Airborne Pheromone Quantification in Treated Vineyards with Different Mating Disruption Dispensers against Lobesia botrana

Aitor Gavara1,*; Sandra Vacas1,*; Ismael Navarro2; Jaime Primo1; Vicente Navarro-Llopis1,*

1 Centro de Ecología Química Agrícola – Instituto Agroforestal del Mediterráneo. Universitat Politècnica de València – Edificio 6C - Camino de Vera s/n 46022 Valencia, Spain; vinallo@ceqa.upv.es
2 Ecología y Protección Agrícola SL, Pol. Ind. Ciutat de Carlet, 46240 Carlet (Valencia), Spain
* Correspondence: aigavi@etsiamn.upv.es (A.G.), sanvagon@ceqa.upv.es (S.V.), vinallo@ceqa.upv.es (V.N.-L.)

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Abstract: Mating disruption (MD) is widely used against the European grapevine moth (EGVM), Lobesia botrana Denis & Schiffermüller (Lepidoptera: Tortricidae), by installing passive dispensers or aerosol devices. The present work reports a new sampling and quantification methodology to obtain absolute data about field airborne pheromone concentration based on air samplings and sensitive chromatographic-spectroscopic methods. Samplings were performed in fields treated with passive dispensers or aerosol devices at different moments throughout the crop cycle to study how they act and how the disruption is triggered. Moreover, pheromone adsorption and releasing capacity of vine leaves were studied to elucidate their role in the disruption. Although both types of dispensers were effective in controlling the damage inflicted by EGVM, they performed differently and provided different airborne pheromone concentration profiles. Results also proved that leaves were able to adsorb and release airborne pheromone acting as buffers. This fact could explain the different concentration profiles. Moreover, our results suggest that lower pheromone emission than that of the current passive dispensers still could provide an adequate performance in field. Competitive mechanisms involved in MD using both dispensers, the dynamics of the airborne pheromone throughout the time and the importance of the canopy are discussed.

Keywords: European grapevine moth; Lepidoptera; Tortricidae; passive dispensers; aerosol devices; air samples; GC-MS/MS

1. Introduction

Mating disruption (MD) is widely used as an environmentally friendly pest control method in economically important crops due to its high effectiveness, specificity and the lack of toxic residues on fruit, in contrast to the conventional methods based on the use of pesticides [1,2]. Nevertheless, the higher cost of mating disruption compared with the traditional control in some crops is sometimes detrimental for its implementation [3-6]. For this reason, the studies focused on the optimization of the pheromone used, quantity and releasing mode are essential to reduce the implementation costs of this technique and make it more available to growers. This is important for species that are currently controlled by this method and even to introduce its use for species in which the cost used to be too high to be assumed by the crop.

Once mating disruption is proven effective against a pest, the pheromone cost is crucial for the implementation of this technique. The final cost of mating disruption depends on pheromone production costs [7,8], which are related with: (1) chemical synthesis and economies of scale, (2) the quantity of pheromone needed and (3) the dispensers’ emission rate and their number required per hectare. All these parameters depend on the biology of the target pest and the mechanisms that
trigger the mating disruption. In general, mating disruption may be governed by different mechanisms acting almost simultaneously but there is usually a principal mechanism responsible for the disruption. These mechanisms can be classified as competitive or non-competitive. In the case of competitive disruption, males, females and their signals have no impairments, therefore, females compete with the artificial pheromone sources (dispensers) and the efficacy of the treatment will depend mainly on pest density. On the other hand, when non-competitive disruption is acting, the signal, males or females are impaired since the beginning of sexual activity, making the efficacy pest-density-independent [9].

Hand applied passive dispensers are traditionally the most commonly employed devices to implement mating disruption as a control pest technique [1,2,10,11]. They consist of little containers of plastic materials filled or impregnated with the pheromone, which is released passively through their walls. These dispensers are deployed at high densities of more than 300 units/ha [9]. Using this type of dispensers, competitive attraction seems to be the main mechanism acting in disrupted fields [12]. Alternatively, automatic aerosol devices are being increasingly used with good efficacy against many moth pests [2,13-16]. They are mechanical devices connected to a pressurized aluminum can, which is loaded with an appropriate formulation of the pheromone and a propellant, usually an inert gas. These devices can be programmed for releasing equal amounts of the formulation containing the pheromone via shots at regular time intervals when moths are active. Unlike passive dispensers, these devices are deployed at very low densities (2-5 units/ha) due to their high pheromone release [9]. Non-competitive disruption triggered by desensitization of males along their wide plumes was an explanation of their performance [7], although a competitive mechanism cannot be dismissed and most current studies report that males are drawn away from females towards the pheromone sources in a mechanism called “induced allopatry” [9,14,15].

The European grapevine moth (EGVM), Lobesia botrana Denis & Schiffermüller, is a key pest in vineyards which has been traditionally controlled using mating disruption [16,17,18] by employing hand applied passive dispensers at high densities providing a continuous emission of (E,7,9-dodecadien-1-y) acetate [19,20]. Besides passive pheromone dispensers, aerosol devices are also available nowadays to apply mating disruption against EGVM. In this pest, the efficacy of aerosols is scarcely reported in the scientific literature but with promising results [2,21]. In addition, these studies highlighted three inherent advantages of this technique: the lower number of dispensers per hectare needed, the reduction of labor costs and the reduction of plastic disposal compared with passive dispensers.

Despite the effectiveness reported for both types of dispensers, the reduction of their cost and improvement of their efficacy are still necessary to spread the use of mating disruption. For this reason, a better knowledge of the mode of action of each one must be accurately studied. Most of the studies performed on different pests are based on empirical and indirect measures of the efficacy based on parameters such as fruit injury levels, dispenser load and distribution, etc. [5,22-24]. The quantification of airborne pheromone concentrations present in the field, the study of pheromone distribution from the time of their releasing and the interaction with the canopy could offer an interesting approach with valuable information to improve the efficacy of pheromone-releasing systems [25].

