with an increase of temperature (in the range from 15 to 25 °C) and wind speed in the laboratory. Moreover, studies with different types of polymeric dispensers (including polyethylene and nylon tubes, polyethylene vials, and glass fiber disks) loaded with the sex pheromone of the summerfruit tortrix moth, Adoxophyes orana (Fischer von Röslerstamm), concluded that all dispensers were temperature-dependent (Hofmeyr and Burger, 1995).

Other weather variables, such as wind (Daterman et al., 1982; Hofmeyr and Burger, 1995) and humidity, are considered of minor importance (Wiesner and Silk, 1982). However, certain levels of wind speed have been reported to directly affect pheromone emissions (Hofmeyr and Burger, 1995; Femenia, 2010). Increased wind speeds facilitate the releasing of the pheromone placed on the dispenser's surface, increasing the mobility of the inner molecules (Femenia, 2010; Alfaro et al., 2008; Hofmeyr and Burger, 1995; Leonhardt et al., 1989). A decrease of dispenser emission has also been demonstrated at higher humidity levels (Zhu et al., 2015; Shem et al., 2009; Johansson et al., 2001).

These laboratory studies have provided valuable information on dispensers' performance subjected to specific weather parameters. However, dispensers are simultaneously exposed in the field to different and variable conditions, and may be temporarily subjected to extreme conditions that are not considered in laboratory experiments. Considering the existing laboratory results and the lack of studies under field conditions with other parameters, such as humidity, the present work aimed to study the influence of different weather parameters

(temperature, wind speed, relative humidity, and precipitation) that simultaneously affect MD dispensers under field conditions to better understand their emission profile. For this purpose, we correlated the emission rates of two types of MD passive dispensers currently used in Spain against EGVM with different weather variables. The efficacy provided by these dispensers was also studied in terms of EGVM male catch inhibition and damage reduction in grapes. This information can estimate how weather conditions affect pheromone emissions and dispensers' lifespan to make future improvements.

#### 2. Materials and methods

#### 2.1. Mating disruption pheromone dispensers

The Isonet® L rope dispensers (Shin-Etsu Chemical Co., Tokyo, Japan) (Rope) and Lobetec dispensers (SEDQ SL, Barcelona, Spain) (Ampoule) were used to test MD in field trials. Both MD dispensers release the pheromone through polymeric plastic walls. They contain synthetic (*E,Z*)-7,9-dodecadienyl acetate, the main component of the sex pheromone released by EGVM females. The Isonet® L dispensers consist of plastic twist-tie ropes on which pheromone (172 mg) is loaded inside capillaries. The Lobetec dispensers comprise small plastic ampoules loaded with 210 mg, according to the manufacturer's technical sheet.

#### 2.2. Mating disruption experiments

Three experimental replicates (trials) were conducted in two locations in the Valencian Region (eastern Spain); Requena (location 1; UTMs 39.482608, -1.318822) and Mogente (location 2; UTMs 38.808779, -0.815917): Mogente 2018 (trial 1), Requena 2018 (trial 2), and Mogente 2019 (trial 3). Winery vineyards at both locations were grown on a trellis and have been treated with MD against EGVM for more than 10 years. Each location had homogenous conditions and wine grape varieties:  $2.5\times1.5$  m planting pattern for the mid-season cultivar Bobal (location 1) and  $2.5\times1.5$  m planting pattern for the mid-season cultivar Monastrell (location 2).

Each experimental replicate consisted of a 10-ha vineyard divided into three plots to apply three different treatments. Randomly assigned treatments corresponded to: (1) MD treatment with the Isonet® L rope dispensers (Rope) at a density of 500 dispensers/ha; (2) MD treatment with the Lobetec dispensers (Ampoule) at a density of 400 dispensers/ha; (3) (UTC) untreated control with no type of treatment against EGVM. This last plot was located upwind from the MD treatments in each trial. During the trials, no additional insecticide or fungicide treatment was applied in any plot.

EGVM male flight was monitored from mid-March and MD treatments were deployed when the first catch was detected in each trial. Accordingly, MD dispensers were deployed on April 24, 2018, April 23, 2018, and April 26, 2019 in trials 1, 2, and 3, respectively.

