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Suspension of pheromone microcapsules on vine leaves acting as passive dispensers against pests

Valérie Desauziers¹ · Elodie Sicre² · Jane Vignau-Laulhère² · Sylvain Bourrigaud² · Hervé Plaisance¹

Abstract

Pheromones are increasingly used as alternatives to pesticides to protect vineyards against *L. botrana*, a key grape pest. To diffuse (7E,9Z)-7,9-dodecadien-1-ylacetate, the *L. botrana* pheromone, passive, or aerosol dispensers are commonly applied. This paper deals with another method based on spraying an aqueous formulation, Lobesia Pro Spray, containing the pheromone encapsulated in a resin. The objectives were to assess the ability of vine leaves to act as pheromone dispensers and to check that encapsulation protects the plant from pheromone penetration. Laboratory testing based on an emission cell combined with airborne pheromone measurements by active sampling on sorbent tubes followed by ATD-GC-MS analysis was developed to accurately characterise the release of the pheromone into the air. Release kinetics analysis performed on the vine leaves showed a high pheromone release (about 30% of the sprayed quantity) the first day of the test. The release rate then decreased rapidly to reach about 650 mg/day/ha after 4 days. Kinetic modelling showed that it would be possible to maintain an effective airborne concentration of pheromone for approximately 12 days. Release tests were also carried out on glass, PVC and blotting paper. The results obtained showed that the vine leaves behaved as a non-absorbent material, implying that the pheromone used in the Lobesia Pro Spray formulation did not penetrate the plant. These first results prove the feasibility of using vine leaves as pheromone dispensers for a sprayed formulation and the ability to optimise the treat-ment conditions (dose and frequency) through laboratory testing.

Keywords Grapevine moth control · Spray formulation · Volatile semiochemicals · Release kinetics · Adsorption · Laboratory testing · Environmental chamber · GC-MS

Introduction

Semiochemicals are volatile or semi-volatile organic compounds (VOCs or SVOCs) emitted in air at low concentrations by many species, including pest species, and used as communication signals. Among these molecules, odorants that mediate intra-species behaviours are called “pheromones” (Brezolin et al. 2018). Insects, which have a highly developed olfactory sense, are particularly affected by these pheromone-mediated behaviours (UGVB 2011).

Since their first chemical identification in the early 1960s (Karlson and Lüscher 1959), a large number of pheromones have been identified. Their relatively easy synthesis led to their growing use in agriculture for pest-insect management as an eco-friendly alternative to intensive pesticide use. This approach is strongly encouraged by European regulations to limit health and environmental effects (Hillocks 2012; Desneux et al. 2007). Two main types of pheromone use have been developed: the attraction and capture of individuals (mass-trapping) and that based on confusing the insects, such as mating-disruption (MD) (Brezolin et al. 2018). MD is currently applied to about 800,000 ha worldwide (Benelli et al. 2019) and is particularly used against the European Grape Moth *Lobesia botrana* (Denis and Schiffmüller) (Lepidoptera: Tortricidae), a key grape pest in most vineyards (Lucchi et al. 2018a). For the field treatment, various pheromone dispenser technologies have been developed (Ioriatti and Lucchi 2016; Miller and Gut 2015; Lucchi et al. 2018b). The main commercially available devices are passive

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dispensers that continuously release pheromones during the total flight period of the target pest. The release rate depends on various parameters such as the initial content of pheromone in the formulation, diffusion in the formulation, diffusion in air, volatility, dispenser geometry, temperature, and wind velocity effects. For *L. botrana*, the dispensers release plumes of (7E,9Z)-7,9-dodecadien-1-ylacetate, which is the main component of the female pheromone (Ioriatti and Lucchi 2016). The number of units distributed across an agricultural field is rather high, from 200 to 600 per hectare (Lucchi et al. 2018b, Altindisli et al. 2016; Wilson and Daane 2017; Ioriatti et al. 2011). As an example, for the RAK® dispensers, registered in France since 1995 (UGVB 2011) and purchased by BASF, a density of 500 units per ha is recommended. The effectiveness of such devices has been proven but their number for the treatment of one field requires supplementary labour costs for the installation and removal of the dispensers (Lucchi et al. 2018b; Gavara et al. 2020). Moreover, with non-biodegradable devices, plastic residues may lead to soil pollution. To overcome these limitations, aerosol dispensers were proposed as alternative to passive devices (Lucchi et al. 2018b; Gavara et al. 2020; Benelli et al. 2019). Pheromones are released in puffs at programmed time intervals enabling optimisation of the time of use and the amount applied (Benelli et al. 2019). The efficacy of aerosol dispensers was assessed in vines for *L. botrana* management and studies showed that only 2 or 3 devices per hectare are sufficient to observe disruption (Lucchi et al. 2018b; Benelli et al. 2019). Indeed, pheromone was shown to travel over hundreds of meters downwind from the source (Benelli et al. 2019). The leaves and aerosols contained in the atmosphere are assumed to enhance this long distance and persistence efficacy, by ad(ab)sorption–desorption of pheromones (Wall and Perry 1983; Schmitz et al. 1997b; Suckling et al. 2007) and particles (Jami et al. 2020). To combine the advantages of passive dispensers and aerosol puffers while overcoming the costly need for extra labour time to distribute and remove classical dispensers in the field, a sprayable dispersion of passive dispensers has recently been developed by the company M2i. Commonly available machines already used for conventional treatment can be used to spray these passive dispensers in the form of microscopic, isolated capsules suspended in an aqueous solution. For such a novel form of dispenser, classical methods to measure the release rate such as gravimetry are not applicable. The airborne concentration resulting from the emission of pheromones from the microscopic dispensers needs to be measured directly.

