Spray Deposition and Control of *Botrytis cinerea* on Grape Leaves and Bunches: Part 2 (Wine Grapes)

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Poor control of fruit and foliar diseases in vineyards is often attributed to insufficient spray deposition of susceptible tissue. To optimise spray deposition, a deposition assessment protocol using fluorometry, photomicrography and digital image analyses was developed to determine minimum spray deposition quantity and quality levels needed for effective *B. cinerea* control in wine grapes (Chenin blanc). Leaves and bunches were sprayed at different growth stages with different volumes of a mixture of fenhexamid and fluorescent pigment. Pigment deposition quantity and quality were determined from photos of pedicels and leaves taken with a digital camera under a stereo microscope and black light illumination at ×30 and ×10 magnification, respectively. After inoculation with dry airborne conidia of *B. cinerea* infection levels on pedicels, receptacles and leaves were determined and infection levels and deposition data were subjected to sigmoidal and Hoerl regression analyses, respectively. From these biological efficacy curves the deposition levels that affected 75% control of *B. cinerea* infection (FPC_{75} values) were calculated for leaves and for each growth stage for pedicels and receptacles. Deposition measurements on sprayed leaves and bunch parts correlated favourably with *Botrytis* infection levels. An increase in spray volume resulted in higher deposition quantity and improved quality values with a reduction of *B. cinerea* infections. However, at a certain point, deposition quality remained constant and infection levels did not decrease significantly with increasing spray volume. Susceptibility of pedicels and receptacles to *B. cinerea* decreased with maturity. FPC_{75} values can be used as benchmarks to evaluate spray application in wine grape vineyards.

INTRODUCTION

Botrytis cinerea Pers.:Fr causes grey mould (Nair & Hill, 1992) on grapevines (*Vitis vinifera* L.) in all vineyards of the world and can severely reduce crop quality and yield. In wine grape production, juice from *Botrytis* infected grapes is darker, with higher volatile acids, pectin and mucins and bitterer than the must from healthy grapes (Bulit & Dubos, 1994). Low amino nitrogen and high sugar levels can lead to slow fermentation (Somers, 1984). Wines from *B. cinerea* infected grapes have off-flavours and are sensitive to oxidation and secondary contamination by bacteria making the wine unsuitable for aging (Bulit & Dubos, 1994).

Substantial research was conducted on aspects of *B. cinerea* in South African vineyards (Coertze *et al.,* 2001; Van Rooi, 2001; Coertze & Holz, 2002; Holz *et al.,* 2003; Van Schoor, 2004). Collectively, these studies found that *B. cinerea* was most frequently found in the air and on/in plant parts during the pre-bloom until bunch closure stage. Various studies (Coertze *et al.,* 2001; Coertze & Holz 2002; Holz *et al.,* 2003) have found that *B. cinerea* symptom expression was predominantly associated with the bases of the berry and the pedicel. The next prominent positions occupied were rachises and laterals and not the berry cheek. Young grape leaves were highly susceptible and were infected especially at the leaf base, which often remained asymptomatic (Holz *et al.,* 2003). As the leaves matured, they got increasingly resistant to infection due to a thicker cuticle layer and the presence of inhibitory compounds (Langcake & Pryce, 1976).

The control of *B. cinerea* infection by chemical, cultural and biological means can only be achieved by reducing inoculum on susceptible plant parts at the appropriate growth stage when propagules are present (Van Rooi & Holz, 2003). The control of plant diseases with a fungicide depends on delivering the chemical to the site of infection (*i.e.* the structural bunch parts and leaves), at the appropriate time and at a cost that is reasonable to the economics of the crop being

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produced (Brandes, 1971). Therefore, the main objective is not in spraying a crop *per se*, but rather in effectively covering established or potentially infected targets (Fulton, 1965). Knowledge of the duration and dynamics of fruit susceptibility to infection is critical for intelligent use of fungicides in managing grape diseases (Weed, 1903).

Identification of target sites naturally revolves around the susceptibility of various plant parts at different phenological stages. *B. cinerea* (Pearson & Goheen, 1988; Holz *et al*., 2003), powdery mildew (*Erysiphe necator* [Schw.] Burr.) (Chellemi & Marois, 1992; Delp, 1954; Gadoury *et al*., 2003) and downy mildew (*Plasmopara viticola* [Berk. & Curt.] Berl. & De Toni) (Kennelly *et al*., 2005) are linked to key phenological stages during which susceptibility to infection is maximal. As for *Botrytis*, structural bunch parts also appear to be essential in the disease expression of powdery mildew (Gadoury *et al*., 2001; Gadoury *et al*., 2003) and downy mildew (Kennelly *et al*., 2005). This is also true for other bunch rot fungi, such as *Penicillium*, *Aspergillus*, *Alternaria*, *Mucor* and *Rhizopus* spp. (Hewitt, 1974; Holz *et al.,* 2003). It is therefore essential that spray assessments should be made directly from host tissue and specifically from the susceptible plant parts. This is also important in choosing a suitable spray assessment protocol.