# 2.3. Weather data

The weather data were obtained from the Associació Valenciana de Meteorologia (AVAMET) from the nearest station to each location. The location 1 data were obtained from the IVIA weather station of the Los Ruices Irrigation Association (5 km from the field), whereas the station of the solar farm at Font de la Figuera (5 km from the field) provided the location 2 data.

#### 2.4. Dispenser release kinetic studies

Fifty additional dispensers of each type (Ropes and Ampoules) were simultaneously aged with the MD experiments, under the same field conditions in areas near each trial location, to study their release profiles and life span. Four dispensers of each type were randomly collected once a month, from April to October, and stored at  $-20\,^{\circ}\text{C}$  inside airtight bags

until extracted in the laboratory.

To extract residual pheromone, each dispenser was cut into pieces and individually soaked in  $24\,\mathrm{mL}$  of dichloromethane (DCM) inside a 50-mL glass centrifuge tube for  $2~\mathrm{h}$  at room temperature with magnetic stirring. One mL of an internal standard n-dodecane solution was then added to each extract for the final chemical analysis and quantification.

Residual pheromone content determination was done by gas chromatography with a flame ionization detector (GC/FID) in a Clarus 500 GC apparatus (PerkinElmer Inc. Wellesley, MA, USA). All injections were performed in a ZB-5 (30 m  $\times$  0.25 mm  $\times$  0.25 µm) column (Phenomenex Inc., Torrance, CA, USA), held at 100 °C for 1 min, and then raised from 25 °C/min to 250 °C, and maintained for 3 min. The carrier gas was helium (1.5 mL/min). The amount of pheromone and the corresponding chromatographic peak areas were connected by fitting a linear regression model, y=a+bx, where y is the ratio between pheromone and n-dodecane areas, and x is the amount of pheromone. Occasionally, the re-extraction of previously extracted dispensers was done to corroborate that there was no pheromone left in the residue.

To obtain the release kinetics, multiple linear regression analyses were run to relate the quantified residual pheromone loads (mg) to the corresponding field exposure days for each dispenser type in every location and during each season. The polynomial terms of time (days, days<sup>2</sup>, days<sup>3</sup>) were introduced as independent variables to model emissions. The first derivative of the resulting equations then provided estimations of the mean daily emission rates. These analyses were performed using R version, 4.0.3 (The R Foundation for Statistical Computing 2020).

#### 2.5. Population monitoring

Four delta traps provided with a sticky base and baited with a "Grapemone" monitoring lure (OpenNatur, Lleida, Spain) were placed in the center of each plot (treated and untreated) in a square arrangement ( $70 \times 70$  m) to record EGVM male flight from April to October in each trial. Traps were placed 1 m above the ground inside the canopy. On account of the low EGVM populations in the study areas, traps were visited fortnightly to replace sticky bases and count moth catches. Septum lures were replaced every 42 days as the effective period assured by the manufacturer. The number of males captured was compared among treatments. The absence or reduction of male captures in MD-treated plots compared to those recorded in the untreated ones was taken as preliminary indicator of the technique's efficacy.

## 2.6. Damage assessments

Crop damage inflicted by EGVM was scouted after each male flight, according to the population monitoring data of the corresponding untreated plot, as additional evidence of MD efficacy. At least 200 bunches were randomly selected and visually examined in 50 vines around all four delta traps installed in the center of each plot to look for damaged or occupied flower clusters or bunches (% damage). In the first assessment (June), a flower cluster was considered damaged when nests, larvae or pupae were found. In the second (July) and third assessments (September), a bunch was considered damaged when nests, eggs, larvae or pupae were found.

## 2.7. Statistical analysis

Regarding pheromone emission, the main goal was to evaluate dispensers' dependence on the main field weather variables: temperature (minimum (Tmin), mean (Tmean), maximum (Tmax)), relative humidity (minimum (Hrmin), mean (Hrmean), maximum (Hrmax)), wind velocity (mean (Wm) and maximum (Wmax)) and precipitation (Pp). After applying multiple linear regression (MLR) to obtain the release kinetics, the mean daily emission rates for each dispenser type in all locations were obtained deriving the resulting equations from the MLRs.