Unfortunately, there is scarce information regarding airborne pheromone concentrations due to the difficulty of its quantification. Three techniques were described in the past to measure airborne pheromone [26]: (1) field electroantennography (EAG) measurements, (2) single sensillum recordings and (3) air sampling by collecting airborne volatiles on filters and their chemical analysis. Field EAG offers more instantaneous and quick measurements that allow studying temporal and spatial pheromone fluctuations. However, data obtained with these methods could be misinterpreted due to the interaction of pheromone and host-plant detection, differences between individual antennae and the lack of linearity of the detector [26]. Moreover, this procedure needs precise calibrations due to the complexity of the environmental matrix and insect fitness, and it also depends on a proper handling of insect preparations. Single sensillum recordings have the advantage of the elimination of other semiochemicals effect in the signal. However, it is more difficult to carry out than field EAG.
due to the fragile and laborious preparation of the antennae, besides not being effective for the interpretation of the signal when a pheromone blend triggers an insect response. There are works that attempted to study airborne pheromone concentration in fields treated against EGVM using field EAG in the 1990s [26-29]. In these experiments, they were capable to obtain relative data to see differences in airborne pheromone concentration by studying different parameters such as different seasons (spring and summer), height of dispensers deployment, their density, presence of developed foliage, distance from the center of the plot, etc., but they had several difficulties due to the field EAG drawbacks.

Air sampling followed by chemical analysis of collected samples for pheromone quantification is a technically feasible method that can provide the most accurate and absolute data about airborne pheromone concentration in treated fields. However, it provides time-averaged data because relatively long sampling periods are needed to detect the minute quantities of pheromone collected [26]. The low detection level thresholds required were, in the past, the main difficulty for these measurements, but nowadays, due to the lower limits of detection achieved by analytical instruments, an accurate quantification of airborne substances is possible. Many methods have been developed to detect environmental and health-damaging substances at very low concentrations, i.e. dioxins and furans are quantified in air at concentrations of femtograms (10\(^{-15}\) g) per cubic meter [30-32]. Airborne psychotropic substances were quantified at nanograms (10\(^{-9}\) g) per cubic meter in different Italian cities [33] as an example of substances at similar concentration levels to airborne pheromones. These quantifications can be achieved with high volume collectors, effective adsorbents and more sensitive chromatographic and spectroscopic methods such as gas chromatography coupled with triple quadrupole mass spectrometers (GC/MS/MS). Although there are many protocols for quantifying environmental and health damaging substances, the similarities of pheromone chemical structures with organic compounds present in the environmental matrix and the lack of characteristic functional groups still make their identification and quantification more difficult.

In this work we have developed a method that allowed us to successfully quantify airborne concentrations of EGVM pheromone in vineyards treated with two commercial systems: passive dispensers and aerosol devices. These quantifications have been performed in three different moments throughout the vineyards crop cycle for both types of dispensers while comparing their efficacy. In addition, foliar samples of these treated vineyards were taken to analyze their pheromone content and check their role as buffers. All the data collected has been employed to discuss the operating mode of both dispensers and the competitive mechanisms governing the mating disruption of the EGVM with each pheromone emission system.

2. Materials and Methods

2.1. Field locations and mating disruption systems

Studies were conducted between March and October 2018 in two 2-ha plots of vineyards for winery trained onto trellises and located in two municipalities of Valencia, Spain. Field 1 was located in Venta del Moro (UTMs 39.482608, -1.318822) with a plantation pattern of 2.5 x 1.5 m (cultivar Bobal) and was treated with 500 passive dispensers/ha (Isonet® L, Shin-Etsu Chemical Co., Tokyo, Japan), installed on 24 April 2018. Field 2 was located in Moixent (UTMs 38.808779, -0.815917) also with a plantation pattern of 2.5 x 1.5 m (cultivar Monastrell) and was treated with three aerosol devices per ha (CheckMate®Puffer® LB; Suterra LLC, Oregon, USA), following manufacturer instructions. Aerosol devices were installed on 23 April 2018 and programmed to release the pheromone via shots each quarter from 18:00 h to 06:00 h. An untreated control plot was also included in both areas, located 500 m upwind from the treated plots.

2.2. Airborne pheromone quantification

2.2.1. Chemicals and reagents

In addition, foliar samples of these treated vineyards were taken to analyze their pheromone content and check their role as buffers. All the data collected has been employed to discuss the operating mode of both dispensers and the competitive mechanisms governing the mating disruption of the EGVM with each pheromone emission system.
All solvents and chemical reagents used (HPLC and Synthesis grade, respectively) were purchased from Merck (Darmstadt, Germany). All reactions were carried out under a nitrogen atmosphere with dry solvents, unless otherwise noted. Solvents were dried under standard procedures. All purchased chemicals were used without further purification. Macherey-Nagel Silica gel 60 F254 TLC plates (Fisher Scientific SL, Madrid, Spain) were used for analytical thin layer chromatography (TLC) technique. Plates were visualized by using a UV lamp or using an appropriate stainer (p-anisaldehyde in ethanol/aqueous H$_2$SO$_4$/CH$_3$CO$_2$H or 10 % solution of phosphomolybdic acid in ethanol and heat as developing agents).

(E,Z/E)-7,9-dodecadien-1-yl acetate was supplied by Ecología y Protección Agrícola S.L. (Carlet, Valencia, Spain). High purity standard of (E,Z)-7,9-dodecadien-1-yl acetate (ca. 95 %) was obtained after purification of the supplied sample by gravity column (silica gel containing 10 % of AgNO$_3$; eluent: 3 % EtO/Hexane), which had spectroscopic properties identical to those described in the literature [34].

Due to the abundance of pheromone structurally related compounds in air collected samples, a straight chain fluorinated hydrocarbon ester (heptyl 4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononanoate), was selected as an internal standard in order to improve both sensitivity and selectivity for MS/MS method optimization. See next section for experimental details.

### 2.2.2. Synthesis of heptyl 4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononanoate (TFN)

To a solution of 4-dimethylaminopyridine (DMAP) (15 mg) in dichloromethane (DCM) (30 mL), 4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononanoic acid (500 mg, 1.3 mmol) was added. After stirring during 60 min at room temperature, 1-heptanol (0.21 mL, 1.5 mmol) was added and the mixture was refluxed for 24 h. After this period, the solvent was removed under vacuum and the crude was purified by column chromatography (silica gel; eluent: 1 % EtO/Hexane) to yield heptyl 4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononanoate (281 mg, 45 %) as a colorless oil. MS (70 eV, m/z): 393 (10 %), 375 (40 %), 373 (5 %), 132 (10 %), 98 (30 %), 83 (15 %), 70 (100 %), 69 (70 %), 57 (90 %), 56 (90 %).