Artificial targets, such as water-sensitive papers, can be used for spray assessment, but is not ideal as it does not have the same orientation and surface properties of a 3-dimensional natural target site (Holownicki *et al*., 2002), such as structural grape bunch parts target. Fluorescent tracers and residue recovery techniques (Cunningham & Harden, 1999; Gil *et al.*, 2007; ISO, 2007; Fourie *et al.*, 2009; van Zyl *et al.*, 2010ab; Pergher *et al.*, 2013; van Zyl *et al.*, 2013, 2014) are more suitable, but do not always give a good indication of application quality such as uniformity or spray distribution on the leaves and bunch parts. Visual assessment of fluorescent pigment deposition gives an indication of the quality of the application, but the human eye lacks deposition quantity measuring and speed of measurement (Derksen & Jiang, 1995). At the time of this study, there was no appropriate spray assessment protocol that could be used to assess quantity and quality of spray deposition on a susceptible bunch part on a 3-dimensional microscopic level. Measurement of deposition quality is an important aspect as "a high-level deposit badly distributed is less efficient than a low-level deposit well distributed" (Frick, 1970). A good spray assessment protocol should therefore consist of spray deposition quantity and quality measurements. The importance of including a deposition quality assessment when research is conducted to improve contact fungicide disease control has also been shown by Van Zyl *et al.* (2010a, 2010b), Chaim *et al*. (2003) and in Brink *et al*. (2016). Brink *et al*. (2004) developed a spray assessment protocol that gives an indication of quantity of spray deposition in grape pedicels using fluorescence microscopy and digital image analyses. This protocol was improved in Brink *et al*. (2016) to also include deposition quantity and quality assessments from grapevine leaves. These were used to determine benchmark values for effective control of *B. cinerea* infections on Waltham Cross table grape bunch and leaf tissue (Brink *et al*., 2016). Van Zyl *et al*., (2010a, 2010b), Chaim *et al*. (2003) and Brink *et al*. (2016) indicated that there are optimal deposition levels for disease control on grapevine leaves, and increasing the spray volume and deposition quantity or quality levels past this point might not significantly improve disease control. Similar to those developed by van Zyl *et al.* (2013) for Alternaria brown spot control on mandarin citrus leaves, spray deposition benchmark values were determined for table grapes (Waltham Cross, Brink *et al*., 2016) that could be used to optimise spray application studies in table grape vineyards. However, due to the differences in trellis and canopy management, benchmark values are also needed specifically for wine grapes.

The aim of this study was therefore to use the spray assessment protocol (Brink *et al*., 2004, 2006, 2016) to enable spray deposition quantity and quality measurements on wine grape bunches and leaves, and to determine the minimum effective spray deposition levels needed for effective *B. cinerea* control on susceptible Chenin blanc grapevine tissue.

MATERIALS AND METHODS

Spray application

Grape bunches (2004-2005) and leaves (2005-2006) were selected from the wine grape cultivar Chenin blanc in two vineyards in the Paarl region (Western Cape province, South Africa). Grape bunches were selected at berry set, pea-size, bunch closure and pre-harvest, while the 4th or 5th leaves from the apical ends of green shoots were selected between pea-size and bunch closure. Bunches were cut into a 2-dimensional shape to minimise variation in conidium and spray deposition, as recommended by Brink *et al.* (2006). Bunches and leaves were sprayed with six different application volumes ranging between 1-11 mL and 0.25-7 mL, for bunches and leaves respectively. Control treatments were left unsprayed. Sprays consisted of a mixture of fenhexamid (Teldor® 500 SC, Bayer Cropscience, Isando, South Africa) at the recommended concentration (75 mL/100 L) (Nel *et al.,* 2003) and Yellow Fluorescent Pigment® (400 g/L, EC) (South Australian Research and Development Institute, Loxton SA 5333 Australia) at 200 mL/100 L (Furness, 2000). Brink *et* al. (2005) indicated that growth of *B. cinerea* was not affected by the fluorescent pigment. Sprays were applied by means of a gravity feed mist spray gun (ITW DEVILBISS Spray Equipment Products, 195 Internationale Blvd, Glendale Heights IL 60139 USA) with a fluid nozzle tip of 1.5 mm in diameter in a spray chamber, which consisted of a steel framework (800 × 1410 × 660 mm; L × H × W). Application was conducted at 75 kPa at a spray angle of 45° and 1.4 m from the plant part. For each treatment replicate and assessment purpose, one detached leaf or bunch were positioned horizontally on a mesh tray with the upper or lower leaf surface facing upward. Bunches were sprayed on both sides, whereas leaves were sprayed on one side only.

Image capturing and analysis

From each sprayed bunch, three pedicel samples were taken for spray efficiency assessment. Sprayed pedicels from bunches and leaves were illuminated under black light [six BLB T5/6W fluorescent tubes (Lohuis, Kruisweg

18, Netherlands)], as described in Brink *et al.* (2004). The fluorescent tubes were installed in a custom-made hexagonal illumination box that fitted closely around the P-Plan 1X lens (×10.0-63.0 magnification) of a 800 stereoscopic zoom microscope (Nikon SMZ, www.nikon.com). Images were captured with a digital camera (Nikon DMX 1200, www.nikon.com) through a stereoscopic microscope at ×30 (pedicels) and ×10 (leaves) magnifications. Image analysis was conducted by means of Image-Pro Plus version 6.2 software for Windows (Media Cybernetics, www.mediacy.com). Two images were taken in the middle part of each leaf from the sprayed sides of leaves, one on the L1 (closer to the main vein) and the other on the L2 (closer to the leaf margin) sinus lobes (Guisard & Birch, 2005). The other half of the leaf was used for *B. cinerea* infection assessment. Images could be analysed for deposition quantity and quality by using the measurement tool in Image-Pro Plus.

Deposition quantity analysis involved removal of green channels from the original colour image. A threshold was done to binarise the image into foreground objects (*i.e.* fluorescent particles) and background (Brink *et al*., 2016). An area of interest (AOI) of 1/100 the size of the image was placed in the top left corner of the image and moved across the image. A count of foreground elements (deposited pigment) was done at each iteration and the total area quantified and expressed as percentage area of pigment particles in relation to the AOI. The median deposition quantity of 100 measurements was used for further analysis.

As demonstrated in Brink *et al.* (2016), an Euclidian distance map of the binarised image was created for deposition quality analysis of spray deposition on leaves. The max-white pixel indicated the point furthest removed from any foreground objects (*i.e.* pigment particles). A thinning filter was used to attain the skeleton of the background. Using the AND function between the distance map and the skeletonised image, a new distance skeleton image was created. By analysing a histogram of the grey-scale values of the distance skeleton, the statistics of the distances in pixels between foreground objects was expressed. The black (0 value) was ignored in the statistics. Higher values indicated larger distances between objects, while smaller values indicate smaller distances between objects, and therefore a better quality spray deposition.