In most papers in the literature, the effectiveness of pheromone treatments on pest management is assessed by indirect methods based for example on determining the abundance of infested bunches (Lucchi et al. 2018a, b). The identification and quantification of airborne pheromones is little-reported in the literature due to the very low concentration

levels, which are difficult to quantify using current sampling and analytical techniques (Barbosa-Cornelio et al. 2019). Electroantennography (EAG) is one of the few techniques enabling sufficiently sensitive and rapid measurements to study spatial and temporal variations in the field, but precise pheromone quantification is difficult due to the variability of the method, its preparation time, the lack of detector linearity and possible interferences with other semiochemicals (Gavara et al. 2020; Brezolin et al. 2018). Active sampling on solid sorbents followed by gas chromatography (GC) with flame ionisation detection (FID) or mass spectrometry (MS) is therefore preferred for accurate quantitative analysis (Brezolin et al. 2018). However, very long sampling times (several hours to several days) are required to reach the target concentration levels of airborne semiochemicals, leading to time-weighted average results and hence a lack of precision on kinetic processes. For example, a recent paper described a comparative study of passive and aerosol dispensers for *L. botrana* population control in vines (Gavara et al. 2020). For the first time, high-volume sampling was applied at a flow rate of 15 m³/h for 48 h to quantify (7E,9Z)-7,9-dodecadien-1-ylacetate in field air. For aerosol-treated plots, the concentrations measured showed that approximately 5 ng/m³ is sufficient for effective control of the pest population. Additionally, the total concentration of pheromone in the leaves was determined by solvent extraction. The decrease in concentration from 312.4 µg/g to 38.7 µg/g 4 h after treatment demonstrating that the leaves can retain and release the pheromone with a high release rate.

In this framework, the presented study deals with a sprayable suspension of microcapsules for direct application of the *L. botrana* pheromone in vineyards for mating disruption. Sprayable microencapsulated formulations are easily synthesised by interfacial polymerisation (Dubey et al. 2009). Their application started in the mid-1970s for orchard protection (Beroza et al. 1974; Cardé et al. 1975) and the technique continued to be developed due to the advantage of being easily implemented by farmers using conventional agricultural equipment (Campion 1976). Conversely, numerous application parameters such as rate, frequency, emission profile over time, and sticker adjuvant are involved in the treatment efficacy (Knight and Larsen 2004; Stelinski et al. 2007). The use of sprayable microencapsulated sex pheromones is reported in the literature for various pests (Stelinski et al. 2007; Chen et al. 2018). However, the literature related to *L. botrana* is scarce. The formulation studied here is Lobesia Pro Spray, developed by M2i (France) and composed of an aqueous encapsulated formulation containing (7E,9Z)-7,9-dodecadien-1-ylacetate. The aim of the polymeric resin encapsulating the pheromone is to protect the molecule, controlling its release in air and preventing the pheromone from entering the leaf. The objective of this work was therefore to study whether vine leaves

can act as a carrier for passive dispensers and to check that the synthetic pheromone is not sorbed by the vine leaves in order to prevent possible damage to the plant. For this purpose, laboratory testing using an environmental chamber under controlled conditions was developed to precisely determine the pheromone release kinetics in air. Previously reported chamber tests mostly involve the assessment of pheromone release rates by weighing the residual load of dispensers (Zhu et al. 2015; Correia et al. 2019). This can lead to misinterpretation because of the presence of isomers, the volatilisation of other components from the commercial formulation and the possible pheromone transformations (degradation, etc.). Identification and quantification of (7E,9Z)-7,9-dodecadien-1-ylacetate in air was therefore carried out by active sampling on adsorbent tubes followed by automated thermal desorption (ATD) coupled to GC/MS/FID analysis (Plaisance et al. 2017a, b; Costarramone et al. 2017). Firstly, to achieve realistic conditions, the chamber test parameters were optimised using the RAK 1 + 2 MIX® passive dispenser whose release rate is known. Then, the release kinetics of the pheromone was studied after spraying the formulation on vine leaves and on three other materials (glass, PVC, and blotting paper) for comparison of sorption and release behaviours. The results obtained for vine leaves were then extrapolated to field application conditions and compared to recent literature data to estimate the potential of the leaves to act as pheromone dispensers.

Materials and methods

Passive dispenser and sprayed formulation

The RAK 1 + 2 MIX® BASF was used as reference dispenser. It protects the grapes from two different moths: *L. botrana* and *E. ambiguella* (*Eupoecilia ambiguella* (Hübner) (Lepidoptera Tortricidae). This dispenser consists of two plastic reservoirs containing respectively 226 mg of (E,Z)-9-dodecen-1-yl acetate (E7Z9-12Ac), the *E. ambiguella* attractant, and 217 mg of (E,E/Z)-7,9-dodecadien-1-yl acetate, the *L. botrana* attractant. The two molecules were analysed using the analytical method described in the “Air sampling and analysis” section but only results obtained for E7Z9-12Ac are presented for reasons of concision.

The sprayed formulation studied for release kinetics was Lobesia Pro Spay (M2i, Lacq, France). It consists of an aqueous based suspension of an encapsulated organic phase containing 3% (w/w) of (E,Z)-7,9-dodecadien-1-yl acetate (isomeric purity: 67.3%). The diffusion of the active substance inside the micrometric particles is regulated by a specific beeswax formulation whereas the encapsulation layer protects plant leaves from direct contact with the active substance. The formulation's overall specific gravity is

0.97 ± 0.01 (mean \pm SD). Usually, the formulation is diluted 250 times in water before being sprayed in vineyards (the treatment dose is equivalent to 1L of the pure formulation per ha). For the kinetic study, the same dilution of the formulation in MilliQ water (deionised water) was performed to be representative of the actual concentration applied to the vines.

Tested materials and formulation deposit method

For this study, daily cut mature vine leaves (Cabernet Franc variety) with a cumulative area of 130 cm² placed on the bottom of a Petri dish were used. Three other materials were tested to compare their sorption properties to those of the vine leaves. A glass plate (petri dish of 314 cm²) was chosen to represent an inert surface. In contrast to glass, a sheet of blotting paper placed in the bottom of a petri dish was selected to mimic an absorbent material. A PVC sheet was also tested since this material is assumed to have surface properties close to vine leaves and is used for testing the spraying of plant protection products (Decourcelle 2013).

The deposit on the material carriers was carried out by spraying about 600 mg (weighed exactly) of the diluted solution using a basic PET spray bottle for houseplants (1L). The repeatability of weighing was assessed over 15 replicates on a glass surface. The relative standard deviation is 2%.

Emission chamber tests

The principle consists of introducing the diffusion system to be tested in a laboratory-made cylindrical glass emission cell inspired by previous works (Mocho and Desauziers 2011; Bourdin et al. 2014). The cell was subjected to a flow of humidified clean air ($50 \pm 3\%$) produced by a dry zero air generator (Claind, Marcq en Baroeul, France) and a humidifier made of a water bubbler, regulated by mass flow controllers (Bronkhorst, Montigny-les-Cormeilles, France). The temperature was maintained at 20 ± 3 °C. Two airflow rates through the cell were tested: 0.5 and 10 L.min⁻¹. The air velocity in the cell near the sample surface was measured with a Testo 480 anemometer (Testo, Forbach, France). Experimental conditions were kept constant throughout the duration of the test. A diagram showing the principle of the device is given in Fig. 1.

