THE USE OF ADJUVANTS TO IMPROVE FUNGICIDE SPRAY DEPOSITION ON GRAPEVINE FOLIAGE

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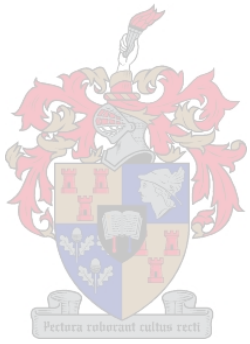

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SUMMARY

Sufficient fungicide deposition on the target site is an essential requirement for effective chemical management of fruit- and foliar diseases such as grey mould of grapevines. Control failure is often attributed to insufficient quantitative deposition on susceptible grapevine tissue. However, in high disease pressure situations control failure might also be attributed to poor qualitative deposition. The primary objective of spray technology is to optimise deposition, of which the plant surface is a critical component in the spray application process, specifically in the retention of spray droplets. Adjuvant technology is reported to improve the wettability and spread of droplets by surface-acting-agents on the target surface and thereby improve deposition and retention of the fungicide active ingredient. However, this relatively new spray technology on viticulture and horticultural crops, and possible effects of adjuvants on epicuticular wax affecting plant disease development, needs to be investigated. Moreover, the development of useful prescriptions for adjuvants by determining water volumes and adjuvant dosages is required for different pesticide tank mixes. The aims of this study were, firstly to determine the effect of selected adjuvants on quantitative and qualitative spray deposition on grapevine leaves and subsequent biological efficacy of a fungicide, and secondly to evaluate selected adjuvants under field conditions and determine the effects of adjuvant dosage and spray volume on deposition.

Leaves were sprayed under similar laboratory conditions to pre-run-off with 1 mL of a mixture of fenhexamid (Teldor® 500 SC, Bayer) at recommended dose, a fluorescent pigment (SARDI Fluorescent Pigment, 400 g/L EC; South Australian Research and Development Institute) at 0.2 L/100 L, as well as 15 selected commercial adjuvants to manipulate the deposition quality of a given quantity of deposited spray. Spray deposition on leaves was illuminated under black light (UV-A light in the 365 nm region) and visualised under a stereo microscope (Nikon SMZ800) at 10× magnification. Photos of sprayed leaf surfaces were taken with a digital camera (Nikon DMX 1200). Digital images were quantitatively and qualitatively analysed with Image-Pro Discovery version 6.2 for Windows (Media Cybernetics) software, to determine spray deposition. The sprayed leaves were inoculated with 5 mg dry airborne conidia of *Botrytis cinerea* in a spore settling tower and

incubated for 24 h at high relative humidity (≥ 93%). Leaf discs were isolated onto Petri dishes with paraquat-amended water agar and rated 11 days later for development of *B. cinerea* from isolated leaf discs. *B. cinerea* incidence on the upper and lower surfaces of water sprayed leaves averaged 90.4% and 95.8%, respectively. Despite full spray cover of leaves, applications with fenhexamid alone did not completely prevent infection and resulted in 34.6% and 40.8% *B. cinerea* incidence on the upper and lower surfaces of leaves, respectively. Through the addition of certain adjuvants, *B. cinerea* incidences were significantly lower (2.9-17.1% and 10.0-30.8%, respectively), while some adjuvants did not differ from the fungicide-only treatment, even though they might have improved spray deposition. The effects of Hydrosilicote and Solitaire alone and in combination with fenhexamid on germinating Botrytis conidia on leaf surfaces were studied in a histopathology study using epifluorescence microscopy. Distinct differences were observed in conidium mortality, germination and germ tube lengths between adjuvants alone and in combination with the fungicide, which might be attributed to indirect effects of the adjuvant mode of action on *B. cinerea*. The laboratory study clearly demonstrated the potential of adjuvants to improve the bio-efficacy of a fungicide directly through improved deposition on grapevine leaf surfaces.

For the vineyard evaluations, the same fluorometry, photomicrography and digital image analysis protocol were used to assess quantitative and qualitative spray deposits under varying adjuvant dosage and volume applications. The Furness visual droplet-rating technique was initially included to determine optimum spray volume with a STIHL SR400 motorised backpack mistblower by assessment of pigment deposition on Chardonnay leaves under illuminated black light. Both assessment protocols showed that quantitative spray deposition increased with increasing spray volume applications of 40 L/ha to 750 L/ha, but decreased at 900 L/ha, possibly due to run-off. The addition of selected adjuvants at recommended dosage and at 600 L/ha demonstrated the potential of adjuvants to increase quantitative and qualitative deposition significantly on upper and lower leaf surfaces. Agral 90, BB5, Nu-film-P, and Solitaire significantly improved deposition on upper and lower leaf surfaces compared with the fenhexamid only and water sprayed control. Break-thru S 240 and Villa 51 did not improve quantitative deposition, although remarkably better qualitative deposition was obtained. An adjuvant dosage effect (within the registered dosage range) was evident, especially those retained on the upper leaf surfaces. Agral 90 and Nu-film-P affected significant improvement of spray deposition at the higher, but not at the lower dosage tested. Solitaire improved deposition at the lower dosage tested, whereas reduced deposition at the

higher dosage was attributed to excessive spray run-off. No significant improvement of spray deposition was observed for both dosages tested with Villa 51. Spray mixtures with adjuvants Agral 90 and Solitaire yielded similar deposition values at 600 L/ha compared with the fenhexamid only control at 900 L/ha, but reduced deposition at the higher spray volume, possibly due to spray run-off. This study clearly demonstrated the potential of adjuvants to improve quantitative and qualitative deposition, but highlights the necessity to match adjuvant dosages and application volumes on the spray target to achieve maximum spray deposition.

DIE GEBRUIK VAN BYVOEGMIDDELS OM FUNGISIED DEPONERING OP WINGERDLOWER TE VERBETER

OPSOMMING

Effektiewe beheer van vrug- en blaarsiektes soos vaalvrot op wingerde benodig voldoende deponering van die swamdoder op die teikenoppervlak. Verlies aan beheer word gewoonlik aan onvoldoende kwantitatiewe deponering op vatbare wingerddele toegeskryf. Onder ’n hoë siektedruk kan mislukte beheer ook moontlik toegeskryf word aan swak kwalitatiewe deponering. Die primêre doelwit van spuittegnologie is om deponering te optimaliseer met die plantoppervlak as ’n belangrike komponent in die spuittoedieningsproses, spesifiek in die retensie van spuitdruppels. Byvoemiddel tegnologie het bewys dat oppervlak-aktiewe-agente verbeterde benatting en verspreiding van druppels op die teiken oppervlakte tot gevolg kan hê, en verder ook die deponering en retensie van die aktiewe fungisied bestanddele kan verbeter. Hierdie relatiewe nuwe spuittegnologie op wingerd- en hortologiese verbouing, asook die moontlike effekte van byvoegmiddels op epikutikulêre waks om siekte ontwikkeling te beïnvloed, moet ondersoek word. Verder word nuttige aanbevelings benodig vir byvoegmiddel toedienings by verskillende spuitvolumes en dosisse van die betrokke spuitmengsel. Die doelwit van hierdie studie was, eerstens om die effek van sekere byvoegmiddels op kwantitatiewe en kwalitatiewe spuitbedekking van wingerdblare te bepaal en dan te vergelyk met die biologiese effektiwiteit van ’n fungisied, en tweedens om van die byvoegmiddels onder veldtoestande te evalueer, asook die effek van byvoegmiddel dosisse en spuitvolumes te bepaal.

Blare is onder dieselfde laboratorium toestande tot net voor-afloop met 1 mL van ’n spuitmengsel, bestaande uit fenhexamied (Teldor® 500 SC, Bayer) teen die aanbevole dosis, ’n fluoreserende pigment (400 g/L EC; Suid Australiese Navorsing en Ontwikkeling Instituut) teen 0.2 L/100 L, sowel as 15 geselekteerde kommersiële byvoegmiddels gespuit om die kwalitatiewe deponering, vir ’n gegewe kwantiteit van spuitdeponering, te manipuleer. Die fluoreserende pigment is op die blaaroppervlak belig met ’n swart lig (UV-A ligbron in die 365 nm golflengte) en deponering is onder ’n stereo mikroskoop (Nikon SMZ800) teen 10× vergroting waargeneem. Die gespuite blaaroppervlaktes is op die manier met ’n digitale kamera afgeneem (Nikon DMX 1200), waarna die digitale foto’s kwantitatief

en kwalitatief deur die gebruik van ‘Image-Pro Discovery version 6.2 for Windows (Media Cybernetics)’ sagteware geanaliseer is om spuitbedekking te bepaal. Na elke blaarspuit is die blare met 5 mg droë konidia van *B. cinerea* in ’n inokulasietoring geïnokuleer en daarna vir 24 h onder hoë relatiewe humiditeit (≥ 93%) geïnkubeer. ’n Aantal skyfies vanuit elke blaar is op Petri bakkies met paraquat medium geïsoleer en 11 dae later is die persentasie van *B. cinerea* ontkieming bepaal. Die gemiddelde voorkoms van *B. cinerea* op die blare wat slegs met water gespuit is, was 90.4% op die boonste en 95.8% op die onderste blaaroppervlaktes. Spuitbehandelings met slegs fenhexamied, ongeag goeie blaarspuitbedekking, kon nie die *B. cinerea* infeksie ten volle voorkom nie, en infeksie van gemiddeld 34.6% en 40.8% is onderskeidelik op die boonste- en op die onderste blaaroppervlaktes waargeneem. Met die byvoeging van sekere byvoegmiddels het die voorkoms van *B. cinerea* betekenisvol verminder (2.9-17.1% en 10.0-30.8%, onderskeidelik), terwyl ander byvoegmiddels nie van die fenhexamied behandeling verskil het nie, hoewel hierdie middels meestal wel spuitdeponering verbeter het. Die effek van slegs Hydrosilicote en Solitaire, en in kombinasie met fenhexamied op ontkiemende Botrytis conidia, is bestudeer in ’n histopatologiese studie deur middel van die gebruik van epifluoresensie mikroskopie op die blaaroppervlak. Duidelike verskille in die aantal dooie konidia, ontkiemingpersentasies en kiembuislengtes is tussen die byvoegmiddels en in kombinasie met fenhexamied waargeneem, waar sommige waarnemings moontlik aan die indirekte effek van die byvoegmiddel op *B. cinerea* toegeskryf kan word. Hierdie laboratoriumstudie wys duidelik dat byvoegmiddels oor goeie potensiaal beskik om die bio-effektiwiteit van die fungisied te verbeter deur die direkte verbetering van deponering op die wingerdblaaroppervlak.

Dieselfde fluorometrie, fotomikrografie en digitale foto-analise protokol is in ’n wingerd evaluasie om die kwantitatiewe en kwalitatiewe spuitdeponering van verskillende byvoegmidel dosisse and spuitvolumes te bepaal, gebruik. Die Furness visuele druppel meting tegniek is aanvanklik ingesluit om die optimale spuit volume met ’n ‘STIHL SR400 motorised backpack mistblower’ te bepaal deur visuele meetings van gedeponeerde pigment op Chardonnay blare onder ’n swart ligbron. Beide protokolle wys dat kwantitatiewe spuitbedekking met ’n toename in spuit volumes 40 L/ha tot 750 L/ha verbeter het, maar afgeneem het teen 900 L/ha, moontlik as gevolg van druppel-afloop. Die byvoeging van ’n byvoegmiddel teen die aanbevole dosis en 600 L/ha wys uitstekende potensiaal om kwantitatiewe en kwalitatiewe deponering betekenisvol op boonste en onderste blaaroppervlaktes te verbeter. Agral 90, BB5, Nu-film-P, en Solitaire het deponering

betekenisvol op boonste en onderste blare in vergelyking met die fenhexamied alleen en die water kontrole verbeter. Break-thru S 240 en Villa 51 het nie kwantitatiewe deponering verbeter nie, alhoewel verbeterde kwalitatiewe bedekking met hierdie produkte waargeneem is. ’n Byvoegmiddel dosis effek (binne die registreerde dosis reeks) was duidelik waarneembaar, veral vir druppel retensie op die boonste oppervlak van blare. Agral 90 and Nu-film-P verbeter die spuit deponering betekenisvol met die hoër getoetste dosis, maar nie teen die lae dosis nie. Solitaire verbeter egter die deponering teen die laer dosis, maar minder deponering teen ’n hoër dosis kan moontlik toegeskryf word aan oormatige druppel-afloop. In die geval van Villa 51 was geen betekenisvolle verbetering van spuitdeponering vir beide die behandelingsdosisse waargeneem nie. Spuitmengsels met byvoegmiddels, Agral 90 en Solitaire, het soortgelyke deponerings gelewer teen 600 L/ha in vergelyking met die fenhexamied kontrole teen 900 L/ha, maar deponering neem af teen hoër spuitvolumes met byvoegmiddels moontlik as gevolg van druppel-afloop. Hierdie studie wys duidelik die uitstekende potensiaal van Byvoegmiddels om kwantitatiewe en kwalitatiewe deponering te verbeter, maar beklemtoon die noodsaaklikheid van die korrekte gebruik van byvoegmiddel dosis en volume om die maksimum spuitdeponering op die teiken te verkry.

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CONTENTS

1. THE USE OF AGRICULTURAL SPRAY ADJUVANTS IN FRUIT AND FOLIAR DISEASE CONTROL

INTRODUCTION

The primary objective of spray technology is to optimise deposition and activity of the fungicide or pesticide active ingredient, of which the plant surface is a critical component in the spray application process, specifically in the retention of spray droplets (Bukovac *et al.*, 1986; Holloway; 1993; Wagner *et al.*, 2003; Hunche *et al.*, 2006). Good deposition of active ingredient on the target site is an essential requirement for effective disease management (Brink *et al.*, 2004, 2006).

The target sites for most fungicide or pesticide spray applications are fruit or leaves. The cuticle is a non-living, lipoidal membrane, which covers all aerial plant surfaces (Bargel *et al.*, 2006) of leaves, stems, flower parts and fruit. This outer dermal layer forms the interface between the plant and the environment in waterproofing and protection, providing reduced transpiration with effective transport in and out of the plant part (Bargel *et al.*, 2006), while protecting plant parts against penetration of pathogens (Bukovac and Petracek, 1993; Mlikota Gabler *et al.*, 2003). The microstructure of the cuticle surface has a great influence on its wettability, and thus, on the deposition of water-based sprays (Wagner *et al.*, 2003). The wax embedded in the outer layer of the cutin matrix of higher plants represents a hydrophobic layer on which foliar sprays need to be retained. This comprises the cuticular- and epicuticular wax layers with hairy filaments and can be a ‘difficult-to-wet’ surface area (Bargel *et al.*, 2006). Studies done on its relation to plant surface wetting showed that microstructures i.e. cuticular foldings and epicuticular waxes minimise the contact area between water droplet and the plant surface by the combination of hydrophobic chemistry and micro-roughness, and form an enlarged air-water interface, which constitutes a composite surface with air enclosed between epicuticular crystals (Holloway, 1970; Wagner *et al.*, 2003; Bargel *et al.*; 2006). On such low energy surfaces, water forms spherical droplets owing to surface tension, which rest on the outermost tips of the wax crystals, with minimised contact area, a phenomenon called water repellency (Wagner *et al.*, 2003). Therefore, the water repellant cuticular waxes (Bargel *et al.*, 2006) are an important site of action for agrochemical wetting-spreading adjuvants and retention of active ingredient (Holloway, 1970; Bukovac *et al.*, 1986; Bukovac and Petracek, 1993; Holloway, 1993; Wagner *et al.*, 2003), especially when contact fungicides are being used on the plant target surface.

Waxy leaf surfaces are readily wetted by aqueous sprays containing a suitable surface-acting-agent (Foy, 1964; Stevens *et al.*, 1993). Adjuvants are additives with surface-acting-agents commonly applied with pesticide to improve spray performance (Ryckaert *et al.*, 2007), which include deposition and retention (De Ruiter *et al.*, 1990; Hall *et al.*, 1993, 1998; Hart *et al.*, 1992), penetration (Screiber, 1995; Thompson *et al.*, 1996), persistence on foliage (Kudsk *et al.*, 1991; Hart *et al.*, 1992; Reddy and Locke, 1996) and efficacy (Percich and Nickelson, 1982; Grayson *et al.*, 1996a, 1996b; Young and Hart, 1998; Holloway *et al.*, 2000). There are various types of adjuvants, but adjuvant research has predominantly focused on herbicide performance (Foy, 1993; Steurbaut, 1993). An exhaustive review of the relevant literature done by Stevens (1993) found that only 2% of publications on the use of organosilicone surfactant adjuvants were associated with fungicides or disease control. Similarly, Steurbaut (1993) noted that only 3% of the literature on adjuvants referred to fungicides and improved retention has been demonstrated in relatively few field applications with fungicides. Amber *et al.* (1993) reported that adjuvants improved control of powdery mildew of wheat by 30%. Field studies done by Sieverding *et al.* (2008) showed that the adjuvant Break-thru S 240 in fungicide applications improved the efficacy against powdery mildew, downy mildew and grey mould. Elad *et al.* (1990) showed that several film-forming polymers reduced amount of grey mould on various crops. Gaskin *et al.* (2002, 2004a) showed that adjuvant technology can improve application, and may improve disease control on grapevines tested in a field study. Adjuvants can improve pesticide application from a preventative high-dose and high volume applications to improved preventive low-dose low volume specifically targeted applications (Gaskin *et al.*, 2002; Ryckaert *et al.*, 2007). There is now considerable interest in using adjuvants for fungicides to enhance activity (Green, 2000; Keegan, 2004) and possibly reduce dose rates for application (Underwood, 2004). Moreover, this has beneficial economical (Gaskin *et al.*, 2004a, 2004b) and environmental implications (Kirkwood, 1993; Knowles, 2008).

