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Aerial spraying for downy mildew control in grapevines using a remotely piloted aircraft

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ABSTRACT. Downy mildew is a major problem for grape growers, as this disease is difficult to control. Synthetic fungicides are used to treat downy mildew with handheld backpack and tractor sprayer applications, with high chemical exposure by operators. As important tools for maximising yield, application technologies must be studied to optimise control efficiency. The objective of this study was to evaluate the efficiency of fungicide spray application using Remotely Piloted Aircraft (RPA) for the control of downy mildew in grapevine, with different spray volumes. The study was divided into two experiments using 4 vine lines, 10 blocks and 5 treatments with different mixture volumes: Experiment 1 with RPA application of 5 mixture volumes - 0, 22, 44, 66, and 88 L ha^{-1}; Experiment 2 with RPA application of 3 mixture volumes - 44, 66, and 88 L ha^{-1} and a backpack application of one mixture volume - 800 L ha^{-1}. Coverage percentage, droplet density and volume median diameter (VMD) were evaluated. Downy mildew severity on grapevine leaves was assessed using visual analysis and a diagrammatic scale. The application of 44 L ha^{-1} provided the greatest coverage and droplet density in the upper and middle strata; however, the backpack application had a better droplet distribution than the RPA application. Treatments of 44 L ha^{-1} with RPA and backpack application (800 L ha^{-1}) provided the best disease control. In the trellis system, RPA application must be improved because of the low coverage in the lower parts of the plant, and further studies with different spray nozzles and application heights are needed.

Keywords: *Plasmopora viticola*; aerial spraying; low volume.

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Introduction

In Brazil, grapes are among the top three most produced fruits, behind only oranges and bananas. With a domestic revenue increase of 2.9% in value (R$) in the 2021/2022 season, this crop is third in the export ranking, with a variation of 42% in value (US$) compared with 2020/2021 (Kist, Carvalho, & Beling, 2022).

Brazilian viticulture is an important source to complement the income of farms and has contributed to employment by means of investments made by producers and entrepreneurs in the wine sector (Mello, 2020). However, the plant has disease problems that cause great economic losses to farmers. Some of these diseases are caused by fungi, and their control is usually carried out with synthetic fungicide applications (Peruch, Medeiros, Della Bruna, & Stadinik, 2007).

Among the fungal diseases of economic importance for the crop is downy mildew caused by the fungus *Plasmopora viticola*. Downy mildew damages leaves, flowers and berries, with losses of up to 100% of production. High relative humidity, high rainfall and temperature favour the fungus to develop powdery white patches, indicating the existence of fungal mycelium (Garrido & Sônego, 2007).

In southern Brazil, numerous vineyards grow on slopes with limited mechanisability; thus, fungal mixtures are frequently applied using a backpack sprayer but with low efficiency and high chemical exposure to the operators. Even if mechanisation is possible, tractor spraying is expensive, requiring a mixture volume many times greater than 400 L ha^{-1} and possible high drift losses.

Compared with ground spraying, agricultural aircraft provide high rates of droplet penetration in the canopy because of the motion of the propellers. However, the vortex at the tip of the wings of the aircraft causes a great loss of the mixture by drift; in addition, the need for higher flight altitudes causes greater losses by evaporation (Coimbra & Catalano, 1999).

Due to the complexity and number of variables involved in pesticide application in vineyards, Remotely Piloted Aircraft (RPA) systems have become feasible, mainly because they provide excellent operability in sloping terrain, as well as minimal pesticide exposure to the operators (Carvalho, Chechetto, Mota, & Antuniassi, 2020; Giles & Billing, 2015; Martin, Woldt, & Latheef, 2019).

Viticulture in the southern region of Brazil is predominantly located on sloping lands of difficult mechanisation; therefore, RPA systems for spraying pesticides are promising. However, important issues, such as the volume and concentration of the sprayed solution, losses and environmental impact, and uniformity of the deposition of the mixture on crop leaves, still need to be addressed.

It is important to consider that the use of RPA for spraying in vineyards is a recent technology and is currently in the process of regulation by relevant authorities. Recently, in 2021, the Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2021) launched Ordinance No. 298 regarding the use of RPA for spraying. This regulation establishes the rules for the operation of RPA intended for the application of pesticides, adjuvants, fertilisers, inoculants, soil correctives and seeds.

In this sense, it is necessary to develop protocols that attest to the safety and efficiency of the technique. The objective of the present study was to evaluate the efficiency of RPA application of a fungicide mixture with different spray volumes to control downy mildew on grapevine.

Material and methods

Two experiments were carried out in an experimental vineyard in the *Universidade Tecnológica Federal do Paraná* (UTFPR), Pato Branco Campus, Paraná State, Brazil, at coordinates 26°10' S and 52°41' W and 765 m of altitude. The climate of the area is Cfa (Koppen classification), with an average annual temperature around 20°C and average annual rainfall around 2,250 mm, with good rainfall distribution throughout the year and a tendency for rainfall to concentrate in the summer months (Bhering et al., 2008).