### 2.2.3. Field air sampling

Air sampling was carried out with a high-volume air sampler (CAV-A/Mb, MCV, Barcelona, Spain) provided with an adsorbent sandwich composed by alternate layers of 20 g/L polyurethane foam (MCV, Barcelona, Spain) and adsorbent resin (XAD-2, Sigma-Aldrich, Madrid, Spain). The sandwich structure was designed according to literature [35,36] and preliminary laboratory experiments.

The air samples were taken during three different plant growth phenological stages (BBCH) according to Lorenz et al. [37] throughout the crop cycle: (1) 4 samples after dispenser setting at BBCH 00-15 (May-June) (according to Lorenz et al., 1994) for both the aerosol devices and passive dispensers; (2) 4 samples for aerosol devices and 3 samples for passive dispensers at the middle of the crop cycle at BBCH 68-75 (last week of July and first of August); (3) 3 more samples for each one of both types of dispensers at the end of the crop cycle, before the harvest BBCH (81-89) (between 11 and 27 September 2018). Samplings were initiated at 12:00 h and the sampling time was established at 48 hours with a flow of 15 m$^3$/h to cover two nights because of the aerosol devices activity. Once the samples were collected, the different parts of the sandwich were stored individually in a freezer at 3 ºC in airtight bags until their extraction.

### 2.2.4. Sample extraction and filtration

Foam-resin adsorbent sandwiches were extracted using a 250-mL Soxhlet apparatus, bringing 300 mL of hexane to boil. Temperature was set at 90°C for 6 hours obtaining approximately 15 Soxhlet cycles. Each extract was concentrated up to 5 mL using a rotary evaporator set at 30 ºC and finally to 1 mL, blown down with a gentle stream of nitrogen. Then, the samples were cleaned up through a gravity column (0.5 x 15 cm) packed, from bottom to top, with a glass wool plug and 500 mg of silica...
gel pre-eluted with hexane. The column was sequentially eluted with hexane (5 mL) and 2% EtO/Hexane (12 mL), collecting three fractions of 5 mL. Fractions two and three containing the pheromone were concentrated up to ca. 1.5 mL using a rotary evaporator set at 30 °C, and finally to 0.5 mL in a 2-mL GC screw-cap (Fisher Scientific SL, Madrid, Spain) blown down with a gentle stream of nitrogen. Occasionally, the column was rinsed with 100% EtO in order to corroborate the complete elution of the pheromone from samples.

2.2.5. Pheromone quantification

Ten-µl of an internal standard solution of TFN were added to the abovementioned fraction containing the pheromone for the final chemical analysis and quantification. Each extract was then analyzed using a TSQ 8000 Evo triple quadrupole MS/MS instrument operating in SRM (selected reaction monitoring) mode using electron ionization (EI+), coupled with a Thermo Scientific™ TRACE™ 1300 GC. All injections were made onto a ZB-5 (30 m × 0.25 mm ×0.25 mm) fused silica capillary column (Phenomenex Inc., Torrance, CA). The oven was held at 60 °C for 1 min then raised at 10 °C/min up to 110 °C, maintained for 5 min, raised at 3 °C/min until 150 °C and finally raised at 35 °C/min up to 300 °C held for 5 min. The carrier gas was helium at 1.5 mL/min.

For each target compound —TFN and the main component of EGVM pheromone, (E,Z)-7,9-dodecadien-1-yl acetate—, the MS/MS method was optimized by selecting the precursor ion and the product ions that provided the highest selective and sensitive determinations (Table 1). TFN transition 2 and EGVM transition 3 were the ones employed to obtain the chromatographic areas. In the case of EGVM signal, the other transitions were monitored for confirmatory purposes to have increased selectivity when several peaks appear near to the pheromone peak retention time.

The amount of pheromone and the corresponding chromatographic areas were connected by fitting a linear regression model, \( y = a + bx \), where \( y \) is the ratio between pheromone and TFN areas and \( x \) is the amount of pheromone.

The percentage of pheromone recovery after the extraction was checked by spiking known pheromone concentrations to a sandwich previously exposed to the same procedure of air sampling in pheromone-free places and, thus, containing environmental matrix. The chemical analysis showed a mean pheromone recovery rate of 92.8%.

Table 1. Optimum values of the MS/MS parameters for each target compound.

<table>
<thead>
<tr>
<th>Transition</th>
<th>Precursor ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>375</td>
<td>263</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>393</td>
<td>373</td>
<td>5</td>
</tr>
<tr>
<td>EGVM pheromone (main component)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>79.1</td>
<td>77</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>93.1</td>
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<td>10</td>
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<tr>
<td>3</td>
<td>164.2</td>
<td>79</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>164.2</td>
<td>94.1</td>
<td>5</td>
</tr>
</tbody>
</table>

2.3. Adsorbed pheromone on leaves

2.3.1. Adsorption capacity and emission assays

The potential of vine leaves for airborne pheromone adsorption was explored in an experimental plot in Experiment 1 and 2 and later in the commercial field conditions of Fields 1 and 2 (Experiment 3).

In Experiment 1, a single aerosol shot was made 2 m away from vine leaves —simulating the distance between two rows in a vineyard—. Then, 8 g of leaves in the shot influence area were randomly collected immediately (09:00 h) and 4 h, 8 h and 24 h after application. The collected leaf samples were extracted to quantify their pheromone content and to study the release profile. This experiment was replicated 4 times in different points along the vine row (at least 2 m between shots).
In Experiment 2, an aerosol installed 2 m away from vine leaves was programmed to deliver a shot every 15 min from 18:00 h to 06:00 h. Then, 8 g of leaves were collected at three different times: 09:00 h, 13:00 h and 17:00 h (3, 7 and 11 h, respectively, after the last shot), and their pheromone content was quantified. Four replications of this experiment were done in previous non-treated areas separated at least 2 m among them.

Once the potential for pheromone adsorption was demonstrated, the Experiment 3 was carried out in real field conditions in mid-August. Given that passive dispensers are evenly distributed along the plot in Field 1, leaves were sampled only in the center of this plot. However, as the aerosols were deployed along the perimeter of the plot in Field 2, we decided to sample leaves randomly in the border — along the row where the aerosol was installed — and in the center of the plot (70 m from the closest aerosol device) to check differences. Each sample consisted of 8 g of leaves, and three replicates were taken in all cases. All samples were taken at 12:00 h, 6 hours after the last puff shot.

