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Evaluation of the distribution of spray deposits within a vine canopy from measurements on artificial targets and real leaves

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ABSTRACT

Understanding the distribution of intercepted spray deposits is important for the study of the dose-response relationship of spraying a targeted pathogen and for the optimisation of the spraying process. However, carrying out exhaustive measurements of canopy spray deposits is difficult, particularly in production situations. This new experimental method for use in commercial vineyards was based on the installation of artificial targets (PVC collectors) within the canopy. To evaluate the quality of this experimental method for estimating the statistical distribution of deposition it was compared to an intensive manual method of foliar deposition measurements on real leaves. Intercepted deposition data on the real leaves and artificial targets were collected in a regular grid pattern within 12 non-contiguous vegetation sections. The results showed that although the means were similar, the variance in deposition appeared to differ between the distributions on artificial targets and real foliage, with CV values of between 37.4-52.7 % and 69.4-80.5 % respectively. Therefore, any central statistics must be supplemented with a statistical distribution analysis to account for the dispersion of deposition values within the vegetation. The results from comparisons between the cumulative distributions of intercepted deposition on the real leaves and on the PVC collector sections showed that the deposition estimates averaged over three-vine sections gave relevant, repeatable estimates for both approaches. The results also showed that for low deposition values, the experimental method led to a correct estimation of deposition on real leaves. However, above 230 ng dm² per 1 g/ha, the experimental method underestimated the deposition on real leaves by 13.6 %. Using these methodological results, it may be possible to develop models capable of predicting the distribution of deposition within the plant canopy. It would thereby be possible to develop an approach for variable-rate sprayer control that takes into account the phytosanitary risk and the site-specific variable structure of the vegetation during the season.

INTRODUCTION

The application of plant protection products (PPPs) is the predominant method used for crop protection in viticulture. The study of spray deposits on vegetation is of major interest for the evaluation of the agro-environmental performance of sprayers (Codis *et al.*, 2018), as well as the study of the dose-response relationship when targeting a pathogen (Koch and Knewitz, 2006). The generally admitted objective of PPP spray applications is to ensure sufficient foliar deposition and an even distribution in the vegetation (Felber, 1997).

Different protocols are used to quantify foliar deposition in vineyards (Giles and Downey, 2003; Pergher and Lacovig, 2005; Codis *et al.*, 2018; Salcedo *et al.*, 2020), often leading to incomparable results (Koch and Knewitz, 2006; Forster *et al.*, 2014). Published papers on the characterisation of foliar spray deposition report very different measurement protocols using either metal tracers (Llorens *et al.*, 2010), food dyes (Catania *et al.*, 2011; Codis *et al.*, 2018; Michael *et al.*, 2020) or fluorescent dyes (Siegfried *et al.*, 2007; Sinha *et al.*, 2019). In addition, the use of different types of artificial targets positioned in vegetation has been documented in the literature (Matthews, 1992; Codis *et al.*, 2018; Allagui *et al.*, 2018). Artificial collectors, which replace natural foliage, have been used for some time. The reason for this is that the recovery of the sprayed tracer retained on natural plant surfaces is more difficult and expensive than retrieval from artificial targets. In addition, research using natural targets is always limited by the size and spatial heterogeneity of the sample, and these parameters play an important role in the impartiality of the results. Finally, there is a limit to the accuracy of surface quantification of natural plant targets (Forster *et al.*, 2014). PVC collectors have been shown to have a good recovery rate and to be effective in recovering deposits (Garcera *et al.*, 2012).

Recent research has been conducted with collectors made of polyvinyl chloride (PVC) (Bastianelli *et al.*, 2017; Codis *et al.*, 2018), which are positioned in the vegetation and then collected after spraying. They provide quantitative information on the actual deposition that reaches the target. In particular, the use of PVC collectors has been promoted as a means of measuring spray deposits in the vineyard, including commercial vineyards (Codis *et al.*, 2018), without the need for destructively removing samples from the canopy. This has been done under the assumption that the mean deposition on PVC collectors reflects the mean depositions on the leaves in the canopy. In order to check this assumption, mean deposition measurements made on PVC collectors and real leaves have been performed at the upscale, aggregated sections (equivalent to the idea of trios or the control unit presented hereafter in the paper). Such aggregation minimises small-scale stochastic variance effects. At this scale of measurement, the mean deposition on PVC collectors has been shown to be relevant for modelling the mean depositions in the actual canopy (Llorens *et al.*, 2010; Siegfried *et al.*, 2007; Bastianelli *et al.*, 2017).

However, while this aggregated comparison supports the idea that the mean depositions on PVC collectors and real leaves are in accordance, no attempt has previously been made to precisely compare deposit measurements from individual PVC collectors with individual samples taken on leaves. However, the consideration of the statistical distribution of deposits in the canopy rather than an average of deposits for the whole canopy would result in a progressive shift in the spray management paradigm. With a model that estimated mean deposits per unit of leaf area, it would be possible to create a sprayer-sensitive dose management rationale in order to, for example, achieve a constant average deposit per leaf area throughout the season (if leaf area can be estimated). With a deposit distribution prediction model, it would be possible to develop a new site-specific management of the amount of PPPs to be applied. For example, a target distribution could be reasoned with regards to reference data in relation to crop protection needs. Such target distribution could be defined as the minimum to be ensured at any time of the season. Alternatively, one may consider evolving target distributions during the season in order to optimise the protection against one pathogen. With this paradigm shift it would also be possible to develop spray management that takes into account the variability of locally intercepted deposits, thus avoiding PPP underdosing, regardless of the vegetation zone (depending on depth and height) where this underdosing may occur. According to this vision, the study of foliar deposition requires a clear description of the sampling procedures. The sampling units for vegetation deposition must also be clearly described in the procedure.

Therefore, the main objective of this study was to evaluate both at an advanced vegetation stage and at an operational scale in the vineyard the distributions of depositions on the foliage and on artificial targets. The artificial targets are a requirement for designing formal experimental methods for conveniently measuring deposits in research conditions. The specific objectives of this research were as follows:

- To study the statistical distribution of leaf deposits within a vine canopy at a fine spatial scale on real leaves and artificial collectors, both being spatially registered within a grid according to depth and height dimensions.