The mean daily emission rates of the last month of each trial (from 140 to 170 days of field exposure), corresponding to pheromone loads <10% of the initial load, were removed from the MLR analyses by considering that the effect of dispenser depletion could be confounded with weather effects from this point. The daily mean emission rates were then correlated with the corresponding values of the abovementioned different climate variables by means of MLRs. These analyses were performed using R version 4.0.3 (The R Foundation for Statistical Computing 2020). The variables were selected by stepwise removal and the quality of models was assessed by the Akaike Information Criterion (AIC) indices Posada and Buckley, 2004. The emission rates data did not need transformation because they fulfilled normality requirements.

The number of captured males was summed across the four traps of each plot for each trapping period (fortnightly); these values were subjected to analysis by generalized linear mixed models (GLMM) to assess the significance of the differences observed among treatments. For this purpose, the *glmer* function from the *lme4* package was employed by assuming the poisson error distribution. Models were constructed with the fortnightly summed captures as the dependent variable, treatment and time (week of the study period) as fixed factors and trial (experimental replicate) as random factor. Different models were obtained for the whole data set (capture data throughout the entire trial periods) and separately for the first flight capture data.

Similarly, GLMM was used for the damage assessment data. Poisson distribution was assumed for the number of damaged bunches found around all the four delta traps installed in the center of each plot. An offset vector was introduced to relate these numbers to the corresponding total number of sampled bunches. The fixed factor treatment and the random factor trial were included in the model. In all cases, the significance of treatment effects was assessed by removing them from each model and comparing models with likelihood ratio tests. The *glht* function in the *multcomp* package was then used to perform Tukey HSD tests for *post hoc* pairwise comparisons.

# 3. Results

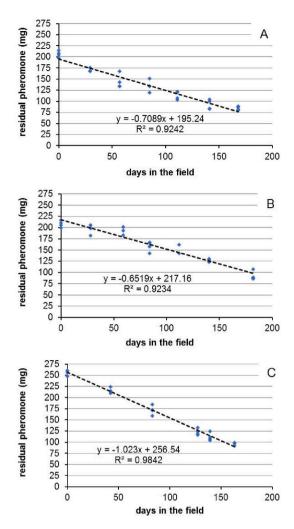
# 3.1. Dispensers release kinetics

After extraction of the field-aged dispensers and GC analysis, the quantified residual pheromone contents were related to the corresponding field exposure days by fitting regression models ( $R^2 > 0.92$  and P < 0.0001 in all cases) depicted in Figs. 1 and 2. In all cases, the residual pheromone load of the Ampoule dispensers was faithfully described by linear models regardless of location (Fig. 1). The quadratic and cubic effects of time initially included in the models were not significant (P > 0.1 in all cases). Accordingly, the slopes of the fitted linear models provided mean emission rates of the Ampoule dispensers throughout the study period in trials1, 2 and 3 (0.71, 0.65 and 1.02 mg/day, respectively). Besides having a higher mean emission rate, the results highlighted that the Ampoule dispensers during the 2019 season (trial 3) also had a higher mean initial load (256 mg), compared to the 2018 data (206 mg) (trial 1).

In contrast, the release profile of the Rope dispensers was not constant and the residual pheromone loads fitted polynomial equations represented in Fig. 2. The fitting of polynomial models indicated the presence of different slopes in the release profile, which meant variable pheromone emission rates throughout the study period.

Dispensers differed in their initial content, emission rates and residual pheromone loads at the end of the crop cycle (Table 1). Although the Rope dispensers contained a smaller initial amount of pheromone than the Ampoule dispensers (196 mg vs. 206 mg), their emission was higher throughout the growing season, as was the total pheromone applied, when considering the larger number of installed dispensers (503.82 vs. 254.01 mg/ha/day). The pheromone content at the end of the crop cycle was nearly half the initial content (ca. 40%) in the Ampoule dispensers, whereas the Rope dispensers were almost empty

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**Fig. 1.** Pheromone release profiles of the Ampoule dispensers in the three experimental replicates: (A) Mogente 2018; (B) Requena 2018 and (C) Mogente 2019. Residual mean pheromone load (mg) contained in dispensers vs. field exposure time (days). Release kinetics fitted the linear models given by their equations (P < 0.0001 in all cases).