The vineyard was planted in early 2019 in an area of 1,440 m², trained in the espalier system, with the 'BRS Magna' cultivar grafted onto 10 different rootstocks as follows: '1103 Paulsen', 'IAC 572 Jales', 'IAC 766 Campinas', 'IAC 313 Tropical', 'Freedom', 'Harmony', '420A', '101-14 MGT', 'SO4', and 'Kober 5BB'. The vines were planted in a north–south orientation and spaced 1 m within the row and 4 m between the rows. Each row was 90 m long and arranged in a contour line.

Experiment I: The spraying experiment was arranged in a randomised block design, with four treatments and one control (untreated plants), with ten replications. The experiment was composed of four rows, and each row received a treatment (Figure 1). Each row comprised 10 blocks with an area of 36 m², and each block consisted of a different rootstock. The treatments consisted of RPA fungicide applications with spray volumes of 22, 44, 66, and 88 L ha^{-1}. Each block contained nine plants. The 2 plants at both ends of the plot were taken as absolute controls (0 L), receiving no treatment, and were covered by a plastic sheet. The remaining seven plants in the middle of each plot received the treatments. The four treatments had the same concentration of active ingredient (a.i.) per hectare (Table 1).

The experiment started on October 5, 2020, and ended on February 9, 2021, when the plants were at stage 43 of the Eichhorn and Lorenz (1984) scale, and there were 7 total applications. Application intervals were not defined, ranging from 15 to 30 days because the climatic conditions (humidity and rainfall) were not favourable for disease occurrence. The a.i. in the mixture were alternated, with Chlorothalonil 500 g L^{-1} and Azoxystrobin 200 g L^{-1} + Difenoconazole 125 mg L^{-1} and with water and the Weitec® adjuvant.

Spray applications were carried out with the remotely piloted Align Demeter E1 helicopter, which had the following parameters: 2 rotors, 15 L liquid chemical capacity, four 90 degree hollow cone nozzles, operating at a spray rate of 1.29 L min.$^{-1}$, spaced at 50 cm, flight duration of 12 minutes, flight speed of 3 m s^{-1}, average flight altitude of 2 m from the top of the vine canopy, and coverage of about 4 m.

The helicopter was operated in manual mode because of the crop line contour arrangement, which made it difficult to operate in automatic mode. The fungicide was sprayed over the rows (treatment), and to meet the desired volume of the applied mixture, we increased the number of spray passes the helicopter made over the row. Thus, for volumes of 22, 44, 66, and 88 L ha^{-1}, the helicopter flew over the rows 1, 2, 3, and 4 times, respectively.

Applications took place on Oct. 5, 2020; Oct. 20, 2020; Nov. 3, 2020; Dec. 1, 2020; Dec. 21, 2020; Jan. 19, 2021; and Feb. 9, 2021, with alternate application of the a.i. Spray pressure remained the same for all treatments.

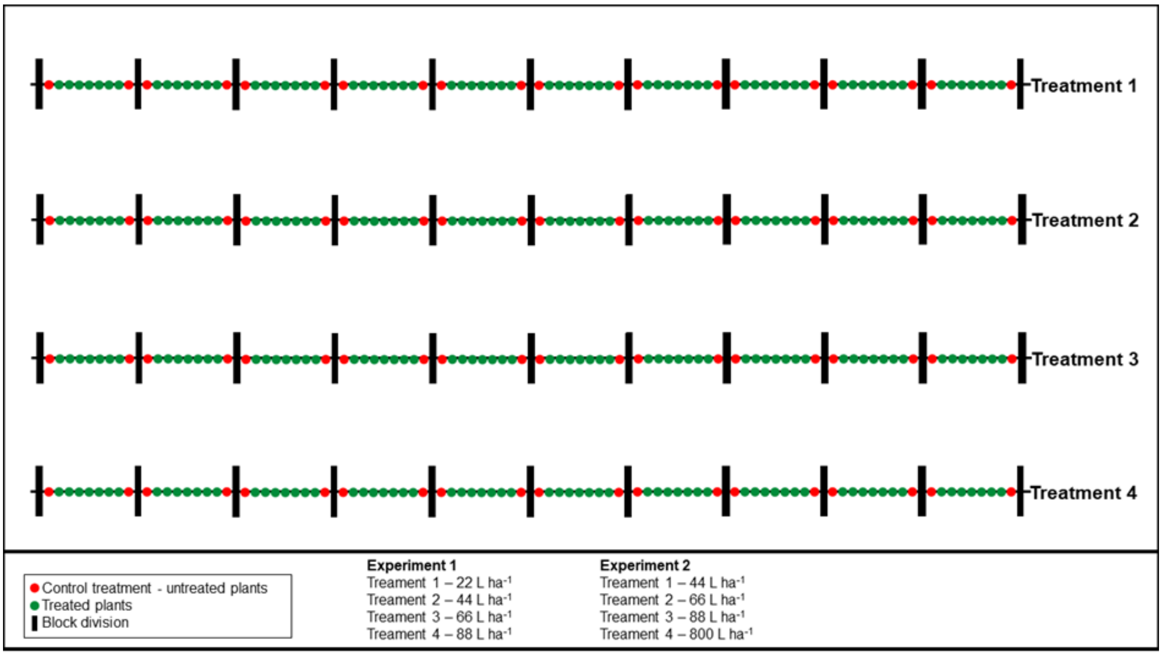

Figure 1. Representative scheme of the experiments. Each row consisted of 10 blocks, with each block comprising treated plants (green circle) and untreated plants (red circle). The experimental design was the same for both experiments, with only the spray volumes altered.