2.3.2. Leave sample extractions

Each 8 g sample was soaked in 300 mL hexane inside a 500-mL round-bottom flask. Each flask was partially immersed in an ultrasonic bath for 30 min and the hexane extract was filtered and concentrated using a rotary evaporator up to 1.5 mL. The solution was transferred to an Eppendorf to be centrifuged for 5 min at 3000 rpm. The supernatant was recovered, and to ensure the complete extraction of the pheromone the residue was re-extracted with 1 mL of hexane. Both solutions were concentrated separately, under a gentle nitrogen stream, up to 0.5 mL and first analyzed by gas chromatography using a flame ionization detector (GC/FID) in a Clarus 500 GC (PerkinElmer Inc. Wellesley, MA) using n-dodecane as an internal standard. All injections were made onto a ZB-5 (30 m × 0.25 mm × 0.25 µm) column (Phenomenex Inc., Torrance, CA), held at 100 ºC for 1 min and then, raised at 25 ºC/min up to 250 ºC, maintained for 3 min. The carrier gas was helium at 1.5 mL/min. The amount of pheromone and the corresponding chromatographic 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. The samples whose pheromone concentration was below the quantification limit of the GC/FID, were analyzed using a TSQ 8000 Evo triple quadrupole mass spectrometer (GC-MS/MS) coupled with a Thermo Scientific™ TRACE™ 1300 GC. These analyses were performed as described previously in section 2.2.5. Moreover, the leaves were re-extracted and analyzed to ensure the absence of pheromone in the samples’ residues. Commercial pheromones are usually mixtures of (E, Z)-7,9-dodecadien-1-yl acetate. The initial proportion observed between geometrical isomers remain constant along the different measures, showing no isomerization to the more stable (E/E) isomer during this period.

2.4. Release profiles studies of the emission systems

In parallel with the field trials, the pheromone release profiles of the different systems were studied.

Additional passive dispensers were simultaneously aged in nearby areas of Field 1 for approximately 150 days to obtain the release kinetic in a similar way than other studies [38]. Four dispensers were taken from the field each month and their residual pheromone content was extracted and quantified by GC/FID, using the same capillary column and conditions described above in section 2.3.2. For residual pheromone extraction, each dispenser was cut in pieces and soaked in 25 mL dichloromethane, inside a 50-mL glass centrifuge tube, for 2 h with magnetic agitation. Residual (E, Z)-7,9-dodecadien-1-yl acetate contained in the extracts was then measured by GC/FID, using n-dodecane as an internal standard. Occasionally, the dispensers were re-extracted to verify the complete extraction of the pheromone.

In the case of the aerosol devices, pheromone release was estimated by the gravimetric method. Six devices were monthly weighted with a scale in the field. According to the composition label of the aerosols used, the pheromone is present as a 9.11% w/w. The amount of pheromone released over a period was calculated after applying that percentage to the weight differences.
2.5. Efficacy of the Treatments

Population was monitored to check the efficacy of each type of dispenser and relate efficacy of mating disruption with airborne pheromone concentration measured in the same fields. For this purpose, four delta traps baited with a “Grapemone” lure (OpenNatur, Lleida, Spain) and provided with a sticky base were set in the center of each plot inside the vine canopy at 1 m above the ground. Captures were revised fortnightly and lures were replaced every 42 days. The absence of catches in mating disruption plots was considered as mating disruption success performance.

In addition, crop damage assessment gave the final proof for the treatment efficacy. For this purpose, at least 200 bunches were inspected in 50 vines surrounding each delta trap to look for damaged bunches. A bunch was considered damaged when eggs, nests or larvae were found in the visual inspection.

2.6. Data analysis

All the statistical analyses were performed using SPSS v. 16 (Armonk, NY, USA). The analysis of variance (ANOVA) followed by post-hoc Fisher’s least significant difference (LSD) test at P < 0.05 was used to study the differences observed in absolute airborne pheromone concentrations among mating disruption dispensers’ air samplings. The analysis was applied to these data without transformation, as they fulfilled the homoscedasticity requirements and the residuals of the ANOVA fitted a normal distribution. In the case of the leave adsorption experiments, data was normalized using $\sqrt{x}$, whereas data from the crop damage assessments were subjected to the angular transformation, $\arcsin(\sqrt{x})$, prior to the ANOVA. Due to the impossibility of normalizing the distribution and homogenize the variance of male moth captures in both locations, the non-parametric Kruskal-Wallis test ($P = 0.05$) was employed to determine if there were statistically significant differences between the male catches obtained with each treatment throughout the main flight peaks [2].

3. Results

3.1. Airborne pheromone quantification

Field volatile collections and the following chemical analysis showed that airborne pheromone concentrations throughout the season differed significantly between the treatments deployed ($F=28.592; df= 5, 20; P<0.005$). The mean concentration in fields treated with passive dispensers was 4.84 ng/m$^3$ in May and the quantity increased up to 42.20 ng/m$^3$ in the middle of the crop cycle, significantly differing from the initial concentration (Figure 1). Finally, the last sampling highlighted a decrease in the concentration up to the initial levels of 4.76 ng/m$^3$. By contrast, the mean pheromone concentration in aerosol treated fields was 0.44 ng/m$^3$ at the beginning of the season. This concentration also increased significantly in the middle of the crop cycle (22.34 ng/m$^3$) but not as much as with the passive dispensers. Then, the airborne pheromone concentration was maintained until the end of the trial in the last sampling period (30.83 ng/m$^3$) (Figure 1).

The main difference between both releasing systems is that in the field treated with aerosol devices, the total amount of airborne pheromone at the end of the crop cycle remains with the same levels that in the second sampling period, whilst in the case of passive dispensers it decreases until the first basal levels shown in the first sampling period.
Figure 1. Mean (±SE) absolute airborne pheromone concentrations (ng/m³) in vineyards treated with passive dispensers and aerosol devices. Air samples were collected at three moments during the crop cycle. The beginning of the crop cycle (second week of May to middle June). In the middle of the crop cycle (last week of July-first week of August). At the end of the crop cycle (second to third week of September). Bars labelled with different letters are significantly different, ANOVA followed by Fisher LSD test ($F = 28.592; df = 5, 20; P < 0.005$).