#### (3-6%).

In accordance with the data from both seasons, dispensers' release kinetics differed during the 2019 season as dispensers belonged to a distinct production batch than those of the 2018 season (Table 1). First, the initial pheromone loads were higher during 2019, especially in the Ampoule dispensers, whose mean load reached 256 mg. These higher initial contents may be related to the higher emission rates (more than 620 mg/ha/day vs. 560 mg/ha/day for the Rope dispensers, and 380 vs. 280 mg/ha/day for the Ampoule dispensers).

# 3.2. Dispensers' climate dependency

Both locations share the characteristics of Mediterranean climate conditions with a mild winter and a marked increase in summer temperatures. The data obtained throughout the vine crop cycle showed a progressive increase in temperatures from March, with an average of around 10 °C, to more than 25 °C in July (Fig. 3). After August, temperatures declined until harvest (September–October) to around 15–20 °C. The average wind speed during the study periods was maximum in April with more than 4 m/s, just after deploying MD dispensers, while the average wind speed remained at 2–3 m/s with only limited variation in the rest of the cycle.

According to the results of the release profile studies, the mean

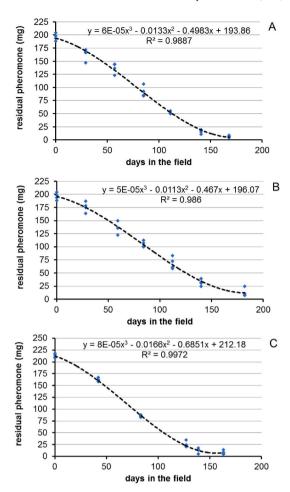


Fig. 2. Pheromone release profiles of the Rope dispensers in the three experimental replicates: (A) Mogente 2018; (B) Requena 2018 and (C) Mogente 2019. Residual mean pheromone load (mg) contained in dispensers vs. field exposure time (days). Release kinetics fitted the polynomial models given by their equations (P < 0.0001 in all cases).

emission rate of the Ampoule dispensers remained constant throughout the study period, which suggests that their performance was independent of the weather variables registered in the study area. In contrast, the pheromone emission rates of the Rope dispensers were not constant and were likely affected by weather variables. The simplest model that significantly fitted our field data for the Rope dispensers was given by Equation (1) (P < 0.0001;  $R^2 = 0.359$ ; AIC = 145.2) as:

ER (daily emission rate) = 
$$0.238 + 0.043 \times \text{Tmean} + 0.013 \times \text{Wmean}$$
 (1)

Accordingly, the regression analysis confirmed that the emission rate of the Rope dispensers depended on both the mean daily temperature and the mean daily wind speed. However, the AIC indices showed that the daily pheromone emission rates could be slightly better predicted by including the minimum and maximum daily temperatures (P < 0.0001;  $R^2 = 0.378$ ; AIC = 74.0), as shown in Equation (2):

$$ER = 0.246 - 0.031 \times Tmin + 0.105 \times Tmean - 0.031 \times Tmax + 0.012 \times Wmean$$
 (2)

All independent variables included in both models were highly significant (P < 0.0001). Hence, the mean temperature and wind conditions likely affected the Rope dispensers by increasing emission rates when the values of these parameters rose.

When the mean temperatures rose to 20  $^{\circ}$ C in June, the slope of the Rope dispensers curve became steeper, indicating that the mean emission rates increased with temperature. In contrast, the slope of the

Table 1
Initial pheromone content, total pheromone released per dispenser and per ha/day and percentage of residual pheromone at the end of the season.