- To compare, at an operational scale in the vineyard, these statistical distributions of deposits and to provide guidelines for the deployment of artificial collectors in vineyards to estimate foliage depositions in vineyards.

MATERIALS AND METHODS

1. Study site

A vineyard that is characteristic of wine production in the south of France, both in terms of the varieties it contains and method of cultivation, was chosen to carry out this experiment: Domaine du Chapitre, Hérault, France, Lat. 43°31'55.9 "N; Long. 3°51'50.3 "E° - geodetic system WGS 84. The study was carried out on a plot of *Vitis vinifera* L. cv Caladoc. The vines were trained in a Royat cordon with a support wire

FIGURE 1. Air assisted tunnel sprayer (Acrobaleno, Bertoni®) fitted with air injection flat fan nozzles.

and a trellis wire. The study was conducted at an advanced growth stage of vegetation development (BBCH 77, bunch closure) (Lorenz *et al.*, 1994) on the 11 July, 2018. The spacing between grapevines was 1 m intra-row and 2.50 m inter-row. A 15 m long plot was chosen for the study site due to its high homogeneity in terms of canopy size and vegetation density. Two days before the study, the vine row was mechanically topped and trimmed to obtain a uniform canopy along the 15 m section.

2. Sprayer characteristics

A recycling tunnel sprayer was used (Acrobaleno, Bertoni, Castel Bolognese, Italy) to ensure symmetrical spraying. It consisted of two vertical booms arranged on each side of the vine row, with each boom carrying a set of nozzles aligned on a vertical plane. The nozzles had outlets for high-speed airflow that brings kinetic energy to the spray they produce. The vertical booms were each equipped with four IDK (IDK Lechler, Metzingen, Germany) low-pressure air injection flat fan nozzles (Figure 1). The number of open nozzles and their direction were adjusted in the field while the sprayer was stationary to adjust the spray cloud and achieve a cross spray distribution compatible with the vegetation profile (carefully following general good practice for calibrationing and setting a sprayer). The flow rate of the nozzles was evaluated using graduated cylinders connected to the nozzles by flexible hoses at an operating pressure of 5 bars. The total flow rate was 5.5 L/min. At a forward speed of 1.39 m/s, the spray volume was 250 L/ha. The spraying operation was performed on 12 July between 11:30 and 12:30, the average air temperature was 27.6 °C, the relative humidity 46 % and the average wind speed 1.5 m/s.

3. Sampling strategy for foliar spray deposition in vegetation

Within the 15 m section, 12 vegetation segments (S1-12) of 0.2 m wide were selected and these were separated by a 0.7 m gap. In order to assess the variability of spray deposition, each of the 12 vegetation segments was subdivided into a regular grid perpendicular to the vine row. Each grid cell

was 0.4 m high and 0.1 m deep. This resulted in a total of 36 grid cells (parallelepipedal volume of 0.2 m wide, 0.4 m high and 0.1 m deep) in each of the 12 vegetation segments. The four heights were denoted A to D (bottom to top) and 9 depth classes denoted from P1 to P9 across the width of the canopy (Figure 2).

Artificial collectors, referred to as S1, S2, S3, S4, S5 and S6, were placed in 6 of the 12 vegetation segments. These 6 vegetation segments were aggregated into trios, with S1, S2 and S3 forming Trio 1 and S4, S5 and S6 forming Trio 2 (Figure 3). Trios were used because they correspond to the scale at which measurements of vegetative indicators are generally made in vineyards by professionals and that is found in experimental protocols. Analysis at the trio scale also allows deposition to be characterised at the operational scale (with implications for dose management), while observations of the individual segments allows deposition to be characterised at a fine scale.

In the remaining six segments (S7-12), leaf tissue samples were taken. These six segments were aggregated to provide a 'control' unit within the 15 m section, which formed a reference measurement of deposition.

4. Data collection: spray deposit measurement

Prior to the sprayer run, three PVC collectors were randomly placed in the canopy within each of the 36 predefined grid cells (Figure 2) in segments S1 to S6. However, it was decided to keep only one PVC collector per grid cell in the subsequent analyses, in order to investigate the possibility of less labour intensive sampling when using PVC collectors. To this end, one of the three collectors was randomly selected in each grid cell. In total, 216 collectors were then analysed across the six vegetation segments, representing a total measurement area of 1.72 m². Collectors of full size 0.08 × 0.05 m were folded over both sides of the leaves into a 0.04 × 0.05 m rectangular shape, so that both its sides represented the area that collected the deposits (i.e., a collection area of 0.004 m²).
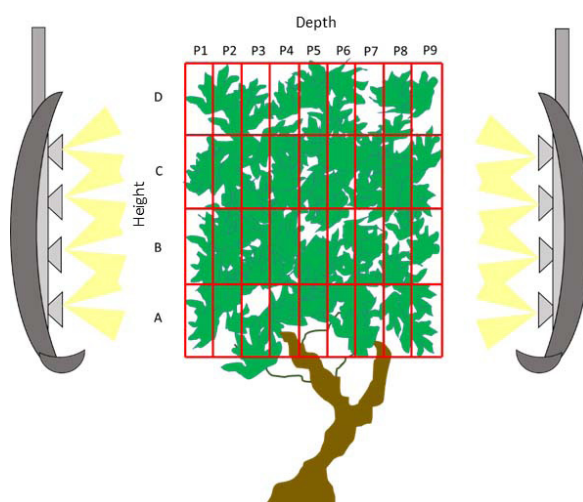
FIGURE 2. Cross-section of a vegetation segment and the grid used to measure the deposition within each of the 12 vegetation segments. Four height classes (A - D; bottom - top) and 9 depth classes (P1 - P9; P1 and P9 are exterior and P5 central interior) were defined. Grid height was 0.4 m and grid depth was 0.1 m. An over-the-row recuperating panel-type sprayer was used to spray each side of the vegetation row simultaneously.
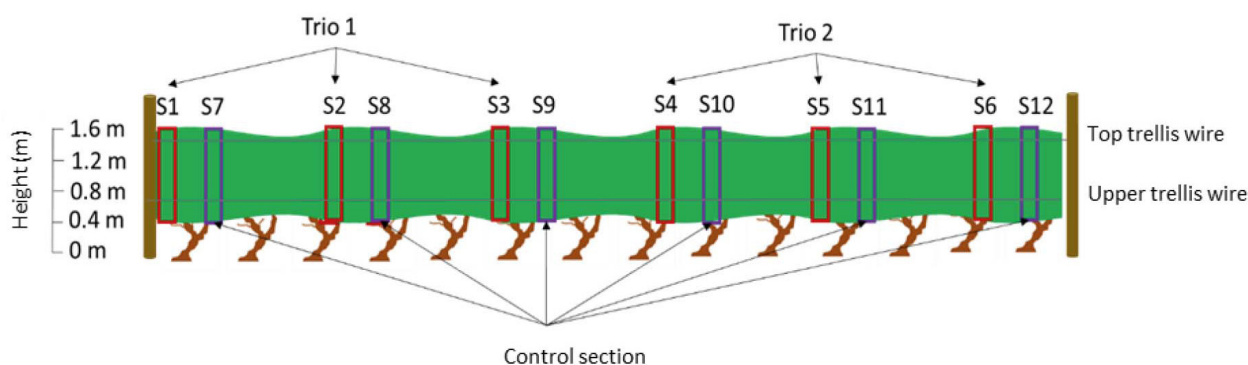