3.1. Pheromone leaves adsorption capacity

3.1.1. Experiment 1

Immediately after the single aerosol shot, the total amount of pheromone quantified on leaves was 312.4 µg/g, which decreased significantly up to 38.7 µg/g in just 4 h after the application (Figure 2) ($F = 48.996; df = 3, 15; P < 0.001$). This amount represented approximately a decrease of almost 88% of the initial content. Then, this content kept on reducing, but at a slower rate, up to 8.3 µg/g in the third sample taken at 17:00 h (8 h after the application). Finally, the last sample taken 24 h after the application showed 5.6 µg/g. Thus, the quantified values suggest that vine leaves can release the previous adsorbed pheromone probably acting as a buffer.

Figure 2. Mean (±SE) quantity of pheromone (µg pheromone/g leaves) adsorbed on leave samples of Experiment 1, after one aerosol shot. Bars labelled with different letters are significantly different, ANOVA followed by Fisher LSD test ($F = 48.996; df = 3, 15; P < 0.05$).
3.1.2. Experiment 2

When leaves were treated throughout the evening-night, despite the different mean amounts quantified (383 µg/g, 239.9 µg/g and 177.6 µg/g, 3 h, 7 h and 11 h after the last shot, respectively), these were not significantly different (Figure 3; F=0.560; df= 2, 13; P > 0.05). We observed high variability in the different samples for every treatment, as can be observed in the wide error bars of Figure 3. This could be possibly explained by the different leave position on the plant and the difficulties to establish an “aerosol action area” due to the irregularity and diffusion of the plume and the wind effect. Despite the lack of statistical support, data suggest a similar decreasing trend to that observed in Experiment 1 (Figure 2). Connecting the mean amounts of pheromone with the time passed after the last shot by fitting a linear regression model, \( y = a + bx \) (\( y = -28.61x + 390.89; R^2=0.91 \)), the mean pheromone emission from leaves could be established at 28.61 µg/h.

![Figure 3. Mean (±SE) quantity of pheromone (µg pheromone/g leaves) adsorbed on leave samples of Experiment 2, after overnight aerosol shooting (from 18:00 to 6:00). Bars labelled with different letters are significantly different, ANOVA followed by Fisher LSD test (F = 0.560; df = 2, 13; P > 0.05).](image)

3.1.2. Experiment 3

When leaf samples were taken from the field plots treated with mating disruption, (Figure 4) no statistically significant differences were observed (F = 1.287; df = 2,8; P > 0.05). The results highlight that leave samples reached the same mean quantity of pheromone (0.07 µg/g) when taken in the center of the plots treated either with aerosols or passive dispensers. In the border of the aerosol-treated plot, this quantity reached a mean of 0.14 µg/g, although this measure had a high variability —the sample collected closest to the aerosol reached 0.26 µg/g, while the furthest contained only 0.06 µg/g—.
Figure 4. Mean (±SE) quantity of pheromone (µg pheromone/g leaves) adsorbed on leaf samples of Experiment 3, collected in actual field conditions: along the row where aerosol devices were deployed (Aerosol devices border), 70 m away from the aerosols row (Aerosol devices center) and at the center of the passive dispensers treated field (Passive dispensers center). Bars labelled with different letters are significantly different, ANOVA followed by Fisher LSD test (F = 1.287; df = 2, 8; P > 0.05).

3.2. Dispensers’ pheromone release profile

3.2.1. Passive dispensers’ release profile

The quantified residual pheromone contents fitted the linear regression profile depicted in Figure 5 until the end of the growing season (140 days), when their useful life started to deplete. This means that pheromone load decreased at a constant rate and the slope of the resulting equation gives a mean pheromone release rate of about 1.2 mg/day/dispenser. Thus, the total amount of pheromone emitted is estimated at 526.29 ± 76.30 mg/day/ha from May to September (Table 2). Then, the release rate decreased in the period from early September to mid-October (from 140 to 180 days) up to one third of the previous level (226.85 mg/day/ha).

Figure 5. Pheromone release profile of passive dispensers, as residual mean pheromone load (mg) contained in the dispensers vs. the time of field exposure (days). Release kinetics fitted the linear model given by the equation.
Table 2. Passive dispensers aging. Residual means of pheromone content (mg) throughout the time in field (days). Point values of Figure 5 and the total mean of pheromone used (mg/day/ha).

<table>
<thead>
<tr>
<th>Days in field (d)</th>
<th>Pheromone content (mg)</th>
<th>Dispensers/ha</th>
<th>Pheromone emission (mg/day/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>196,02 ± 3,39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>176,04 ± 4,74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>135,82 ± 5,58</td>
<td>500</td>
<td>356,71</td>
</tr>
<tr>
<td>84</td>
<td>105,08 ± 2,92</td>
<td></td>
<td>648,67</td>
</tr>
<tr>
<td>112</td>
<td>68,81 ± 6,99</td>
<td></td>
<td>614,85</td>
</tr>
<tr>
<td>140</td>
<td>31,68 ± 3,03</td>
<td></td>
<td>647,80</td>
</tr>
<tr>
<td>182</td>
<td>12,63 ± 4,03</td>
<td></td>
<td>662,89</td>
</tr>
<tr>
<td>Mean</td>
<td>526.29 ± 76.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2.1. Aerosol devices’ profile

The resulting release profile of the aerosol devices also fitted the linear equation depicted in Figure 6, which means a significantly constant mean release rate of 0.15 g/day/dispenser and a total pheromone emission of 602.49 ± 37.15 mg/day/ha (Table 3). In this case, aerosols did not show a decrease in their emission, in comparison to the passive dispensers, because of the dispensers’ nature.

Figure 6. Pheromone release profile of aerosol devices, as residual mean pheromone load (mg) contained in the dispensers vs. the time of field exposure (days). Release kinetics fitted the linear model given by the equation.

Table 3. Aerosol devices pheromone content. Residual means of pheromone content (mg) throughout the time in field (days). Point values of Figure 6 and the total mean of pheromone used (mg/day/ha).