Trial	Location	Dispenser	Initial content (mg/dispenser) <sup>a</sup>	Released (mg/dispenser) <sup>b</sup>	Released (mg/ha/day) <sup>c</sup>	Residual <sup>d</sup> %
1	Mogente (2018)	Rope Ampoule	$196.04 \pm 3.39$ $206.05 \pm 3.70$	183.39 115.57	503,82 254,01	6.44 44.39
2	Requena (2018)	Rope	$196.04 \pm 3.39$	189.69	564.56	3.23
3	Mogente (2019)	Ampoule Rope Ampoule	$206.05 \pm 3.70$ $211.83 \pm 2.16$ $256.09 \pm 2.99$	121.53 204.06 155.46	289.36 625.94 381.50	41.02 3.67 39.29

<sup>&</sup>lt;sup>a</sup> Initial pheromone content (mg/dispenser) quantified by dispenser extraction and GC/FID analysis.

<sup>&</sup>lt;sup>d</sup> % Residual pheromone, total pheromone remaining in the dispenser at the end of the trial.

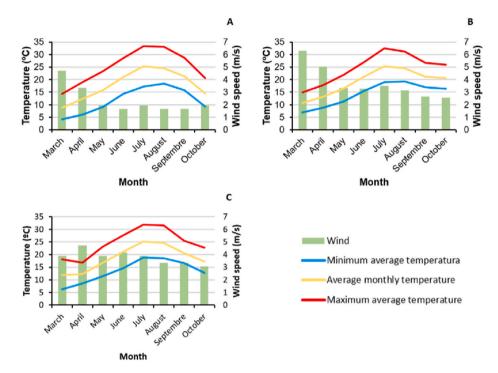
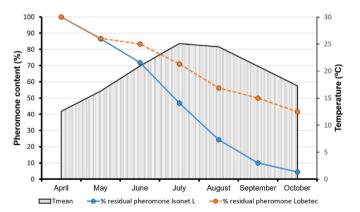


Fig. 3. Average temperatures (minimum, mean, maximum in °C) and wind speed (m/s) parameters identified as statistically significant in the Rope dispensers emissions after the regression analyses in the three experimental replicates (A: Requena 2018, B: Mogente 2018, and C: Mogente 2019).

Ampoule dispensers remained more constant with no substantial changes with rising temperature (Fig. 4).



**Fig. 4.** Mean residual pheromone content (%) of each dispenser type throughout the crop cycle and monthly average mean temperatures from the three experimental replicates.

# 3.3. Field efficacy of the MD dispensers

The male catches in monitoring traps (Fig. 5) were low throughout the trials, which is likely due to the use of MD against EGVM in the study areas during the last 10 years. In fact, no male catches were recorded in any plot treated with the MD Ampoule dispensers and the data of this treatment were not included in the analysis. Despite the low values recorded both in UTC plots and those treated with Rope dispensers throughout the trials, the results showed that the applied treatment had significant effects ( $\chi^2=272.9;$  df = 1,59; P < 0.0001), and the treatments with Rope dispensers significantly reduced the male captures compared to the untreated controls. The fixed factor week was also significant ( $\chi^2 = 220.3$ ; df = 11,59; P < 0.0001). Upon the first flight, no captures were obtained in the plots treated with the Ampoule dispensers, whereas a 96.4% inhibition was recorded for the Rope dispensers, and the male captures significantly reduced vs. the UTC plots  $(\chi^2 = 36.8; df = 1,19; P < 0.0001)$ . The week factor effect on moth captures was also significant during the first flight ( $\chi^2 = 20.9$ ; df = 3,19; P = 0.0001). Finally, no males were captured during the second and third flights with both MD treatments, indicating very high effectiveness against the UTC plots.

<sup>&</sup>lt;sup>b</sup> Pheromone released (mg/dispenser), total pheromone released until harvest.

<sup>&</sup>lt;sup>c</sup> Pheromone released (mg/ha/day), total pheromone released per day and ha until harvest. Rope dispensers are deployed at 500 dispensers/ha, whereas Ampoule dispensers at 400 dispensers/ha.

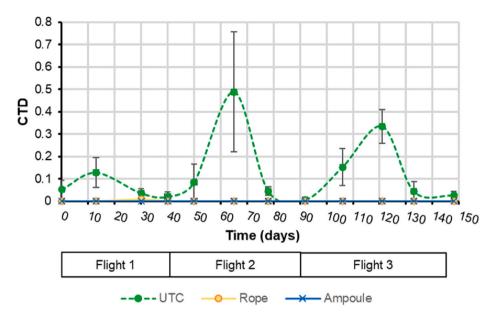


Fig. 5. Mean (±SE) male captures per trap per day (CTD) throughout the study period of the three experimental replicates in the plots with different treatments: (UTC) untreated control, (Rope) MD treatment with the Isonet® L dispensers, and (Ampoule) MD treatment with the Lobetec dispensers.