Emission cell

The cell used was equipped with an air inlet and outlet. Samples were introduced through the open bottom which was then tightened on a clean glass surface for release testing. A Viton® fluoroelastomer O-ring covered with Teflon® (Dimatrap, Pau, France) ensured air tightness, which was higher than 95%. This was checked for each trial using

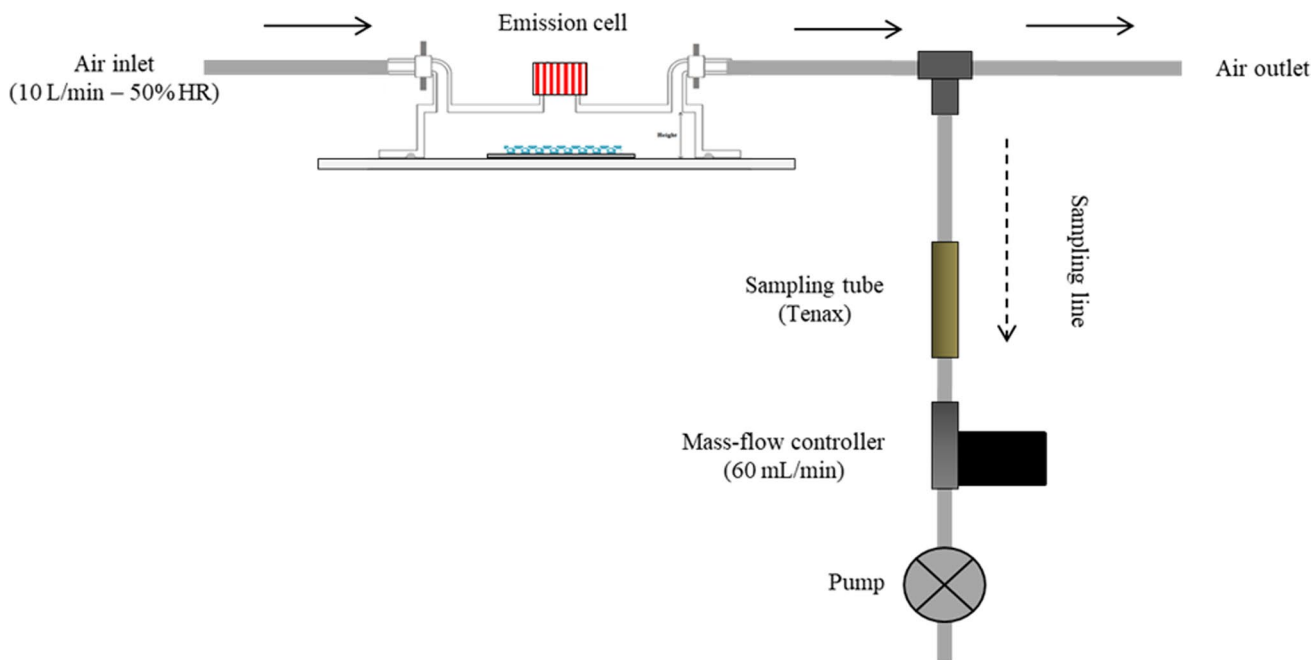


Fig. 1 Experimental device for evaluating the release kinetics of deposited pheromone

Table 1 Geometry of the emission cell

Characteristics of the cell used	
Volume (mL)	620
Diameter (cm)	14.8
Height (cm)	3.6

a soap film flowmeter (Model M-30, AP BUCK, USA). A polytetrafluoroethylene-silicone septum was screwed in the middle of the cell for RAK® dispenser exposure. It was replaced by a plug for the tests on the sprayed formulation. The cell characteristics are reported in Table 1 and Fig. 2.

Adsorption of pheromone on cell walls

Because of their low vapour pressure, SVOCs could be partially adsorbed on chamber walls at ambient temperature, affecting the results of emission tests (Ghislain et al. 2017). To evaluate the adsorption capacity of the pheromone on cell walls, a mass balance was performed after 15 days of the RAK® exposure test in the cell. The cumulated mass of the Lobesia pheromone released in air after 15 days was estimated at 14.2 ± 1.8 mg from the daily release rates determined in the “Results and Discussion” section (Cf. 3.1). The remaining mass in the dispenser, measured by weighing it before and after the test, was 214.1 mg leading to a mass

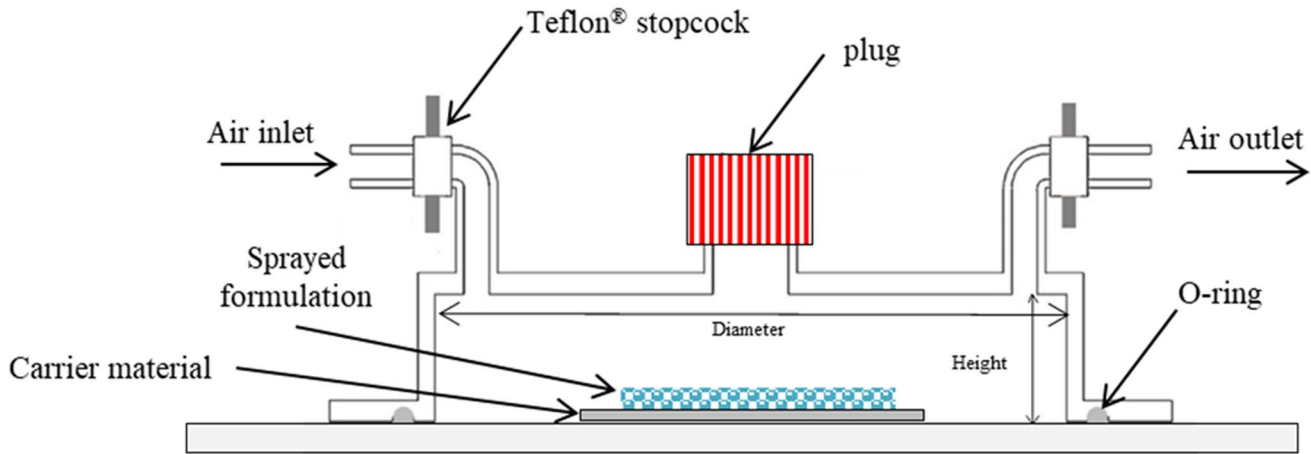


Fig. 2 Design of the emission cell

balance of 228.3 mg. The initial amount of pheromone in the dispenser being 226 mg and considering measurement uncertainties, it can be assumed that there is a negligible loss of compound by adsorption on the device walls.