<table>
<thead>
<tr>
<th>Days in field (d)</th>
<th>Weight (g)</th>
<th>Pheromone content (9.11% w/w) (g)</th>
<th>Devices/ha</th>
<th>Pheromone emission (mg/day/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>375,25</td>
<td>34.19 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>334,75</td>
<td>30.50 ± 0.07</td>
<td></td>
<td>508.90</td>
</tr>
<tr>
<td>57</td>
<td>282,75</td>
<td>25.76 ± 0.16</td>
<td>4</td>
<td>676.74</td>
</tr>
<tr>
<td>85</td>
<td>243,75</td>
<td>22.21 ± 1.10</td>
<td></td>
<td>507.56</td>
</tr>
<tr>
<td>111</td>
<td>191,83</td>
<td>17.48 ± 0.79</td>
<td></td>
<td>727.63</td>
</tr>
</tbody>
</table>
3.3. Population monitoring

Male catches per trap and day (CTD) recorded throughout the assay were low in both locations and not significantly different between them (H = 2.724, P > 0.05), which leads to conclude that the infestation level of both locations was similar. These low catches can be explained by the use of mating disruption against EGVM in these areas during the last years. However, population dynamics were somewhat different. The most abundant peak was the third in Field 1 and the second in Field 2 (Figures 7 and 8).

In Field 1 (Figure 7), the plot treated with passive dispensers did not obtain any male in the monitoring traps throughout the trial, so flight inhibition was complete (100%). However, in the case of the aerosol devices, some male captures were recorded, mainly in the first flight. During this flight, these dispensers reached an inhibition of 53% compared with the captures in the control plot, without showing statistical differences between them (H = 0.886, P > 0.05), neither with the passive dispensers performance (H = 1.940, P > 0.05). No males were captured during the second flight in both plots, showing a total male capture inhibition and significantly differing from the control plot (H = 3.200, P < 0.05), neither with the passive dispensers plot (H = 0.938, P > 0.05).

Aerosol devices achieved 97% capture inhibition regarding the control plot during the third flight and the number of captures was significantly different from those recorded in the control plot (F = 15.378, P < 0.05) but not from the passive dispensers plot (H = 0.938, P > 0.05).

In Field 2 (Figure 8), passive dispensers also showed a total flight inhibition (100%) compared with the control plot, with no male captures throughout the study period. The absence of captures along the peaks was reflected in statistical differences against the control plot male captures in the first (H = 6.650, P < 0.05), second (H = 12.754, P < 0.05), and third flight peak (H = 11.713, P < 0.05).

In the case of the plot treated with the aerosol dispensers, some captures were registered during the first flight, in which they performed only 64% capture inhibition, showing significant differences against the passive dispensers treatment (H = 8.064, P > 0.05) but not with the control plot captures (H = 0.055, P > 0.05). No captures were obtained during the other two flight peaks (100% inhibition),

### Table 1

<table>
<thead>
<tr>
<th>Location</th>
<th>Male Catches</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field 1</td>
<td>139.83</td>
<td>631.63</td>
</tr>
<tr>
<td>Field 2</td>
<td>98.16</td>
<td>562.48</td>
</tr>
</tbody>
</table>

Mean 602.49 ± 37.15

### Figure 7

Male captures per trap per day (CTD) throughout the season in Field 1 in the plots with different treatments.

<table>
<thead>
<tr>
<th>Date</th>
<th>Flight 1</th>
<th>Flight 2</th>
<th>Flight 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-5</td>
<td>0.35</td>
<td>0.25</td>
<td>0.15</td>
</tr>
<tr>
<td>23-24</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>12-13</td>
<td>0.2</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>2-7</td>
<td>0.1</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>11-8</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>31-38</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>20-29</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

- Control
- Passive dispensers
- Aerosol devices
significantly differing from the control plot in the second (\(H = 13.467, P < 0.05\)) and the third flight (\(H = 11.713, P < 0.05\)).

Figure 8. Male captures per trap per day (CTD) throughout the season in Field 2 in the plots with different treatments.

3.4. Crop damage prospections.

As it is summarized in Table 4 and Table 5, damage assessments in the control plots showed similar low values in both locations, with about 5% of affected bunches during early and mid-summer (assessments 1 and 2). However, just before the harvest (assessment 3), these data increase at very high levels — exceeding 30% in Field 1 and near 15% in Field 2. In particular, the damage level in Field 1 was statistically reduced using both MD dispensers compared to non-treated plot damage. Moreover, there were no statistical differences between MD treated plots with both types of dispensers, until the assessment done immediately before the harvest when aerosol devices reduced the damage until lower values than passive dispensers. Nevertheless, the control plot reached 35% damage (Table 4). Thus, both treatments achieved high efficacy, reducing crop damage by 97.14% in the case of aerosol devices and 81.43% in the case of passive dispensers.
Table 4. Percentage of damaged bunches obtained with each treatment in the three damage assessments carried out in Field 1.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Control (C)</th>
<th>Aerosol devices (A)</th>
<th>Passive dispensers (P)</th>
<th>Comparisons</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7,5</td>
<td>1,25</td>
<td>1,25</td>
<td>C-A*</td>
<td>7,470</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-P*</td>
<td>7,470</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A-P</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>C-A*</td>
<td>5,490</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-P*</td>
<td>10,244</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A-P</td>
<td>2,010</td>
<td>0.249</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>1</td>
<td>6,5</td>
<td>C-A*</td>
<td>78,320</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-P*</td>
<td>49,394</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A-P*</td>
<td>8,381</td>
<td>0.006</td>
</tr>
</tbody>
</table>

1 Damage assessments were performed after the first, second and third male flights. 2 Percentage of damaged bunches. 3 Results of the statistical analysis (χ² test, at P < 0.05), statistical differences are marked with asterisks.

In Field 2, passive dispensers’ treatment reached 100% efficacy in all the assessments throughout the crop cycle. In the case of aerosol devices, their efficacy varied throughout the assessments (Table 5). In the first assessment, the efficacy of aerosol devices was about 73% but no statistical differences were seen against control plot damage. In the second assessment, their damage reduction was 72.72%, and the damage was statistically lower than in the control plot. Finally, this treatment achieved a 96.55% damage reduction compared with the control at the end of the crop cycle showing statistical differences.