Although, adjuvants may improve deposition and disease management with reduced fungicide rates, the knowledge on adjuvants and their role in disease management is limited (Knowles, 2008). Research on foliar application of adjuvants has rarely been analysed, although this information would be of significance (Screiber, 2006). This can possibly be attributed to the lack of adequate methods for analysis. The objective of this literature study is to provide an overview on the theoretical possibilities to improve spray deposition through the use of different agricultural adjuvants, with specific focus on fungicide performance on horticulture and viticulture crops.

ADJUVANT CATEGORIES

There is much disagreement regarding how spray adjuvants should be categorised, and to complicate matters further some adjuvants perform more than one function and thus really do fit in more than one category. They may affect a complex of interlinked events from droplet transport and active ingredient movement on or in the plant (Hall *et al.*, 1993). Depending on functionality, adjuvants can be divided into two broad categories, namely activator adjuvants and utility adjuvants (Hazen, 2000).

Activator adjuvants will be discussed in a conceptual and literal sense, for better understanding of the main functional mode of action on the plant surface itself. For this purpose, the main effects of activator adjuvants will be addressed, although, more than one action may reside within a particular product (Stock and Briggs, 2000). Activator adjuvants are the most difficult to validate for performance. There are many interacting contributors that enhance the efficacy of a pesticide (Hazen, 2000).

Activator adjuvants

Activator adjuvants maximise the biological efficacy of the pesticide (activity) once the spray mixture is deposited on the target (McMullen, 2000). These tank mix adjuvants include a wide variety of surfactant chemistries (Penner, 2000). The efficiency of the adjuvant can be influenced by a combination of different factors present, i.e. the adjuvant, the pesticide and the plant species (Steurbaut, 1993; Hess and Foy, 2000). Modes of action of activator adjuvants can be the reduction of droplet surface tension to enhance contact area for improved wetting and spreading action (increased spray retention), promotion of rain fastness, protection of the pesticide in the spray solution, emulsifier action, wax solubilisation of the leaf cuticle, and enhancement on the foliage surface areas for improved absorption (Penner, 2000). The latter two modes of actions are especially designed for the use of adjuvants in mixture with herbicides to enhance foliar uptake and biological efficacy of the herbicide active ingredient (Kirkwood, 1993; Stevens *et al.*, 1993; Kirkwood, 1999; Hess and Foy, 2000).

Wetter-Spreader adjuvants. Wetting and spreading agents (surfactants) lower internal surface tension in the spray droplet, spreading the volume over a large thin layer on the target surface (Foy, 1964; Stevens *et al.*, 1993). The droplet is less spherical with more contact area on the plant surface (Hazen, 2000; Wagner *et al.*, 2003; Gaskin *et al.*, 2005).

Normal surface tension by inwardly directed forces tends to make the spray droplet become more spherical in shape. A natural spherical shape prevents the droplet from contacting a very large area of hydrophobic surface (Wagner *et al.*, 2003; Bargel *et al.*, 2006). Surface tension influences the sizes of droplets in the spray and the likelihood that droplets will roll off a leaf (Watanabe and Yamaguchi, 1992). Surfactants at the right concentration reduce droplet equilibrium surface tension (EST) that allow the droplet to lie flat in a relatively thin layer on the waxy leaf surface (Hazen, 2000). Further reduced tension to a very low level will spread the droplet, which allows the crop production chemical to be distributed more broadly over the target surface (Gaskin *et al.*, 2002). However, too much surfactant may cause negative effects like run-off, especially on 'easy-to-wet' surfaces. The correct adjuvant concentration is therefore of the outmost importance for maximum pesticide performance (Green, 1999; Gaskin *et al.*, 2000, 2002).

Sticker adjuvants. Stickers reduce losses of formulation due to droplets evaporating from the target surface or due to beading-up and run-off (Hazen, 2000; Hunche *et al.*, 2006). Their viscous nature allows them to adhere with pesticide deposits, and prolong activity. These adjuvants typically keep the pesticide in contact with the plant tissue, and resist being washed off during rain or irrigation (Roggenbuck *et al.*, 1990). Many crop production formulations already contain polymers or polymeric surfactants that improve sticking character of the chemical deposit (Hazen, 2000).

Humectants. These additives have the primary function of slowing down the dry up rate of a pesticide on the plant surface (Hazen, 2000). When droplets dry up, only the crystallised form of the active ingredient will remain on the target area. This crystal form of an active ingredient is the least available form for absorption and uptake. Some humectants, however, have inherent liquidity and like salts, will draw moisture from the atmosphere (Hazen, 2000). This maintains a higher humidity level near the spray deposit, which reduces evaporation and improves retention, more specifically adsorption and/or uptake (Mac Manus, 2000).

Penetration agents. These adjuvants enhance the penetration of pesticides from the target surface through the natural barrier or cuticle for uptake (Foy, 1964; Gauvrit and Cabanne, 1993). Cuticular waxes may be softened or dissolved or the stomata infiltrated (Stevens *et al.*, 1991), allowing diffusion movement of the pesticide to more hydrophilic structures

beneath (Foy, 1964). Enhanced efficacy may result in increased bio-availability of an active ingredient, improving biological control of the pesticide.

Utility adjuvants (including spray modifiers)

Utility adjuvants are added in the tank-mix to improve the application of the formulation to the target plant surface. By themselves, they do not directly enhance pesticide activity, but generally work on the properties of the spray solution or spray mixture to improve the application process (McMullen, 2000). These adjuvants change the physical or chemical properties of the tank mix for easier application to the target plant. They may be a spray modifier agent for improved deposition (Hall *et al.*, 1993) and may also function as agents for compatibility, defoaming, drift control, water conditioning, acidifying or buffering (McMullen, 2000). Some wetting or spreading agents within the utility adjuvant category may affect only the physical properties of the spray droplets, and do not affect behaviour of the formulation once it is in contact with plants.

A subgroup of utility adjuvants, spray modifiers, should also be recognised, which include some activator deposition characteristics for horticultural and viticulture crops. These adjuvants include activator characteristics of wetters, spreaders, stickers and may also enhance some penetration with systemic pesticide active ingredient at a higher concentration (Hall *et al.*, 1993; Kirkwood, 1993; Hazen, 2000). Products like Break-thru S 240 claim to increase penetration by stomatal flooding and better uptake through cuticular penetration, which is caused by ultra low surface tension of the spray droplet (www.break-thru.com). These adjuvants should not disrupt or solubilise the cuticle and plant surface wax (Hall *et al.*, 1993), which is a requirement for herbicide activator adjuvants (Kirkwood, 1993; Stevens *et al.*, 1993; Kirkwood, 1999; Hess and Foy, 2000). At lower concentration, spray modifiers will cause improved wetting and spreading and are regarded as more important for non-systemic agrochemicals to be applied uniformly to the target surface (Kirkwood, 1993; Hazen, 2000). Spray modifiers can enhance the application process (utility characteristics) by adjusting droplet surface tension for improved deposition, drift control and impaction of spray droplets on the target surface (Hall *et al.*, 1993; Stock and Biggs, 2000). These adjuvants normally do not influence the active ingredient directly, but may enhance biological activity of the fungicide by physicochemical characteristics of the agrochemical upon any of the stages of the application process (Hall *et al.*, 1993).

Combinations of activator and utility adjuvants

The classification of adjuvants in main functional groups may sometimes overlap when adjuvants can be classified in utility and activator adjuvant categories. It must be remembered that adjuvants are a diverse group of active ingredient surfactant chemistries (Stock and Briggs, 2000). Therefore, it can be expected that a particular adjuvant may influence more than one application function, which can be beneficial or negative to agrochemical performance. Hall *et al.* (1993) demonstrated the complexity of various effects of adjuvants and outlined how beneficial effects at one level in the application process may be detrimental at another.

ACTIVATOR ADJUVANT COMPOSITION

Adjuvants may include an agent or normally a variety of agents as active ingredients to improve pesticide application and deposition performance. In the next section, these surface-acting-agents (i.e. surfactants) for activator and spray modifier adjuvants will be discussed, particularly their function as activator adjuvants for improved active ingredient performance.

For a fungicide to perform its function properly, a spray droplet must be able to wet the foliage and spread out evenly over the target surface. Surfactants enlarge the area of fungicide deposition, thereby increasing the pathogen's exposure to chemical. Surfactants are particularly important when applying a fungicide to waxy or hairy leaves (Gaskin *et al.*, 2005). Surfactants have a major effect on the surface tension of the spray droplet at the air-water interface and on the contact angle at the water-plant interface (Holloway; 1970; Kirkwood, 1993). They can influence the droplet spectrum, spray drift and the efficacy of delivery to the leaf surface, as well as adhesion, spreading and wetting (Hall *et al.*, 1993; Kirkwood, 1993). Without proper wetting, sprays may run-off due to weaker retention, and result in inadequate deposition (Watanabe and Yamaguchi, 1992). Too much surfactant, however, can also cause excessive run-off (Kirkwood, 1993; Holloway, 1994), thus reducing fungicide efficacy.

On the basis of their ability to ionise in aqueous solutions they can be grouped into non-ionic, cationic and anionic surfactants (Parr and Norman, 1965; Kirkwood, 1993; Hazen, 2000). These molecules are similar to soaps with a non-polar, lipophilic (oil soluble) and a polar hydrophilic group (water-soluble). Most types of surfactants can act as emulsifiers as well as wetters and spreaders (Hall *et al.*, 1993; Kirkwood, 1993), but compatibility with ionic pesticides is important (Hazen, 2000; Bunting *et al.*, 2004). The surfactant molecule's

hydrophilic side can associate with the water phase and the lipophilic portion must have strong affinity for nonaqueous substrate (agrochemical crystal, an oil or solvent/agrochemical solution) (Kirkwood, 1993; Hazen, 2000). Gaskin and Holloway (1992) stated that a surfactant that is incorrectly matched with an agrochemical might also deactivate performance.

Non-ionic surfactants consist of a molecule that combines both hydrophilic and lipophilic groups (polar and non-polar) and is the balance of the size and strength of the two opposite groups, which is called the hydrophilic-lipophilic balance (HLB). The HLB scale is a range between the numbers 1 (lipophilic surfactant) and 20 (hydrophilic surfactant), which indicates the ability of a surfactant's performance with a chemical (Griffin, 1949; Griffin, 1954; Hess and Foy, 2000). The surfactant can approach the full range by having an extreme affinity for lipid or water. In general, surfactants with lower HLB numbers (4-6) are mostly used as emulsifiers (water in oil), while those with higher HLB numbers between 7 and 9 are superior candidates for wetting agents (Griffen, 1949). Attaining the optimum surfactant HLB for a specific agrochemical will optimise the formulation's spread on plants (Hazen, 2000).

Ionic surfactants (anionic and cationic) can readily pair with oppositely charged pesticides, increasing the solubility of polar pesticides in water. However, some ionic surfactants may form complex structures with other compounds/contaminants in agrochemicals, and this may interfere with their function. For this reason, non-ionic surfactants are more commonly recommended (Parr and Norman, 1965; Hazen, 2000).

Wetting agents. These agents typically consist of nonionic surfactants in water, alcohol, or glycols (Stock, 1997; Hazen, 2000). The commercially available surfactants are the alkylphenol ethoxylate (nonylphenol or octylphenol) (APE) surfactants (Knowles, 1995; Green, 1999; Hazen, 2000). This group is widely used as wetters and emulsifiers. The most important APE's in this group are the nonylphenols (Green, 1999). They are, however, slow degradable endocrine disrupters and will be removed from the adjuvant market (Stock, 1997; Bialek, 2004; Ryckaert *et al.*, 2007). More recently, there has been a trend towards introducing surfactants that are more environmentally friendly in terms of biodegradability and ecotoxicity.

Spreading agents. Many surfactants allow a spread diameter increase of 2 to 3 times. Concentration is a secondary key for spreading (Green, 1999; Gaskin *et al.*, 2000; Gaskin *et al.*, 2002; Spanoghe *et al.*, 2006). Typically, the alcohol ethoxylates will spread well (Green,

1999). Certain triloxane ethoxylate organosiloxane derivatives cause phenomenal spreading. Triloxane alcoxylates have the ability to cause superspreading far exceeding the capability of traditionally organic surfactants. The superspreading is rapid and succeeds in dramatically better coverage (Hazen, 2000; Gaskin *et al.*, 2002).

Sticking agents. Deposition stickers decrease losses of formulation from targeted plants due to beading-up and run-off (Hazen, 2000). Higher molecular weight surfactants, such as ethylene oxide/propylene oxide, have an affinity to stick onto the sprayed surface (Hazen, 2000). The degree of sticking can be measured to a rain wash-off parameter, which may differ according to the water solubility, and changed by the concentration of the sticker relative to the pesticide. Many adjuvants are formulated as a spreader-sticker, which is blended for improved deposition of the pesticide before dry-down, whereafter it can become adhesive (Hazen, 2000).

Penetration agents. These adjuvants can increase the penetration of pesticides into plants (Gauvrit and Cabanne, 1993; Steurbaut, 1993; Foy, 1964). They are derived from either refined petroleum (mineral) oils or from vegetable oils (seed oils) (Hamilton, 1993). These oils do not readily mix with water. A surfactant emulsifier is often needed to disperse these oil-in-water micelles (Hamilton, 1993; Kirkwood, 1993; Stock and Briggs, 2000).

Petroleum oils. Mineral oil adjuvants are used in low quantities as carriers of oil-soluble pesticides. These adjuvants can reduce surface tension (Foy, 1964), increase wetting and spreading (Gauvrit and Cabanne, 1993), give quicker absorption (Hess and Foy, 2000) and improve rain fastness (Gauvrit and Cabanne, 1993). Mineral paraffinic oils may soften waxes, or break up some of the cuticle, allowing better leaf penetration (Gauvrit and Cabanne, 1993).

Vegetable oils. Vegetable oil (canola or soybean oil) concentrate consists of an emulsifiable vegetable oil product, containing a surfactant and vegetable oil. This can be triglyceride or methylated vegetable oil (Hazen, 2000). Triglycerides are pressed and extracted out of plant tissue, and have higher viscosity than methylated oils (Hamilton, 1993). Triglycerides usually contain only 5 to 7% surfactant emulsifier. Methylated seed oils are chemically modified from seed extracts and derived to methyl and alkyl esters (methylated soybean or ethyl canolate) (Hamilton, 1993). The composition of these oils varies depending on the seed source, which influence efficacy.

ADJUVANT USE FOR IMPROVED FUNGICIDE CONTROL IN HORTICULTURE AND VITICULTURE

Deposition

The wetting of plant surfaces by agrochemical spray solutions is one of the most important factors affecting the ultimate bioavailability of these chemicals through the quantity retained and the quality of its distribution. The plant surface can be viewed as a hydrophobic layer of material that covers much of the aerial plant surface (Wagner *et al.*, 2003). When small droplets of water are placed on the leaves it is most often attached to the hairs (specialised epidermal cells) or lie on the tips of epicuticular wax crystals, often without contact with the true leaf surface (Holloway, 1970; Watanabe and Yamaguchi, 1992; Grayson *et al.*, 1993; Dickinson, 2000; Wagner *et al.*, 2003). Microscopic roughness (i.e. surface contours, trichomes and waxes) influences plant surface wettability (Wagner *et al.*, 2003). Gaskin *et al*. (2005) used contact angles of acetone droplets on surfaces to compare and group leaves by their "surface roughness factor" in differentiated classes between 'easy', 'difficult' and 'very-difficult-to-wet' species. Higher contact angles indicate 'difficult-to-wet surfaces' (Gaskin *et al.*, 2005), with minimised droplet plant surface contact area (Wagner *et al.*, 2003). Grapevine leaves, which retain less water than other foliage, are classed as moderately 'difficult-to-wet' on the upper, and 'very-difficult-to-wet' on the lower surfaces. Since the minimisation of the contact area is one of the major reasons for extreme water repellency, Wagner *et al.* (2003) made an attempt to determine the contact area between a water droplet and rough surface base compared with the project area of a droplet on a smooth surface. In relation to a smooth surface the contact areas of these droplets can decrease by 95%. Surfactants in adjuvants have the ability to reduce droplet surface tension, which decreases the contact angle between the droplets and the epicuticular wax layer for better droplet contact and wetting and spreading properties (Stevens *et al.*, 1993; Hess and Foy, 2000; Wagner *et al.*, 2003) on the spray target surface. Additionally, there will be an increase in the retention of spray droplets on the plant surface (Foy, 1964; Watanabe and Yamaguchi, 1992; Hall *et al.*, 1993; Stevens *et al.*, 1993). However, results for improving spray droplet adhesion and retention may vary with surface wettability or surfactant type and concentration (Wirth *et al.*, 1991; Gaskin *et al.*, 2005). Very 'easy-to-wet' surfaces have no requirement for surfactants, but when surface roughness increases, the addition of a surfactant, and in increased amounts, may improve droplet adhesion (Gaskin *et al.*, 2005).