Table 1. Fungicide combinations, spray volume (L ha^{-1}), and rates of application and adjuvant used in the treatments of 'BRS Magna' vines grown on espaliers. (A.I) Active ingredient. (A) 200 g L^{-1} Azoxystrobin + 125 mg L^{-1} Difenoconazole and (B) 500 g L^{-1} Chlorothalonil.

Spray volume (L ha^{-1})	Spray volume 360 m^{-2} (mL)	Chemical (A.I)	A.I mL ha^{-1}	AI mL 360 m^2	Mineral oil mL
0	-	-	-	-	-
	-	-	-	-	-
22	792	A	500	18	25
		B	1666	60	25
44	1584	A	500	18	25
		B	1666	60	25
66	2376	A	500	18	25
		B	1666	60	25
88	3168	A	500	18	25
		B	1666	60	25

Water-sensitive paper (WSP) was applied to assess the percent coverage, droplet density and volume median diameter (VMD%) in the spraying carried out on February 9, 2021. The atmospheric conditions at the time of application were a wind speed of 5.0 km h^{-1} and a temperature of 28°C. The WSP cards were positioned in the upper, middle and lower leaf strata, in four blocks of each treatment. After collecting and storing the cards, we scanned them using an EPSON® L396 scanner at 600 ppi (pixels per inch resolution) and analysed them with the DepositScan® software.

The downy mildew severity in the 2020/2021 season was assessed biweekly after the first symptoms appeared on 10 leaves from four branches in each experimental unit. The severity assessment was conducted using the diagrammatic scale proposed by Buffara et al. (2014). The incidence and severity were determined and the area under the progress curve for mildew severity was calculated.

Experiment II: The experiment was conducted in randomised blocks, with four treatments and one control (untreated plants) with ten replications. The mixture was applied by RPA AGRAS MG-1S, with volumes of 44, 66, and 88 L ha^{-1}, and a conventional application with a backpack sprayer with a volume of 800 L ha^{-1}. Control plants were also included without fungicide treatment. The applications were carried out from September 2021 to February 2022.

The spraying was carried out with an AGRAS MG-1S crop sprayer with the following parameters: 10 kg tank capacity, maximum flight speed 7 m s^{-1}, four extended range nozzles (XR11001VS) with a flat spray pattern, spray rate of 1.2 L min.$^{-1}$, spaced at 50 cm, and flight duration of 10 min. During application, the RPA was operated at a flight speed of 3 m s−1, pressure of 1.2 bar, average flight altitude of 2 m from the vine canopy, and coverage of about 4 m range. The RPA was operated in manual mode using the same settings as in the previous experiment to meet the different mixture volumes without changing the operating parameters. Thus, for the volumes of 44, 66, and 88 L ha^{-1}, the AGRAS MG-1S flew over the rows 2, 3, and 4 times, respectively (Figure 1). A backpack JACTO PJH20 sprayer of 20 L with a maximum working pressure of 6.8 bar and a hollow cone nozzle was used for the manned spraying operation. The four treatments that received fungicides had the same a.i. concentration per hectare.

Percent coverage, droplet density and VMD% were assessed on two different days using WSP cards arranged in the upper, middle, and lower leaf strata in five blocks of each treatment. The collection and analysis of the WSP were conducted as in the first experiment. The first WSP collection was carried out on January 20, 2022, with the application of systemic fungicide. The weather conditions at the time of application were as follows: wind speed 10 km h^{-1}, temperature 32°C, and relative humidity 67%. The second collection was on February 8, 2022, with the application of contact fungicide, but we could not read the WSP cards of the 800 L ha^{-1}, as they were heavily stained by the mixture. Climate conditions at the time of application were as follows: wind speed 15 km h^{-1}, temperature 27°C, and relative humidity 50%.

The assessment of downy mildew severity on leaves during the 2021/2022 season was carried out, as in the previous experiment.

Data were analysed for normality of errors and homogeneity of variances. The Box–Cox transformation was used when these assumptions were not met. Analysis of variance and linear regression were performed using R (R Core Team, 2021).

Results and discussion

Experiment I

Interactions were found between strata and spray volume for percent coverage and droplet deposition (Figure 2). No significant differences were found between the strata at a spray volume of 22 L ha^{-1}; however, the percent target coverage in the upper stratum of the canopy increased with the increase in spray volume (Figure 2A). The middle and lower strata were not significantly different, but both showed less coverage in relation to the upper stratum of the vines.