Inoculation and incubation

A virulent isolate of *B. cinerea,* obtained from a naturally infected grape berry, was maintained on potato dextrose agar (PDA; Biolab, Midrand, South Africa) at 5°C. For the preparation of inoculum, the isolates were first grown on tomato (surface sterilised in 70% alcohol for 30 s) quarters. Grape medium (GM) was made up (1000 mL water, 1.95 g fructose, 0.25 g sucrose, 0.15 g malic acid, 5 g peptone, 5 g NaCl, 15 g bacteriological agar, 1.85 g glucose and 2 g yeast extract), after which conidiophores from the colonised fruit were transferred to the medium in Petri dishes and incubated at 22°C for 7 days. Dry conidia were harvested with a suction-type collector and stored at 5°C until use. Storage time did not affect germination of dry conidia (Spotts & Holz, 1996). Bunches and leaves were inoculated with 3 mg dry conidia, which was dispersed by air pressure into the top of an inoculation tower (Plexiglas, 3 × 1 × 1 m [height × depth × width]) according to the method of Salinas *et al*. (1989). The conidia were allowed 20 minutes to settle onto bunches and leaves that were positioned on two screens on the floor of the inoculation tower. By using this inoculation technique, approximately three conidia were evenly deposited as single cells on each mm^2 of plant surface (Coertze & Holz, 1999). Petri dishes with water agar (WA) were placed next to the bunches or leaves and percentage germination was determined 6 h post inoculation (100 conidia per Petri dish, two replicates). Following inoculation, the plant parts were placed on sterile epoxy-coated steel mesh screens (53 × 28 × 2 cm) in ethanol-disinfected perspex (Cape Plastics, Cape Town, South Africa) chambers lined with a sheet of chromatography paper with the base placed in water to establish high relative humidity (≥93% RH). The chambers were incubated for 24 h at 22°C. According to Gütschow (2001), sufficient germination, surface colonisation and

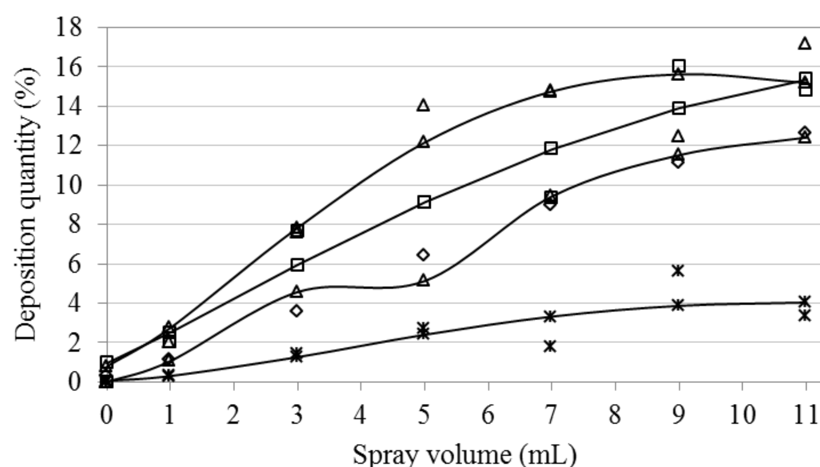

FIGURE 1
Mean deposition quantity (percentage area covered by fluorescent pigment) values on pedicel surfaces and Hoerl regression lines at berry set (□), pea size (Δ), bunch closure (◊) and pre-harvest (*) stages following spray application with SARDI Yellow Fluorescent Pigment and fenhexamid at volumes ranging from 1 to 11 mL.

penetration of grapevine leaves will occur within this period. These conditions are similar to what the pathogen commonly encounters on grape bunches or leaves in nature; namely, dry conidia on dry plant surfaces under high relative humidity (Gütschow, 2001). Non-inoculated bunches or leaves were used to determine the natural infection levels of *B. cinerea.*

Assessment of *B. cinerea*

Incidence of viable *B. cinerea* conidia, germlings and/or infections, occurring on leaves, receptacles and pedicels was determined by means of isolations onto paraquat medium as per methods described by Brink *et al.* (2006). Twenty receptacles and 20 pedicels per cluster and 25 leaf discs (5 mm in diameter) per leaf were isolated on Petri dishes containing water agar medium supplemented with paraquat (Grindat & Pezet, 1994). Paraquat terminates host resistance in the cells of the cuticular membrane without damaging host tissue (Grindat & Pezet, 1994), and allows the development of conidia and mycelia on the surface, as well as mycelia in the tissue in the absence of active defence (Coertze & Holz, 1999; Coertze *et al.*, 2001). The plates were incubated at 22°C under diurnal light and the sections were monitored daily for symptom expression and the development of *B. cinerea*. After 11 days, *B. cinerea* typically sporulated on infected sections. The number of sections yielding sporulating *B. cinerea* colonies were recorded, and used to determine the percentage incidence of *B. cinerea*.

Minimum spray deposition levels for effective *B. cinerea* control

For upper and lower leaf surfaces, and each bunch part and stage (berry set, pea-size, bunch closure and pre-harvest) combination, regression analyses were conducted for volume *vs.* spray deposition and spray deposition *vs.* percentage *B. cinerea* incidence. The fluorescent pigment deposition needed for 75% control of *B. cinerea* infection (FPC_{75} values) on bunch parts and each leaf side was subsequently calculated for each stage (berry set, pea-size, bunch closure and pre-harvest).

Experimental design and statistical analyses

Median values of deposition quantity (percentage area covered by fluorescent pigment) and deposition quality (grey-scale values of skeleton of Euclidian map of binarised images) as well as infection data, were subjected to the appropriate analysis of variance using SAS v. 8.2 statistical software (SAS Institute, 1999). Student's t-Least Significance Difference was calculated at the 5% significance level to compare treatment means of significant effects (Snedecor and Cochran, 1967). Hoerl regression analyses of deposition quantity ($y = \mathrm{A}x^{\mathrm{B}}e^{\mathrm{C}x}$) and deposition quality [$y = \mathrm{A}(x+1)^{\mathrm{B}}e^{\mathrm{C}(x+1)}$] and sigmoidal regression analyses [$y = \mathrm{A}+\mathrm{B}/(1+e^{(-1(x-\mathrm{C})/\mathrm{D})})$] of infection data related to spray volumes, were done to demonstrate treatment effects and trends (Daniel & Wood, 1971). Pearson's correlation analysis was also conducted to compare mean infection values for pedicels and receptacles with deposition data.

