

# A Protocol To Quantify Spray Deposits In Grape Bunches

## Introduction

Various studies revealed that *Botrytis cinerea*, the causal pathogen of Botrytis bunch rot, is mostly associated with rachises, laterals, pedicels and berry bases, and not with berry skins as previously conceived (Holz et al., 2003). Provided that sufficient coverage of inner bunch parts was achieved, laboratory studies have shown that fungicides almost completely reduce the amount infection and symptom expression of *B. cinerea* at all growth stages. The same efficacy was, however, not achieved with the same fungicides when using conventional spraying methods in vineyards (Van Rooi & Holz, 2003).

Failure to control Botrytis and other fruit and foliar diseases in vineyards is often attributed to insufficient coverage of susceptible tissue. Research regarding spray application to ensure efficient spray coverage is therefore needed improve disease management of fruit and foliar diseases in vineyards. Previously, water-sensitive papers were used in spray application experiments in South Africa. However, to give a true indication of spray deposits and penetration on certain critical positions in grape bunches, cards need to be the same size and orientation as the target, and this method does therefore not give a good indication of the spray coverage on the 3-dimensional target sites in bunches (Holownicki et al., 2002). Furthermore, the target to which fungicides are applied changes constantly, because of the transformation of grape bunches. Residue recovery techniques were also used, but did not give a good indication of application quality such as uniformity or spray distribution (Holownicki et al., 2002). As part of a research programme aiming at optimising spray application in vineyards, the Department of Plant Pathology at the University of Stellenbosch developed a spray cover assessment protocol using fluorometry, photomicrography and digital image analyses to measure spray coverage on susceptible grape bunch parts (Brink et al., 2004). Spray Cover Assessment Protocol

Bunches are sprayed with a fungicide and Yellow Fluorescent Pigment® (400 g/L, EC) (South Australian Research and Development Institute) at 2L/100L mixture (Fig. 1) mixture at the recommended dose. Sprayed parts are illuminated under six black lights which are installed in a custom-made hexagonal illumination box (Fig. 2A) that fits closely around a Nikon SMZ 800 stereoscopic zoom microscope (Fig. 2B). Images are digitally captured through a stereoscopic microscope at 20 x magnification using a high-quality photomicrographic Nikon DXM 1200 digital camera (Fig. 2C). Image analysis and enhancements are done with Image-Pro Discovery version 4.5 for Windows (Media Cybernetics) software. In order to reduce background noise (Fig. 3A) and enhance fluorescent pigment, brightness, contrast and gamma settings are adjusted (Fig. 3B). The total areas of deposited pigment in selected areas of interest (AOI) are calculated (Fig. 3C) and the percentage area covered is subsequently calculated for each AOI.

Figure 1. Mixture of SARDI Yellow Fluorescent Pigment and the fungicide fenhexamid.

Figure 2. Bunch parts were illuminated in a

hexagonal box fitted with 6 black light tubes (A), visualised under a stereo microscope at 20x magnification (B) and photographed with a digital camera (Nikon DMX 1200) (C).

Figure 3. Image processing and analysis of a digital photo taken of a berry skin from a grape bunch that was sprayed with a mixture of fenhexamid and Yellow Fluorescent Pigment. (A) Selected objects are UV-illuminated and digitally photographed at 20x magnification, (B) subjected to several image contrasting and filtering processes, (C) an AOI selected and the total area of deposited pigment calculated for each AOI using Image-Pro Discovery image analysis software.

Figure 4. Gravity feed mist spray gun.

Figure 5. Mean fluorescent pigment coverage (% area) on berry skin, pedicel and rachis (bunch closure stage only) at pea size and bunch closure stages and linear regression lines fitted on spray volume for part x stage combinations.

#### Validation Of Spray Application Protocol

Dauphine grape bunches (sampled at pea-size and bunch closure) were sprayed with a mixture of fenhexamid (Teldor® 500 SC, Bayer) at the recommended dose (75 ml/100 l) and Yellow Fluorescent Pigment® at 2L/100L (Furness, 2000). Spray volumes ranging from 1 to 15 ml were applied by means of a gravity feed mist spray gun [Fig 4 (ITW DEVILBISS Spray Equipment Products)]. Fluorescent pigment coverage data for each spray volume and growth stage were subjected to the appropriate analysis of variance, linear regression analysis and variance component analysis using SAS v 8.2 statistical software. Statistical analyses clearly showed that the described protocol could be used to accurately determine coverage on the susceptible bunch parts in grape bunches. Fluorescent pigment coverage had a significant linear fit on spray volume (Fig. 5). An increase in spray volume generally led to an increase in coverage. Coverage was significantly influenced by growth stage and bunch parts. In general, pea size bunches had a higher mean

percentage area coverage on the different bunch parts than bunches sprayed at bunch closure. This can be explained by higher porosity of bunches at pea size compared with more compact bunches at bunch closure. Structural bunch parts were furthermore up to three times more difficult to cover than berry skins at both stages. Variance component analysis revealed that variation can be reduced by increasing the number of bunches, rather than the samples per bunch or measurements per image.

Conclusion Collectively, these results clearly showed that spray applications earlier in the season will result in higher and more effective spray deposition on the susceptible bunch parts. Disease management would thus be most effective since structural bunch parts are most susceptible and pathogen inoculum most abundant during pre-flower to pea-size stages in vineyards (Holz et al., 2003). The described protocol provides an essential tool that can be used to study the optimisation of spray application of agro-chemicals and/or biological control agents in vineyards. Hence, adequate deposition of active ingredient on the susceptible vegetative and reproductive parts of grapevines for effective pathogen or pest control can be facilitated. In future studies, minimum coverage levels for effective pathogen control will be determined and subsequently be used as benchmarks to evaluate spray application in vineyards. The technology developed in the *Botrytis*-grapevine model will directly benefit the management of other foliar and fruit diseases of grapevine, such as powdery and downy mildew as well as diseases or pests in other cropping systems.

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