FIGURE 3. Diagram of the vegetation sampling procedure within the 15 m vineyard plot. The segments in which the PVC collectors were placed (S1-6) are in red and the defoliated segments (S7-12) are in purple.

In order to quantitatively assess the distribution of the spray in the canopy on the artificial collectors, as well as on the leaf samples, the sprayer was filled halfway with pure water, and the necessary amount of a colorimetric tracer (tartrazine, E-102, Sigma, St. Louis, MO, USA) was added to obtain a target concentration of 10 g/L. Spraying was carried out on the vine plot in a single journey of the sprayer following the generally established spraying procedure.

After the tracer had completely dried, all PVC collectors placed in the vegetation of segments S1 to S6 were collected and placed in an individual bag. At the same time, in the six vegetation segments (S7-12), the vines were totally defoliated and all leaf material (i.e., whole leaves and leaf fragments) within each grid cell in each section was collected and placed in individually labelled plastic bags. The leaf and PVC bags were transferred to the laboratory for analysis.

For the leaf samples, the total mass of plant material in each grid cell was determined using a precision balance (Kern ADB 600-C3, Kern®). A 3.6 cm diameter cutter was used to extract the maximum number of leaf discs from the leaf

material collected in each grid cell. The mass of some of the leaf disc samples was also determined and recorded. From these data it was possible to calculate that 98 % of the leaf area in the six defoliated segments were extracted as leaf discs, representing a vegetation area of 3.4 m². The remaining 2 % of the plant material was in the form of fragments. These fragments of plant material were not included in the quantification of the foliar spray deposition, but were taken into account in the assessment of the leaf area present in each grid cell. In total, 1766 leaf discs were collected from the six selected vegetation segments (Table 1).

In the laboratory, the natural leaf discs or the artificial PVC collector corresponding to each grid cell of the sampling scheme were individually rinsed in a known volume of distilled water to extract the tartrazine. The concentration of the tracer in the rinsed solution was then measured with a spectrophotometer (Uviline 9100, resolution: 0.001, accuracy $\pm$ 0.003, Secomam, Champigny sur Marne, France) at a wavelength of 427 nm. The deposition on both natural and artificial collectors in each grid cell was evaluated and

TABLE 1. Number of leaf discs collected from the defoliated segments.

Segments	Number of leaf discs
S7	249
S8	295
S9	282
S10	361
S11	358
S12	221
Total (Control)	1766

normalised according to the collector area and the tartrazine dose rate per hectare. It should be noted that both sides of the leaf discs were taken into account when calculating their surface area, as was the case for the PVC collectors. Foliar deposition was expressed in nanograms per square decimetre of leaf area per 1 g sprayed per hectare (ng dm² per 1 g/ha).

5. Statistical analysis

5.1. Total area of vegetation removed within the defoliated vegetation segments

In order to describe the vegetation removed within the defoliated segments (S7-12), histograms representing the distribution of the total vegetation area removed (m²) as a function of height class (A - D) and depth (P1 - P9) were made. To investigate the effect of the longitudinal position (segment), height and depth of the vegetation grids collected, a three-factor ANOVA was performed. In the case where an explanatory variable had a significant effect on average, a Tukey post-hoc test was used to compare all means in pairs and distinguish groups. This was done to identify variation in leaf area in vegetation, as this is known to have an impact on the spray deposition (Pergher *et al*., 1997; Pascuzzi *et al*., 2017). As the vegetation area data were non-normal, a log normalisation transformation was performed to obtain residual normality and homoscedasticity.

5.2. Descriptive and exploratory statistics of foliar spray deposition

Descriptive statistics (mean and coefficient of variation) were derived for each study segment (S1-12), for the trios (aggregated segments with PVC samples) and the control section (the aggregated segments with real leaves samples) as an exploratory analysis of deposition in the different vegetation segments studied. Empirical foliar spray deposition density curves were generated for each segment and modelled as a Poisson distribution. The differences between the vegetation segments are discussed below. Because the collectors and leaf samples data were normalised according to their surface, they could be compared despite their differences in size.