Air sampling and analysis

The concentration of pheromone in the air in contact with the sample was evaluated by a series of air samplings at the cell outlet. Active sampling was carried out with stainless steel tubes containing 250 mg of Tenax TA (PerkinElmer, Waltham, MA, USA). Sampling tubes were cleaned before and after each use with pure helium at 100 mL/min at 320 °C for 15 min. The sampling rate ranged from 60 to 80 mL/min and was checked before and after each pumped sample using a gas flow meter (DryCal DC-Lite, Butler, NJ, USA). The sampling time varied from 5 min to several hours depending on the expected concentration level and the number of data required for accurate determination of release kinetics.

The Tenax tubes were analysed by thermal desorption using a TurboMatrix 650 ATD thermal desorption system (PerkinElmer, Waltham, MA, USA). The Tenax tubes were heated at 320 °C for 15 min using a helium flow rate of 100 mL/min without inlet split to desorb the analytes and focus them into a cold trap kept at 0 °C. The desorption trap was ramped at 99 °C/min to 350 °C, held for 10 min, with an outlet split of 5 mL/min. The transfer line to the Clarus 680 gas chromatograph (Perkin Elmer, Waltham, MA, USA) and the valve were maintained at 250 °C. The carrier gas was helium at a flow rate of 1.3 mL/min. A 5% phenyl Elite capillary column (PerkinElmer, Waltham, MA, USA) of 60 m × 0.25 mm i.d. and 0.25 µm film thickness was used. The oven temperature was programmed as follows: 50 °C for 4 min, 20 °C/min to 250 °C held for 10 min. The gas chromatograph was equipped with dual MS/FID detection. The mass spectrometer used for the identification of compounds was a Clarus SQ 8 T (PerkinElmer, Waltham, MA, USA). The MS transfer line and the ion source were maintained at 180 °C. The acquisition was performed in full scan (50 to 300 amu) using electron impact mode (70 eV). Quantification was carried out by FID and based on external calibration. The Tenax tubes were loaded with 2 µL of standard solutions of (E,Z)-7,9-dodecadien-1-yl acetate in acetonitrile using a GC syringe and then purged for 10 min with a helium flow of 35 mL/min to remove the solvent. The calibration range was from 10 to 350 ng and the calibration curve with six standard levels showed a squared correlation coefficient of $R^2 > 0.98$. According to this methodology, the limit of detection is 1.8 ng, the limit of quantification is 6.1 ng (corresponding to a measurable concentration at the exit of the exposure cell of 1.8 µg/m³ for 1-h sampling time) and the reproducibility is assessed as 4.6%.

Release kinetics tests

Tests were carried out on glass, PVC, blotting paper, and vine leaves. For all the materials, the tests were performed under realistic dilution conditions of the pheromone (dilution 250 times of Lobesia Pro Spray) to assess the actual amount released in air. A volume equivalent to that of a spray (weighed precisely) was deposited on the material surface which was placed in a 620-mL emission cell. The mass of the major isomer of the pheromone deposited on the carrier was between 140 and 170 µg corresponding to 70 to 85 µg/g of leaf (the average mass of one leaf was estimated to be 2.1 g from 6 independent weighings). This is in the same order of magnitude as the concentration reported in the leaves after aerosol treatment, which ranges between 312 µg/g just after aerosol emission and 38 µg/g 4 h afterwards (Gavara et al. 2020). The inlet air flow rate was 10 L/min and the other experimental conditions are described in “Air sampling and analysis” section. Sampling times on Tenax tubes ranged from 5 min (at the beginning of the kinetics) to 16 h (at the end of the kinetics). Measurements were stopped when the concentration reached the quantification limit of the method (i.e., approximately 100 ng/m³ for 16-h sampling). Three or four test replicates were performed, in function of the material, to estimate variability.

Estimation of the cumulated amounts of pheromone released in air

For each kinetics trial, the total amount of emitted pheromone Q (µg) was estimated by integration of the pheromone concentration using the trapezoid method. It was determined as follows (Eq. 1):

$$Q = D \int_0^t C \cdot dt \quad (1)$$

where C is the concentration of E7Z9-12Ac in air (µg/m³), D the air flow rate through the emission cell (m³/min) and t the sampling time (min).

The application of the trapezoidal method to integrate C leads to (Eq. 2):

$$Q = D \int_0^t C \cdot dt = D \cdot C_0 \cdot t_0 + D \sum_{n=1}^N (t_n - t_{n-1}) \left(\frac{C_{n-1} + C_n}{2} \right) \quad (2)$$

With C_0 and t_0 respectively the initial concentration and the initial time, $(t_n - t_{n-1})$ the time interval between two measurements, N the total number of time intervals.

Results and discussion

Optimisation of the release tests

To determine experimental settings representative of the actual conditions, the RAK 1 + 2 MIX® was used as a reference. The release rate of E7Z9-12Ac was estimated in the field by weighing the remaining pheromone and is between 1.2 and 1.4 mg/day for each dispenser (Ioriatti et al. 2011). A similar emission rate of 1.2 mg per day was also estimated for another passive diffuser, Isonet L, delivering the same pheromone (Gavara et al. 2020).

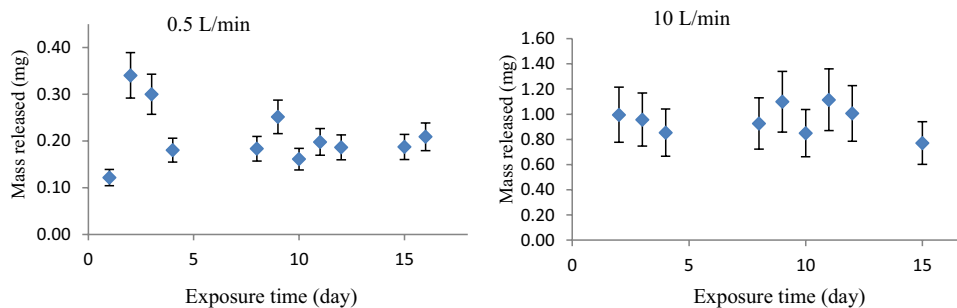
One RAK 1 + 2 MIX® dispenser was exposed in the cell for 15 days under two air flow rates: 0.5 L/min and 10 L/min. Each experiment was carried out once. The other experimental conditions were as described in “Air sampling and analysis” section. The airborne concentration of E7Z9-12Ac was monitored by two samplings per day with Tenax tubes at a rate of 60 mL/min for 20 min. For each test, the average daily release rate was determined by averaging the release rates after their stabilisation. The results are summarised in Table 2. The release profiles obtained are shown in Fig. 3.