Table 5. Percentage of damaged bunches obtained with each treatment in the three damage assessments carried out in Field 2.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Control (C)</th>
<th>Aerosol devices (A)</th>
<th>Passive dispensers (P)</th>
<th>Comparisons</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,22</td>
<td>0,60</td>
<td>0</td>
<td>C-A*</td>
<td>1,574</td>
<td>0.374</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-P</td>
<td>3,710</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A-P</td>
<td>4,003</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>4,29</td>
<td>0,95</td>
<td>0</td>
<td>C-A*</td>
<td>4,574</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-P*</td>
<td>9,197</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A-P</td>
<td>2,010</td>
<td>0.249</td>
</tr>
<tr>
<td>3</td>
<td>14,50</td>
<td>0,50</td>
<td>0</td>
<td>C-A*</td>
<td>28,252</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-P*</td>
<td>31,267</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A-P*</td>
<td>1,574</td>
<td>0.374</td>
</tr>
</tbody>
</table>

1 Damage assessments were performed after the first, second and third male flights. 2 Percentage of damaged bunches. 3 Results of the statistical analysis (χ² test, at P < 0.05), statistical differences are marked with asterisks.

All these data agree with the information provided after the flight curve in both locations. The lack of captures in passive dispensers’ plots corresponds with the absence of fruit damage. The lower inhibition achieved with the aerosol devices, which at the first flight peak produces little damage in the first harvest, possibly allowed the remaining of little population during the crop cycle.

4. Discussion

Despite its low concentration and the complexity and diversity of substances present in the environment [38], in this work, we have developed a new methodology capable of quantifying airborne pheromone concentrations. Due to the increasing concerns about the impact of
471 semiochemicals into the environment [40], this methodology could be useful to detect and quantify
472 their presence and elucidate potential consequences.
473
474 In our case, this methodology has been used to study the differences in the airborne pheromone
475 concentrations present in fields treated with different types of MD dispensers. The increasing use of
476 mating disruption to control insect pests, and the rapid development of new tools for its
477 implementation, have made necessary to gain insight into the mechanisms governing the disruption
478 and how these devices act. Several studies have hypothesized about the different mechanisms
479 involved when using aerosol devices or the traditional passive dispensers. However, just a few of
480 them are based on pheromone distribution in crops and the interaction with the canopy [21,41,42].
481 The methodology employed in the present work has allowed the detection of different airborne
482 pheromone concentrations throughout the season between aerosol and passive dispensers’ treated
483 fields. These data provide a new approach that helps to understand differences and similarities in
484 both systems in addition to support other studies in this field.
485
486 Although portable EAG can help to understand the variations of airborne pheromone along the
487 day and their dynamics in front of episodes as winds, dispensers’ distribution, etc. [26-28], it does not
488 provide absolute data. Despite the large sampling periods required (48 h), our results provide actual
489 pheromone concentration measures that can be related with the efficacy of mating disruption
490 treatments (expressed as male flight) providing valuable information for a better understanding of
491 how the pheromone remains in the field and to reduce pheromone wasting. Moreover, it may help
492 to establish a relationship with dispenser characteristics to improve their density and emission and
493 so reducing the cost of implementation of the technique.
494
495 The results from the field air collections and quantifications showed differences between the two
496 systems tested for the mating disruption of EGVM. In the case of the passive dispensers, lower mean
497 quantities (4.84 ng/m$^3$) of airborne pheromone were detected at the beginning of the crop cycle,
498 whereas the concentration reached a maximum (42.20 ng/m$^3$) in the middle of the cycle during
499 summer. Similar results were obtained in relative data by Sauer et al. [28] in which significant
500 differences were found between EAG measurements done in spring and summer, showing lower
501 EAG signals in the spring studies. Later in the season, the concentration values decreased at the end
502 of the crop cycle (4.76 ng/m$^3$), coinciding with the end of the dispensers’ lifespan. The concentration
503 profile was quite different in the field treated with the aerosol devices, showing a minimum mean
504 concentration at the beginning of the crop cycle (0.44 ng/m$^3$) and maintaining higher quantities for
505 the rest of the season (22.34 - 30.83 ng/m$^3$). In both cases, the initial low quantity of pheromone
506 detected could be explained by the lack of a developed canopy. When the canopy is scarce, the wind
507 velocity increases and consequently the pheromone may be washed away. In this regard, it has been
508 reported that foliage is able to act keeping the pheromone in the crop environment reducing the
509 convective air streams [28,43]. This effect could explain why the mean pheromone concentrations
510 detected in the aerosol treated field at the beginning of the season are lower than those achieved with
511 the passive dispensers. The latter system does not stop emitting pheromone during the day and is
512 able to maintain a certain airborne pheromone concentration despite the lack of plant foliage.
513 However, aerosol devices are designed to release the pheromone only at programmed intervals
514 during the evening-night. Thus, airborne pheromone is not continuously supplied, making this
515 system more sensitive to pheromone washing. This phenomenon agrees with the lower male flight
516 inhibition (first flight) achieved in fields treated with the aerosol devices, as shown by our monitoring
517 data and, also, by other technical reports [44,45]. The high number of passive dispensers installed per
518 hectare makes the competitive attraction ‘females-dispensers’ possible, preventing males from female
519 finding. In contrast, the low density of pheromone sources in the case of aerosols and the lack of
520 canopy lead the pheromone to the soil, where the degradation is triggered as described by Shaver
521 [46].
522
523 The highest mean airborne pheromone concentration was obtained in the second volatile
524 sampling (July) with both emission systems coinciding with the highest mean temperatures. When
525 these samplings were performed, all vines had a well-developed canopy, supporting the hypothesis
526 of the role of the canopy in regulating field air streams. Moreover, several studies describe leaves as
pheromone sources (by adsorption and release) [47-50], based on the reported adsorption of the pheromone components on their waxy surfaces [48]. This “adsorption-releasing” mechanism is supported by the results of our experiments. When the aerosol was shooting over the vines during the whole night (Experiment 2), leaves sampled 3 h after the last shot still had 383.1 µg/g in their surface. Then, a decreasing trend in the quantity of pheromone is observed throughout the day, suggesting that pheromone is released from the leave surface at an estimated rate of about 28.6 µg/h per gram of leaves. This value is comparable with those obtained in similar studies done by McGhee [15], in which 15 apple leaves receiving five emissions from a CM MIST emitted codlemone at 4 µg/h in apple orchards. In our Experiment 1, we have also observed that this release rate can be higher immediately before the aerosol shot, as the quantified amounts of pheromone decrease from 312 to 38 µg/g in 4 h (approximately 68 µg/h). Despite the lack of detection of degradation products of the pheromone in the leaf experiments, leaves are not probably free of these degradation processes as they are also exposed to climate conditions, as it occurs on the soil. These adsorption-releasing dynamics are not exclusive of the aerosol devices system, as the leave samples collected in the plot treated with passive dispensers (Experiment 3) showed similar quantities of pheromone (70 ng/g) than those collected in the aerosols plot. Considering that the quantity of pheromone emitted by L. botana females has been estimated near 0.3 ± 0.1 ng/h as a maximum in the first hour of the scotophase [51] and the amount of pheromone adsorbed on leaves and their proved dynamics, our data supports that vine leaves could imply countless pheromone point sources capable of competing with females.