5.3. Attribute variability of intercepted deposition on actual leaves

In order to test whether samples originate from the same distribution, collected data from defoliated segments (control, segments S7-12) were analysed using a non-parametric method. The data were resampled into deciles and the data

were ranked from the lowest (1st decile; D1) to the highest (10th decile; D10). For each segment (S7-12), the mean value of each decile class was plotted against the decile number (D1-10) and, in addition, the overall aggregated mean for each decile was obtained across the control. The Kruskal-Wallis test was used to determine whether there were significant differences (p-value < 0.05) in the resampled decile data between the statistical distributions of the deposits observed on the six defoliated segments. For the overall aggregated decile control data set, each observed leaf disc was assigned a decile ranking and its positioning in the grid cell from where it was sampled in the canopy (4 heights (A - D) and 9 depths (P1 - P9)). This allowed the decile information to be plotted in the grid space to provide a preliminary indication of the overall spatial distribution of deposition and its variability on actual leaves in this study. It is important to note that this analysis, which does not involve geostatistics is merely indicative of the spatial distribution within the canopy and the variance of the deposition within a grid cell. The positioning of a given leaf in the canopy should affect the average deposition on the leaf.

To identify whether there was a significant difference in mean deposition between leaves located in height (A - D) or depth (P1 - P9) layers, a two-factor ANOVA was performed. As the deposition data were non-normal, a log normalisation transformation was performed to obtain residual normality and homoscedasticity. The "classes" (height and depth) were also unbalanced (unequal sizes), due to the different leaf areas in the different canopy zones, so the group means were compared in pairs using the Tukey post-hoc test to identify significantly different groups.

5.4. Comparison of statistical distributions of deposits observed on real leaves and on artificial PVC collectors, at an operational scale in the vineyard

To evaluate the ability of the PVC collectors to properly describe the statistical distribution of deposition in the foliage, a Kruskal-Wallis test was performed to investigate whether there was a significant difference between the statistical distributions observed in the control section, Trio 1 and Trio 2. This test only approaches the comparison from a "qualitative" point of view, indicating whether at least one group is stochastically dominant in the population. The test does not identify where this stochastic dominance occurs or for how many pairs of groups stochastic dominance occurs.
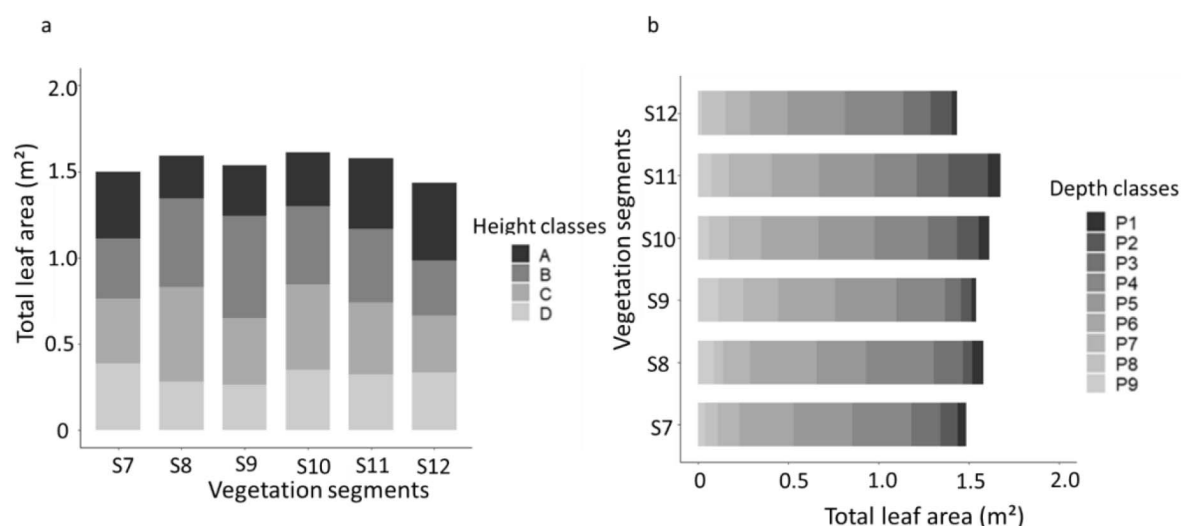
FIGURE 4. Histograms of the distribution of the total vegetation area (m²) as a function of the vegetation segment considered (S7-12) for (a) the height class (A - D) and (b) the depth classes (P1-9). Note that the orientation of the histogram reflects the direction of measurement (height or depth of the canopy).

In order to perform a pairwise comparison and more precisely compare the shape of the deposition distributions in the trios (observations on PVC collectors) and the control section (observations on real leaves), the Kolmogorov-Smirnov test was also used. This test determines whether the deposition distribution functions observed on Trio 1 and Trio 2 follow the same law as that defined on the control section. This test measures the fit between the compared distribution functions by means of the Kolmogorov-Smirnov distance (denoted D) and relative to a threshold distance Dα(n) that is a function of the sample size (n) and a specified significance level (α). Graphically, D is a measure of the largest vertical difference in absolute value between the two distribution functions. This test thus makes it possible to quantitatively assess any over- or underestimation that exists between the two compared distribution functions. These distribution functions for the two trios and the control section were plotted to compare the trend of the observations made on the PVC collectors and real leaves.

All statistical analyses were performed using the open source statistical software R (version 1.2.5001) (R Development Core Team, 2019). Graphs were made with MS Excel (Microsoft Office, Windows 10 2015, USA) and R. All statistical tests were performed using a significance level (α) of 0.05.

RESULTS AND DISCUSSION

1. Total area of vegetation removed within the defoliated vegetation segments

Regarding the six defoliated segments (S7-12), the total leaf area (TLA) removed within each of the meshes was measured. There was no significant difference (α = 0.05) in the mean TLA removed between the six defoliated segments ($p = 0.21$) or the mean TLA removed in relation to each

of the different height strata (A – D) in the sampling grid ($p = 0.12$) (Figure 4a). The aim was to select a homogenous section of canopy for the study and these results validated the study site selection. It may also reflect the efficiency of the canopy trimming process performed two days before the start of the study. In contrast, there was a significant effect on the mean TLA removed at different depths of the canopy (P1-9) ($p = 0.02$) (Figure 4b). Three groupings of leaf area relative to depth were observed: an exterior grouping (P1 and P9) with a very low mean TLA, an interior grouping (P4-6) with the highest mean TLAs that accounted for 57 % of the TLA, and a transitional grouping (P2, P3, P7, P8) with an intermediate mean TLA. These results illustrate that at a fine spatial scale, there is an asymmetry in terms of TLA along a depth gradient within a segment of vine vegetation. In order to maximise crop protection, this asymmetry in the vegetation structure should be taken into account when adjusting the PPP application rate.