Many adjuvants are reported to improve disease or pest control, because they increase droplet spread that increases active ingredient deposition (Hall *et al.*, 1993; Gaskin *et al.*, 2004a). A variety of methods can be used to assess spray deposition in vineyards, however appropriate technology is required for accurate assessment of improved deposition of fungicide active ingredient through the use of adjuvants. Visual observation of droplets on target sites immediately after spraying gives a rough indication of the quality of spray deposits. Visual assessment was greatly improved by adding a fluorescent dye to the spray mixture and illuminating deposits under black light (Furness, 2000). A droplet rating chart was recently developed by Furness *et al.* (2006), and uses a fluorescent pigment to estimate the number and size of droplet deposition and coverage per cm^2. The advantage of this method is that it is quick, cheap and easy to use. Water-sensitive papers are widely used for visual assessment as well as for image analyses in spray application experiments (Holownicki *et al.*, 2002). The card is very sensitive to moisture and high relative humidity and spray droplets impacting the surface of the card can show stains bigger than the real droplet size (card spread factor) (Anonymous, 1999). On the other hand, droplets smaller than 50 μm may evaporate before leaving a stain (Anonymous, 1999; Brink *et al.*, 2004). Visual assessment gives an indication of the quality of the application, but the human eye lacks quantitative measuring and speed of measurement (Derkson and Jiang, 1995). Bioassay and chemical residue recovery techniques provide an overall assessment of the quantity of spray deposits, but residue levels alone do not give a good indication of application quality such as uniformity of spray distribution (Holownicki *et al.*, 2002). The knowledge on adjuvant deposition and their role in disease management is limited (Knowles, 2008). Research on foliar application of adjuvants has rarely been analysed, although this information would be of significance (Screiber, 2006). This requires accurate quantitative and qualitative measurement analysis for agrochemical adjuvant deposition. Quantification of active ingredient (amount of dose deposited), is an important component in predicting improvements in pesticide dose efficacy (Abbott *et al.*, 1990; Brink *et al.*, 2005; Ryckaert *et al.*, 2007). Manipulation of the formulation components by an adjuvant can improve deposition quality, most probably due to improved spread (distribution of the deposited active ingredient) (Hall *et al.*, 1993). Improved quality of deposition might compliment the quantity of active ingredient in terms of biological efficacy. Therefore, it will be of great value to combine a quantitative and qualitative deposition assessment protocol when research is conducted with agrochemical spray adjuvants. In laboratory evaluations, Brink *et al.* (2005) demonstrated good correlation between quantitative spray deposition values and *B. cinerea*

incidence on inoculated structural grape bunch parts. These authors also developed and exhibited the value of qualitative deposition assessment where an improved quality of spray deposition was correlated with improved *B. cinerea* control on grapevine leaves (Fourie *et al.*, 2007). The use of adjuvants in the application of pesticides is becoming a common means of improving deposition of active ingredient, specifically by overcoming the physicochemical properties of the cuticle barrier (Stock and Briggs, 2000). If these changes, by the correct selection of an adjuvant can improve active ingredient deposition, quantitative and qualitative deposition parameters might give a good prediction of performance in horticulture and viticulture crops.

Certain factors can decrease spray deposition. If the velocity and dynamic surface tension are high, the spray may rebound from the droplet striking the hydrophobic plant surface and decrease deposition (Hall *et al.*, 1993; Webb *et al.*, 1999; Brazee *et al.*, 2000). Webb *et al.* (1999) conducted a trial with monosized droplets in which the behavior of droplet bounce trajectories were studied from point of first impact to final retention on, or escape from, a leaf and yielded velocity threshold for capture or bounce following impact. Water droplets on water-repellant leaves were only captured better at lower pre-impact velocities, which reduced the number of bounced droplets before finally being retained. The addition of a surfactant to water invariably reduced the numbers of bounced droplets between first impact and retention, and increased the velocity threshold for capture following impact (Webb *et al.*, 1999). Dynamic surface tension is strongly related to droplet reflection (Brazee *et al.*, 2000). Lower dynamic surface tension by surfactant composition may reduce the number of bounces and increased droplets finally retained (Webb *et al.*, 1999). Higher concentrations of surfactant may suppress reflection completely for improved droplet retention (Brazee *et al.*, 2000). On the other hand, higher concentration of adjuvant in the spray mix may also increase droplet spread, which can increase spray run-off, as previously explained. Some surfactants work by increasing the dynamic surface tension and are thus less prone to drift. If too much surfactant is added, these larger spray droplets may roll or fall off, therefore being less likely to adhere to a leaf surface (Holloway *et al.*, 2000). Hence, higher adjuvant dosage does not necessarily translate into better deposition. Gaskin *et al.* (2002) illustrated the risk of spray run-off when using a superwetter adjuvant at high volume sprays, as retention was reduced. It is important to match adjuvant dosage with application volume to achieve improved spray retention in viticulture and horticultural crops (Gaskin *et al.*, 2002, 2004a, 2004b). The stability of the pesticide can also be influenced by the choice of adjuvants. Physical and chemical incompatibility may influence pesticide performance

(McMullen, 2000). Limited research is done on the use of adjuvants and fungicides, but some research shows that additives that produce alkaline solutions can reduce the effectiveness of some fungicides such as captan, possibly due to the instability of the active ingredient under these conditions (Lukens, 1969). Gaskin *et al.* (2000, 2002) found that superspreading properties of organosilicone can be reduced or enhanced by different agrochemical formulations. A possible antagonistic effect was observed between captan+choropyrifos and a superspreading organosilicone adjuvant that resulted in less than expected spray coverage of foliage. However, mancozeb+sulphur provided excellent coverage with the superspreading adjuvant at the applied dosage of 0.2%, whereas captan combined with sulphur may double spreading properties of the spray adjuvant solution (Gaskin *et al.*, 2002). The latter may result in run-off, and hence reduce efficacy. It is therefore important to take this into account when organosilicones or other adjuvants dosages are prescribed for a specific agrochemical. Elliott chemicals, New Zealand, summarised some of their adjuvant products' recommendations in table format, with suggested dosage rates for different fungicides, insecticides, foliar fertilisers and other additives applied at varying spray volumes on grapevine foliage (www.elliottchemicals.co.nz).

Adjuvant-plant-interaction and biological response

Although, improved pesticide deposition has been the focus of most studies to indicate the effectiveness of the agrochemical, it should be judged by the biological response from the plant and/or effect on the pathogen or pest. A significant factor affecting this is the sensitivity of the plant surface area to phytotoxicity. The plant cuticle is the first barrier to overcome by fungal pathogens, such as *Botrytis cinerea*.

Surfactants in adjuvants that reduce the surface tension of the sprayed droplets making the plant cuticle more wettable, might also reduce wax viscosity for agrochemical penetration. Activator adjuvants were originally designed to influence the physicochemical properties of the cuticle surface area to improve droplet deposition of herbicides (Kirkwood; 1999; Stock and Briggs, 2000) and therewith the rate and efficiency of cuticle penetration (Foy, 1964; Kirkwood 1993; Kirkwood, 1999; Hess and Foy, 2000). It has been reported in viticulture crops that some adjuvants may increase fungal infection, which can be attributed to the disruption of the cuticle (Blakeman, 1973; Marois *et al.*, 1987; Knoche *et al.*, 1992; Rogiers *et al.*, 2005). Rogiers *et al.* (2005) and Marois *et al.* (1987) showed that berry cuticle disruption might occur with certain adjuvant applications. Rogiers *et al.* (2005) showed that under ideal conditions, waxes of berries have upright platelets and are intricate with a fine

frill-like fringe. Some herbicide adjuvants, which were not recommended for use on sensitive crops like grapevines, showed more disruption of epicuticular wax morphology, and severity of this disruption was dependent on the particular adjuvant used. Loss in wax platelet sharpness and fine structure was least for the wetter-spreader recommended for sensitive crops, and the greatest for the crop oil concentrate and the activator-penetrant. These authors hypothesise that adjuvants may also increase the porosity of the cuticle that may lead to greater exudation rates of nutrients and sugars that are used by germinating *B. cinerea* conidia. Marois *et al.* (1987) found an increase of *B. cinerea* development on grapes after they had been treated with spreaders and stickers. Surfactants may also decrease the cuticle thickness. Cuticle thickness has been demonstrated to have an effect on the ability of some pathogens to successfully penetrate the host cell (Blakeman, 1973; Mlikota Gabler *et al.*, 2003); for example, young leaves are highly susceptible and are mostly infected at the leaf base (Holz *et al.*, 2003). However, as leaves mature, they get increasingly resistant to infection where a thicker cuticle layer plays an important role (Langcake and Pryce, 1976). Elad and Ayish (1990) showed that some film-forming polymers, such as Biofilm, may alternatively reduce germination of conidia and germ tube length of *B. cinerea*, and therefore demonstrated that some adjuvants may also have fungistatic effects in disease reduction.

Some organosilicone superspreaders were identified as a class of adjuvant with the potential to reduce spray volumes without adversely affecting agrochemical performance on viticulture and horticultural crops (Gaskin *et al.*, 2004a). Although, these surfactants were primarily developed as adjuvants for herbicides (Stevens, 1993; Hess and Foy, 2000), some of their physical properties were less desirable as horticultural adjuvants. Recent studies in Australia and New Zealand showed potentially improved deposition with "modified organosilicones" suitable for use in horticulture and viticulture (Gaskin *et al.*, 2000, 2004a, 2004b). Research in grapevines showed that using these prescribed adjuvants throughout a full season gave a consistent trend of improved disease control, compared to the standard pesticide program (Gaskin *et al.*, 2004a). The superspreader adjuvants provided better coverage and spray deposition on bunches at as little as 200 L/ha, compared with traditional high volume spray applications. Both incidence and severity of Botrytis, and incidence of powdery mildew, were reduced. No adverse effects on fruit or wine quality, grape fermentation or pesticide residues were recorded (Gaskin *et al.*, 2004a). Few growers use adjuvants in avocado spray programmes due to anecdotal evidence suggesting phytotoxicity might occur on these highly susceptible fruit. However, these adjuvants are designed for use with non-systemic pesticides in horticulture and viticulture. No evidence of phytotoxicity on

any fruit or foliage on avocados or grapevines was observed (Gaskin *et al.*, 2004a, 2004b). It is important to find adjuvants that are less 'aggressive' on the outer layers of the plant deposition surface. Spray modifiers, as previously described, may improve the application and deposition of pesticides on the target plant surface, without significantly influencing of the plant surface structure. Therefore, spray modifiers are preferred to improve quantitative and qualitative deposition on viticultural and horticultural crops.

CONCLUSION

Better understanding of adjuvants will certainly come with ongoing research, and lead to the development of superior additive products for fungicide technology on viticulture and horticultural crops. The leaf structure is, however, complex and it is only by using real surfaces that progress has been made towards understanding the interactions that take place between the deposit and plant surfaces (Stevens and Baker, 1987; Stevens *et al.*, 1988). Many speculate that adjuvants interact differently with each pest, crop, and pesticide (Steurbaut, 1993), but results often showed strong similarities (Green, 2000). More research is needed to optimise the specific application conditions for each adjuvant/fungicide/crop/fungus combination (Steurbaut, 1993).

Biological effectiveness needs to be included in adjuvant research (Hall *et al.*, 1993, Rogiers *et al.*, 2005) in combination with quantitative and qualitative deposition that will give better understanding of the potential control of agrochemical and adjuvant combinations. The effect of spray adjuvants on epicuticular wax effecting plant disease development needs to be investigated on viticulture and horticultural crops. Moreover, the development of useful prescriptions for adjuvants by determining water volumes and adjuvant dosages is required for different pesticide tank mixes.

Therefore, it was decided to conduct two laboratory and field trials to demonstrate the effect of adjuvants on deposition and biological efficacy of fenhexamid fungicide sprays on Chardonnay grapevine foliage.

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2. THE USE OF ADJUVANTS TO IMPROVE SPRAY DEPOSITION AND *BOTRYTIS CINEREA* CONTROL ON CHARDONNAY GRAPEVINE LEAVES

ABSTRACT

Sufficient fungicide deposition on target sites is an essential requirement for effective chemical management of fruit and foliar diseases, such as grey mould. Control failure is often attributed to insufficient quantitative deposition on susceptible grapevine tissue. However, in high disease pressure situations control failure might also be attibuted to poor qualitative deposition. Spray adjuvants have the potential to improve the quality of fungicide applications by effecting uniform distribution of fungicide on plant surfaces. In order to study whether such a qualitative improvement of spray deposition would lead to improved disease control, a laboratory experiment was conducted on artificially inoculated grape (cv. Chardonnay) leaves. Prior to inoculation with *Botrytis cinerea* conidia in a spore settling tower, leaves were sprayed to pre-runoff with 1 mL of a mixture of fenhexamid, a fluorescent pigment, and one of 15 selected commercial adjuvants to manipulate the deposition quality of a given quantity of deposited spray. Following an incubation period of 24 h at high relative humidity, leaf discs were plated onto Petri dishes with paraquat-amended water agar and rated for development of *B. cinerea* from isolated leaf discs. Spray deposition on leaves was assessed with a spray assessment protocol using fluorometry, photomicrography and digital image analyses. *B. cinerea* incidences on the upper and lower surfaces of water sprayed leaves averaged 90.4% and 95.8%, respectively. Despite full spray cover of leaves, applications with fenhexamid alone did not completely prevent infection and resulted in 34.6% and 40.8% *B. cinerea* incidences on the upper and lower surfaces of leaves, respectively. Through the addition of certain adjuvants, *B. cinerea* incidences were significantly lower (2.9-17.1% and 10.0-30.8%, respectively), while some adjuvants did not differ from the fungicide only treatment, even though they might have improved spray deposition. The effects of Hydrosilicote and Solitaire alone and in combination with fenhexamid on germinating *Botrytis* conidia on leaf surfaces were observed in a histopathology study using epifluorescence microscopy. Distinct differences were observed in conidium mortality, germination and germ tube lengths between adjuvants alone and in combination with the fungicide, which might be attributed to indirect effects of the adjuvant mode of action on *B. cinerea*. The study clearly demonstrated the potential of adjuvants to

improve the bio-efficacy of a fungicide directly through improved deposition on grapevine leaf surfaces.

INTRODUCTION

Botrytis cinerea Pers: Fr. is a common destructive pathogen causing grey mould (Nair and Hill, 1992). *B. cinerea* is not specific to grapevines (*Vitis vinifera* L.) where it causes serious quality and quantity loss of grapevine yield (Bulit and Dubos, 1994), but also attacks many nursery plants, vegetable, ornamentals, field and orchard crops worldwide (Coertze and Holz, 2002; Elmer and Michailides, 2004). In South Africa, this is an economically important disease on grapevines (*Vitis vinifera* L.) (Holz *et al.*, 2003). Grey mould symptoms generally become prominent in vineyards after bunch closure (Holz and Volkmann, 2002; Van Schoor, 2004). In table grape production, the most serious damage is the loss of fruit quality due to pre-harvest or post-harvest berry rots. Fruit wetness and raised temperatures during storage or transit favour disease expression (Fourie, 1992). In wine grape production, the fungus causes a serious decrease in quality of juice and wine. The pathogen converts simple sugars (glucose and fructose) to glycerol and gluconic acid and produce enzymes that catalyse the oxidation of phenolic compounds. Wines produced from *B. cinerea* infected berries have off-flavours and are sensitive to oxidation and bacterial contamination, making them unsuitable for aging (Bulit and Dubos, 1994, La Guerche *et al.*, 2007).

Identification of spray application target sites naturally revolves around the susceptibility of various plant parts at different phenological stages, but also requires an in-depth knowledge of the pathogen's ecology in vineyards, as well as its infection pathways at the various phenological stages (Holz *et al.*, 2004; Elmer and Michailides, 2004). The pathogen exists in grapevine as sclerotia, conidia and mycelia from floral debris whereas conidia are the major dispersal and infectious unit of the fungus in the field (Holz *et al.*, 2004; Elmer and Michailides, 2004). Splashing water (Coertze and Holz, 2002) or insects (Michailides and Spotts, 1990; Louis *et al.*, 1996; Holz *et al.*, 2003, 2004; Elmer and Michailides., 2004) can also displace or transport inocula. Germination occurs at temperatures between 3-30ºC (15-20ºC is the optimum) with wetness duration periods of 15 h (Holz, 2001). In water, germination can be stimulated by exogenous nutrients from pollen or leaf exudates (Fourie and Holz, 1998; Coertze and Holz, 2002; Van Schoor, 2004). Studies on the ecology of *B. cinerea* in South African vineyards have shown that conidia levels in air currents and within bunches, are high from pre-bloom to late pea size, after which it declines

to very low levels (Holz *et al.*, 2003, Van Schoor, 2004). Van Rooi and Holz (2003) recommended an early-season suppression of *B. cinerea* inoculum build-up with a botryticide application preventing primary leaf infection. Grape leaves may carry high amounts of *B. cinerea* early in the season. Soft immature leaves can show symptom development (Hill *et al.*, 1981; Van Schoor, 2004), but as the leaf matures they become increasingly resistant to infection due to thicker cuticle and inhibitory compounds (Holz, 2001). Therefore, primary infection on young leaves may play an important role in the first generation of conidia on grapevines (Holz *et al.*, 2003, Van Schoor, 2004). Following infection, *B. cinerea* penetrates flower parts or structural bunch parts (rachis, laterals and pedicel) of the developing fruit to establish latent infections (Gessler and Jermini, 1985; Nair *et al.*, 1995; Elmer and Michailides, 2004). Verhoeff (1980) stated that fungitoxic compounds exist that disappear during the ripening process of grapes (Elmer and Michailides, 2004), whereas Vercesi and Bisiach (1997) proved that an increase in nutrients (sugars, malic acid, potassium and sodium) later in the season promoted mycelia growth of *B. cinerea* on grape berries. *B. cinerea* also opportunistically infects wounds, causing necrosis. After infection and death of the host tissue, the pathogen survives and sporulates as a saprophyte on the necrotic tissue (Holz, 1999; Keller *et al.*, 2002; Elmer and Michailides, 2004).