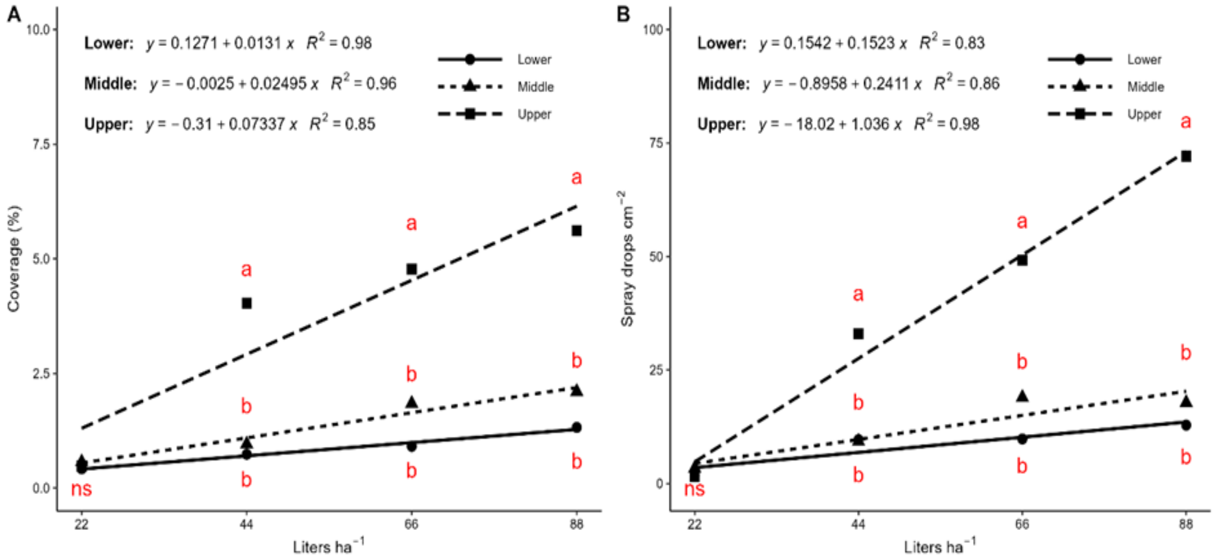

Figure 2. Percent target coverage (%) and droplet deposition (droplets cm^2) for different spray volumes – 22, 44, 66, and 88 L ha^{-1} applied by helicopter sprayer on 'BRS Magna' grapevines on an espalier. Means with different letters between strata are different by Tukey's test ($p \leqslant 0.05$). ns = means are not different between strata.

Similar behaviour was observed for droplet deposition (droplet cm^{-2}). In the application of the different volumes on the strata, the upper stratum received the highest deposition in relation to the others, proportional to the increase in the volume of the applied mixture (Figure 2B).

For VMD50%, differences were found only between spray volumes without interactions between the plant strata evaluated. Consequently, the evaluation was carried out only between the different volumes (Figure 3). The increase in spray volume also promoted an increase in VMD50%, assuming an average droplet spectrum of VMD at 266 – 339 µm (Figure 3).

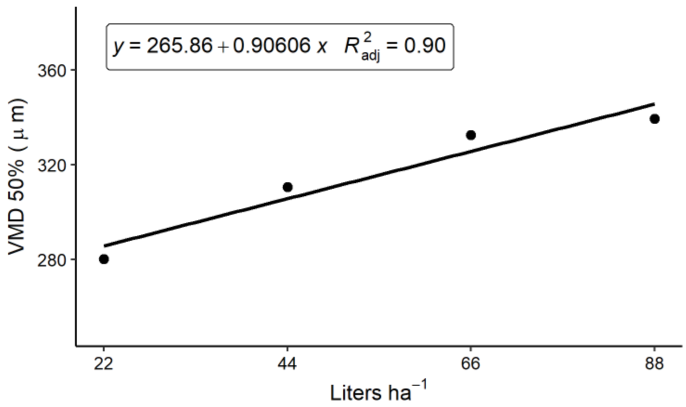

Figure 3. Volume median diameter (VMD50%) in different volumes of mixture (22, 44, 66, and 88 L ha^{-1}) applied by helicopter sprayer on 'BRS Magna' grapevines trained on an espalier. Each spray volume represents the average across strata.

The assessment of downy mildew in 'BRS Magna' showed that the climatic conditions of the 2020/2021 season were not optimal for the appearance of the disease until December 2020. The first symptoms were identified in the second half of January 2021, when rainfall increased (Figure 4). For this reason, no symptoms of the disease were found on the berries since the fruit clusters were harvested on December 15, 2020.