2. Descriptive and exploratory statistics of foliar deposition

The normalised mean depositions observed on Trio 1 and Trio 2 differed little, with values of 250.9 and 246.8 ng dm² per 1 g/ha respectively and almost identical coefficient of variations, 34.3 and 31.2 % respectively (Table 2). The mean trio depositions were also very similar to the control mean depositions (observations on real leaves) (238.9 ng dm² for 1 g/ha). This reinforces the assumptions made by Codis *et al.* (2018) that the PVC collector methodology produces a good estimation of the mean foliage deposition. However, the dispersion around the mean of these data was different, with the control section having a much larger CV (74.9 %) than the trios (Table 2). Thus, while the means were similar, the variance of depositions appears to differ between distributions on the PVC collectors and the actual foliage.

TABLE 2. Mean (ng dm² per 1 g/ha) and coefficient of variation (%) observed at the scale of individual segments (S1-12) (ordered according to positioning along the canopy section), Trio 1 (S1-3), Trio 2 (S4-6) and the control section (S7-12). See Figure 3 for segment ordering and trio and control locations.

Sampled Segments	Collector Type	Normalised Mean (ng dm² per 1 g/ha)	CV (%)
S1	PVC	252.1	39.1
S7	Vegetation	243.6	69.4
S2	PVC	256.3	52.7
S8	Vegetation	242.4	69.5
S3	PVC	244.2	47.0
S9	Vegetation	236.7	79.9
S4	PVC	237.6	40.6
S10	Vegetation	229.6	80.5
S5	PVC	244.9	37.4
S11	Vegetation	239.6	77.7
S6	PVC	257.9	45.5
S12	Vegetation	241.5	72.6
Trio 1	PVC	250.9	34.3
Trio 2	PVC	246.8	31.2
Control	Vegetation	238.9	74.9

Figures 5a and 5b show the empirical density curves of the normalised depositions for the vegetation segments S1-6 (PVC) and S7-12 (real foliage) respectively. The shape of the statistical distributions follows a Poisson distribution, regardless of whether the deposits were sampled from PVC collectors (Figure 5a) or from real leaves (Figure 5b), with both exhibiting a positive skewness. However, the distributions from the foliage segment (S7-12) showed higher variability and a stronger positive skewness (Figure 5b). This indicates that higher deposit values were sampled on discs cut in the real leaves compared to the PVC collectors. Please note that there are differences in size in discs and collectors,

discs being smaller. Furthermore, it was observed that the probability density for very low deposition values (between 0 and 60 ng dm² per 1 g/ha) was higher for the measurements on real leaves than on the artificial PVC collectors.

Given the shape of the distributions and the dispersion of the deposition values, a single, central statistic (mean or median) for foliar spray deposition does not appear to sufficiently describe the deposition of the spray on the vegetation. Therefore, any central statistics should be complemented with a statistical distribution analysis to take into account the dispersion of deposition values within the vegetation.
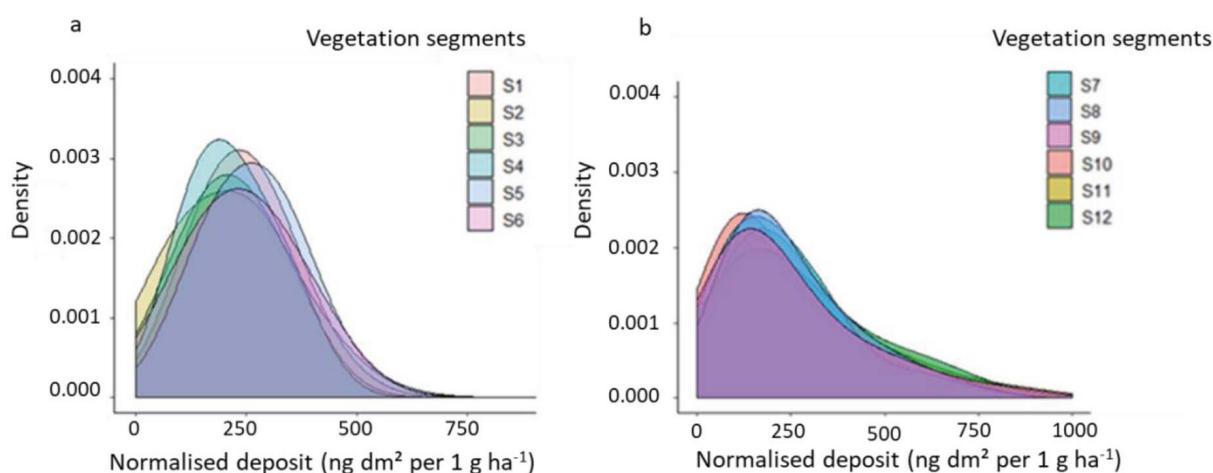