During the second sampling (end of July), the airborne pheromone concentration was significantly higher in the plot treated with passive dispensers than in the aerosols plot. Taking into account that the canopy is acting in both plots and the release profiles of both systems are similar, this difference can be attributed to the distribution of the pheromone sources. Given that the volatile collections are performed in the center of each plot, it is possible that the passive dispensers ensure a better airborne pheromone distribution inside the plot. Nevertheless, the airborne pheromone concentrations obtained in both plots (42.2-22.3 ng/m³) provided effective disruption levels (total male capture inhibition compared to control plots).

When the end of the crop cycle was near (September), airborne pheromone concentration with the passive dispensers dropped up to initial levels, which agrees with their reduced mean emission rate (226 mg/day/ha) at the end of their lifespan. At this moment, the quantity of pheromone emitted by the dispensers is too low and the buffer effect of the leaves is not able to compensate airborne concentrations because the adsorption-release dynamics of the leaves might be too quick [29]. On the contrary, aerosols were able to maintain high airborne pheromone concentrations because their emission is programmed.

The distribution of the quantified airborne concentrations throughout the crop cycle suggests that the plant canopy is not a requirement to achieve disruption when using passive dispensers. However, it helps keeping the pheromone within the field area by reducing pheromone washing, distributing the pheromone more uniformly and increasing the number of pheromone sources along the crop, and thus improving the competitive mechanisms of disruption. On the contrary, the automatic aerosol system is somewhat canopy-dependent. As obtained in the Experiment 3, leaves randomly sampled in the center of the plot treated with aerosols contained significantly the same amount of pheromone than those from the center of the passive dispensers’ plot. This might explain how mating disruption works when installing aerosols, as leaves may be acting as additional point sources within the vineyards. Moreover, the variability in the data quantified in samples from the border of the aerosol plots, highlighted by the wider error bars, might respond to a gradient of pheromone content according to the distance to an aerosol. The nearer the leave samples from an aerosol device, the higher the pheromone quantified from those leaves. This progression in the amount of pheromone adsorbed on the leaves according to distance would be the result of the plumes generated by the aerosols, which can reach more than 460 m long and 90–150 m wide as Welter et al. [7] outlined. In this way, the nearest vines of the aerosol devices would act as high releasing dispensers during the periods between the shots. These results support the reported theory of
induced allopatry in which males are attracted to the pheromone sources following an increasing concentration of pheromone when using aerosol devices [9,14,15]. At the same time, leaves loaded gradually along the distance would act as temporary plumes between aerosol shots.

Considering male flight inhibition values in our trials, passive dispensers were capable of inhibiting male captures during the whole crop cycle in both locations, whereas aerosol devices were unable to inhibit totally the first flight peak performing 53% and 64% inhibition in Field 1 and Field 2, respectively. Taking into account these results and the different pheromone airborne concentrations found, we might estimate that the lowest airborne pheromone concentration in which male flight was effectively disrupted matches the mean concentration found in vineyards treated with passive dispensers at the beginning of the growing season, approximately 5 ng/m³. The inhibition values would confirm that mating disruption might be not properly performing as such in our assays with pheromone concentration of approximately 0.5 ng/m³. Further trials would be necessary to reduce this range of values and determine an optimal pheromone concentration in air. Considering competition as the main mechanism acting in mating disruption treated fields and leaves acting as countless pheromone sources, overcoming this concentration could result in pheromone wasting. Nevertheless, this kind of studies should be carried out in areas with different pest populations and different climate conditions to draw more powerful conclusions.

5. Conclusions

In the present work, a new methodology based on high volume air samplings and current sensitive chromatographic and spectroscopic methods —GC/MS/MS— has been developed to detect and quantify airborne pheromone concentrations. This new methodology has been employed to gain insight into the mode of operation of two different releasing systems —passive dispensers and aerosol devices— employed to apply MD against EGVM. The results found can be used to optimize and reduce pheromone waste in MD treated fields.

Although both releasing systems released the same average quantity of pheromone and displayed similar control of fruit damage, spatial and seasonal differences in pheromone distribution have been demonstrated. Passive dispensers kept lower airborne pheromone concentrations at the beginning and the end of the crop cycle with a maximum in mid-season, the treatment significantly reduced male catches compared to non-treated vineyards during the whole crop cycle. By contrast, aerosol devices were not capable of fully inhibiting male catches at the beginning but kept effective high amounts of airborne pheromone at the end. The canopy role as pheromone concentration buffer —adsorbing and releasing previously emitted pheromone— has been demonstrated and it is suggested as an important factor affecting the efficacy of the aerosol devices. In addition, this capacity would explain how competitive mechanisms act when using both types of dispensers. Airborne pheromone data collected demonstrates that in our experimental conditions, airborne pheromone values just over 5 ng/m³ effectively inhibited male captures, reflecting a good performance. Although this must be proven in similar studies made in locations with different climate conditions and pest-density populations, our results highlight that there is still room for improvement to optimize pheromone use. Similar efficacies might be obtained with lower emission rates and lower initial pheromone content in the case of passive dispensers and adjusting releasing time of automatic aerosol devices. Moreover, it could be interesting to test if a delayed deployment of aerosol devices after the first flight could obtain the same efficacy.

Author Contributions: A.G., S.V. and V.N.-L. designed and performed the field experiments; A.G., I.N. and J.P. designed and performed air sampling, sample preparation and chemical analysis. All authors analyzed the results and contributed to the writing of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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