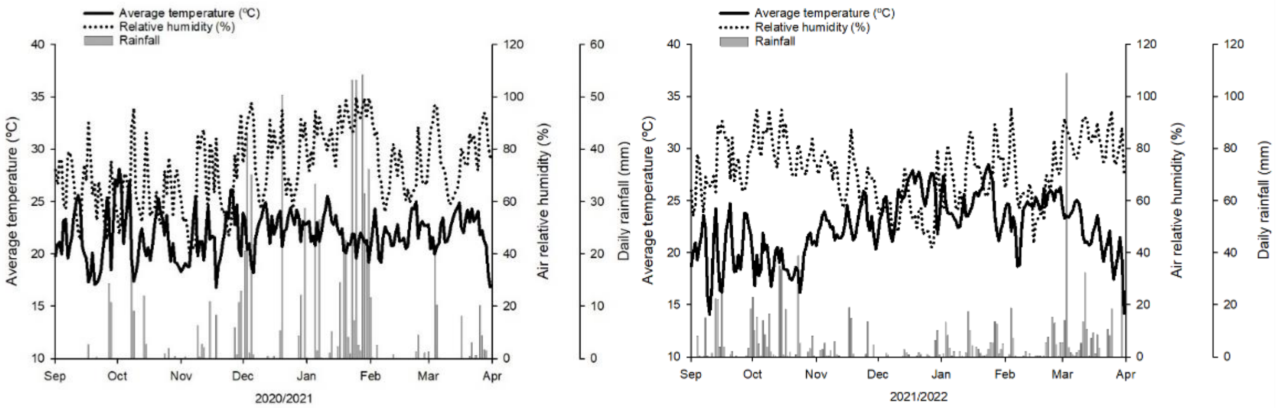

Figure 4. Average temperature, relative humidity and total rainfall from September to March of the 2020/2021 and 2021/2022 harvests in the municipality of Pato Branco, Paraná State, Brazil.

After detecting the first symptoms of downy mildew in the vines, interactions were found between the mixture volume and the assessment days (Table 2).

No significant differences were found for severity until the third assessment (Table 2). At 45 days after the first symptom was recorded, the spray volume 22 L ha^{-1} showed the lowest efficiency in controlling downy mildew in the vines and did not differ from the control. The non-treated plants did not differ significantly from the plants treated with a spray volume of 88 L ha^{-1}. Plants treated with volumes of 44 and 66 L ha^{-1} were not different among themselves and showed less downy mildew spread at 45 days of observation (Table 2).

The area under the disease progress curve (AUDPC) showed significant differences between the spray volumes. Plants treated with a spray volume of 44 L ha^{-1} presented lower severity despite not differing from the volumes of 66 and 88 L ha^{-1}. The volumes 0 and 22 L ha^{-1} were not significantly different, nor were the volumes 66 and 88 L ha^{-1} (Figure 5).

Li et al. (2021) evaluated the application with an RPA sprayer and found that the upper stratum of the canopy concentrated the largest volume of the mixture, and the lower stratum had the smallest volume, which is similar to the results of the present work. The spray application of ultra-low-volume insecticide in a high-density olive orchard with RPA showed the highest average droplet deposition in the upper stratum of the canopy, with 25 drops cm^{-2} and 13 drops cm^{-2} in the middle stratum, while in citrus, the percent coverage and drop diameter were higher than in olive trees (Martinez-Guanter, Agüera, Agüera, & Pérez-Ruiz, 2020).

Table 2. Average incidence and severity of downy mildew on leaves of grapevine cv. 'BRS Magna' grown on espaliers as a function of the different mixture volumes applied with a helicopter sprayer.

Treatment	Incidence			
	0 days	15 days	30 days	45 days
0	0.386 cC	0.636 bB	0.816 bA	0.87 bA
22	0.528 bC	0.763 aB	0.904 abA	1.00 aA
44	0.507 bC	0.788 aB	0.934 aA	1.00 aA
66	0.631 aB	0.704 abB	0.980 aA	1.00 aA
88	0.504 bC	0.774 aB	0.972 aA	0.99 aA
CV= 10.6%				
Treatment	**Severity**			
	0 days	15 days	30 days	45 days
0	15.31^{NS}	32.00^{NS}	69.14^{NS}	79.49 ab
22	18.30	33.69	65.77	82.34 a
44	16.80	27.17	57.69	62.08 c
66	15.98	25.11	58.58	64.83 c
88	9.64	29.25	63.41	70.49 bc
CV (%)	46.75	29.65	14.73	9.82

Means followed by different small letters in the column and capital letters in the row are significantly different according to Tukey's test (p ≤ 0.05). ns – non-significant.