Both MD dispenser types (Ampoules and Ropes) reduced damaged bunches below 5% (Fig. 6). Both treatments significantly reduced damage against the UTC plots in the first ( $\chi^2=27.6$ ; df = 2,34; P < 0.0001), second ( $\chi^2=62.8$ ; df = 2,34; P < 0.0001) and third assessments ( $\chi^2=166.7$ ; df = 2,34; P < 0.0001). No significant differences were observed between the MD treatments in any assessment (P>0.28).

#### 4. Discussion

In Mediterranean vine production areas, the damage inflicted by the third EGVM generation is usually the most important because it coincides with the end of the crop cycle, just before harvest (Vassiliou, 2009; Ioriatti et al., 2004). Consequently, ensuring pheromone emissions covering the third male flight until the end of September is a necessary condition for MD dispensers to be used in these production areas, especially with late season varieties. Our results demonstrate that

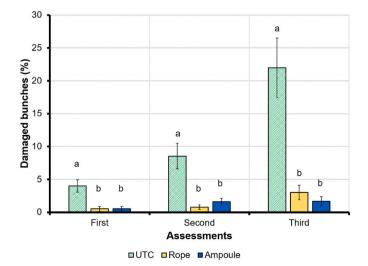


Fig. 6. Mean ( $\pm$ SE) percentage of the damaged bunches obtained with each treatment (untreated control (UTC), MD with the Rope dispensers, MD with the Ampoule dispensers) after the first, second and third male flights. Bars labeled with different letters are significantly different (GLMM, Tukey HSD, at P < 0.05).

the life span of both dispensers accomplished this requirement with different emission rates obtained for the mid-season wine cultivars. However, a late fourth generation can infest grapes that remain in the field until November, mainly in the case of table grape varieties (Coscollá, 1997). The Isonet® L rope dispensers used under the conditions of our study could not ensure effective pest control beyond 120 days and only the lifespan of the Lobetec Ampoule dispensers would be suitable for late varieties.

Regarding environmental humidity, our results confirmed that this weather variable did not affect dispensers' emission like in other studies with polyethylene dispensers (Zhu et al., 2015; Wiesner and Silk, 1982). Wind speed can also be an influential variable for semiochemical dispensers (Suckling et al., 1999; Sauer and Karg, 1998). An increase in this parameter has been shown to increase release rates in laboratory experiments; for example, trimedlure emission, the synthetic parapheromone of the tephritid Ceratitis capitata Wiedemann, increased in the laboratory following a linear correlation with wind speed (Alfaro et al., 2008), especially when using polymeric dispensers. Ogawa (1997) reported a 13% increase in the pheromone release rate of the Isonet® L rope dispensers when wind speed increased from 1 to 2.5 m/s under controlled conditions. In our case, multiple regression suggested that the mean wind speed significantly affected the Rope emission rates. The higher emission rates recorded in our study in the first month immediately after being installed (April to May with the highest wind speed values, 4 m/s) could be due in part to this wind-dependence effect.

Many studies relate higher emissions to higher temperatures in laboratory experiments (Pop et al., 1993; Weatherston, 1992; McDonough et al., 1989). In the present study conducted under field conditions, while increasing mean temperatures appeared to impact the Rope dispensers by increasing their release rate from June to the end of their life span, temperatures did not significantly affect the Ampoule dispensers, which had similar emission rates throughout the crop cycle. Femenia (2010) found that the Rope dispensers aged in a thermostatic chamber at 30  $^{\circ}$ C at a constant airflow of 0.3 m/s presented higher emission values than field-aged dispensers, which were exposed to fluctuating temperatures. The differences in dispensers' climate dependence can be partially attributed to the nature of their polymeric wall (material, porosity, thickness, etc.), which can condition not only permeability, but also the exposure surface of each dispenser type (Femenia, 2010). Reduced dependence on temperatures ensures, in principle, pheromone waste reduction. Greater temperature dependence means wasting A. Gavara et al. Crop Protection 155 (2022) 105926

pheromone in both warmer months and the warmest hours of the day when no male moth activity occurs (Lucchi et al., 2018a). Therefore, in an extreme case, a highly temperature-dependent dispenser may release enough mean daily amounts of pheromone, but provide suboptimal release rates to achieve the disruption effect from sunset to sunrise.