Chemical control is the main way to reduce grey mould on crops (Leroux, 1995, 2004). Producers in South Africa invest heavily in chemical products and routine spray applications each year (Holz *et al.*, 2003). The control of *B. cinerea* by chemical approach can only be achieved by reducing inoculum on susceptible target tissue (Van Rooi and Holz, 2003). Laboratory studies showed that improved quantitative (Brink *et al.*, 2006) and qualitative (Fourie *et al.*, 2007) deposition with the fungicide fenhexamid, gave better *B. cinerea* control on susceptible bunch parts. Similarly, laboratory studies conducted by Van Rooi (2001) showed that when fungicides were properly applied to target sites, the amount of *B. cinerea* was reduced, and infection and symptom expression were prevented at all growth stages. However, the same efficacy was not achieved with the same fungicides when using conventional spraying methods in vineyards (Holz *et al.*, 2003). Therefore, insufficient deposition of fungicides on the target sites, coinciding with favourable conditions for pathogens, might result in disease development and large economical losses of yield and grape quality. Optimisation of spray deposition on target sites is therefore an essential requirement for effective disease management. New spray technology showed that certain adjuvants can improve fungicide spray deposition in vineyards (Gaskin 2002; 2004a),

whereas some may reduce *B. cinerea* development by using the adjuvant alone (Elad and Ayish, 1990).

Adjuvants are powerful tools used to improve uniform deposition of the fungicide active ingredient (Hall *et al.*, 1993; Holloway *et al.*, 2000; Penner, 2000). Improved leaf wetting, spreading, sticking and penetration are important modes of action of adjuvants (Abbott *et al.*, 1990; Knowles, 1995; Penner, 2000; Ryckaert *et al.*, 2007). Grapevine leaves, which retain less water than other foliage, are classed as moderately 'difficult-to-wet' on the upper, and very 'difficult-to-wet' on the lower surfaces (Gaskin *et al.*, 2005). Surfactants in adjuvants have the ability to reduce droplet surface tension, which decrease the contact angle between the droplets and the epicuticular wax layer for better droplet contact (wetting and spreading properties) (Stevens *et al.*, 1993; Hess and Foy, 2000; Wagner *et al.*, 2003) on the spray target surface. A variety of methods can be used to assess spray coverage in vineyards, however, appropriate technology is required for accurate assessment of adjuvant-fungicide deposits. Methods such as visual assessment on water-sensitive paper, bioassays and chemical residue recovery techniques are used to determine coverage in vineyards (Holownicki *et al.*, 2002). Visual assessment was greatly improved by adding fluorescent dyes to the spray mixtures, followed by illumination of deposits under black light (Furness, 2000). However, visual assessment may give an indication of the quality of the application, but the human eye lacks quantitative measuring and speed of measurement (Derkson and Jiang, 1995). Bioassay and chemical residue recovery techniques provide an overall assessment of the quantity of spray deposits, but residue levels alone do not give a good indication of application quality such as uniformity of spray distribution (Holownicki *et al.*, 2002). Efficacy of agricultural chemicals is influenced by both quantitative- (amount of deposit) and qualitative deposition (distribution of deposit). If the quality of the deposited dosage is poor, efficacy may also be poor, even if the correct quantity or chemical was deposited. It will be of great value to include a quantitative and qualitative deposition assessment protocol when research is conducted with spray adjuvants for improved contact fungicide disease control. Brink *et al.* (2005) showed good correlation between quantitative spray deposition values and *B. cinerea* incidence on susceptible grapevine tissue. These authors also developed a qualitative deposition assessment to compliment quantitative deposition assessment (Fourie *et al.*, 2007). However, the exact mode of action of adjuvants can be complex (Abbott *et al.*, 1990; Steurbaut, 1993; Stock and Briggs, 2000) in combination with fungicides, and may not only be a reflection of improved deposition to control pathogens. Sticker and spreader surfactants increased bio-availability of the active

ingredients, especially for contact fungicides. However, research also showed that adjuvants may alter the cuticle wax components of the targeted area for increased wettability and spreading (De Ruiter *et al.*, 1990; Stevens *et al.*, 1993; Hess and Foy, 2000; Penner, 2000). Penetrator adjuvants normally increase wax permeability for better penetration of the fungicide active ingredient (Foy, 1964; Hamilton, 1993). It has been reported in viticulture that some adjuvants may increase fungal infection, which can be attributed to the disruption of the cuticle (Blakeman, 1973; Marois *et al.*, 1987; Knoche *et al.*, 1992; Rogiers *et al.*, 2005). Biological efficacy needs to be included in adjuvant research (Hall *et al.*, 1993, Rogiers *et al.*, 2005) to support quantitative and qualitative deposition for the specific fungicide evaluated.

Quantitative and qualitative retention efficiency of different adjuvants and fungicides on plant surfaces and the effect thereof on biological efficacy is poorly described in literature (Holloway *et al.*, 2000). For this purpose, a laboratory study was conducted to examine qualitative and quantitative deposition of different commercial adjuvants and its effect on *B. cinerea* infection on Chardonnay grapevine leaves.

MATERIALS AND METHODS

Biological efficacy study

Grapevine leaves. Fresh unsprayed leaves [cv. Chardonnay (*Vitis vinifera* L.)] were collected early in the growing season from a commercial vineyard. Leaves were carefully selected to be of similar age and size (5^{th} exposed leaf per shoot). These leaves were kept at relatively cool temperatures (*circa* 18°C) and were transported to the laboratory. Prior to further treatment, leaves were not surface-sterilised, in order to keep the natural epicuticular wax layers intact.

Spray applications. Leaves were sprayed with a mixture of fenhexamid (Teldor® 500 SC, Bayer CropScience, Bayer Ltd [P.O. Box 143, Isando, 1600, South Africa]) at the recommended dose (75 mL/100 L water) (Nel *et al.*, 2003) and fluorescent pigment (400 g/L, EC) (South Australian Research and Development Institute) at 2 mL/100 L (Furness, 2000). The fluorescent pigment does not influence *B. cinerea* growth (Brink *et al.*, 2005). Selected

adjuvants were added to the spray mixture to manipulate the deposition quality of a given quantity of deposited spray (Table 1).

Spraying was conducted in a spray chamber, which consist of a steel framework (800 × 1410 × 660 mm; L × H × W). For each treatment replicate, one detached leaf, positioned horizontally on a mesh tray with the upper or lower leaf surface facing upward, was sprayed at 1 mL (to pre-run-off) with a gravity feed mist spray gun (ITW DEVILBISS, Spray Equipment Products, 195 International Blvb, Glendale Heights IL 60139 USA) with a fluid nozzle tip of 1.5 mm in diameter. Application was conducted at 0.75 bars at a spray angle of 45° and 1.4 m from the leaf surface. The spray droplets were allowed 1 minute to settle on the leaflets where-after trays were removed from the chamber and leaves allowed to air-dry. This ensured proper coverage according to Van Rooi and Holz (2003). The spray gun was cleaned with soap-water and triple rinsed with de-ionised water and air dried between treatments.

Inoculum and inoculation. A virulent isolate of *B. cinerea*, obtained from a naturally infected grape berry, was maintained on potato dextrose agar (PDA; 12 g Biolab agar, 200 g potatoes, 20 g sucrose, 1000 ml H_2O) 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) were made up (GM; 1000 mL H_20, 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. After 14 days, dry conidia were harvested with a suction-type collector and stored at 5°C. According to Spotts and Holz (1996), germination was not affected by storage of dry conidia over time. This allowed the use of the same conidia in all the experiments over this time.

Sprayed leaves were each placed in its own Petri dish and then inoculated with dry *B. cinerea* conidia (Salinas *et al.*, 1989) with 5 mg spores in a spore settling tower (Plexiglas, 3 × 1 × 1 m; H × D × W); Coertze and Holz, 1999). Conidia were dispersed by air pressure into the top of the spore settling tower and allowed to settle onto the leaves positioned on mesh trays on the tower floor. Petri dishes with water agar (WA; 12 g Biolab per 1000 mL water) were placed next to the leaves on the floor and the percentage germination was determined after 6 h incubation at 28°C (100 conidia per Petri dish, two replicates). A conidium was considered as germinated when a germ tube was as long as, or longer than the conidium diameter.

Each Petri dish with a sprayed leaf, was placed in perspex chambers (Cape plastics, Cape Town, South Africa) and incubated at 28°C for 24 h to establish high relative humidity (RH) ≥ 93%. According to Gütschow (2001), sufficient germination, surface colonisation and penetration of grapevine leaves will occur within this period. Non-inoculated leaves were used to determine the natural infection levels of *B. cinerea.*

B. cinerea *assessment*. Following incubation, one half of each leaf was used to assess *B. cinerea*. The amount of *B. cinerea* (viable conidia, germlings and/or infections) occurring on leaves was determined by means of isolations onto paraquat medium as per methods described by Brink *et al.* (2005). Twenty leaf discs (5 mm diameter) were removed from one half of each leaf and plated onto Petri dishes (5 leaf discs per plate) containing paraquat selective medium (Grindat and Pezet, 1994). The plates were incubated at 22°C and rated after 11 days. A leaf disc was recorded as infected when it yielded sporulating *B. cinerea* colonies. The percentage incidence of discs infected by *B. cinerea* occurring was calculated.

Spray deposition assessments. The other half of each leaf was used to assess deposition. A quantitative and qualitative protocol was used to assess spray deposition by fluorometry, photomicrography and digital image analyses (Brink *et al.* 2005; Fourie *et al.* 2007). The sprayed plant material was illuminated under black light and visualised using a Nikon SMZ800 stereomicroscope at 10× magnification. Digital photos were taken with a Nikon DMX 1200 camera and image analyses performed with Image-Pro Plus version 5.0 for Windows (Media Cybernetics, www.mediacy.com) software. By using the measurement tools, these images could be analysed quantitatively (Brink *et al.*, 2004; 2005) and qualitatively (Fourie *et al.*, 2007). Quantitative analysis involved removal of green channels from the image, followed by quantification of the percentage area covered by the foreground elements (deposited pigment) in each of 100 equally sized squares spanning the binarised image. From these 100 measurements, the median quantitative measurement was used for further analysis. Qualitative analysis indicates uniformity of spray deposition on leaf surfaces. As for quantitative analysis, green channels are removed and the image binarised in fore- and background colour. An Euclidian distance map is created with max white pixel furthest away from deposition of pigment, after which a thinning filter creates a skeleton of the image. The relation between distance map and skeleton is created and by analyses of a histogram of grey-scale values of the distance skeleton, the statistics of the distances between foreground elements can be expressed (Fourie *et al.*, 2007).

Experimental layout. Experimental layout was a randomised block design with all treatments replicated 6 times for each leaf side in three block-repeats for spray deposition assessment. In two of these blocks, biological efficacy was included.

Histopathology study

Two adjuvants were selected according to similar effect on improvement of spray deposition, but differences in effect on biological efficacy. This disparity was microscopically investigated on sprayed glasshouse leaves.

Chardonnay vines were potted and cultivated under glasshouse conditions at 27°C. Leaves (5^{th} stage) were collected from unsprayed plants. The leaves were cut in the shape of a 90 mm petri dish whereafter adjuvant spray application followed. Spraying, leaf inoculation and spray deposition assessments were done as described previously. After 24 and 48 h incubation at a RH of > 93%, two thin hand sectioned pieces (7 × 5 mm) of leaf were removed with a razor blade from each leaf. The sections were stained for 5 minutes in a differential stain containing fluorescein diacetate ([FDA] Sigma Chemical Co., St Louis, MO, USA), aniline blue ([AB] B.D.H. Laboratory Chemicals Division, Poole, England) and blankophor ([BP] Bayer), mounted on a glass slide in 0.1 M KH_2PO_4 buffer (pH 5.0) and covered with a cover slip. FDA (2 mg/mL acetone) and AB (0.1% in KH_2PO_4, pH 5.0) were prepared as stock solutions and stored at -20°C and 5°C, respectively. Before a microscopy session, BP (0.5%) was added to the AB solution and a fresh stain prepared by mixing 25 µL of the FDA stock solution with 1 mL of the AB/BP stock solution in a 1.5-mL polypropylene Eppendorf tube, which was then kept on ice. Germination, germ tube lengths and mortality of fungal structures were examined with the aid of a Zeiss Axioskop microscope equipped with an epifluorescence condenser, a high-pressure mercury lamp, Neofluar objectives and Zeiss filters 02, 06 and 18. These sets included excitation filters G 365, BP 436/8 and BP 395-425, respectively. With this set-up, protoplast of viable fungal structures fluoresced brilliant yellow-green with filter No. 02, 06 and 18. Protoplasts of dead cells were blue black (Filter 06, 18), whereas cells without protoplast fluoresced white (filter 02) or yellow (filter 18) (O'Brien and McCully, 1981).

Statistical analyses

Analyses of variance were conducted on the quantitative and qualitative assessment data and *B. cinerea* incidence data on upper and lower leaves. Student's T-tests were performed to compare treatment means using SAS v. 8.2 statistical software (SAS Institute, 1999).

RESULTS

Conidia used for inoculation were highly viable as determined on water agar and germination varied between 86-94%.

Biological efficacy study

Assessment of spray deposition on leaves. The addition of fluorescent pigment to the spray mixture allowed clear visualisation of spray deposition when sprayed leaves were illuminated with black light (Fig. 1 and Fig. 2). Without the addition of an adjuvant, aggregation of pigment particles in remnants of droplets on leaf surfaces resulted in a distinct droplet effect (Fig. 1 A and B; Fig. 2 A and B). When certain adjuvants were included, the droplet effect was not as distinct and improved qualitative deposition was observed as an even spread of pigment particles across the leaf surface (Fig. 1 C-D; Fig. 2 C-D). However, with other adjuvants, deposition also resulted in a distinct droplet effect (Fig. 1 E; Fig. 2 E) or possible spray run-off (Fig. 1 F; Fig. 2 F).

Analysis of variance of median values for quantitative and qualitative deposition assessments on upper and lower leaf surfaces indicated significant effects for treatments ($P < 0.05$; Table 2). For mean infection values, analysis of variance also indicated a significant treatment effect ($P < 0.0001$; Table 3). Pearson's correlation procedure conducted on mean infection values with median quantitative deposition values of all treatments with fenhexamid in the spray mixture indicated reasonably good correlation on upper leaves [$r^2 = -0.538$ ($P = 0.0317$)] and lower [$r^2 = -0.560$ ($P = 0.0242$)] leaf surfaces (Table 4). However, correlation

between mean infection and median qualitative deposition values was higher for both upper leaves [r^2 = -0.585 (P = 0.0173)] and lower [r^2 = 0.703 (P = 0.0024)] leaf surfaces.

Upper leaf surface

Quantitative deposition. Although not significant, addition of fenhexamid to the fluorescent pigment and water mixture improved quantitative deposition (14.04%) compared to the water only controls (11.26% and 10.78%; Table 5). The addition of Biofilm significantly improved quantitative deposition (18.63%) compared with the fenhexamid alone treatment. Agral 90, BB5, Hydrosilicote, LeafCote, Li 700 and Solitaire yielded deposition values (14.80% to 17.77%) that were statistically similar to that effected by Biofilm, although not statistically better than fenhexamid alone. The remaining adjuvants, Biodew, Break-thru S 240, Buffernat, Designer, Nu-film-17, Nu-film-P, Villa 51 and WetCit, yielded deposition values similar or lower than the fenhexamid alone spay application (9.22% to 14.23%).

Qualitative deposition. Fenhexamid alone improved the quality of deposition markedly compared to the water sprayed controls, as the mean distances between fluorescent particles was shorter, measuring 35.66 pixels compared with 41.39 and 43.91. Addition of an adjuvant to the fungicide spray mixture did not significantly improve the deposition quality, although Agral 90, BB5, Biofilm, Hydrosilicote, Leafcote, Li 700, Solitaire and Villa 51 (29.08 to 31.32) representing improved deposition quality from 5.9% to 18.5% when compared to the fenhexamid only spray. The remaining adjuvants, Biodew, Break-thru S 240, Buffernat, Designer, Nu-film-17, Nu-film-P, and WetCit (33.93 to 40.87) yielded qualitative deposition values statistically similar to that of fenhexamid and the water sprayed controls.

B. cinerea assessment. Relatively low natural infection levels were measured on leaves that were not inoculated (6.67%) compared with leaves sprayed with water and inoculated with dry conidia of *B. cinerea* (90.42%). The fenhexamid only spray significantly reduced the infection level of the inoculated leaves to 34.58%. Through the addition of most adjuvants, *Botrytis* levels were significantly reduced (2.92% to 17.08%) from that obtained with fenhexamid alone. Biodew, Hydrosilicote, Nu-film-17 and WetCit (19.58% to 32.50%) caused slight to moderate improvement in the biological efficacy of the fungicide, although not statistically significant.

Lower leaf surface

Quantitative deposition**.** The fenhexamid only treatment showed significantly better quantitative deposition (13.62%) than the water only sprayed controls (8.93% and 8.59%). Treatments that included adjuvants showed no significant spray improvement compared with the fenhexamid only treatment. Agral 90, BB5, Biodew, Hydrosilicote, Li 700 and Solitaire yielded deposition values higher (14.07% to 17.24%) than the fenhexamid only spray, with a proportional increase between 3% and 27%. Biofilm, Break-thru S 240, Buffernat, LeafCote, Nu-film-17, Nu-film-P, and Villa 51 yielded deposition values (10.26% to 13.30%) that were proportionally 2% to 25% lower than that of the fenhexamid only spray, whereas only Designer and WetCit yielded significantly less (8.30% to 9.83%) quantitative deposition.

Qualitative deposition. Although not significant, the fenhexamid only application showed an improved qualitative deposition with a markedly lower pixel value (45.36) than the water only sprayed controls (53.25 and 50.88). When Agral 90, BB5, Biodew, Break-thru S 240, Hydrosilicote, Li 700, Solitaire and Villa 51 were included in the spray mixture qualitative deposition was improved further (36.13 to 43.16). Biofilm, Buffernat, Designer, LeafCote, Li 700, Nu-film-17, Nu-film-P, and WetCit, yielded pixel values higher (46.29 to 57.89) than that of fenhexamid, with only Designer resulting in significantly poorer qualitative deposition.