The results presented in Table 2 show that under our experimental conditions, the release rate is obviously influenced by the air flow rate. At 10 L/min, a release rate of E7Z9-12Ac of 1 mg/day was obtained, similar to that reported in the literature for the RAK 1 + 2 MIX® diffuser. This flow rate, corresponding to an air velocity of 0.29 m/s (1.04 km/h) (see Table 2), was thus chosen for the rest of the study. Additionally, the time to reach a stable rate was lower for 10 L/min than for 0.5 L/min (Fig. 3).

Table 2 Average release rates of (E,Z)-7,9-dodecadien-1-yl acetate emitted by the RAK 1 + 2 MIX® according to the air flow-rate

Air flow rate (L/min)	Air exchange rate (per h)	Air velocity (m/s)	Average release rate of E7Z9-12Ac (mg/day)
0.5	48	0.12 ± 0.02	0.20 ± 0.03
10	967	0.29 ± 0.02	0.95 ± 0.12

Fig. 3 Time variation of daily release rate of E7Z9-12Ac by the RAK 1 + 2 MIX® obtained in the cell for different air flow rates: 0.5 L/min and 10 L/min



Release kinetics of E7Z9-12Ac from Lobesia Pro Spray—Influence of the carrier material

Kinetic profiles of pheromone concentration in air

Release kinetics tests were performed on glass, blotting paper, PVC and vine leaves as described in “Release kinetics tests” section. Kinetics and sorption behaviours were compared.

The complete set of data is presented in Fig. 4.

To ensure efficient pest management, extended release at a constant rate is required (Atterholt et al. 1999). This is generally achieved with passive diffusers containing the pheromone whose release rate is controlled by diffusion through the plastic reservoir wall (Atterholt et al. 1999). This is typically the case of the RAK 1 + 2 MIX® for which a zero-order release rate was effectively observed for at least 15 days (Fig. 3). For the sprayed emulsion studied here, zero-order release kinetics were not obtained. Whatever the carrier material, the initial concentrations of the released pheromone were between 20 and 60 µg/m³ and decreased sharply until about 500 min to reach 100 ng/m³ or less at the end of the test (6000 min). The best adjustment of the experimental data was obtained by applying two first order kinetic models (Eq. 3) in function of the release time.

$$C = C_0 \cdot e^{-kt} \quad (3)$$

By applying these models to the kinetic data of each material (Fig. 5), the equations presented in Table 3 were obtained. Model 1 corresponds to data obtained before approximately 200 min and model 2 to those obtained between 200 and 6000 min. To check whether these two models are consistent with the complete dataset for each material, the concentrations obtained from Model 1 were added to those obtained from Model 2 over the entire period of the kinetic test. The corresponding curves called “Model 1 + 2” are shown in dashed lines in Fig. 5. The squared correlation coefficients (R^2) are between 0.86 and 0.96, the best fits being observed for PVC, which presents less scattered results compared to vine leaf, for example. The good fit between experimental data and the combination of two

Fig. 4 Release kinetics profiles of E7Z9-12Ac in air in function of deposit medium on glass, blotting paper, PVC sheet and vine leaf (in the box: focus on the first 500 min)

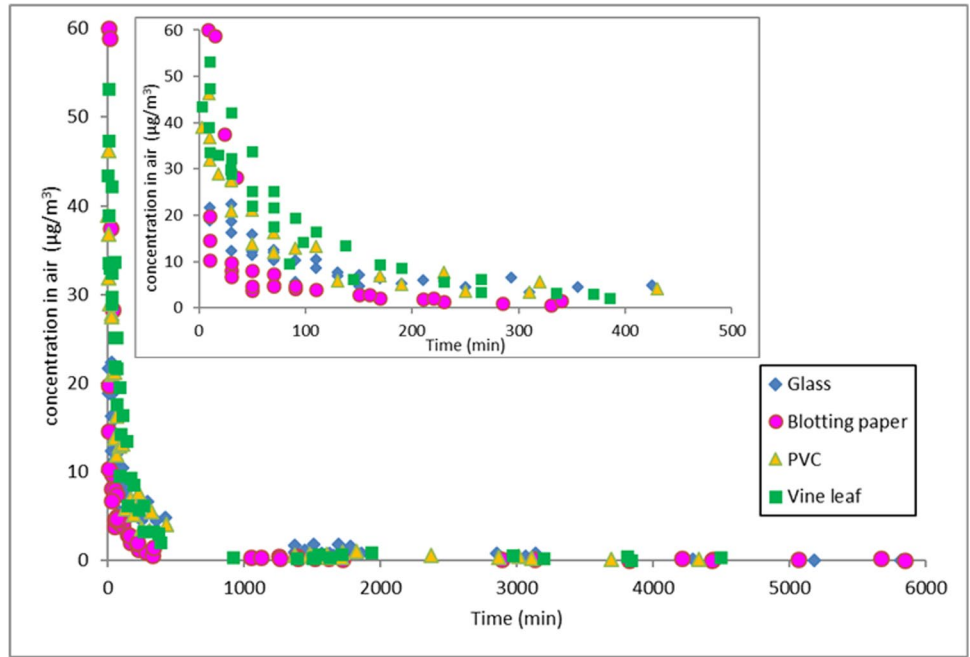
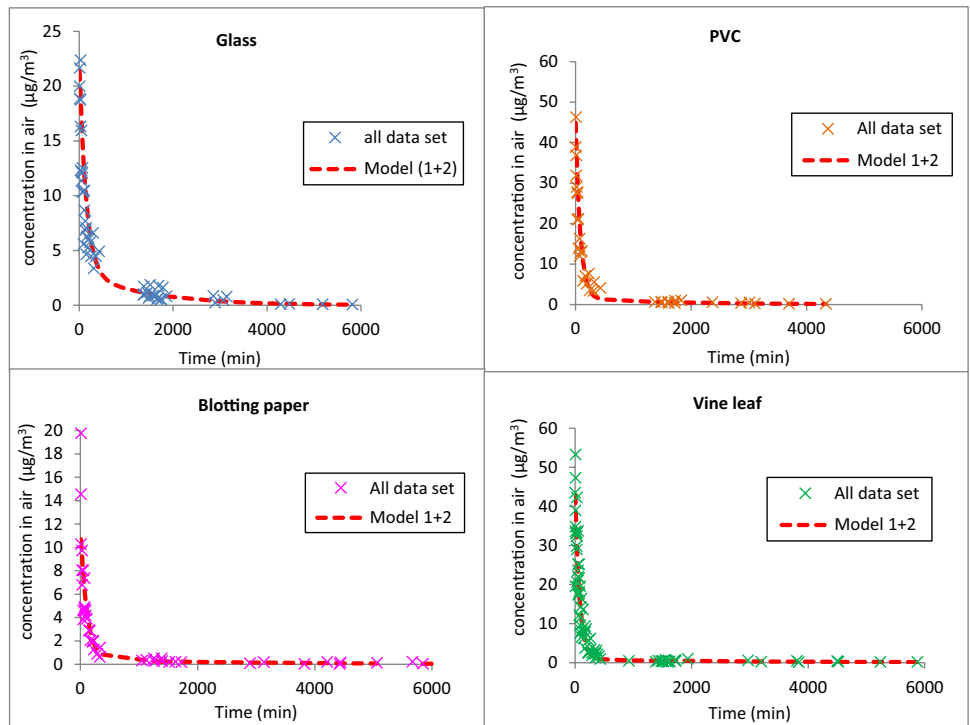