FIGURE 5. Empirical density curves of the normalised deposition values as a function of vegetation section for (a) artificial PVC collectors (S1-6), and (b) real leaves within the canopy (S7-12).
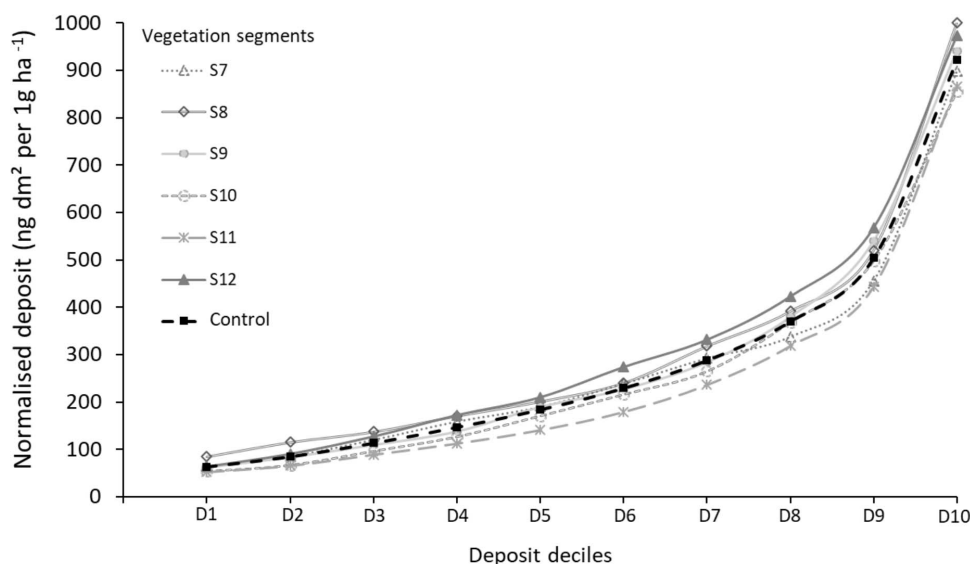
FIGURE 6. Statistical distributions distinguishing the ten deciles (D1 - D10) of the deposition values obtained for the six vegetation segments (empty triangle = S7; empty diamond = S8, full circle = S9, empty circle = S10, cross = S11 and full square = S12) and the aggregated control section (dotted line in black).

3. Variability of intercepted deposits on the real leaves

As the aim was to study in detail the variability in intercepted spray within the vegetation, a more detailed investigation of the deposition data acquired for real leaves (segments S7-12) was performed. The Kruskal-Wallis test showed that the statistical distributions of deposits obtained for the six segments (S7-12) did not differ significantly ($p = 0.24 > 0.05$). Figure 6 illustrates this graphically by plotting the normalised mean deposition for each decile in the six distributions. The aggregated mean response for these six distributions is also shown (Control).

Given the similarity in response of the individual segments with real spray depositions, the data of all S7-S12 segments were regrouped into a unique distribution, regardless of the segment label, but keeping grid cell information. This is shown in Figure 7 to illustrate the spatial distribution of the intercepted deposition deciles within the whole control area. Overall, the observed deposition was very variable, even within a grid cell. However, there was a trend in the data, with weaker deposition deciles (red colour) positioned in the centre of the vegetation, where the total leaf area was higher (Figure 4a) and where the leaves were furthest from the sprayer (Figure 2). Despite this, there were still some leaf discs that were associated with high deciles (D8-10) located in the P5 section. Thus, while there is a trend in decreasing levels of deposition towards the centre of the canopy, there are many physical effects at play that influence the deposition values on individual samples, and therefore its associated decile. These effects include variation in spray path angle, anisotropic leaf area distribution (Walklate *et al.*, 2011), leaf arrangement relative to airflow (Raupach *et al.*, 2001) and small-scale aerodynamics of spray droplets near collector surfaces (May and Clifford, 1967). These effects are either constant or difficult to measure and characterise due to the complexity of geometry and physics at this fine scale. Thus no attempt was made to disentangle these effects within this study.

A two-factor ANOVA test was performed to investigate whether there was a significant difference ($\alpha = 0.05$) in terms of the mean depositions observed between the different classes in height (A - D) and depth (P1 - P9). The result was that there was no significant difference in the average deposition observed across the height classes ($p = 0.37$) (Figure 8a), but that there was a significant difference in the mean deposition observed across the depth classes ($p = 0.019$) (Figure 8b). This follows the same trend as the previous TLA analysis. The Tukey test distinguished two significantly different groupings according to the depth positioning of the grid cells; an exterior grouping (P1, P2, P3, P7, P8, P9) and an interior grouping (P4, P5, P6) (Figure 8b). On average, foliar spray deposition was 1.5 times higher for the exterior group than the interior group. There was no intermediate grouping, as indicated from the TLA analysis. This result is characteristic of this type of over-the-row spray equipment, which treats both sides of the vegetation row simultaneously (symmetrically) and shows a tend in depletion of the spray droplets with increasing depth into the canopy.

For all the defoliated vegetation segments, the deposition distribution showed a strong positive asymmetry, associated with very high deposition values in the outermost layers of the vegetation. The 10th decile consistently exhibited a saturation level of deposition. In contrast, the lowest deposition values were found in the area in the centre of the vegetation, where where the vegetation was the most vigorous. By characterising the attribute variability, which is determined from the statistical distribution of the deposits, the lowest decile of deposits corresponding to the zones that are the least well treated during a spraying operation can be taken into account and identified.
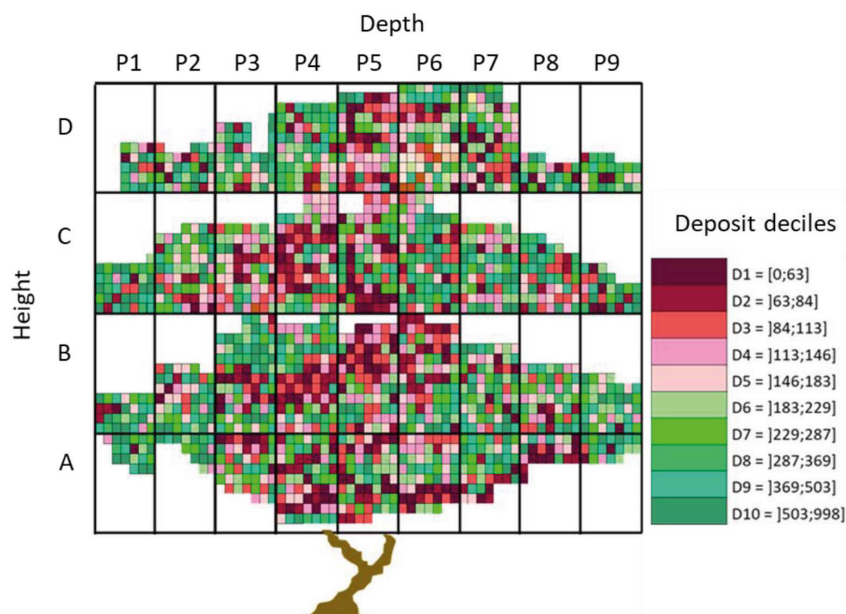
FIGURE 7. Cross-section of the whole control section deposition data expressed as deciles. Each coloured square represents a leaf disc from 1 of the 6 vegetation segments. The colour indicates the decile associated with its deposition value in the distribution that regroups all 6 segments. The location corresponds to the grid from where the leaf disc was taken (height and depth of the canopy). The positioning of the squares within a given grid cell is meaningless and was done randomly.