B. cinerea assessment. Natural *Botrytis* incidence was low (3.33%), whereas much higher levels of *B. cinerea* incidence occurred on the inoculated water-sprayed leaves (95.83%). Fenhexamid alone reduced levels of *Botrytis* incidence significantly to 40.83%. When adjuvants were included, all treatments, except Nu-film-17 (37.08%) resulted in significant reduction of *B. cinerea* levels (10.00% to 30.83%) compared with the fenhexamid only treatment. Fenhexamid sprays including Agral 90, BB5, Biodew, Break-thru S 240, Li 700, Solitaire or Villa 51 resulted in the lowest *B. cinerea* incidence (10.00% to 19.17%).

Histopathology study

Analysis of variance of median quantitative and qualitative deposition assessments on upper leaf surfaces indicated significant effects for treatments ($P < 0.0001$; Table 6). For mean values of histopatology parameters (germination, mortality germ tube growth) analysis

of variance also indicated a significant treatment effect ($P < 0.0001$; Table 7).

Quantitative and qualitative deposition. Quantitative and qualitative values of the fenhexamid only spray (1.71% and 56.28, respectively) were statistically similar to deposition following the water only spray (1.34% and 60.86, respectively). With the addition of Hydrosilicote or Solitaire alone or with fenhexamid in the spray mix, significantly improved quantitative (2.2% to 2.6%) and qualitative deposition values (39.89 to 44.41) were observed.

Histopathology parameters. The stained fungal structures and fluoresecnt pigment in the sprayed deposit could clearly be visualised using epifluoresence microscopy (Fig. 3 AB). Mean measurements for germination, spore mortality and germ tube length are summarised in Table 8.

Germination. With the fenhexamid only application, significantly less germination was observed (57.85%) compared with the water only spray (77.34%). The addition of Solitaire to fenhexamid further showed markedly less germination (51.37%) and statistically less germination than the Hydrosilicote-fenhexamid treatment (60.38%). The adjuvant only sprays indicated no major differences in germination (75.73% to 76.73%).

Mortality. Dead conidia or germ tubes were observed as blue-black structures whereas conidia were sometimes obviously distorted (Fig. 3 B). The fenhexamid only application significantly increased mortality (28.14%) when compared to the water only spray (6.45%). The addition of Solitaire to fenhexamid further markedly increased mortality (31.19%), although not significantly more than with the fenhexamid only application. However, when Hydrosilicote was added to fenhexamid, statistically less mortality was obtained (20.52%) than the fenhexamid control. The adjuvant only sprays did not influence mortality (7.07% and 8.84%) of *B. cinerea* conidia.

Germ tube growth. With the fenhexamid only application, significantly shorter germ tubes were observed (35.20 µm) when compared to the water only spray (71.50 µm). Addition of Solitaire or Hydrosilicote to fenhexamid resulted in a further reduction in germ tube length (19.72 to 27.83 µm, respectively) (Fig. 3 A and B), with the reduction effected by Solitaire proving to be statistically significant. Moreover, the adjuvant only sprays also resulted in statistically shorter germ tube lengths (56.10 to 59.10).

DISCUSSION

These studies showed that the addition of adjuvants may increase quantitative and qualitative deposition. Although a strong correlation exists between the quantitative and qualitative protocols, Pearson's correlation between deposition and infection values indicated that the qualitative deposition assessment correlated more accurately with biological efficacy of fenhexamid deposition on upper and lower leaf surfaces. Brink *et al.* (2005) also demonstrated a good correlation between quantitative spray deposition values and *B. cinerea* incidence on inoculated structural grape bunch parts. Spray application to individual leaves in this study resulted in very good quantitative and qualitative deposition. Van Rooi (2001), using a similar spray application system, reported that good deposition of fungicide reduced the amount of *B. cinerea* efficiently on the target surfaces, even when a high inoculum dosage of 3 mg spores was applied, which resulted in 3.88 conidia dispersed per mm^2 on berry surfaces (Coertze and Holz, 1999). Therefore, the improved biological efficacy of these high-quality sprays following the addition of certain adjuvants in this study is remarkable and clearly shows the potential beneficial use of this technology.

When a droplet is impacted on a leaf, it may adhere, spread or run-off. The wettability of the leaf surfaces has a large effect on the initial droplet adhesion (Bargel *et al.*, 2006). The fenhexamid only treatment showed good improved quantitative and qualitative deposition relative to the water only sprayed controls, however, it still resulted in a distinct droplet effect. Surfactants in adjuvants have the potential to lower surface tension of the aqueous solution applied on the target surface for improved droplet wettability and distribution of the active ingredient (De Ruiter *et al.*, 1990; Steurbaut, 1993, Stevens, 1993; Stevens *et al.*, 1993; Penner, 2000; Bargel *et al.*, 2006). Generally, when adjuvants were sprayed with or without fenhexamid, the droplet effect was less distinct and fluorescent pigment particles were most often spread in amorphous groups. This can be attributed to the surface-acting-agents. The improvement of spray deposition obtained on leaf surfaces when sprayed with an adjuvant (De Ruiter *et al.*, 1990) included in the fenhexamid mixture, may explain the decrease in *B. cinerea* incidence observed under the extremely high inoculation dosage applied (5 mg spores which might result in approximately 6 to 7 spores per mm^2 [Coertze and Holz, 1999]).

An exception was observed in the case of the super-spreader Hydrosilicote, which showed good qualitative and quantitative deposition values, but without the concomitant reduction in *Botrytis* incidence. The histopathology study indicated that this phenomenon might be caused by the indirect or direct influence of Hydrosilicote on *B. cinerea* on the grape leaf surfaces. Significantly shorter germ tubes developed on leaves treated with Hydrosilicote and Solitaire alone, compared with those on water-sprayed leaves. Elad and Ayish (1990) found that polymer adjuvants most often reduced germ tube development of *B. cinerea*. With only fenhexamid in the spray mixture, fungicide exposure on the leaf surface caused significantly collapse of the conidia and germ tubes. Germination and germ tube growth were inhibited when conidia came in contact with fenhexamid active ingredient. These modes of action of fenhexamid are in agreement with Hänßler and Pontzen (1999. However, similar to the biological efficacy study, less than expected *Botrytis* inhibition was achieved when Hydrosilicote was applied with fenhexamid in the histology study. Quantitative and qualitative deposition was similar when Solitaire or Hydosilicote was added with fenhexamid in the spray mix, but showed significantly more germination and longer germ tube lengths with the latter adjuvant in the spray mixture. Furthermore, significantly lower mortality occurred when Hydrosilicote was included with fenhexamid in the spray mixture compared with the Solitaire-fenhexamid or fenhexamid alone treatment. This might be attributed to some antagonistic effect in the fenhexamid and Hydrosilicote mixture. Rogiers *et al.* (2005) also showed that adjuvants might increase susceptibility to infection by *B. cinerea* by counteracting the positive effects of the fungicide by facilitating infection of *B. cinerea*. They demonstrated with scanning electron microscope images that some adjuvant-fungicide applications might disrupt epicuticular wax and that the severity of this disruption was dependent on the particular adjuvant used. Therefore, it might have been easier for *B. cinerea* to penetrate the cuticle and infect the leaf once some of the delicate leaf wax was damaged (Hall *et al.*, 1965; Blakeman, 1973; Knoche *et al.*, 1992; Rogiers *et al.*, 2005). It can be hypothesised that the epicuticular wax layer is a less effective protective barrier against invading pathogens when the orientation, composition or size of the surface wax platelets are disturbed (Rogiers *et al.*, 2005), while grapevine leaves may also exude some nutrients that promote *B. cinerea* development (Blakeman, 1975, 1993).

The concentration of the adjuvant surface-active-agents influences the potential outcome of droplet deposition on targeted surfaces (Holloway *et al.*, 2000; Gaskin *et al.*, 2005). Poor quantitative and qualitative deposition with adjuvants applied on upper and

lower leaves might be attributed to a wrong concentration of wetting and spreading agents when the given volume of spray solution with fenhexamid was applied. Designer`s main components belong to organosilicones, known to spread exceptionally well (Stevens, 1993; Gaskin *et al.*, 2000; Roggenbuck and Penner, 2000). These types of adjuvants are sensitive to the dosage applied (Gaskin *et al.*, 2004a). Too high concentrations inflict much lower surface droplet tension, which may cause excessive spreading with droplet run-off (Stevens, 1993; Gaskin *et al.*, 2000, Holloway *et al.*, 2000; Gaskin *et al.*, 2004b; Spanoghe and Steurbaut, 2004). WetCit applied with fenhexamid, also resulted in poor quantitative and qualitative deposition on leaves, which might be attributed to a sub-optimal dosage. Deposition of the fluorescent pigment following the WetCit treatments was visible as distinct large 'droplets' on upper and lower leaves, which is usually associated with insufficient or no surface-active-agents, as was observed for the fenhexamid- and water only treatments, respectively. Such spray-droplets might have a high contact angle after impaction on the deposition surface, and do not result in optimum wetting (Holloway, 1970; Matthews, 2008) associated with increased droplet repellence and rebound from the leaf surface (Watanabe and Yamaguchi, 1992; Brazee *et al.*, 2000; Stock and Briggs, 2000; Bargel *et al.*, 2006).

In general, quantitative and qualitative deposition were better on upper than lower leaf surfaces. Gaskin *et al.* (2005) reported that grapevine leaf surfaces could be classified in moderately 'difficult-to-wet' on the upper, and very 'difficult-to-wet' on the lower leaf surfaces. Studies done by Combellack *et al.* (2004) support these findings, and less spreading on the lower compared to upper grape leaf surface might be attributed to filamentous wax and sparsely hairy veins. Thus, variation in leaf morphology and epicuticular surface wax on upper and lower grapevine leaves can influence the wettability (Gaskin *et al.*, 2005; Holloway, 1993). This presents a dilemma for the grower as some adjuvant sprays optimised for the upper leaf surfaces may not effect adequate wetting on lower leaf surfaces, and if optimised with surfactants to the target lower leaf surfaces, spray is likely to be lost to run-off from the upper surface (Gaskin *et al.*, 2005). However, deposition following application of fenhexamid in combination with Agral 90, BB5, Break-thru S 240, Nu-film-17 and WetCit showed that in some cases the variation in quantitative coverage between upper and lower leaf morphology can be minimised. In the case of Biodew, quantitative deposition on the lower leaf surface was noticeably (15%) better than on upper leaf surfaces. The reasons for Biodew's disparate results from the other adjuvants are unknown, but might be due to a dosage-spray volume effect and/or the inherent qualities of this adjuvant.

The described quantitative and qualitative protocol showed that most adjuvants have the potential to improve deposition of a given quantity of spray applied to grapevine leaves, and therewith improved biological efficacy of the applied fungicide against *B. cinerea*. However, the potential deposition of spray droplets is a difficult process to predict. Adjuvant-fenhexamid combinations, spray volumes and adjuvant dosages may influence quantitative and qualitative deposition on upper and lower leaf surfaces. More research is therefore required under field conditions to custom-develop adjuvant recommendations for use in viticulture.

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Table 1. Properties of some adjuvants sourced in South Africa[1]

Trade name	Registration holder	Main Components	Possible properties	Chemistry classification	Grams pure active ingredient	Dosage
Agral 90	Kynoch Agrochemicals	Alkylated phenyl ethelene oxide condensate	Surfactants	Ethoxylated alkylphenols	940 g/L	18 mL/100L
BB5	Gouws and Scheepers	Nonyl phenol ethoxylate + acid + colour indicator	Acid + Surfactant	Acidifiers	600 g/L	100 mL/100L
Biodew	Gouws and Scheepers	Alcohol alcoxylate	Surfactants	-	606 g/L	25 mL/100L
Biofilm	Gouws and Scheepers	Propyl alcohol nonyl phenol etoxylate	Sticker	-	976 g/L	50 mL/100L
Break-thru S 240	Degussa Africa	Polyether- polymethylsiloxane-copolymer	Surfactants	Organosilicones	1000 g/L	50 mL/100L
Buffernat	Farmkem	Organic acid + alkali + wetting agents	Surfactants + Buffer	Buffer	536 g/L	50 mL/100L
Designer	UAP Crop Care	Organosilicone/synthetic latex	Surfactants	Latex + Silicone	250 g/L	125 mL/100L
Hydrosilicote	Villa Crop Protection	Silicone polyether copolymer blend	Surfactants	Silicones	1000 g/L	30 mL/100L
Leaf cote	Agrizone	Alkyl phenol ethoxylate	Surfactants	Ethoxylated alkylphenols	940 g/L	20 mL/100L
Li 700	UAP Crop Care	Phosphatidylchloline methylacetic acid and alkylpolyoxyethelene ether	Oil + Buffer	Plant oils	800 g/L	150 mL/100L
Nu-film-17	Miller chemical and fertilizer	Di-1-p-menthene	Sticker	Terpene oils	875 g/L	20 mL/100L
Nu-film-P	Miller chemical and fertilizer	Poly-1-p menthene	Sticker	Terpene oils	875 g/L	30 mL/100L
Solitaire	Safagric	Polyether-polymethylsiloxane-copolymer/vegetable oil	Oil + Surfactants	Silicone/Plant oils	300/650 g/L	50 mL/100L
Villa 51	Villa Crop protection	Isotridecanol	Surfactants	alkylpolyethylene glycol ether	918 g/L	100 mL/100L
WetCit	Citrus Oil Products	Borax/orange oil	Surfactants	-	10/50 g/L	50 mL/100L

[1]Data collected from product label, http:/www/nda.agric.za/act36/AR/Adjuvants.htm

Table 2. Analyses of variance for effects of block, leaf side and varying adjuvant treatments on median values for quantitative and qualitative deposition on upper and lower surfaces of Chardonnay grapevine leaves following spray application with a fluorescent pigment and fenhexamid, with or without selected adjuvants

Source	DF*	Quantitative deposition		Qualitative deposition	
		MS**	P***	MS	P
Upper leaf surface					
Block	2	0.5290	<0.0001	34164.08	<0.0001
Treatment	17	0.0272	0.0003	669.46	0.0094
Experiment Error	34	0.0068		260.81	
Sample Error	594	0.0023		74.61	
Corrected Total	647				
Lower leaf surface					
Block	2	1.3666	<0.0001	110815.82	<0.0001
Treatment	17	0.0268	0.0015	1290.62	0.0125
Experiment Error	34	0.0081		524.62	
Sample Error	594	0.0022		160.58	
Corrected Total	647				

*DF = Degrees of freedom
**MS = Mean sum of squares
***P = Probability

Table 3. Analyses of variance for effects of block, leaf side and varying adjuvant treatments on mean percentage incidence values of *B. cinerea* on artificially inoculated upper and lower surfaces of Chardonnay grapevine leaves following spray application with a fluorescent pigment and fenhexamid, with or without selected adjuvants

Source	Botrytis incidence		
	DF*	MS**	P***
Upper leaf surface			
Block	1	389.35	0.60
Treatment	17	18705.04	<0.0001
Experiment Error	17	1342.30	
Sample Error	828	410.29	
Corrected Total	863		
Lower leaf surface			
Block	1	5201.85	0.0058
Treatment	17	18769.50	<0.0001
Experiment Error	17	524.40	
Sample Error	828	417.71	
Corrected Total	863		

*DF = Degrees of freedom
**MS = Mean sum of squares
***P = Probability

Table 4. Pearson's correlation coefficients of median percentage quantitative and median qualitative spray values, and corresponding incidence of *B. cinerea* on upper and lower surfaces of Chardonnay grapevine leaves

Protocol	Infection	Upper leaf surface*	Lower leaf surface*
Qualitative	***B. cinerea***	0.585 (0.0173)	0.703 (0.0024)
Quantitative	***B. cinerea***	-0.538 (0.0317)	-0.560 (0.0242)

*Values are correlation coefficients and corresponding *P* values (in parenthesis) significant at *P* = 0.05

Table 5. Median values for quantitative (percentage fluorescent pigment deposition) and qualitative (distance in pixels between particles) deposition on upper and lower surfaces of Chardonnay grapevine leaves following spray application with a fluorescent pigment and fenhexamid, with or without selected adjuvants with corresponding mean percentage incidence of *B. cinerea* on these sprayed leaves following artificial inoculation

Treatment	Upper leaf surface			Lower leaf surface		
	Quantitative	Qualitative	Botrytis	Quantitative	Qualitative	Botrytis
Agral 90	17.05abc	29.32e	6.25fg	17.24a	36.13e	10.00gh
BB5	16.53a-d	30.39de	13.33efg	16.42ab	40.46de	15.83fg
Biodew	12.97d-g	35.48b-e	19.58b-f	15.22a-d	41.00de	16.67fg
Biofilm	18.63a	29.74e	10.00fg	13.30a-e	46.29b-e	20.42ef
Break-thru S 240	11.92efg	33.93b-e	16.67d-g	12.14b-f	43.16cde	19.17efg
Buffernat	14.23b-f	35.67b-e	15.83d-g	11.99c-f	49.90a-d	20.00ef
Designer	10.94efg	37.77a-d	15.00efg	8.30f	57.89a	30.83cd
Hydrosilicote	16.22a-d	31.25de	32.50bc	14.08a-e	41.15de	20.83ef
Leaf Cote	14.80a-e	31.32de	17.08c-g	10.26ef	51.07a-d	27.92cde
Li 700	17.29abc	32.36de	8.33fg	16.04abc	41.79de	16.67fg
Nu-film-17	11.78efg	36.23a-e	26.25b-e	11.51def	51.30a-d	37.08bc
Nu-film-P	13.62c-f	34.42b-e	13.33e-g	11.78c-f	54.40ab	30.83cd
Solitaire	17.77ab	29.08e	2.92g	14.07a-e	40.82de	16.25fg
Villa 51	14.06b-f	33.57cde	10.42fg	11.00def	44.39b-e	16.25fg
WetCit	9.22g	40.87abc	31.25bcd	9.83ef	51.35a-d	25.42def
Fenhexamid only	14.04b-f	35.66b-e	34.58b	13.62a-e	45.36b-e	40.83b
Water sprayed control	11.26efg	41.39ab	90.42a	8.93f	53.25abc	95.83a
Water (not inoculated)	10.78fg	43.91a	6.67fg	8.59f	50.88a-d	3.33h
LSD ($P < 0.05$)*	3.96	7.74	15.78	4.31	10.97	9.86