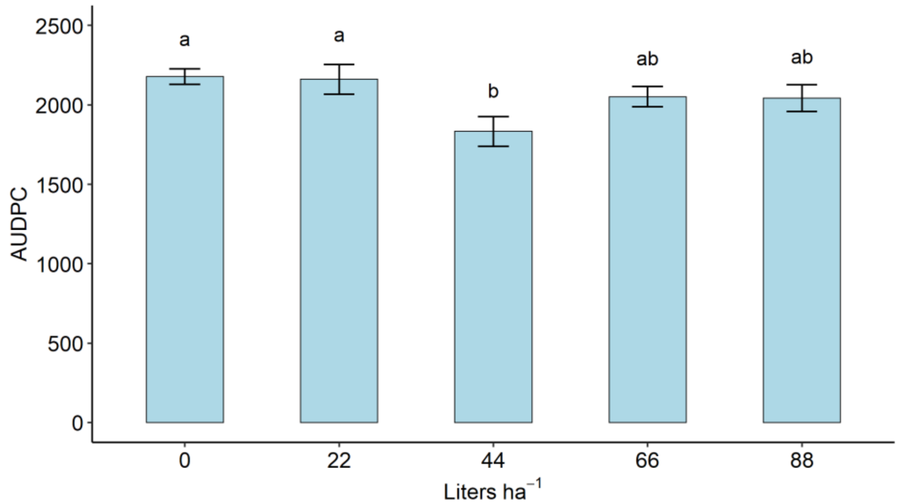

Figure 5. Area under the downy mildew progress curve on leaves of cv. 'BRS Magna' grown on espaliers. Plants were sprayed with different volumes of mixture by a helicopter sprayer from October 5, 2020, to February 9, 2021. Means followed by different letters are significantly different according to Tukey's test (p ≤ 0.05).

In the control of fungal diseases with systemic chemicals, the droplet spectrum should be between 200 and 300 μm (Antuniassi & Boller, 2011). Another factor associated with the success of the application is the characteristics of the crop canopy, which interfere with droplet coverage and penetration. Leaf mass can be a problem mainly if the target is not located in the upper part of the canopy (Cunha, Juliatti, & Reis, 2014). In the case of this experiment, in which a systemic fungicide was used, the VMD50% reached a spectrum of droplets within the recommended range for this class of fungicide.

Characteristics of the espalier system include vertical training and increased canopy density in the planting line. These attributes limit pesticide penetration, especially in the lower stratum of plants (Hernandes, Júnior, & Moura, 2021), when spraying is done over the canopy. In general, it is common for the lower plant parts to have less coverage because droplet penetration into the leaf mass is difficult. Canopy characteristics, such as height, leaf density and fruit bearing, can be obstacles to pesticide droplet penetration (Scudeler, Raetano, Araujo, & Bauer, 2004; Wolf & Daggupati, 2009). Consequently, this part of the plant may have less disease control. This result reinforces concerns about low volumes in the application of pesticides with RPA sprayers in viticulture. In this study, the assumption that, at low spray volume, a finer droplet is needed to succeed in penetrating the plant canopy and reaching the lower parts is clearly demonstrated with the success in controlling downy mildew of the treatment with a spray volume of 44 L ha^{-1}. Thus, it is necessary to investigate different spray nozzles and different working pressures to find the means of penetration of the mixture into the espalier canopy.

Different models of RPA sprayers may have different effects regarding their operation, which may influence the droplet size, coverage rate and spray rate when changing the operating speed (Martin et al., 2019).

Experiment II

The first WSP assessment detected a significant interaction between factors in percent coverage and droplet deposition. In the lower plant stratum, the application of 800 L ha^{-1} by the backpack sprayer provided the highest percent coverage and droplet deposition, and a similar result was observed in the middle plant stratum. However, differences in coverage regarding the three applied volumes were not found in the upper stratum. Overall, evaluating the strata, the greatest coverage and droplet deposition (cm^{-2}) were recorded in the upper stratum, except for the backpack application (800 L ha^{-1}), which showed no differences in percent coverage (Figure 6A and B). The backpack spraying application was performed laterally, whereas RPA spraying was done over the canopy.

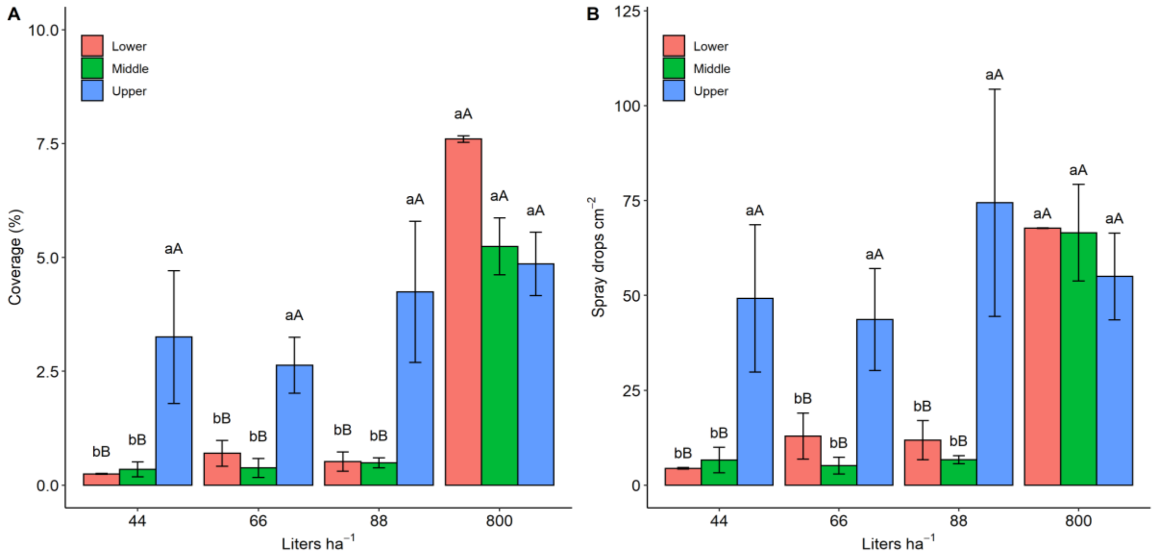

Figure 6. Percent target coverage (%) and droplet deposition (droplets cm^{-2}) in the first evaluation with different spray volumes using an RPA sprayer (AGRAS MG-1S) and a backpack sprayer on vine cv. 'BRS Magna' grown on espaliers. Means followed by different small letters between mixture volumes and different capital letters between strata are significantly different according to Tukey's test ($p \leq 0.05$).