RESULTS

Spray deposition on bunches and leaves

Bunches

Analysis of variance of effects of spray volume on deposition quantity values on pedicels showed a significant 2-factor (volume × stage) interaction ($P < 0.0001$; Table 1). Hoerl regression analyses for deposition quantity on pedicels for this interaction yielded good fits for berry set, pea size and bunch closure (R^2 values of 0.860, 0.861 and 0.958, respectively), but poorer at pre-harvest ($R^2 = 0.450$; Table 2; Fig. 1). There was generally an increase in deposition as spray volume increased for all growth stages. At pea size, the amount of deposition on pedicels decreased with further increase in spray volume probably due to the effects of run-off. However, mean deposition quantity values over all stages were not significantly different following spray volumes between 9 (11.42%) and 11 mL (11.94%; as determined by Student's T-tests). Deposition tended to be higher in the younger growth stages (berry set and pea size) compared with older growth stages (bunch closure and pre-harvest).

TABLE 1

Analyses of variance for effects of spray volume and phenological growth stage on deposition quantity data on pedicels, and infection data on pedicels and receptacles of Chenin blanc grapevine bunch parts following spray application with a spray mixture of SARDI Yellow Fluorescent Pigment and fenhexamid.

Source	Deposition quantity			Pedicel infection			Receptacle infection		
	DF*	MS**	P***	DF*	MS**	P***	DF*	MS**	P***
Model	63	150.609	<0.0001	63	108.676	<0.0001	63	212.005	<0.0001
Volume	6	871.182	<0.0001	6	732.284	<0.0001	6	916.964	<0.0001
Stage (Rep)	36	20.444	0.4538	36	38.492	0.0152	36	150.386	<0.0001
Stage	3	761.881	<0.0001	3	286.904	<0.0001	3	347.470	<0.0001
Volume*Stage	18	68.868	<0.0001	18	11.470	0.9588	18	77.678	0.0291
Error	216	20.158					216	43.650	
Corrected Total									

*DF = Degrees of freedom

**MS = Means Square

***P = Probability

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TABLE 2
Coefficients for Hoerl regression analyses ($y = Ax^Be^{Cx}$) of deposition quantity (percentage area covered by fluorescent pigment) values following spray application with a mixture of SARDI Yellow Fluorescent Pigment and fenhexamid to pedicels of Chenin blanc bunches at volumes ranging from 1 to 11 mL.

Deposition analysis	Variables			
	A ± SE	B ± SE	C ± SE	R^2-value
Berry set	1.064 ± 0.946	1.483 ± 0.875	0.085 ± 0.118	0.860
Pea size	0.930 ± 0.887	2.100 ± 0.982	0.205 ± 0.123	0.861
Bunch closure	0.363 ± 0.289	1.997 ± 0.735	0.117 ± 0.091	0.958
Pre-harvest	0.076 ± 0.278	2.676 ± 3.370	0.223 ± 0.416	0.450

Leaves

Deposition quantity

Analysis of variance of the deposition quantity values on leaves indicated significant leaf side × volume interaction ($P < 0.0001$, Table 3). Hoerl regression analyses for deposition quantity values on upper and lower leaf surfaces (R^2 values of 0.990 and 0.992, respectively) yielded very good fits (Table 4; Fig. 2). Deposition values increased as spray volume was increased on both leaf sides (Fig. 2). Upper and lower leaf surfaces recorded similar deposition values for each spray volume. However, deposition quantity was generally better on upper than on lower leaf surfaces at low spray volumes and *vice versa* at the higher spray volumes (5 and 7 mL; Fig. 2).

Deposition quality

Analysis of variance of deposition quality values on leaves (Table 3) indicated significant effects for spray volume ($P < 0.0001$), but not for leaf side ($P = 0.9521$). Deposition quality improved with the increase of spray volume and yielded very good fits with Hoerl regression for both upper ($R^2 = 0.977$) and lower leaf surfaces ($R^2 = 0.990$; Table 4; Fig. 2). Other than deposition quantity which increased significantly with increase of spray volume. Optimal deposition quality was reached at 3 mL for both leaf surfaces and did not improve significantly afterwards.

Minimum spray deposition levels for effective *B. cinerea* control

Germination of the inoculated *B. cinerea* conidia on water agar plates was between 92% and 98%. Incidence of natural *B. cinerea* infection on bunch parts was very low (< 1%) and the data was not considered in further analyses.

Bunches

Analyses of variance indicated that spray volume ($P < 0.0001$) and growth stage ($P < 0.0001$) had a significant effect on *B. cinerea* infection levels on pedicels (Table 1). Sigmoidal regression analyses for *B. cinerea* infection levels on pedicels over deposition quantity yielded very good fits (R^2 values of 0.966 – 0.978; Table 5 and Fig. 3). *B. cinerea* infection levels on pedicels decreased with the increase in spray deposition quantity on all growth stages (Fig. 3). In general, FPC_{75} values decreased with increased maturity; from 8.9% at berry set to 1.0% at pre-harvest. However, the highest FPC_{75} value was predicted at pea size (13.2%, Table 5).

Analyses of variance for effects of spray volume on infection values on receptacles showed significant 2-factor (stage × volume) interactions ($P = 0.0291$; Table 1). Sigmoidal regression analyses for *B. cinerea* infection levels on receptacles over deposition quantity yielded very good fits (R^2 values of 0.850 – 0.982; Table 6 and Fig. 4). As with pedicels, *B. cinerea* infection levels on receptacles decreased with increase in spray deposition quantity, showing similar trends. However, for receptacle infection, a clear upper asymptote was observed for berry set and bunch closure stages. At these stages, higher *B. cinerea* infections were recorded at lower deposition values (1 - 6%) compared to pea size and particularly pre-harvest. No FPC_{75} values could be calculated from the receptacle treatments, as deposition values could not effect 75% control of infections, therefore FPC_{50} values were calculated. These values showed the same trend as on pedicels, where the FPC_{50} value also decreased from berry set to pre-harvest (8.2% and 0.4%, respectively; Table 6).