*Least significant difference: values in each column followed by the same letter do not differ significantly

Table 6. Analyses of variance for effects of block-, and varying adjuvant treatments on median values for quantitative and qualitative deposition on upper leaf surfaces of Chardonnay grapevine leaves following spray application with a fluorescent pigment and fenhexamid, with or without selected adjuvants

Source	DF*	Quantitative deposition		Qualitative deposition	
		MS**	P***	MS	P
Upper leaf surface					
Block	11	27.60	<0.0001	4095.21	<0.0001
Treatment	5	19.75	<0.0001	6107.75	<0.0001
Experiment Error	53	2.69		291.08	
Sample Error	490	0.00		0.00	
Corrected Total	559				

*DF = Degrees of freedom
**MS = Mean sum of squares
***P = Probability

Table 7. Analyses of variance for effects of block-, and varying adjuvant treatments on mean values for germination, mortality and germ tube length on the upper leaf surfaces of Chardonnay grapevine leaves following spray application with a fluorescent pigment and fenhexamid and/or selected adjuvants

Source	DF*	Germination		Mortality		Germ tube length	
		MS**	P***	MS	P	MS	P
Block	8	437.33	0.5442	352.42	0.0802	853.65	0.2931
Treatment	5	7426.98	<0.0001	6871.03	<0.0001	23193.37	<0.0001
Experiment Error	39	498.75		181.06		678.55	
Sample Error	280	205.51		105.33		425.87	
Corrected Total	332						

*DF = Degrees of freedom
**MS = Mean sum of squares
***P = Probability

Table 8. Median quantitative and qualitative deposition values on upper surfaces of glasshouse-grown grapevine (cv. Chardonnay) leaves after spray application with a mixture of a fluorescent pigment with fenhexamid alone or in combination with Hydrosilicote or Solitaire. Sprayed leaves were inoculated with dry conidia of *B. cinerea* and incubated for 48 hr at high relative humidity. Histological parameters, germination and mortality percentage of spores and germ tube length, were measured using epi-fluorescence microscopy on dissected leaf segments

Treatment	**Deposition**		***Botrytis cinerea* histopathology parameters**		
	Quantitative	**Qualitative**	**Germination (%)**	**Mortality (%)**	**Germ tube (µm)**
Hydrosilicote	2.43a	44.59b	76.73a	7.07c	59.02b
Solitaire	2.20a	44.16b	75.90a	8.84c	56.10b
Hydrosilicote + fenhexamid	2.60a	41.83b	60.38b	20.52b	27.83cd
Solitaire + fenhexamid	2.30a	39.89b	51.37c	31.19a	19.72d
Water sprayed control	1.34b	60.86a	77.34a	6.45c	71.50a
Fenhexamid only	1.71b	56.28a	57.85bc	28.14a	35.20c
LSD (*P* < 0.05)*	0.48	5.02	8.61	5.17	4.01

*Least significant difference: values in each column followed by the same letter do not differ significantly

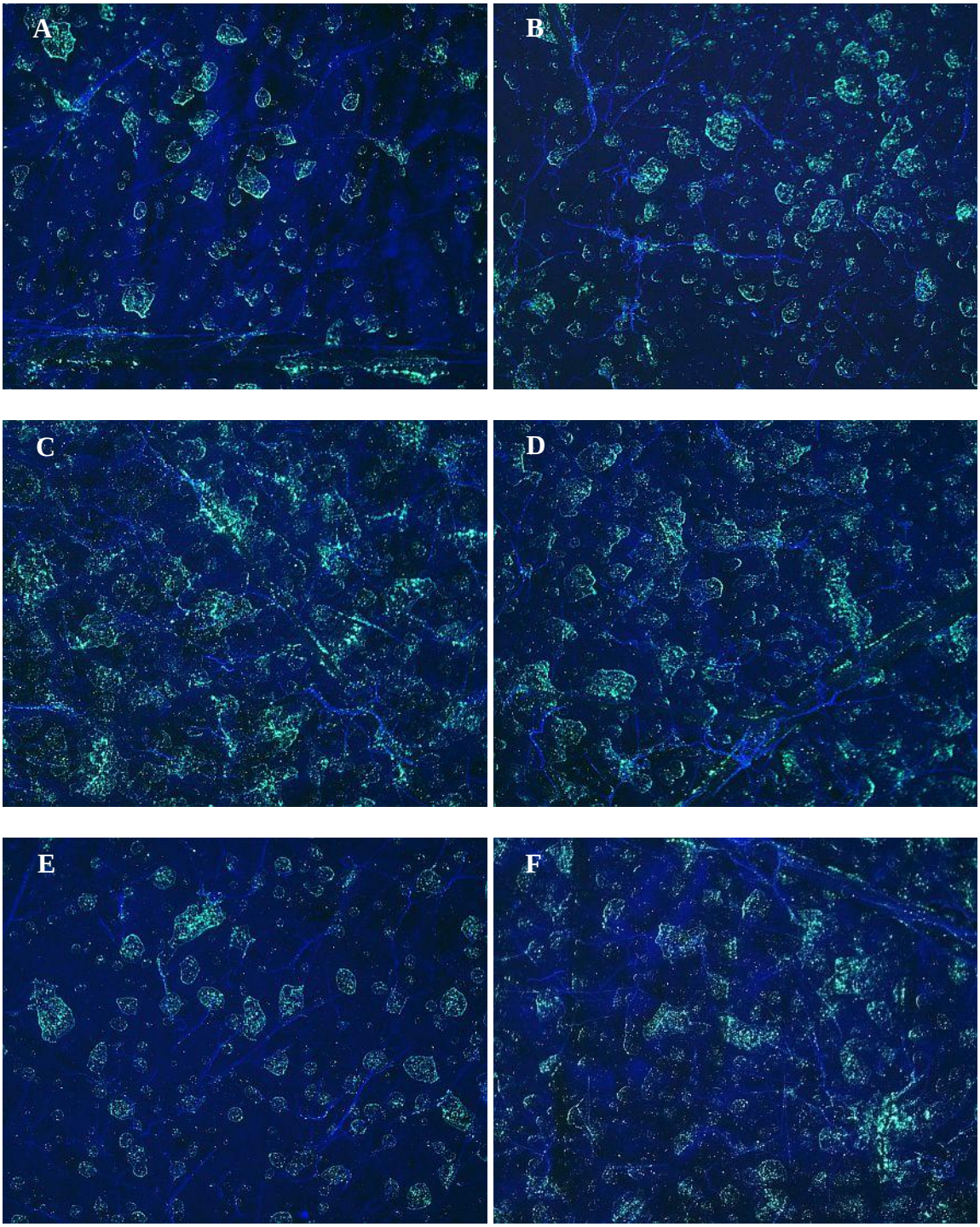

Figure 1. Digital images of upper surfaces of Chardonnay leaves (5th stage) sprayed with 1 mL of spray mixtures excluding adjuvants (**A** water and **B** fenhexamid), and mixtures including adjuvants (**C** Agral, **D** Hydrosilicote, **E** WetCit, and **F** Designer) and the SARDI Yellow Fluorescent Pigment and visualised under black light illumination at 10× magnification.

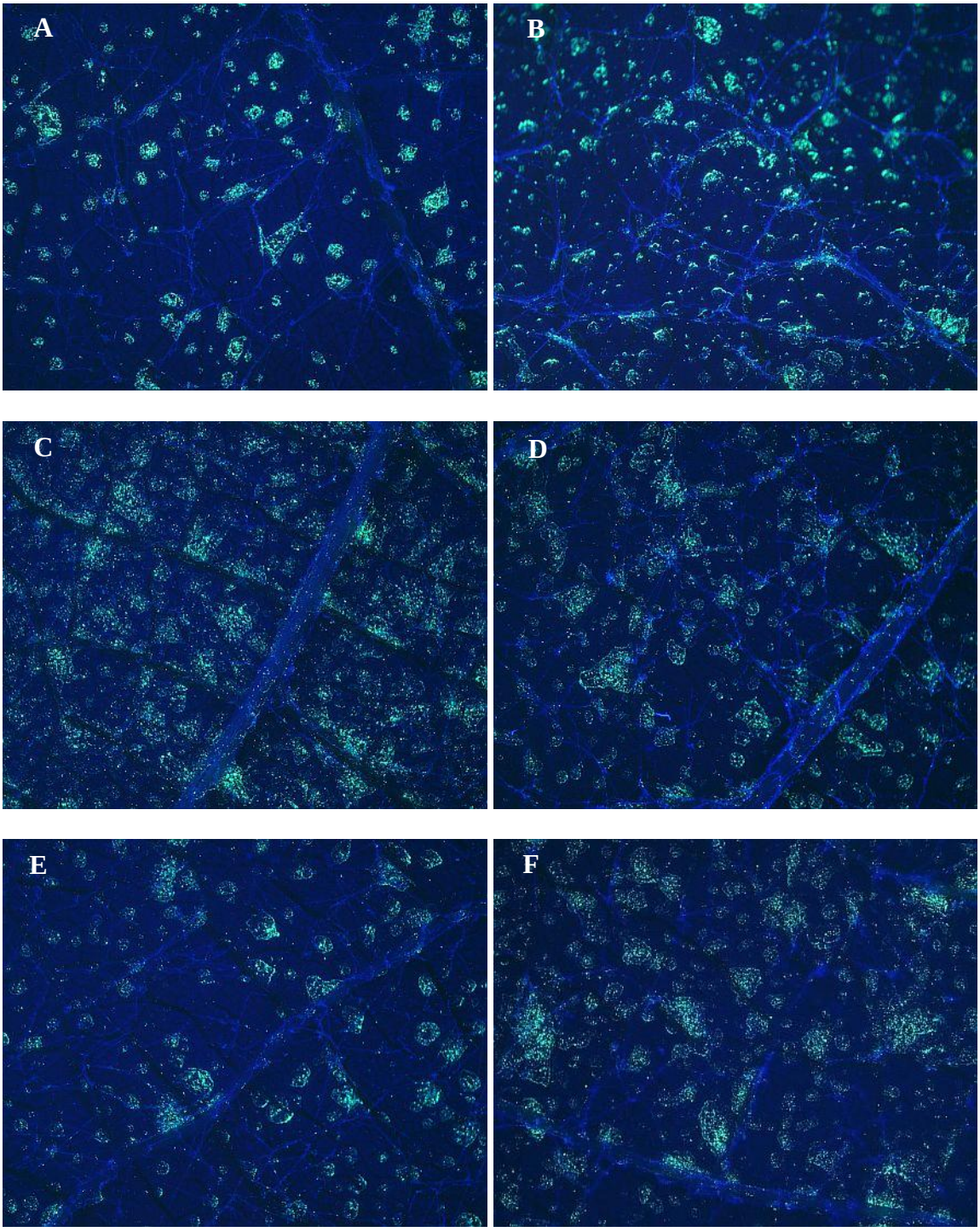

Figure 2. Digital images of lower surfaces of Chardonnay leaves (5th stage) sprayed with 1 mL of spray mixtures excluding adjuvants (**A** water and **B** fenhexamid), and mixtures including adjuvants (**C** Agral, **D** Hydrosilicote, **E** WetCit, and **F** Designer) and the SARDI Yellow Fluorescent Pigment and visualised under black light illumination at 10× magnification.

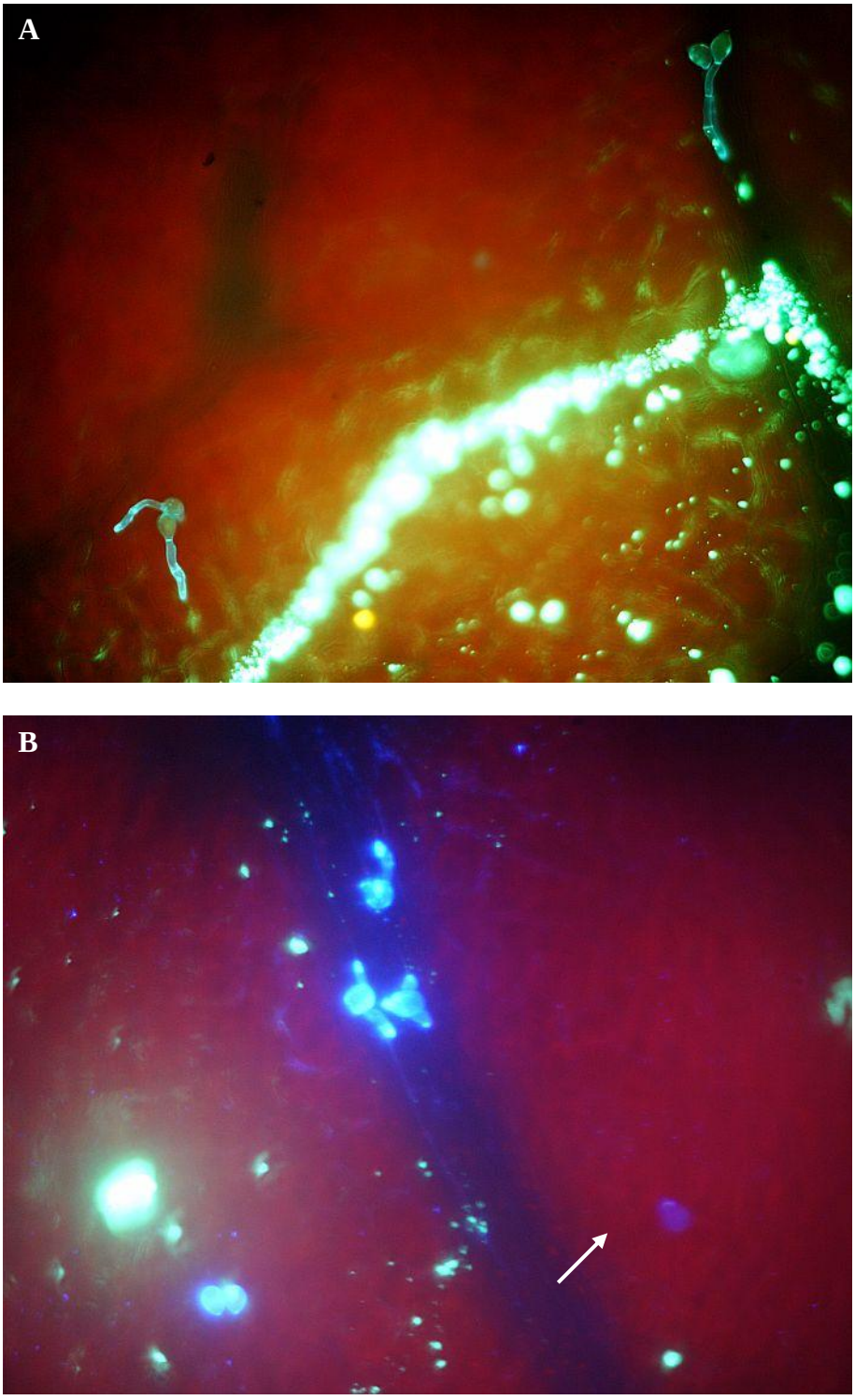

Figure 3. Epifluorescence microscope images (400× magnification) of *B. cinerea* on Chardonnay grapevine leaves that were sprayed with a mixture of fluorescent pigment, fenhexamid and selected adjuvants. **A**: Hydrosilicote + fenhexamid (24 h after inoculation); **B**: Solitaire + fenhexamid (48 h after inoculation) with dead conidia appearing blue-black under filter set 06, 18 (see arrow).

3. EFFECTS OF ADJUVANTS ON DEPOSITION EFFICIENCY OF FENHEXAMID SPRAYS APPLIED TO CHARDONNAY GRAPEVINE FOLIAGE

ABSTRACT

Adequate spray deposition on susceptible grapevine tissue is an essential requirement for effective chemical control of economically important diseases, such as grey mould, powdery mildew and downy mildew. The objective of this study was to evaluate the potential of some agricultural adjuvants to improve foliar spray deposition. Quantitative and qualitative deposition assessment was done by means of a spray assessment protocol using fluorometry, photomicrography and digital image analyses. The Furness visual droplet-rating technique was also included in initial assessments. Both assessment protocols showed that quantitative spray deposition increased with increasing spray volume applications of 40 L/ha to 750 L/ha with a STIHL SR400 motorised backpack mistblower, but decreased at 900 L/ha, possibly due to run-off. Addition of selected spray adjuvants at 600 L/ha volume demonstrated improved quantitative and qualitative deposition. Agral 90, BB5, Nu-film-P, and Solitaire significantly improved deposition on upper and lower leaf surfaces compared with the fenhexamid only and water sprayed control. Break-thru S240 and Villa 51 did not improve quantitative deposition, although remarkably better qualitative deposition was obtained. An adjuvant dosage effect (within the registered dosage range) was evident, especially those retained on the upper leaf surfaces. Agral 90 and Nu-film-P effected significant improvement of spray deposition at the higher, but not at the lower dosage tested. Solitaire improved deposition at the lower dosage tested, whereas reduced deposition at the higher dosage was attributed to excessive spray run-off. No significant improvement of spray deposition was observed for both dosages tested with Villa 51. Spray mixtures with adjuvants Agral 90 and Solitaire yielded similar deposition values at 600 L/ha compared with the fenhexamid only control at 900 L/ha, but reduced deposition at the higher spray volume, possibly due to spray run-off. This study clearly demonstrated the potential of adjuvants to improve quantitative and qualitative deposition, but highlights the necessity to match adjuvant dosages and application volumes on the spray target to achieve maximum spray deposition.