Fig. 5 Kinetic modelling for each material studied (the points marked with an x represent the data of all replicates; dashed lines correspond to Model 1+2)



first order kinetics models shows that the pheromone release depends on different regimes resulting in two main phases (represented by the two models) whose kinetic rate constants differ approximately by one order of magnitude (Table 3). This confirms that permeation of pheromones through complex matrices such as waxes, as in the formulation studied here, is more complicated to describe and understand than

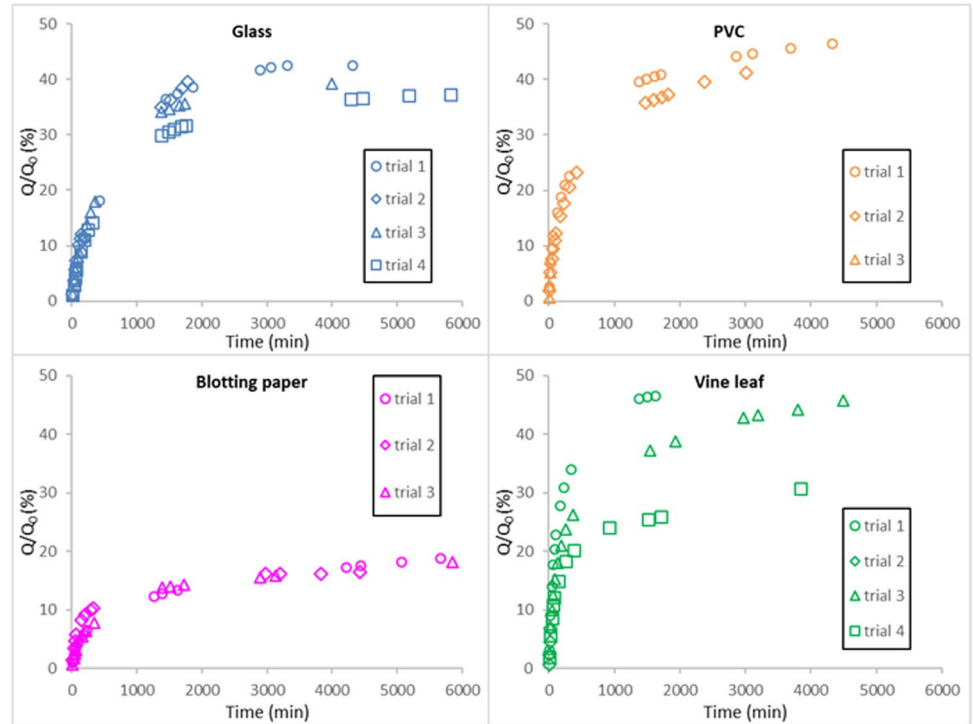
diffusion through homogeneous elastomeric polymers. This is due to the influence on pheromone release of the various physicochemical properties of the pheromone and of the encapsulating matrix (pheromone solubility in waxes, matrix permeability) (Atterholt et al. 1999; Knight and Larsen 2004). In this study, the drastic decrease in release rates occurring after one or two days (1400–3000 min) could

Table 3 Modelling equations obtained for each material studied

Material	Number of kinetic replicates	Model 1 (0–200 min) R^2	Model 2 (1300–6000 min) R^2
Glass	4	$C = 20.0e^{-0.0070t}$ 0.94	$C = 3.0e^{-0.0007t}$ 0.96
PVC	3	$C = 44.9e^{-0.0120t}$ 0.97	$C = 1.3e^{-0.0005t}$ 0.96
Blotting paper	3	$C = 11.3e^{-0.0090t}$ 0.92	$C = 0.4e^{-0.0003t}$ 0.96
Vine leaf	4	$C = 43.6e^{-0.0130t}$ 0.93	$C = 0.8e^{-0.0003t}$ 0.86

C airborne concentration of E7Z9-12Ac ($\mu\text{g}/\text{m}^3$), t release time (min)

Fig. 6 Relative cumulated released amount of E7Z9-12Ac (Q/Q_0) as a function of time



be attributed to a change in release process, an assumption consolidated by the two first order models required to fit the data at the beginning and at the end of kinetics. The size of the sprayed droplets and the diameter of the encapsulating beads, centred on $3 \mu\text{m}$ but ranging from 1 to $10 \mu\text{m}$, could result in different release velocities and explain these release profiles.

Influence of the carrier material on pheromone release

For each release trial, the cumulated amount of E7Z9-12Ac released in air Q was estimated (see method in “[Estimation of the cumulated amounts of pheromone released in air](#)” section).

The following curves (Fig. 6) represent the relative cumulated released amount Q/Q_0 as a function of time, with Q_0 the initial mass of pheromone released in air.