Leaves

The analysis of variance for the *B. cinerea* infection data on sprayed leaves indicated a significant spray volume × leaf side interaction ($P = 0.0325$; Table 3). The highest infection levels were obtained between 0 to 0.5 mL (36.0% – 48.75%) on the lower leaf surfaces. The lowest infection levels were observed on leaves sprayed between 1 and 7 mL (0.5 – 0.75%) and no significant difference was observed in infection levels following sprays with 1 – 7 mL (as per Student's T test; results not shown). Sigmoidal regression analyses of infection values over deposition quantity and quality values indicated fairly good regression fits on upper leaves (R^2 values of 0.652 and 0.696, respectively), and good fits for deposition quantity on lower leaf surfaces ($R^2 = 0.810$), but a poor fit for deposition quality analysis ($R^2 = 0.121$) on the lower leaf surfaces (Table 7; Fig. 5). FPC_{75} benchmarks for deposition quantity and quality levels were calculated at 0.58% and 160, respectively on upper leaf surfaces (approx. at 0.5 mL spray volume) and 0.98% and 164.76, on lower leaf surfaces (approx. at 0.25 mL spray volume). The deposition quality benchmark values for upper and lower surfaces of leaves were therefore comparable, but the deposition quantity benchmark value (FPC_{75}) on lower

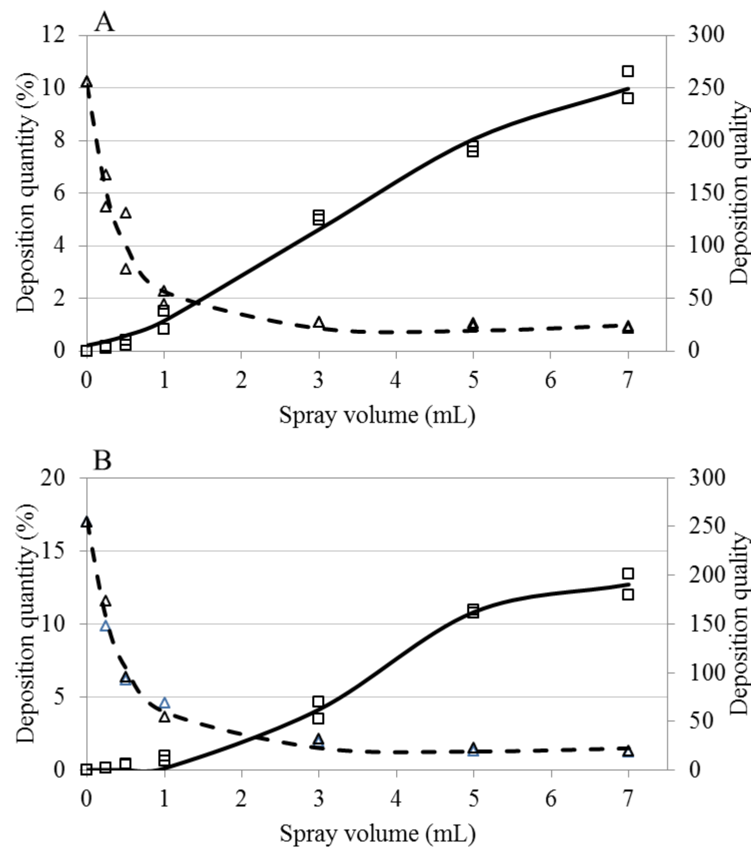

FIGURE 2
Mean deposition quantity (percentage area covered by fluorescent pigment; □) and quality (grey-scale values of skeleton of Euclidian map of binarised images; Δ) deposition values and respective Hoerl regression lines (—, - -) on upper (A) and lower (B) leaf surfaces.

TABLE 3
Analyses of variance for effects of spray volume on deposition quantity and quality data and *B. cinerea* infection data on lower and upper Chenin blanc leaves following spray application with a spray mixture of an aqueous fluorescent pigment and fungicide.

	Deposition quantity			Deposition quality			Leaf infection		
Source	**DF***	**MS****	**P*****	**DF***	**MS****	**P*****	**DF***	**MS****	**P*****
Model	27	209.243	<0.0001	27	69351.548	<0.0001	27	2887.655	<0.0001
Volume	6	907.182	<0.0001	6	307437.88	<0.0001	6	10513.929	<0.0001
Leaf side	1	27.858	0.0017	1	175.729	0.7549	1	5272.232	0.0008
Volume*Leaf side	6	25.409	<0.0001	6	570.241	0.9106	6	932.023	0.0325
Error	252	4.524		252	138.132		252	200.525	
Corrected Total	279			279			279		

*DF = Degrees of freedom
**MS = Means Square
***P = Probability

leaf surfaces was markedly higher compared to upper leaf surfaces (Table 7; Fig. 5).

DISCUSSION

In agreement with previous studies (Brink *et al*., 2004, 2006, 2016) using similar methodology, this study showed that an increase in spray volume resulted in increased deposition quantity and improved quality with a reduction of *B. cinerea* infections on pedicels, receptacles and grapevine leaves. Deposition measurements on sprayed leaves (deposition quantity and quality) and bunch parts (quantity) correlated favourably with *Botrytis* infection levels. Deposition quality on leaf surfaces improved with spray volume, but only to a certain point (approximately 3 mL spray volume), where after no further increase occurred. This correlates well with the 3 mL spray volume determined as the turn-