INTRODUCTION

Grey mould (*Botrytis cinerea*), powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*), which are economically important diseases of grapevines (*Vitis vinifera* L.) (Bulit and Dubos, 1994), are mainly controlled by means of fungicide spray applications (Matthews, 1997). Sufficient deposition of fungicide on grapevine leaves and bunches is an essential requirement for effective chemical control of these pathogens. Grape growers invest heavily in chemical products and routine spray applications each year for disease control (Van Rooi, 2001). However, insufficient deposition of fungicides on susceptible grapevine tissue (i.e. target sites), coinciding with favourable conditions, results in large losses of yield and grape quality.

Holloway (1970) and Gaskin *et al. (*2005) demonstrated that fungicide retention is negatively correlated with surface roughness and epicuticular wax. Gaskin *et al.* (2005) showed that grape foliage is moderately 'difficult-to-wet' on the upper, and very 'difficult-to-wet' on the lower surfaces. They demonstrated that surface roughness increased the contact angle of solution droplets. This can influence the rebound of spray droplets, spray run-off and less contact between the deposit and leaf surface (Wirth *et al.*, 1991; Hunche *et al.*, 2006). The water repellent cuticular waxes (Bargel *et al.*, 2006) are an important site to consider for improvement of agrochemical wetting and retention of active ingredient deposition (Holloway, 1970; Bukovac *et al.*, 1986; Bukovac and Petracek, 1993; Holloway, 1993; Wagner *et al.*, 2003).

Many adjuvants are reported to improve deposition of the pesticide active ingredient (Ryckaert *et al.*, 2007) by the surfactant component in their formulations (De Ruiter *et al.*, 1990; Gaskin *et al.*, 2002), which may increase the wettability of droplets and spread on the target surface (Hall *et al.*, 1993, 1998). Improved spray deposition will undoubtedly improve disease control, as was shown in a recent laboratory study (Chapter 2). This study showed the importance of improved quantitative as well as qualitative deposition, which resulted in a reduction in the incidence of *B. cinerea* on grapevine leaves. In field trials conducted in New Zealand, the inclusion of an adjuvant at reduced spray application volumes improved deposition on a variety of crops (Gaskin *et al.*, 2000a, 2000b, 2001a, 2001b, 2002, 2004a, 2004b). Adjuvants may improve pesticide application from preventative high-dose and high-

volume applications to a more effective preventative low-dose (Ryckaert *et al.*, 2007) low-volume application (Gaskin *et al.*, 2002).

It is estimated that 40-50% of foliar sprays do not reach the target sites with commercial high volume application to the point of run-off (Matthews, 1997). These droplets normally have high contact angles with the hydrophobic leaf surface (Holloway, 1970; Gaskin *et al.*, 2005; Bargel *et al.*, 2006). High droplet tension and poor droplet contact area with the plant surface (possible liquid/air surface tension) means less droplet wettability (Watanabe and Yamaguchi, 1992; Wagner *et al.*, 2003; Bargel *et al.*, 2006). Under such conditions, droplet run-off can be expected to be very high (Holloway, 1970). Lower volume application may influence droplet size, and may increase the quantity of smaller droplet deposits (Fourie *et al.*, 2009). According to Bateman and Jessop (2008), motorised mistblowers can achieve good deposition with the combination of air assistance and production of smaller droplets (i.e. without spraying to run-off). However, spray droplet retention may still be a significant factor on the water repellent plant surface (Wagner *et al.*, 2003). Poor application efficiency might also arise from less contact between fungicide and the leaf surface waxes with low applied volumes, where small droplets can be trapped by hairs (Holloway, 1970; Wagner *et al.*, 2003). Droplet retention can be enhanced by applying an appropriate adjuvant.

Surfactants in adjuvants have the ability to lower droplet surface tension and increase plant cuticle wettability and droplet spreading properties, which results in improved quantity and quality of deposition (Hall *et al.*, 1993; Ryckaert *et al.*, 2007). However, it is hypothesised from previous research that adjuvant dosage may play an important role on deposition (Chapter 2). Too low dosage might not sufficiently reduce droplet surface tension to ensure the spreading effect needed to improve quantitative and qualitative deposition. On the contrary, too high adjuvant dosage might lower droplet surface tension to the extent that run-off is increased. Spray volume might also be an important factor influencing deposition properties of adjuvant spray mixtures. Gaskin *et al.* (2002) found that use of organosilicone adjuvants at higher spray volumes on wine grapes resulted in less retention. Variables, such as larger droplets in combination with reduced surface tension may increase the run-off effect. Gaskin *et al.* (2002) highlighted the importance of matching adjuvant dosage with application volume, spray retention and distribution on grapevine target surfaces. In order to develop useful prescriptions for adjuvants by determining water volumes and adjuvant dosage, an accurate quantitative and qualitative deposition protocol should be employed. A

variety of methods have been used to assess spray coverage in vineyards. These methods include visual assessment on water-sensitive paper, bioassay and chemical residues recovery techniques (Holownicki *et al.*, 2002). Visual assessment was greatly improved by adding fluorescent dyes to the spray mixture, followed by illumination of deposits under black light (Furness, 2000). Furness *et al.* (2006) developed a droplet rating chart, and used fluorescent dye to estimate the number and size of droplets per cm^2. The advantage of this method is that it is quick, cheap and easy to use. However, visual deposition is dependent on human discretion and may lack quantitative measuring and speed of measurement (Derkson and Jiang, 1995). Bioassay and chemical residue recovery techniques provide an overall assessment of the quantity of spray deposits, but residue levels alone do not give a good indication of application quality such as uniformity of spray distribution (Holownicki *et al.*, 2002). Efficacy of agricultural chemicals is influenced by both quantitative- (amount of deposit) and qualitative deposition (distribution of deposit) (Chapter 2). If the quality of the deposited dosage is poor, efficacy may also be poor, even if the correct quantity or chemical dose is impacted. Quantitative and qualitative deposition assessment protocols were developed and validated by Brink *et al.* (2004) and Fourie *et al.* (2007), using fluorometry, photomicrography and digital analyses. Furthermore, the accuracy of these protocols has been proven in a recent study on agricultural spray adjuvants, whereas reduced *B. cinerea* incidence were most often associated with improved quantitative and qualitative deposition (Chapter2).

The objective of this study was to use recently developed deposition assessment protocols to visualise and determine the potential quantitative (Furness, 2000; Brink *et al.*, 2004, 2006; Furness *et al.*, 2006) and qualitative (Fourie *et al.*, 2007) effects of some agricultural tank mix adjuvants on foliar spray deposition as influenced by varying dosage and volume in a Chardonnay vineyard.

MATERIALS AND METHODS

Selected adjuvants were evaluated in commercial Chardonnay vineyards in the Western Cape, Stellenbosch region in the 2006/07 harvest season. The study was divided into four field trials: (A) determination of optimum volume delivery using a STIHL SR400 motorised backpack mistblower (Andreas Stihl AG and Co., Badstr. 115, Waiblingen,

Germany), which was to be used in the subsequent trials; (B) evaluate the vineyard performance of adjuvants that were previously evaluated in a laboratory trial (Chapter 2); and to determine if (C) adjuvant dosage and (D) spray volume influenced deposition on grapevine leaves. Trial (A) and (B) were conducted on a smaller and less dense grapevine canopy [±55 × ±110 × ±840 cm (w × h × l)] than trial (C) and (D) [±75 × ±113 × ±840 cm (w × h × l)].

All sprayed vineyard sections consisted of 6 vines, which were sprayed from both sides of the canopy. Between spray plots, 6 buffer vines were left unsprayed, as well as an unsprayed vineyard row adjacent to each plot. Experimental layout in all trials were randomised complete block designs, where each treatment combination was repeated three times in separated vineyard sections. All trials were repeated once.

(A) Calibration and evaluation of motorised STIHL backpack mistblower

Spray trails were conducted using a STIHL SR400 backpack mistblower, which was used at 7500 rpm (full-throttle). According to the specifications of the STIHL SR400 mistblower, an air velocity of 101m/s (330ft/s) is produced at the nozzle air cap (Andreas Stihl AG and Co., Badstr. 115, Waiblingen, Germany). The air cap was fitted horizontally, blowing upwards. The STIHL metering nozzle settings (sprayer settings) were used in each of the trials to calibrate and evaluate various volume applications in a Chardonnay vineyard. When calibrated with the motor operating at full-throttle, the flow rate was measured for each sprayer setting by determining in triplicate the time needed to empty a spray volume of 1000 mL. The flow rate for setting A, B, C, D, E and F was calculated as 99, 632, 1270, 1818, 2286 and 2603 mL/min, respectively. A typical walking speed of 0.5 m/s was used for treatment application to vineyard sections, which resulted in spray volumes of approximately 40, 225, 450, 600, 750 and 900 L/ha.

Each treatment was applied with SARDI yellow fluorescent pigment (400 g/L, EC; South Australian Research and Development Institute, Loxton SA 5333 Australia) at 2 mL/100 L (Furness, 2000) for subsequent spray deposition analyses. Application were conducted as described above.

Leaf sampling and spray assessment. One hour after application (after leaves had dried off), 5th stage outer canopy leaves (more exposed foliage) were randomly collected from the 3 vines in the middle of a sprayed plot. Each sample consisted of 20 randomly selected leaves,

10 from each side of the canopy. Petioles were then placed in small 14 mL McCartney bottles containing 3% water agar + 100 ppm benomyl. Leaves were then transported in cooled containers, to ensure fresh turgid leaves for deposition assessment on upper and lower leaf surfaces.

Quantitative and qualitative deposition assessments on leaves were done by means of protocols developed and validated by Brink *et al.* (2004) and Fourie *et al.* (2007), using fluorometry, photomicrography and digital analyses. The sprayed plant material was illuminated under black light and visualised using a Nikon SMZ800 stereomicroscope at 10× magnification. Digital photos were taken with a Nikon DMX 1200 camera. Leaves in the first repetition were photographed using the "Norm" sensitivity setting for the camera software (ACT for Nikon DMX 1200) and leaves in the second and third repetition were taken using the "Max" setting.

Image analyses were performed with Image-Pro Plus version 6.2 for Windows (Media Cybernetics, www.mediacy.com) software. By using the measurement tools, these images could be analysed quantitatively and qualitatively. Quantitative analyses involved removal of green channels from the image, followed by quantification of the percentage area covered by the foreground elements (deposited pigment) of the binarised image (Brink *et al.*, 2004, 2006). For qualitative analysis, a combined Euclidian distance map and skeleton is created on the binarised image, with absolute white indicating the furthest distance from a particular foreground element. Subsequent analysis of grey-scale values indicates spray deposition quality. Thus, smaller values (fewer white pixels measured; i.e. particles closer together) indicate a better quality of deposition (Chapter 2).

Spray deposition assessment with the Furness droplet rating chart. For the STIHL volume application trial only, the Furness droplet-rating protocol was included as an alternative spray deposition assessment (Furness, 2000; Furness *et al.*, 2006). Four visual assessments of leaves were made using black light illumination at similar positions to those used for photomicrography. Coverage was rated from a deposition coverage chart 0 to 7, which indicate no coverage to effective visual coverage on grapevine leaves.

(B) Adjuvant application at recommended dosages

Selected adjuvants at recommended and commonly used dosages were applied at 600 L/ha to determine deposition on upper and lower leaf surfaces. Properties of these adjuvants are summarised in Table 1. Each adjuvant was applied with fenhexamid (Teldor® 500 SC, Bayer CropScience, P.O. Box 143, Isando, 1600, South Africa) at the recommended dose (75 mL/100 L water; Nel *et al.*, 2003) and a fluorescent pigment (400 g/L, EC, South Australian Research and Development Institute, Loxton SA 5333 Australia) at 2 mL/100 L (Furness, 2000) at 600 L/ha (i.e. STIHL setting D). The spray reservoir was thoroughly cleaned with a dilution of Scrubbs ammonia in water (1:250), where after a triple rinse with deionised water was conducted prior to the next sprayed treatment. Similarly to (A), leaves were sampled and quantitative and qualitative deposition assessed.

(C) Adjuvant application at varying label recommended dosages

Agral 90, Nu-film-P, Solitaire and Villa 51 were each applied at a lower and higher label recommended dosage to determine the influence of adjuvant concentration on spray deposition. Spray application was conducted as in (B). Leaf sampling and quantitative and qualitative deposition assessment was done as described in (A).

(D) Adjuvant application at different volumes

This trial was conducted to test the deposition effect of Agral 90 and Solitaire at a fixed dosage but with different volume applications. Adjuvant spray mixes were similar as in (B). Spray application were conducted at 225 L/ha, 600 L/ha and 900 L/ha. Leaf sampling and quantitative and qualitative deposition assessment were done as described in (A).

Statistical analyses

Median values of quantitative and qualitative fluorescent pigment deposition on upper and lower leaf surfaces were subjected to the appropriate analysis of variance and linear regression analysis using SAS v. 8.2 statistical software (SAS Institute, 1999). Student`s t-Least Significant Difference were calculated at 95% significance level to compare means of significant effects (Snedecor and Cochran, 1967).

RESULTS

(A) Calibration and evaluation of motorised STIHL backpack mistblower

The addition of the fluorescent pigment to the spray mixture allowed clear visualisation of spray deposition on leaves under illuminated black light (Fig. 1). Remnants of droplets containing the fluorescent pigment on the leaf surface can clearly be seen as an aggregation of pigment particles in distinct circular patterns when sprayed leaves are illuminated with black light. When spray volume was increased from 225 L/ha to 600 L/ha (spray setting B and D, respectively) distinct circular 'droplets' increased in size and became more amorphous (Fig. 1 A and B). When spray volume was increased to 900 L/ha (spray setting F) the droplet effect likewise increased, but signs of run-off (less deposited active ingredient) were clearly visible (Fig. 1 C).

Camera setting had no significant interaction for quantitative data ($P > 0.05$; Table 2), but a significant camera setting × sprayer setting interaction was observed for qualitative data ($P < 0.01$) (Table 2). This interaction was attributed to the more sensitive "Max" camera setting that more clearly showed statistically poorer deposition at the highest sprayer setting, which images taken on the "Norm" camera setting could not discern. This interaction was ignored in the further interpretation of the data and the data for "Max" and "Norm" settings combined. No other significant interactions were observed ($P > 0.05$), and significant effects occurred for sprayer setting ($P < 0.0001$ and $P < 0.0001$) and leaf side ($P = 0.0372$ and $P < 0.0001$) for quantitative and qualitative data, respectively (Table 2). A Pearson's correlation procedure of median quantitative with qualitative deposition values indicated reasonably good correlation on upper [$R^2 = -0.594$ ($P < 0.0001$)] and lower [$R^2 = -0.520$ ($P < 0.0001$)] leaf surfaces (Table 3).

For Furness data, a significant effect was observed for sprayer setting and leaf side interaction ($P < 0.0001$; Table 2). Therefore, Furness data were analysed separately for leaf sides. ANOVA of these data showed significant effects for sprayer settings ($P < 0.0001$ and $P = 0.0054$ for upper and lower leaf surfaces, respectively; ANOVA tables not shown). Pearson's correlation procedure conducted on mean Furness droplet-ratings with median quantitative and qualitative deposition values indicated better correlation on upper [$r^2 = 0.636$

($P < 0.0001$) and $r^2 = -0.669$ ($P < 0.0001$), respectively] than on lower [$r^2 = 0.207$ ($P = 0.0056$) and $r^2 = -0.375$ ($P < 0.0001$), respectively] leaf surfaces (Table 3).

Quantitative deposition. Quantitative deposition on upper (6.22%) and lower (4.02%) leaf surfaces were significantly different ($P < 0.0372$). An increase in spray volume application by sprayer setting generally resulted in an increase of quantitative deposition on leaves (Table 4). Spray application conducted at sprayer settings A, B and C (40, 225 and 450 L/ha, respectively) resulted in significantly lower quantitative deposition (0.85%, 2.61% and 4.64%, respectively) than with higher spray volumes applied at settings D, E and F (600, 750 and 900 L/ha, respectively). Although not statistically significant, an increase in volume application from 600 L/ha to 750 L/ha resulted in slightly improved quantitative deposition (7.53% to 7.81%), while a further increase from 750 L/ha to 900 L/ha proportionally decreased quantitative deposition by 4.7% to 7.45%.

Qualitative deposition. Qualitative deposition on upper (69.30) and lower (116.54) leaf surfaces were significantly different ($P < 0.0001$). Similar to quantitative deposition, an increase in spray volume application generally resulted in improved qualitative deposition on leaves (Table 4). Significantly higher (i.e. poorer) qualitative deposition values were observed for spray application conducted at 40 L/ha and 225 L/ha (192.43 and 91.86) than at 450 L/ha to 900 L/ha (74.26 to 58.09). Although not significant, an increase in volume application from 450 L/ha to 750 L/ha increased quality of deposition (74.26 to 58.09), but a further increase from 750 L/ha to 900 L/ha proportionally decreased qualitative deposition by 22% to 74.29.

Spray deposition assessment with the Furness droplet rating chart. On upper leaf surfaces, an increase in spray volume application generally resulted in an increase in droplet ratings, using the Furness droplet-rating chart (Table 5). At 40 L/ha, fluorescent pigment deposition was not visible to the eye, which resulted in ratings of 0 for both leaf surfaces (Table 5). Significantly lower deposition was rated for spray application conducted at 40 L/ha to 450 L/ha (0.00 to 1.58) than at 600 L/ha to 900 L/ha (3.75 to 4.27). Increased volume application from 600 L/ha to 750 L/ha resulted in significantly increased Furness ratings (3.75 to 4.91). However, when spray volume increased from 750 L/ha to 900 L/ha, deposition as determined by the Furness protocol decreased by 13%.