4. Comparison of the statistical distributions of foliar spray deposition observed on real leaves and on artificial collectors at an operational scale in the vineyard

The results obtained in the previous sections have shown that the defoliated segments (S7-12) were not significantly different from each other in terms of TLA and the statistical distribution of deposition. In view of these results, the data of each grid cell regardless of segments (within S7-12 segments) were averaged to obtain a mean control distribution of deposition as a reference statistical distribution. The Kruskal-Wallis test was used to compare this reference distribution to the mean distributions of Trio 1 and Trio 2 (obtained by averaging data values from the PVC collectors for each cell in a trio, thus an average of 3 values, one for each segment in a trio). The result of this test showed that the statistical distributions of the deposits observed on the reference control section (real leaves) with Trio 1 and Trio 2 were significantly different ($p = 0.019$ and $p = 0.013$) (Table 3). A pairwise analysis using the Kolmogorov-Smirnov tests (Table 3), showed that the distribution functions of depositions observed on Trio 1 and Trio 2 did not differ significantly ($D < D_\alpha$ (n) and $p = 0.99$) and the two distribution functions are very similar (Figure 9). The spatial scale of the vegetation section trio seems relevant for estimating the statistical distribution of deposition at a late vegetation stage in an apparently repeatable way.

In contrast, the pairwise comparisons between the cumulative distribution functions of the control, Trio 1 and Trio 2 showed a significance difference for both trios relative to the control ($D > D_\alpha$ (n) and $p < 0.05$) (Table 3). This result confirms a significant difference between the cumulative distribution functions of deposition on real leaves vs. PVC collector, despite the normalised mean deposition for the control and the trios being very similar (as shown in Table 2).

Figure 9 shows a graphical comparison of these three different cumulative distribution functions. Over the range of deposition values from 63 ng dm² per 1 g/ha (1st percentile of the deposition distribution) to 230 ng dm² per 1 g/ha (60th percentile of the deposition distribution), the cumulative distribution functions for the trios (PVC collectors; blue and green curves) and the control (real leaves; red curve) overlap. In this range, the proposed experimental method of using PVC collectors does not underestimate or overestimate the deposition values compared to measurements on real leaves. However, beyond the 61st percentile (> 230 ng dm² per 1 g/ha), the control cumulative distribution function deviates and the experimental method underestimates the deposition on the real leaves by 13.6 %. This result can be explained by a strong difference in chemical composition, roughness and texture between the surface of artificial targets and real vine leaves, which leads to a lower retention capacity on the artificial PVC targets when deposition rates are very high (Koch and Knewitz, 2008). The effect of spray collecting surface for trios and control should also be checked, as higher collecting surfaces mathematically tend to smooth deposit data. The spray-collecting surface for each collector in a trio is 40 cm² (on both sides) and the total surface involved in the mean for each cell in the trio is 120 cm², while the spray-collecting surface for each leaf disk in the control is 20.4 cm², and the total surface involved in the mean for each cell of control averages 510.4 cm².
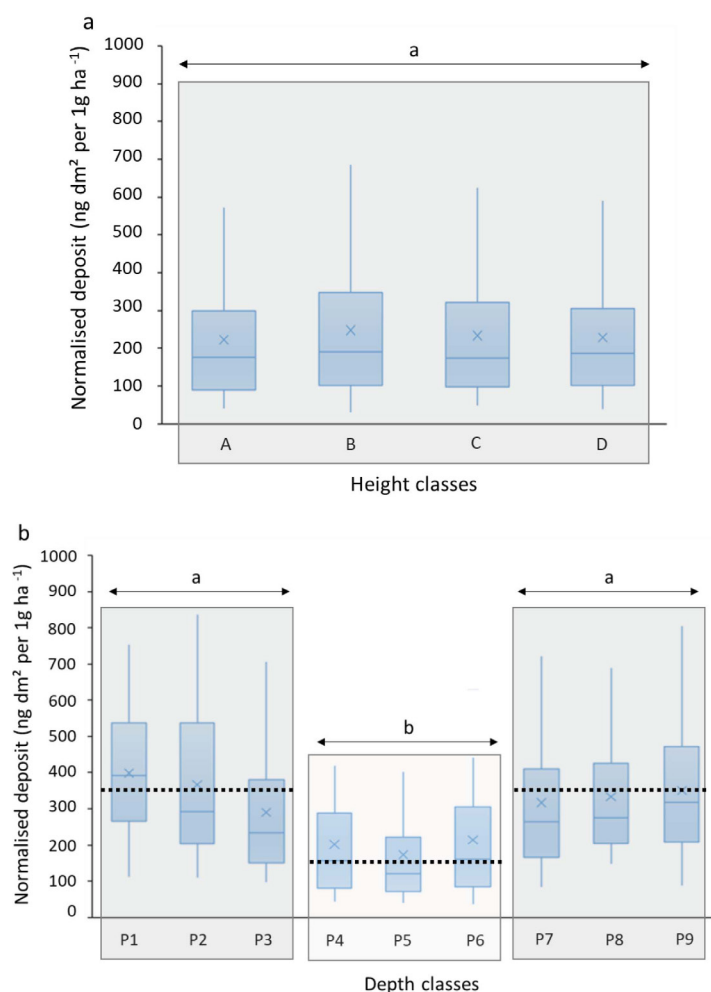
FIGURE 8. Box plots of leaf deposits found within the control section as a function of class, a: height (A - D), and b: depth (P1 - P9). The box plot shows the median (solid line) and mean (cross). The lower and upper limits of the boxes are the first and third quartiles respectively and the error bars show the minimum and maximum values. Values not followed by a common letter indicating depth groups are significantly different according to Tukey's test ($\alpha < 0.05$) in terms of intercepted spray deposition. The mean value of the deposits observed in the discriminating groups is represented by a dotted line.