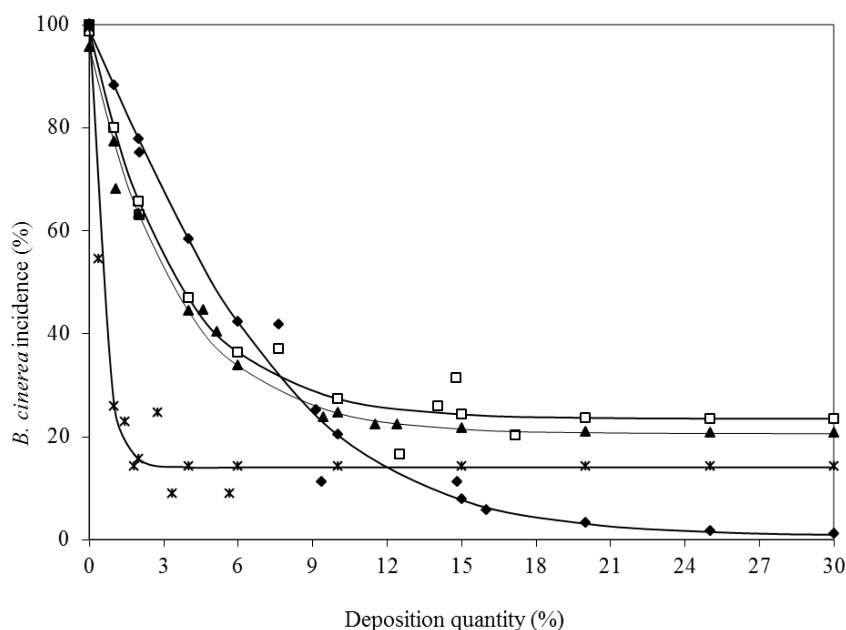

FIGURE 3
Mean percentage *B. cinerea* infection levels on pedicels of Chenin blanc bunches and respective Sigmoidal regression lines on deposition quantity (percentage area covered by fluorescent pigment) at berry set (◊), pea size (□), bunch closure (Δ) and pre-harvest (*) stages.

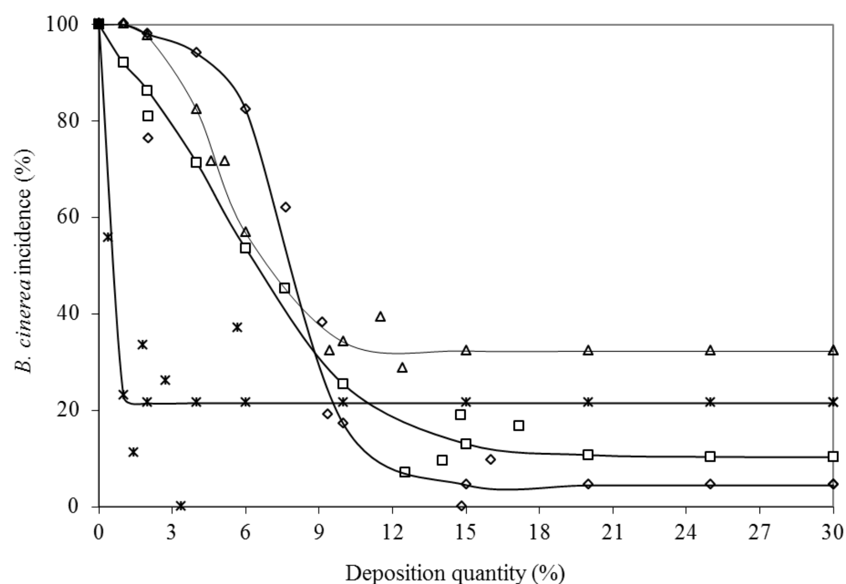

FIGURE 4
Mean percentage *B. cinerea* infection levels on receptacles of Chenin blanc bunches and respective Sigmoidal regression lines on deposition quantity (percentage area covered by fluorescent pigment) at berry set (◊), pea size (□), bunch closure (Δ) and pre-harvest (*) stages.

point for Waltham Cross table grapes (Brink *et al*., 2016) and 750 L/ha from backpack mist blower (Van Zyl *et al*., 2010a) using similar methodology. However, after this point, deposition quantity levels still kept on increasing significantly with spray volume, although infection levels did not decrease further. This was also observed for *B. cinerea* infection on Waltham Cross bunches and leaves (Brink *et al*., 2016) and supports work done on California red scale *Aonidiella aurantii* (Maskell) on citrus, which also indicated that greater coverage does not necessarily result in greater efficacy (Garcerá *et al*., 2011). Similar detrimental effects of runoff on spray deposition as well as control of Alternaria brown spot of mandarins was also demonstrated by Fourie *et al.* (2009). The importance of deposition quality was reported previously (Frick, 1970; Van Zyl *et al*., 2010a, 2010b; Brink *et al*., 2016).

Deposition values affecting 75% control (FPC_{75} values) could not be determined for receptacles as with pedicels,

TABLE 4
Coefficients for Hoerl regression analyses of deposition quantity (percentage area covered by fluorescent pigment; $y = Ax^{B}e^{Cx}$) and quality (grey-scale values of skeleton of Euclidian map of binarised images; $y = A(x+1)^{B}e^{C(x+1)}$) deposition values following spray application with a mixture of SARDI Yellow Fluorescent Pigment and fenhexamid to upper and lower leaves of Chenin blanc at volumes ranging from 0.25 to 7 mL.

	Variables			
Deposition analysis	**A ± SE**	**B ± SE**	**C ± SE**	**R^2-value**
Deposition quantity analysis				
Upper leaf surface	0.286 ± 0.084	2.903 ± 0.397	0.310 ± 0.071	0.990
Lower leaf surface	0.216 ± 0.093	4.572 ± 0.654	-0.689 ± 0.125	0.992
Deposition quality analysis				
Upper leaf surface	147.713 ± 16.892	-2.970 ± 0.309	-0.548 ± 0.122	0.977
Lower leaf surface	157.776 ± 12.051	-2.796 ± 0.198	-0.482 ± 0.081	0.990

TABLE 5
Coefficients for Sigmoidal regression analyses [$y = A+B/(1+e^{(-1(x-C)/D)})$] of *Botrytis cinerea* infection levels on pedicels against deposition quantity (percentage area covered by fluorescent pigment) values following spray application with a mixture of SARDI Yellow Fluorescent Pigment and fenhexamid to Chenin blanc grape bunches. Deposition values that would affect 75% control (FPC_{75} values) of *B. cinerea* were calculated for each stage.