No significant differences were found for VMD50% between the strata evaluated. Thus, the evaluation was carried out only between volumes. The two sprayers produced droplets with an average diameter of 212 – 285 µm, with the largest produced by the backpack sprayer (Figure 7).

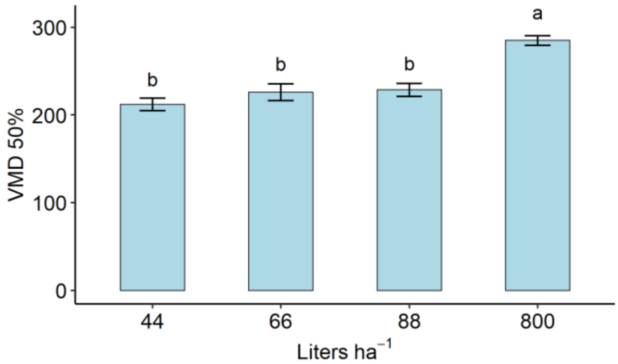

Figure 7. Volume median diameter (VMD%) in the first evaluation with different spray volumes using an RPA sprayer (AGRAS MG-1S) and a backpack sprayer (800 L ha^{-1}) on vine cv. 'BRS Magna' grown on espaliers. Means followed by different letters are significantly different by the Tukey test ($p \leq 0.05$). Each spray volume represents the average across strata.

In the second assessment, an interaction was detected between the mixture volume and stratum for the variables percent coverage and droplet deposition. The percent coverage was not different in the upper and lower strata among the spray volumes. However, in the middle stratum, the volume of 44 L ha^{-1} provided the greatest coverage compared to the other volumes (66 and 88 L ha^{-1}) (Figure 8). Furthermore, at a volume of 44 L ha^{-1}, coverage was not significantly different between the upper and middle strata, whereas the other volumes provided the greatest coverage in the upper stratum.

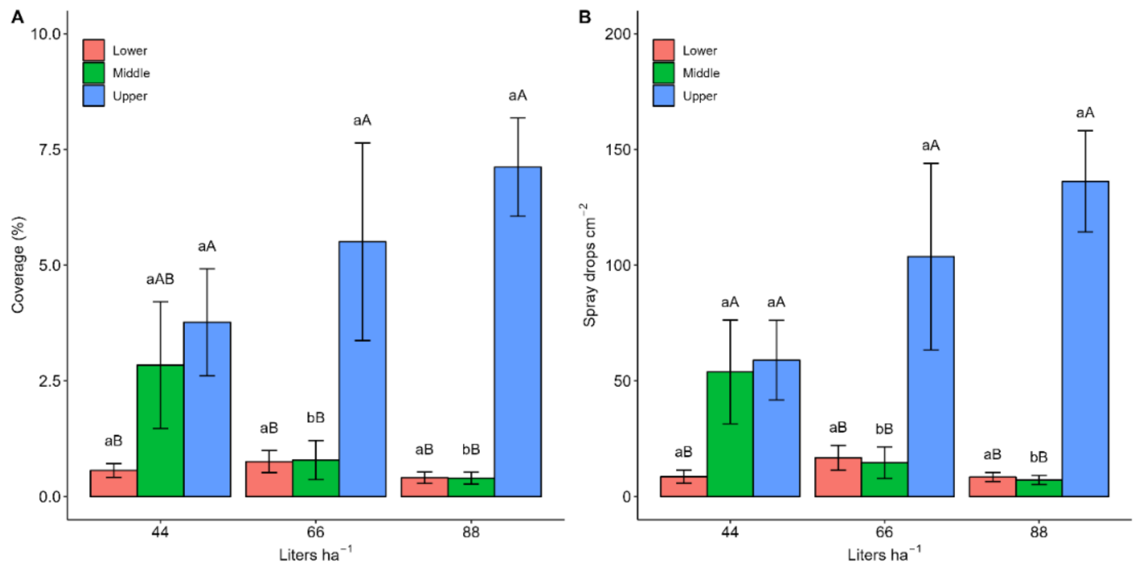

Figure 8. Percent target coverage (%) and droplet deposition (droplets cm^{-2}) in the second evaluation with different spray volumes and strata, using an RPA sprayer (AGRAS MG-1S) in vines grown on espaliers. Means followed by different small letters between mixture volumes and different capital letters between strata are significantly different according to Tukey's test ($p \leqslant 0.05$).