TABLE 3. Results of the comparison between the distribution functions of the deposits observed on the control section, Trio 1 and Trio 2 from the Kolmogorov-Smirnov tests. For each comparison performed, the table shows the total sample size, the D-statistic, the p-value and the critical threshold Dα(n).

Pairwise comparison	n	D	p-value	D_α (n)
Trio 1 - Trio 2	72	0.061	0.987	0.16
Control - Trio 1	72	0.211	0.019	0.16
Control - Trio 2	72	0.246	0.013	

Although the experimental method leads to an underestimation at high deposition values, this was not considered a major drawback, as a modelling approach with "worst case" risk management should encourage underestimation, rather than overestimation, of deposition to ensure that PPPs are applied in sufficient quantities. Furthermore, in crop protection, areas that receive high deposition rates are covered, and it is the vegetation that receives the lowest spray deposition rates that are of more concern and likely to have the highest risk of pathogen occurrence. For the low deposition values (between 63 and 230 ng dm² per 1 g/ha) the experimental method leads to a correct estimation of deposition on the real leaves. In operational conditions, measurements on real leaves are difficult as they are time-consuming and destructive.
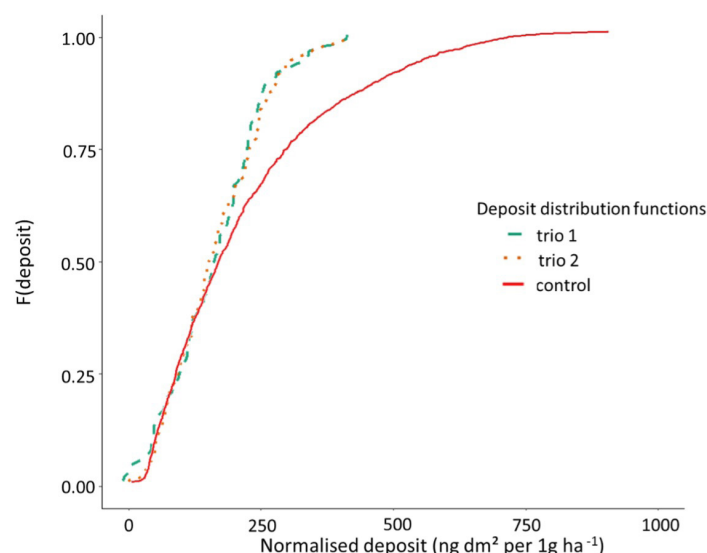
FIGURE 9. Representative curves of the cumulative deposition distribution functions obtained from the control section (solid red line = real leaves), Trio 1 (broken green line = PVC) and Trio 2 (dotted brown line = PVC).

The results here show that the use of PVC collectors is relevant for estimating typical deposition values (63 – 230 ng dm² per 1 g/ha) in well-developed canopies in southern France. For very low deposition values (< 63 ng dm² per 1 g/ha), the error observed is significant at the beginning of the curve. This is probably due to the smaller collection area of the PVC collectors per mesh.

It should be noted that although the collectors and actual leaves were sampled in a regular 2D grid in the canopy, their explicit location was not used. In this paper, the distribution has only been described in the attribute space, not in the geographical space. Further research is certainly needed to develop approaches to spatialise the distribution of deposition in the canopy. Ultimately, the development of statistical models capable of predicting deposition distribution would allow a gradual shift in the paradigm of spray management from deposition management that aims for a constant average deposition to an assumption of deposition distribution; e.g., by referring to the distribution obtained with a given device at a given time of the season. This paradigm shift would allow a pathogen risk management model to be developed that is better adapted to the evolution of the local vegetation structure during the season.

CONCLUSIONS

In this study, an experimental method to estimate the statistical distribution of intercepted foliar deposition within a vine canopy at an operational scale is proposed. This method is based on the positioning of artificial targets in the vegetation according to a regular grid. To evaluate the proposed experimental method, the statistical distribution of foliar deposition intercepted by artificial targets was compared to that intercepted directly by the foliage.

Using the results obtained from data collected at a late stage of vegetation on 12 vegetation segments, the statistical distribution of spray deposition within a vine canopy at a fine spatial scale on real leaves was studied. This analysis shows that it may be relevant to take into account this distribution, instead of simply averaging the deposits, to improve the effectiveness and efficiency of crop protection. The description of the statistical distribution of the overall deposition signal could help determine the areas of plant cover less well-treated during spraying; in turn, this would increase understanding, from an epidemiological point of view, of the resistance and the disease pressure generated by the target pathogens of a spraying. Moreover, this study showed that the proposed experimental method can be used at the operational scale of the trio of grapevines. The empirical results of the study provide clear instructions for the deployment of artificial collectors in vineyards for estimating foliar deposition in vineyards.

It should be noted that although the collectors and actual leaves were placed in regular 2D grids across the vegetation, their explicit location was not used. In this study, the distribution was described only in the attribute space, not in a metric (vegetation) space. Further research is therefore needed to develop approaches to spatialise the distribution of deposits in the canopy. Finally, the development of statistical models capable of predicting deposition distribution would allow a gradual shift in the paradigm of spray management from a constant average deposition to a distribution of the deposition; e.g., by referring to the distribution obtained using a given device at a given time in the season. With such a paradigm shift it would be possible to develop a pest risk management model that is more adapted to the evolution of the local vegetation structure during the season.

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