Variables						
Phenological stage	**A ± SE**	**B ± SE**	**C ± SE**	**D ± SE**	**R^2-value**	**FPC_{75}* (%)**
Berry set	0.74 ± 18.05	195.92 ± 375.63	0.02 ± 15.74	-4.55 ± 4.83	0.967	8.9
Pea size	23.51 ± 6.53	1292.27 ± 37919.83	-9.21 ± 114.24	-3.31 ± 4.27	0.966	13.2
Bunch closure	20.62 ± 8.03	942.87 ± 16562.83	-8.12 ± 70.93	-3.31 ± 3.41	0.978	9.7
Pre-harvest	14.07 ± 4.91	1268.65 ± 29267.05	-1.29 ± 13.49	-0.49 ± 0.55	0.972	1.0

*FPC_{75} = Fluorescent pigment area that effected 75% control of B. cinerea infection

TABLE 6
Coefficients for Sigmoidal regression analyses [$y = A+B/(1+e^{(-1(x-C)/D)})$] of *Botrytis cinerea* infection levels on receptacles against deposition quantity values following spray application with a mixture of SARDI Yellow Fluorescent Pigment and fenhexamid to Chenin blanc grape bunches and the deposition values that would affect 50% control (FPC_{50} values).

	Variables					
Phenological stage	**A ± SE**	**B ± SE**	**C ± SE**	**D ± SE**	**R^2-value**	**FPC_{50}* (%)**
Berry set	4.44 ± 8.56	83.61 ± 12.15	8.41 ± 0.51	-0.92 ± 0.47	0.948	8.2
Pea size	10.27 ± 9.28	98.36 ± 44.47	5.34 ± 2.86	-2.72 ± 2.17	0.971	6.4
Bunch closure	32.26 ± 5.13	71.66 ± 11.69	5.12 ± 0.43	-1.34 ± 1.06	0.982	6.4
Pre-harvest	21.48 ± 9.07	80.48 ± 30920.03	0.34 ± 167.70	-0.09 ± 345.45	0.850	0.4

*FPC_{50} = Fluorescent pigment area that effected 50% control of B. cinerea infection

which is an indication that this part of the bunch is more susceptible and hence more difficult to control *B. cinerea* infections (Holz *et al.*, 2003; Viret *et al.*, 2004; Brink *et al.*, 2016). Deposition quantity measurements on pedicels tended to be higher in the earlier growth stages (berry set and pea size) compared with older growth stages (bunch closure and pre-harvest), which might also be due to the increasing roughness of these bunch parts with increasing maturity that influence image analysis (Brink *et al.*, 2006). Nonetheless, the highest FPC_{75} and FPC_{50} (respectively) values were predicted for earlier phenological stages and it decreased as the season progressed, which could possibly be attributed to the increase in host resistance (Gütschow, 2001; Holz *et al.*, 2003). This finding supports the importance of spray applications early on in the season (Van Rooi & Holz, 2003; Van Schoor, 2004). FPC_{75} values obtained for Chenin blanc pedicels were markedly higher compared to FPC_{75} values from Waltham Cross (Brink *et al.*, 2016), indicating that Chenin blanc bunch parts are more susceptible to *B. cinerea* infections. Therefore larger quantities of fungicide may be required for control. Chenin blanc is known to be particularly susceptible to infection by *B. cinerea* under field conditions due to its tight cluster architecture (Orffer, 1979; Vail & Marois, 1991). However, in this study the grapes

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were cut into two dimensional shapes, therefore cluster tightness could not have had an influence on the observed susceptibility.

Susceptibility of *Vitis* species to *B. cinerea* infections have been established based on the berry and/or leaf cuticle thickness (Bonnet, 1903; Krostanova *et al*., 1989; Mlikota Gabler *et al*., 2003). Direct penetration of the cuticle has been described (Coertze & Holz, 1999; Coertze *et al*.,

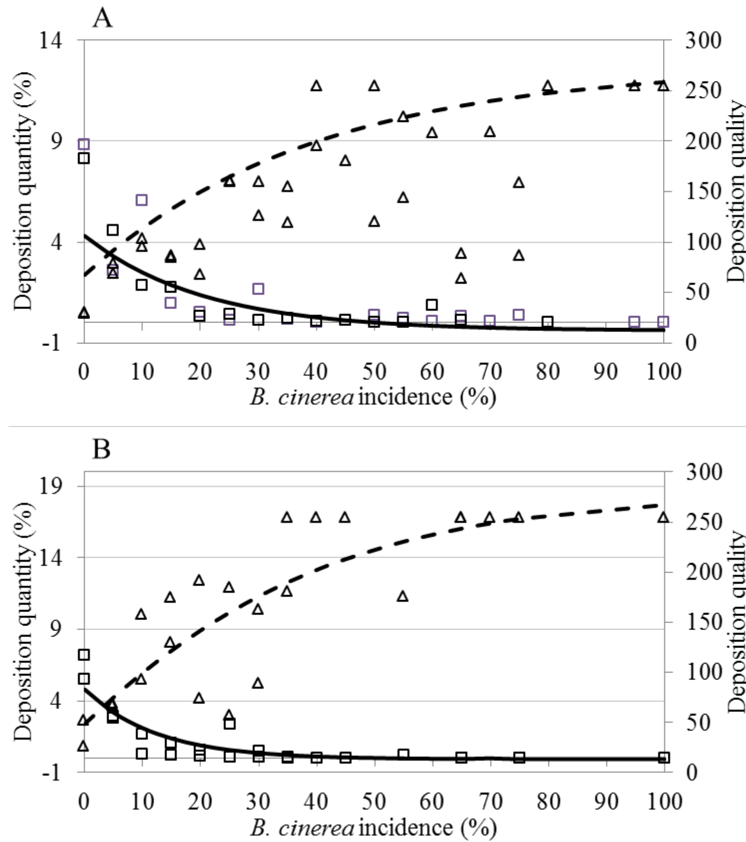

FIGURE 5

Mean percentage *B. cinerea* infection levels on lower (A) and upper (B) leaf surfaces of Waltham Cross leaves following spray application with SARDI Yellow Fluorescent Pigment and fenhexamid at volumes ranging from 0.25 to 7 mL and respective Sigmoidal regression lines (—, - -) on deposition quantity (percentage area covered by fluorescent pigment; □) and the quality (grey-scale values of skeleton of Euclidian map of binarised images; Δ) deposition values.