Table 4 Stabilisation times, experimental and modelled Q/Q_0 after 6000 min

Material	Stabilisation time (min)	Q/Q_0 at 6000 min (Exp.) mean (min–max) (%)	Q/Q_0 at 6000 min (modelled) (%)
Glass	2500	43.5 (38.2–46.6)	43.6
PVC	1500	42.1 (41.2–42.9)	44.1
Blotting paper	3000	17.7 (17.6–17.8)	22.0
Vine leaf	1500	44.9 (44.9–59.1)	48.6

Table 4 summarises the stabilisation times, the Q/Q_0 values reached at the end of the kinetics (6000 min) and the same data obtained by integration of the equations given in Table 3. These modelled data are in good agreement with the experimental ones obtained from the curves given in Fig. 6, thus demonstrating that the empirical power functions

correctly match the experimental kinetics, whatever the carrier material.

For all the materials studied, stabilisation of the relative cumulated released amount is observed after one day (1500 min) for PVC and vine leaf and after 2 days for glass and blotting paper (2500 and 3000 min respectively) (Table 4). The maximum release amount is about 40% for glass, PVC, and vine leaf. This means that after 4 days, a major proportion of the active molecules is still in the sprayed emulsion and remains available for a long release period. The release behaviours for these three carrier materials are similar. Since it was previously assessed that glass does not adsorb E7Z9-12Ac (see “Emission chamber tests” section), it could be thus assumed that the pheromone does not penetrate in the leaf and that the sprayed emulsion should be safe for the plant.

For the blotting paper, only 18% of the initial amount is released, due to absorption of the aqueous formulation in the paper. In this case, absorption strongly contributes to release control, leading to the slowest release rate and the lowest cumulated amounts but also to very reproducible results (Fig. 6 and Table 4). For vine leaves, however, less reproducible results were obtained, probably because of the variability of the surface condition of the leaves, given that a fresh leaf was used for each kinetic trial.

Release rates

The average released amounts (μg) were determined from the experimental data and are shown in Fig. 7 for each day of the kinetic tests. These quantities were related to an initial mass of $150 \mu\text{g}$ of E7Z9-12Ac sprayed on each material.

The first day of test, the amount released for vine leaves was $53 \mu\text{g}$, then dropped to $8 \mu\text{g}$ the second day and reached $3.8 \mu\text{g}$ and $3.4 \mu\text{g}$ the 3rd and 4th days respectively. Considering that the treatment dose is 1 L/ha of pure *Lobesia* Pro Spray containing 3% (w/w) of E7Z9-12Ac, the mass of

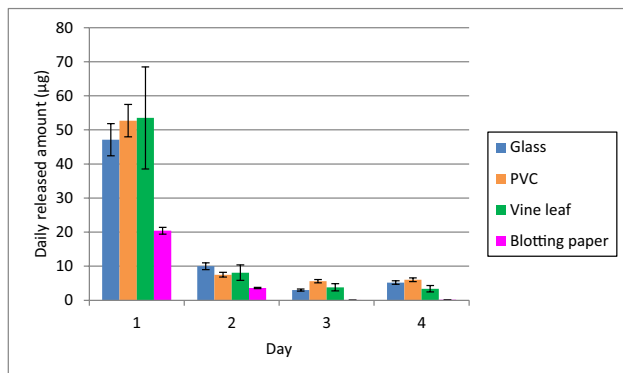


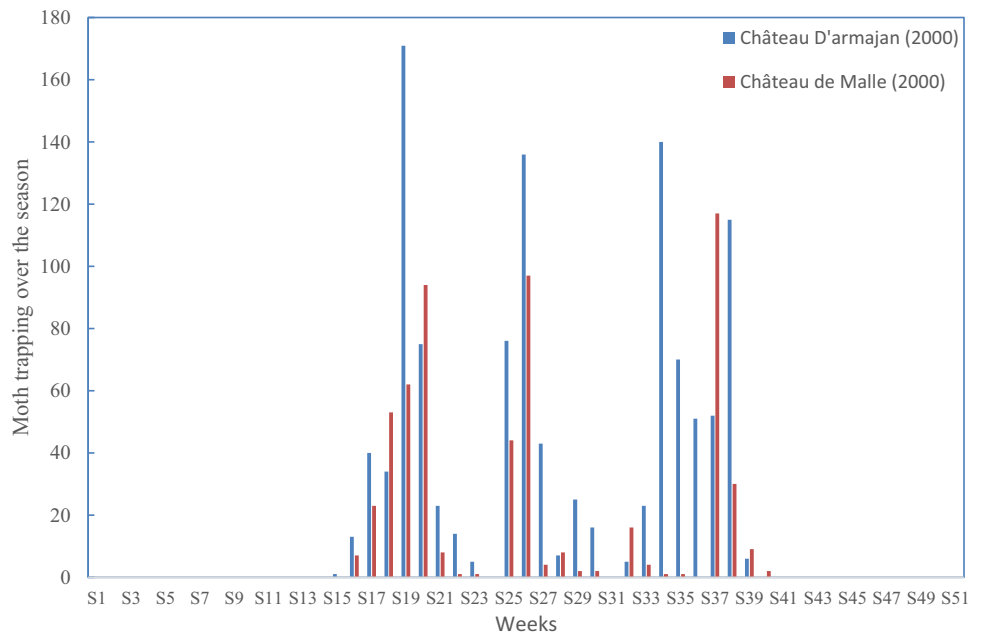
Fig. 7 Average amount of E7Z9-12Ac released per day (bars represent minimum and maximum values)

sprayed pheromone is approximately 29.1 g/ha . Considering also that the spraying conditions used in this study are identical to those applied in the field (same dilution in water), the daily release rate per ha was estimated from the experiments carried out in this study. The estimated initial rate is very high, 10.37 g/day/ha , for the first day, and decreases to 1.57 g/day/ha the 2nd day and then to 740 mg and 650 mg/day/ha the 3rd and 4th days. These last values are in the same order of level as those of the existing treatments recognised as effective, like passive dispensers such as the RAK 1 + 2 MIX® (500 mg/day/ha) (Thiéry et al. 2019), the Isonet L (526 mg/day/ha) (Gavara et al. 2020) and aerosol dispensers (602 mg/day/ha) (Gavara et al. 2020). However, given that the release rate decreases slightly over time, further experiments are required to investigate its evolution over a longer period: typically, the time currently applied by winegrowers between two treatments is 15 days. Laboratory conditions were not randomly selected, since they correspond to those for the release rate of the RAK 1 + 2 MIX® in the field (see “Optimisation of the release tests” section). However, since these laboratory conditions remain unchanged (temperature of $20 \text{ }^\circ\text{C}$, relative humidity of 50% and wind speed of 1 km/h near the leaf surface), results should be considered as a rough estimation and may vary according to vineyard aerodynamics and climatic conditions, rainwater for example being an important factor in the removal of microcapsules from the leaves (Knight et al. 2004).