When considering the number of dispensers installed per ha, their emission rates and the efficacy of each treatment, the Ampoule dispensers released pheromone more efficiently than the Rope dispensers. The Ampoule dispensers provided the same efficacy as the Rope ones by inhibiting male catches and reducing damage with lower mean emission rates and a smaller quantity of dispensers installed per hectare. However, the Ampoule dispensers still contained nearly 40% of their initial mean pheromone load at the end of the crop cycle under Valencian climate conditions, which accounted for significant pheromone waste.

Recent studies based on airborne pheromone quantifications in MDtreated fields showed an increase of airborne pheromone concentration in the middle of the crop cycle, from the last week of July to the first of August (Gavara et al., 2020). This higher concentration in July could be related with the higher emission of Isonet® L rope dispensers in summer that has been demonstrated in the present work. Moreover, the studies by Gavara et al. (2020) suggest that lower airborne pheromone concentrations than those found in the center of a treated field with the Isonet® L rope dispensers could still trigger MD with no loss of effectiveness. This lower airborne pheromone concentration could be achieved following two strategies: reducing the emission rates or reducing the number of dispensers per hectare. The efficacy results obtained with the Lobetec ampoule dispensers with lower emission rates and deployment density (400 vs. 500 dispensers/ha) could confirm this hypothesis by demonstrating that lower emission and reduced number of dispensers/ha can still provide effective MD (inhibiting male catches and reducing damages) in places with similar characteristics to our study areas (with low pest pressure).

According to the efficacy and pheromone emission data obtained in our trials, only 0.65 mg/dispenser/day are required to achieve MD with 400 dispensers/ha. Therefore, to maintain this effect for 150 days, an ideal dispenser with a weather-independent constant release rate would require 97.5 mg of pheromone instead of the reported loads (187 mg or 206 mg). As the pheromone cost could represent 90% of the dispenser price (Vacas et al., 2015), dispensers with half the current load could save 40% of the total treatment cost. However, it is usually not as easy as reducing the dispensers' pheromone load because this can affect release rates. In fact, as seen on our study, the release kinetics of the Lobetec ampoules was somewhat different between the two batches analyzed. The dispensers analyzed in 2018, with a lower amount of pheromone, had lower emission rates, compared to the dispensers' emission in 2019 when they were loaded with more pheromone. This could be due to the internal surface of the dispenser wetted by the pheromone content: less wetted inner surface would mean less emission. For this reason, the impact of lower initial pheromone loads than those reported here on release rates needs to be first explored to elucidate whether lower emission values than those displayed by the currently used Lobetec dispensers would maintain their efficacy or, on the contrary, these values would be unable to disrupt EGVM, especially in high pest pressure areas.

In conclusion, reducing MD implementation costs (i.e., the amount and cost of pheromone used) is crucial for allowing growers to apply this pest control technique. Reducing the amount of pheromone used while maintaining MD efficacy has long been debated. Indeed, making a comparison of different commercial MD dispensers can help to discuss certain parameters, such as emission rates and the effect of weather conditions, which can impact treatment efficacy. The present work included studies carried out with two different MD passive dispensers during two years in locations with similar weather conditions. The results showed that Ropes and Ampoules both have non-ideal characteristics and there is still room for improvement when designing passive MD dispensers. These improvements can be made by avoiding high

residual pheromone contents, reducing climate dependence and ensuring that release rates are implemented to meet actual requirements. Nevertheless, more replicates in different locations with a wider range of weather conditions is needed to help draw more powerful conclusions.

#### Author contribution statement

A.G., S.V. and V.N.-L. designed and performed both, the field experiments, and the laboratory analyses. All authors analyzed the results and contributed to the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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