TABLE 7

Coefficients for Sigmoidal regression analyses [$y = A+B/(1+e^{(-1(x-C)/D)})$] of *Botrytis cinerea* infection levels on leaves against deposition quantity (percentage area covered by fluorescent pigment) and quality (grey-scale values of skeleton of Euclidian map of binarised images;) deposition values following spray application with a mixture of SARDI Yellow Fluorescent Pigment and fenhexamid to upper and lower leaves of Chenin blanc. Deposition values that would affect 75% control (FPC_{75} values) of *B. cinerea* were calculated for each stage.

Variables						
Deposition analysis	**A ± SE**	**B ± SE**	**C ± SE**	**D ± SE**	**R^2-value**	**FPC_{75}* (%)**
Deposition quantity analysis						
Upper leaf surface	768.45 ± 175785	-768.51 ± 175785	-62.197 ± 2887.4	12.30 ± 11.4990	0.652	0.58
Lower leaf surface	494.58 ± 130586	-494.98 ± 130587	-94.42 ± 5567.5	20.32 ± 35.4331	0.810	0.98
Deposition quality analysis						
Upper leaf surface	-536.45 ± 5123.6	814.76 ± 5196.0	-27.90 ± 301.1	29.84 ± 57.00	0.696	160.00
Lower leaf surface	-8046.33 ± 1415242	8320.87 ± 1415373	-141.71 ± 7246.6	38.63 ± 148.8	0.121	164.76

*FPC_{75} = Fluorescent pigment area that effected 75% control of *B. cinerea* infection

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2001; Nelson 1956). Marois *et al*. (1987), Percival *et al*. (1993) and Zoffoli *et al.* (2009) indicated that a reduction in development of the cuticular membrane and deposition of the epicuticular wax layers would lead to increased susceptibility to *B. cinerea* infection of berries. If presumed that there is a positive correlation between berry and pedicel cuticle thickness, Waltham Cross, being a hybrid species (Hyams, 1952) would have a thicker berry cuticle of between 4 to 10 µm compared to 1.5 to 3.8 µm of Chenin blanc (Karadimtcheva, 1981) being from the *Vitis vinifera* species (Maul & Eibach, 1999). Moreover, Waltham Cross would be exposed to more sunlight due to looser bunch architecture, stricter canopy management in table grapes and therewith reduced canopy density. Additional sunlight exposure might further increase resistance to *B. cinerea* by thickening of the epicuticular wax layers (Meadows, 2008; Sholberg *et al*., 2008).

In general, Chenin blanc leaf side did not influence spray deposition or deposition quality benchmarks, as was observed on Waltham Cross (Brink *et al*., 2016). However, deposition quantities needed to control 75% of infections on Chenin blanc were markedly higher on lower leaf surfaces, indicating that lower leaf surfaces may be more susceptible to *B. cinerea*. However, Waltham Cross (Brink *et al*., 2016) had similar FPC_{75} values for deposition quantity on upper and lower leaf surfaces. This observation was also made for Chardonnay leaves (Van Zyl *et al*., 2010a). The internal anatomy and surface features of leaves often determine plant resistance to pathogen infection (Smith *et al*., 1996). Among such characters, aspects of stomata, cuticle and trichome morphology can influence disease resistance (Niks & Rubiales, 2002). Chenin blanc is known for its low number of stomata with only 4 stomata per 100 sq µm compared to the 6 stomata per 100 sq µm of Chardonnay leaves (Manoj *et al*., 2007). In general, stomata number is independent of susceptibility to *B. cinerea* (Bernard and Dallas, 1981). Thickness of epidermal, hypodermal cell layers, cuticle and wax contents are positively correlated with resistance to *B. cinerea* in a wide range of table grape cultivars (Mlikota Gabler *et al*., 2003). Furthermore, following germination of conidia, direct penetration through the intact cuticle and cell wall, rather than via stomata, appears to be the main infection pathway of *B. cinerea* as shown on roses (Elad, 1989). The cuticle of upper leaf surfaces of both cultivars would be thicker than that of the lower leaf surfaces (Boso *et al*., 2010). However, this does not explain the difference in susceptibly of lower leaf surfaces between cultivars. A plausible reason could be higher density of trichomes on the lower leaf surfaces of Chenin blanc compared to that of Waltham Cross. A study by Calo *et al.* (2006) associated the presence of trichomes with enhanced susceptibility to *B. cinerea*, following more opportunities for attachment of the fungal spores to the leaf surface as a possible explanation. This complexity of factors that might influence FPC_{75} values therefore indicate that benchmarks should be obtained for each growth stage and cultivar independently.

CONCLUSION

This study contributes to the understanding of how spray volume influences spray deposition quantity and quality and its influence on disease control of *B. cinerea* on Chenin blanc grapevine leaves and bunches. Increased in spray volumes increased deposition quantity, quality and control of infection levels; however, at a certain point, deposition quality and infection levels did not improve even though deposition quantity was still increasing. Therefore, higher spray volumes may increase deposition quantity levels but might not significantly improve disease control. Certain spray quantity and quality parameters are based on droplet spectra measurements with water, but does not consider the consequent effect following impact and retention, as well as the process of initial deposit formation on natural plant targets such as the effects of sticking and spreading. However, deposition values obtained from this study take in consideration these spray parameters and furthermore demonstrate their effects on biological efficacy. Furthermore, spray volume had similar effect on spray deposition on wine grapes (Chenin blanc) compared to table grapes (Waltham Cross); however, the same spray deposition differs in reaction to infections between the two cultivars. This is an important observation as it is an indication grapevine cultivars have different FPC_{75} values. Therefore to optimize disease control via fungicides, grapevine cultivar cannot be ignored and that spray deposition level that might be sufficient for one cultivar may not be sufficient for another. As this study was done on single leaves and pedicels, the influence of spray volume on deposition throughout the canopy could not be considered. Assessment of spray deposition in vineyards would be considerably more complex than laboratory spray deposition on detached plant parts. This study does, however, indicate that the efficacy of agricultural chemicals is influenced by deposition quantity (amount of deposit), quality (spatial distribution of deposit) and cultivar. FPC_{75} values obtained from this study will be used as benchmarks to evaluate and optimise spray application in vineyards as well as the effect of different spray adjuvants, fungicides and spray parameters such as spray volume and dosage.

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