Passive dispensers and aerosol dispensers, such as *Lobesia* Pro Spray being punctual emitters, should have sufficiently high release rates to ensure an efficient and relatively homogeneous concentration of the pheromone in air over long distances. Recently, in vineyards treated by passive dispensers, an average concentration of 5 ng/m^3 of E7Z9-12Ac was measured and was assessed to be efficient for mating disruption (Gavara et al. 2020). This concentration is in the same order of level (ng/m^3) as those reported in previous studies for mating disruption of *L. botrana* or other pests (Schmitz et al. 1997a; Flint et al. 1990). In the case of spraying treatments, the leaves play the role of countless individual dispensers covering all the area to be protected and may disturb mating (Schmitz et al. 1997b). The concentration of the pheromone in air near the leaf could therefore be assimilated to the field concentration by neglecting, as a first approximation, the dilution related to the distance. From our experiments, a concentration in air of 5 ng/m^3 would be obtained after 11.6 days according to kinetic modelling (Vine Leaf Model 2, Table 3). As mentioned above, this duration corresponds approximately to the time between two sprays and can therefore consolidate empirically defined practices after validation of the laboratory results in open field conditions.

Moreover, *L. botrana* moth flights recorded in several locations (Fig. 8) (data from L. Delbac, INRAe) show that

Fig. 8 Number of moths trapped each week in 2000 on two wineries (Château D'Armajan and Château de Malle)



in most cases the flights are concentrated over a period of 2 weeks which is typically the period covered by the release of the microscopic dispensers of Lobesia Pro Spray. This is especially true for generation 2 (G2) and generation 3 (G3) for which damage to grapes by *L. botrana* moth is the most critical for the harvest. When the G1 pressure level is high it is usually recommended to use an insecticide treatment (ovicide or larvicide) since it has previously been observed that mating disruption is of limited efficiency when dealing with a high pressure of pest population (Feldhege et al. 1993; Cardé and Minks 1995; Louis and Schirra 2001). On the contrary, when the G1 pressure is low, there is no need to treat this generation since damage is well compensated by the plant and does not impact harvest quality and quantity. So, in both cases, whether the G1 pressure is high or low, mating disruption can be applied only to G2 and G3 to protect the culture before the harvest. By using such a method with successive sprayings according to pest flights, winegrowers can fine tune and adapt their treatment to the pest pressure while decreasing the overall treatment dosage and cost over the season.

Although these estimations are extrapolations of laboratory testing, they enable a first evaluation of the potential of a treatment solution and the consideration of possible improvements.

Conclusion

As an alternative to pesticides, the use of pheromones to control crop insect pests is of growing interest and is widely supported by public policies to reduce the environmental

impact of phytosanitary treatments. Several technologies are available to diffuse pheromones, the most common solutions are passive or aerosol dispensers. This study focuses on another method based on the application by spraying an aqueous formulation, Lobesia Pro Spray, containing the pheromone of the European Grape Moth (*L. botrana*) encapsulated in a wax/latex resin. The principle aims to use vine leaves as carriers for microscopic dispensers of pheromone to cover the area to be protected. The objective was therefore twofold: (i) to characterise accurately the release of the pheromone in air and (ii) to demonstrate that the active substance did not penetrate the leaf and therefore did not impact the plant. An experimental device based on an emission cell test associated with airborne pheromone measurements by active sampling on Tenax tubes followed by ATD-GC-MS analysis was therefore developed to study the release kinetics of the pheromone (E7Z9-12Ac). To approach realistic field conditions, this lab test was optimised on the RAK 1 + 2 MIX® passive dispenser releasing the same pheromone at a known emission rate. The limit of quantification was assessed at 1.8 µg/m³ for 1-h sampling time and the air samplings from 20 min to 16 h accurately determined the release kinetics of the pheromone over the first 4 days following spraying on the leaf. Unlike passive dispensers with constant emission rates, the release rate of the sprayed formulation dropped by 75% on the 2nd day of exposure (1.57 g/day/ha) and reached about 650 mg/day/ha the 4th day. Although this release rate is similar to those of passive or aerosol dispensers (500–600 mg/day/ha), further experiments would be necessary to determine the release rate over a longer period and to estimate the influence of various environmental factors on this release. Extrapolation of modelled kinetics showed

that it would be theoretically possible to maintain an effective airborne concentration of pheromone (greater than 5 ng/m³) (Gavara et al. 2020) for approximately 12 days, which corresponds well with the time between two sprays currently applied in field. Due to the high release during the first hours of the kinetics and the remaining concentration of 5 ng/m³ estimated at approximately 12 days, the Lobesia Pro Spray treatment could ideally be applied when it is the most necessary and not over all the breeding season to optimise the cost and the pheromone's release in the environment.

These first laboratory results prove the feasibility of using vine leaves as carriers for microscopic dispensers of pheromone from a sprayed formulation. The accurate kinetic laboratory study would also contribute to the optimisation of the formulation and treatment dosage and would contribute to reducing the number of field validation campaigns.

Release tests were carried out under the same conditions on three other carrier materials (glass, PVC, and blotting paper). The kinetics comparison showed a difference in the behaviour of the active substance according to the material involved. Indeed, the amount of pheromone released from an absorbent material (blotting paper) is half that of an inert one such as glass (respectively 18% and 40% of the initial amount sprayed). The results obtained for these various materials showed that the vine leaf behaved as a non-absorbent material such as glass or PVC and that the pheromone used in the Lobesia Pro Spray formulation did not penetrate the vine leaf, thus protecting the plant through encapsulation of the active substance.

The concept of microscopic passive dispensers sprayed in an aqueous suspension enables treatment dosage to be fine-tuned and applications at of peak pest pressure times, which may help winegrowers to limit the dosage and cost of the overall treatment over the season while keeping their harvest safe.

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Declarations

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