No significant differences were found between the spray volumes for drop deposition in the lower and upper strata. However, in the middle stratum, the application of 44 L ha^{-1} provided the greatest droplet deposition. Furthermore, no significant difference was observed between the upper and middle strata when 44 L ha^{-1} was applied, but for the other volumes, the upper stratum showed the greatest droplet deposition in relation to the middle and lower strata (Figure 8).

The volume median diameter (VMD%) showed a similar behaviour, with the largest VMD occurring in the upper stratum and the smallest in the lower stratum, while the middle stratum was not different from the lower and upper strata (Figure 9).

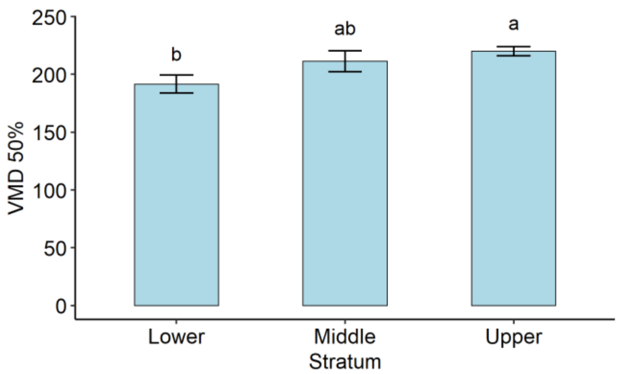

Figure 9. Volume median diameter (VMD%) in the second evaluation in different strata using an RPA sprayer (AGRAS MG-1S) on vine cv. 'BRS Magna' grown on espaliers. Means followed by different letters are significantly different by the Tukey test ($p \leqslant 0.05$). Each stratum represents the average across volumes.

As in the previous experiment, the climatic conditions of the 2021/2022 season did not favour disease occurrence (Figure 4), with the first symptoms observed after the harvest of the fruit clusters. The AUDPC showed less severity in plants treated with a backpack sprayer and the ARP sprayer with a volume of 44 L ha^{-1}, with the greatest control of the disease in relation to the other applied volumes (Figure 10). Lower volumes of fungicidal spray have been shown to be efficient in controlling downy mildew and, consequently, pose less risk to the environment (Bajagic, Sedlar, Latinović, Višacki, & Latinović, 2022; Fachieri, 2013; Michael, Gil, Gallart, Kanetis, & Stavrinides, 2022).

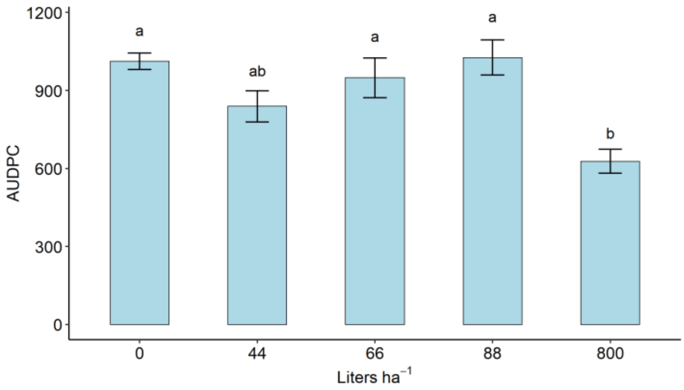

Figure 10. Area under the downy mildew progress curve on leaves of cv. 'BRS Magna' grown on espaliers. Plants were sprayed with different volumes of mixture using an RPA sprayer (AGRAS MG-1S) and a backpack sprayer. Means followed by different letters are significantly different by the Tukey test ($p \leq 0.05$).

Studies on the percent coverage of spraying with an RPA report that the greater the volume of spray applied, the higher the percent coverage (Ahmad et al., 2020; Shan et al., 2021; Wang et al., 2020). Compared with RPA spraying, backpack spraying showed greater coverage and droplet deposition in the upper and middle strata (Wang et al., 2019). This result corroborates the findings of the present study. In addition, the combination of the altitude of the application and the flight speed also influences target coverage. Neto, Sasaki, and Alvarenga (2021) reported that when using a pesticide applied with an RPA at three flight altitudes at an operating speed of 20 km h^{-1}, the highest percent coverage was 3.6% at a flight height of 1 m.

Droplet deposition presented an effect similar to coverage, in which the increase in the spray volume provided a greater number of droplets per area. The spray rate has been proven to influence droplet deposition, since the higher the spray rate, the greater the droplet deposition (Kharin, Wayayok, Shariff, Abdullah, & Husin, 2019). This result is similar to that reported by Shan et al. (2021), who suggested that droplets were more prone to overlap in applications with larger volumes.

Conclusion

In this study, the efficiency of RPA application of a fungicide mixture with different spray volumes was tested to control downy mildew on grapevine. Considering the experimental conditions of the study and the RPA systems used, the spray volume of 44 L ha^{-1} was efficient for controlling downy mildew in 'BRS Magna' grapevines grown on espaliers. The droplet deposition in the lower stratum of the canopy from the application of the RPA sprayers needs to be improved, especially when operating with a low spray volume and over vines with an espalier system, mainly when the targets are the clusters.

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