On lower leaf surfaces, deposition values were remarkably lower than on upper leaf surfaces. Nonetheless, deposition followed a similar trend as on upper leaf surfaces, but a

decrease in deposition was not observed when spray volume was increased from 750 to 900 L/ha. The significant sprayer setting × leaf side interaction observed in Table 2 can be explained by this observation.

(B) Adjuvant application at recommended dosages

Analysis of variance of deposition data indicated a significant treatment × leaf side interaction for quantitative deposition (P = 0.0021), but not for qualitative deposition (P = 0.3949; Table 6). The data for upper and lower leaf surfaces were therefore analysed separately and significant treatment main effects were observed for quantitative and qualitative deposition assessments (P < 0.0001; ANOVA table not shown).

Upper leaf surfaces. The fenhexamid sprayed control showed statistically similar quantitative deposition (1.39%) as the water sprayed control (1.36%; Table 7). When any of the spray adjuvants Agral 90, BB5, Nu-film-P or Solitaire was included in the spray mixture, significantly improved quantitative deposition (2.43% to 2.13%) was observed. Break-thru S 240 and Villa 51 (1.35% and 1.12%, respectively) showed quantitative deposition statistically similar to the fenhexamid and water sprayed control.

Qualitative deposition of the fenhexamid sprayed control (64.66) was statistically similar to the water sprayed control (62.84). All adjuvant treatments improved qualitative deposition (41.85 to 58.56), whereas significantly improved deposition was observed when Agral 90, BB5, Break-thru S 240, Nu-film-P or Solitaire (41.85 to 50.18) were included in the spray mixture. Although not at statistically significant margins, Villa 51 improved qualitative deposition by 9% compared to the fenhexamid control application.

Lower leaf surfaces. Quantitative deposition of the fenhexamid control (0.39%) was similar to the water sprayed control (0.30%). All adjuvant treatments improved quantitative deposition (0.67% to 0.85%), whereas significantly improved deposition was observed when Agral 90, BB5, Nu-film-P or Solitaire (0.70% to 0.85%) was included in the spray mixture. Although not statistically significant, markedly better quantitative deposition was observed for Break-thru S 240 and Villa 51 (0.69% and 0.67%, respectively) compared with the control applications.

Qualitative deposition of the fenhexamid control (109.41) was statistically similar to the water sprayed control (117.82). All treatments with an adjuvant in the sprayed mixture

(76.28 to 88.24) significantly improved qualitative deposition compared with the fenhexamid and water sprayed controls.

(C) Adjuvant application at varying label recommended dosages

With an increase in adjuvant dosage, certain adjuvants visually increased quantity and quality of deposition (Fig. 3 A and B), while other adjuvants visually decreased deposition on upper leave surfaces (Fig. 3 C and D). Villa 51 showed visually similar quantitative and qualitative deposition (Fig. 3 E and F).

Analysis of variance of deposition data indicated a significant interaction between treatment, dosage and leaf side for quantitative deposition ($P < 0.01$), but not for qualitative deposition ($P = 0.3423$; Table 8). The data for upper and lower leaf surfaces were therefore analysed separately and indicated significant interaction between treatments and dosages ($P < 0.0001$ and $P = 0.0106$, respectively; ANOVA not shown).

Upper leaf surfaces. Lower and higher dosages of Agral 90, Nu-film-P and Solitaire influenced quantitative deposition significantly, but not for Villa 51 (Table 9). Quantitative deposition observed with Agral 90 and Nu-film-P was significantly increased at the higher dosages (1.58% to 2.44% and 1.99% to 3.41%, respectively), whereas Solitaire significantly reduced deposition at the higher dosage (2.11% to 1.22%). Villa 51 showed statistically similar quantitative deposition values for the different spray dosages applied (1.51% and 1.82%). Relative to the fenhexamid control (1.69%), Solitaire markedly increased quantitative deposition (2.11%), whereas Agral 90 and Nu-film-P significantly improved quantitative deposition at the higher dosage (2.44% and 3.41%, respectively). Quantitative deposition values for Villa 51 did not differ significantly from the fenhexamid control.

Lower and higher dosages for Agral 90, Nu-film-P and Solitaire influenced qualitative deposition on upper leaf surfaces significantly. The higher dosage significantly improved qualitative deposition of Agral 90 and Nu-film-P compared with the lower dosage (53.41 to 39.87 and 46.99 to 39.48, respectively). However, Solitaire significantly reduced qualitative deposition at the higher dosage (43.36 to 51.98). Villa 51 showed statistically similar qualitative deposition values (48.17 and 44.40) for higher and lower dosages. Qualitative deposition relative to the fenhexamid control (52.46) was significantly improved for Agral 90

(39.87), Nu-film-P (39.48), Solitaire (43.36) and Villa 51 (44.40) at the best application dosages.

Lower leaf surfaces. Quantitative deposition values were much lower than what was observed on upper leaf surfaces (Table 9). Quantitative deposition was significantly increased when the higher dosage was applied for Agral 90 (0.20% to 0.34%) and when the lower dosage was applied for Solitaire (0.23% to 0.40%). Nu-film-P and Villa 51 showed statistically similar deposition for higher and lower dosages (0.40% to 0.36% and 0.27% to 0.40%, respectively). All adjuvant treatments resulted in statistically similar quantitative deposition compared with the fenhexamid treatment (0.29%).

Qualitative deposition significantly increased when the higher dosage was applied for Agral 90 (135.73 to 105.40). All other adjuvant treatments showed statistically similar qualitative deposition values (135.73 to 101.38) compared with the fenhexamid control (120.78).

(D) Adjuvant application at different volumes

In most cases, an increase of spray volume from 225 L/ha to 600 L/ha resulted in increased deposition (example for Agral 90 shown in Fig. 4 A-D). However, at 900 L/ha clear signs of run-off was observed on upper leaf surfaces (Fig. 4 E), which resulted in less pigment deposited compared with 600 L/ha (Fig. C). An increase in spray volume generally showed better visual quantitative and qualitative spray deposition on lower surfaces of leaves (Fig. 4 B, D and F).

Analysis of variance of quantitative and qualitative deposition data showed a significant interaction for treatment, volume and leaf side ($P = 0.0064$ and $P = 0.0157$, respectively; Table 10). Data for upper and lower leaf surfaces were therefore analysed separately. A significant treatment with volume effect was observed for quantitative and qualitative deposition on upper leaf surfaces ($P = 0.0003$ and $P < 0.0001$, respectively; Tabel 11). Treatment with volume interaction for quantitative and qualitative deposition was not significant on lower leaf surfaces ($P = 0.2424$ and $P = 0.0917$; respectively), although a significant volume effect was observed ($P < 0.0001$; Table 11).

Upper leaf surfaces. Quantitative deposition of fenhexamid alone at 225 L/ha (0.50%) was significantly lower compared to applications of fenhexamid with Agral 90 (1.10%) or Solitaire (1.28%; Table 12). Application at 600 L/ha significantly increased quantitative deposition for fenhexamid alone (1.74%), Agral 90 (2.63%) and Solitaire (2.61%). Applications at 900 L/ha showed that only fenhexamid alone (2.42%) significantly improved quantitative deposition compared with 600 L/ha, whereas Agral 90 (1.77%) and Solitaire (1.63%) showed significant less quantitative deposition.

Qualitative deposition of fenhexamid alone at 225 L/ha (83.38) was significant poorer compared to Agral 90 (56.37) and Solitaire (56.92). Application at 600 L/ha significantly increased qualitative deposition for fenhexamid (50.69), Agral 90 (39.56) and Solitaire (40.87). When applied at 900 L/ha, only fenhexamid (42.83) significantly improved qualitative deposition, while Agral 90 reduced qualitative deposition by 15% and Solitaire significantly reduced deposition by 28%.

Lower leaf surfaces. Quantitative deposition on lower leaf surfaces was much lower than levels observed for upper leaf surfaces. Deposition following sprays at 225 L/ha with fenhexamid alone (0.1081%) was lower than for Agral 90 (0.14%) and Solitaire (0.21%; Table 12). When spray volume was increased to 600 L/ha, quantitative deposition increased for fenhexamid alone (0.32%), whereas a significant quantitative increase was observed for the Agral 90 (0.55%) and Solitaire treatments (0.65%). Although not significant, applications at 900 L/ha indicated that fenhexamid (0.51%) and Agral 90 (0.73%) improved quantitative deposition, whereas Solitaire showed statistically similar, although slightly lower quantitative deposition (0.51%).

At 225 L/ha, qualitative deposition was lower for fenhexamid alone (152.25) than for the Agral 90 treatment (137.95) and significantly lower compared with Solitaire (120.63). An increased in spray volume to 600 L/ha significantly increased qualitative deposition for the fenhexamid alone (103.29), Agral 90 (91.68) and Solitaire (93.03) treatments. Although not significant, applications at 900 L/ha indicated improved qualitative deposition for the fenhexamid alone (91.68 to 82.73), Agral 90 (93.03 to 90.27) and Solitaire (103.29 to 99.52) treatments.

DISCUSSION

In this study, two deposition assessment protocols were initially compared. The Furness visual droplet-rating technique (Furness, 2000; Furness *et al.*, 2006) was quick and very user friendly, but this subjective protocol was less sensitive compared to the Brink protocol, which also uses fluorometry, but employs digital photomicrography and image analyses to obtain quantitative and qualitative deposition assessments (Brink *et al.*, 2004; 2006; Fourie *et al.*, 2007). Smaller quantities of deposited pigment were not always easy to observe with the naked eye, whereas the Brink protocol could assess these amounts on leaves (i.e. at 40 L/ha). This might also explain why the Furness droplet-ratings correlated better with the quantitative and qualitative protocol on the upper than on the lower leaves, where less quantities of fluorescent pigment particles were deposited. Visual assessment is dependent on human discretion, and the human eye lacks quantitative (Derkson and Jiang, 1995) and possibly qualitative measuring. Previous laboratory studies showed that fenhexamid deposition as determined by the Brink deposition assessment protocol correlated well with *B. cinerea* control on grapevine leaves and bunches (Brink *et al.*, 2005; Fourie *et al.*, 2007; Chapter 2). Therefore, the Brink protocol can be considered as very accurate to predict fungicide deposition in a disease control model, and was therefore employed to determine further deposition using spray adjuvants under field conditions.

From the evaluation of the STIHL SR400 motorised backpack mistblower, which uses air-shear sprayer technology where the spray mixture is atomised and transported by low volumes of wind at high speed, it was obvious that an increase in spray volume resulted in increased quantitative and qualitative deposition. Relative to the upper leaf surfaces, lower leaf surfaces were generally poorly covered. However, deposition decreased at higher volumes of 900 L/ha, most likely after the possible point of droplet run-off was reached. Fourie *et al*. (2009) made similar observation in a laboratory study using the same deposition assessment protocol on citrus fruit and leaves. Quantitative and qualitative deposition increased with an increase in volume, until a point of run-off was reached, where after these deposition parameters decreased. It was therefore concluded that settings D and E, which resulted in 600 and 750 L/ha, respectively, were the optimal settings of the STIHL-sprayer for dilute fungicide application. From previous grapevine spray application research (Fourie *et al.*, 2007; J.C. Brink, unpublished results), it was obvious that air-shear technology yielded improved deposition at lower spray volumes, 250-500 L/ha. Applications were conducted

with special care, but factors such as canopy size/density and walking speed can be factors influencing some the findings at the different sprayer settings tested. Nonetheless, to our knowledge this is the first published scientific information on the evaluation of the STIHL SR400 motorised backpack mistblower, even though this applicator is commonly used by the agro-chemical industry for evaluation of fungicides and pesticides (Bateman *et al.*, 2005). Spray application with the STIHL sprayer resulted in significant differences in spray deposition between upper and lower leaf surfaces and between the various settings, and it was clear that improper use of this sprayer, for example wrong setting and walking speed, might lead to sub-optimal deposition of the fungicide or pesticide applied. Moreover, should spray application for evaluation and registration trials for agrochemicals be sprayed using such improper methodology, it might lead to the registration of higher dosages than needed for optimal spray application.

Spray deposits on the upper leaf surfaces were generally higher than on the lower leaf surfaces, which can be attributed to the sampling position in the leaf canopy. As only exposed leaves were sampled to minimise variation, upper surfaces on these leaves were directly exposed to the spray application, while deposits on lower surfaces of these leaves mostly depended on spray droplets entering the grapevine canopy from the opposite side of sampling (less exposed to direct spray application). This was especially relevant as the STIHL sprayer does not produce a turbulent air stream, which would have minimised leaf shingling and therewith improved spray deposition in the inner canopy and on lower leaf surfaces (Gan-Mor *et al.*, 1996; Furness and Cambellack, 2000; Furness *et al.*, 2003). In addition, differences in upper and lower grapevine leaf morphology may also have influenced spray deposition. Gaskin *et al.* (2005) classified upper grapevine leaf surfaces as 'difficult-to-wet' and lower leaf surfaces as very 'difficult-to-wet'. A similar observation was made in a previous laboratory study conducted (Chapter 2). Nonetheless, the addition of adjuvants to spray mixtures most often improved deposition on upper and lower leaf surfaces compared with spray mixtures excluding adjuvants (Gaskin *et al.*, 2005; Chapter 2).

In the present study, selected adjuvants, which were previously evaluated in a laboratory trial (Chapter 2), were evaluated at recommended rates at a spray volume of 600 L/ha using the STIHL SR400 motorised backpack mistblower. Most of the adjuvants, viz. Agral 90, BB5, Nu-film-P or Solitaire, improved the quantity and quality of deposition retained on upper and lower leaf surfaces. Break-thru S 240 and Villa 51 did not improve the

quantity of fluorescent pigment measured on leaves compared with the fenhexamid and water control applications, but remarkably improved qualitative deposition. In a previous spray trial conducted under laboratory conditions, good deposition was achieved with fenhexamid alone, but the addition of these adjuvants in the spray mixture most often enhanced deposition quantity and quality (Chapter 2). The wettability of the leaf surfaces has a large effect on the initial droplet adhesion and retention (Bukovac and Petracek, 1993; Gaskin *et al.*, 2005; Wagner *et al.*, 2003; Bargel *et al.*, 2006), which might influence the quantity of deposits. Surface-acting-agents (surfactants) present in adjuvants have the potential to lower surface tension (Stevens *et al.*, 1993) of the aqueous solution applied on the target surface for improved droplet wettability and distribution of the active ingredient (De Ruiter *et al.*, 1990; Steurbaut, 1991; Stevens, 1993; Stevens *et al.*, 1993; Penner, 2000; Gaskin *et al.*, 2005; Bargel *et al.*, 2006). However, it is also known that the concentration of the surfactants (dosage) may influence the retention of droplets on the target surface (Holloway *et al.*, 2000; Gaskin *et al.*, 2005; Spanoghe *et al.*, 2007). The disparate results observed in the cases of Break-thru S 240 and Villa 51 in this study, as well as others in a previous study (Chapter 2), might therefore be attributed to effects of adjuvant dosage and/or spray volume.

Most adjuvants are registered with a range of dosages recommended for a specific application. Certain adjuvants were tested under vineyard conditions at the lower and higher levels of registered dosages, and it was clear that adjuvant dosage at a specific spray volume had a profound effect on the quantities and qualities of spray deposits, especially those retained on upper Chardonnay leaves. Both Agral 90 and Nu-film-P yielded limited to no improvement of spray deposition at the lower dosage, but significantly improved the quantity and quality of spray deposition on upper leaf surfaces at the increased dosage, compared with the no-adjuvant control. These adjuvants have different mechanisms for improvement of spray deposition. Agral 90 surfactant chemistry might decrease the surface tension of the spray mixture, which decreases droplet sizes deposited on the target surface (Holloway, 1994; Spanoghe *et al.*, 2007). Lower surface tension of spray mixtures might increase the number of smaller uniformly deposited droplets, whereas better wetting and spreading on the leaf surface might also increase qualitative deposition (Holloway *et al.*, 2000; Gaskin *et al.*, 2005). The pinolene surfactant chemistry of Nu-film-P might increase the viscosity properties of the spray solution to deposit coarser spray droplets (Holloway *et al.*, 2000; Prokop and Kejklicek, 2002; Spanoghe *et al.*, 2007) that can stick on the leaf surface (Blazquez *et al.*, 1970; Buslig *et al.*, 1971; www.hygrotech.co.za). Moreover, high surface

tension of spray droplets might adversely affect spray deposition on the target, as more droplet rebound can also be expected (Webb *et al.*, 1999; Hall *et al.*, 1993; Brazee *et al.*, 2000). When surfactant concentration was too high, wetting and spreading may take place by decreased surface tension, but it can increase the run-off effect (Holloway, 1994; Kirkwood, 1993) and might also result in inadequate deposition (Gaskin *et al.*, 2004a; 2004b) as was observed with Solitaire, which improved deposition at lower dosages but not at the higher dosage. This adjuvant contains organosilicone surfactant chemistry, known to spread exceptionally well (Stevens, 1993; Stevens *et al.*, 1993; Gaskin *et al.*, 2002). Gaskin *et al.* (2002) highlighted the importance of matching organosilicone adjuvant concentration with application spray volume to achieve improved spray retention and distribution, without unwanted run-off. Hence, higher adjuvant dosage does not necessarily mean better deposition results, especially when not applied at reduced spray volumes. These findings showed that a specific adjuvant might have an optimal dosage at which it can increase fungicide deposition to a maximum. However, no significant improvement of spray deposition was observed with Villa 51 at both dosages tested. This phenomenon might also be explained by a possible dosage effect. Deposition tended to increase following application with the lower to the higher dosage, hinting that an even higher dosage might have yielded better results. However, the possibility also exists that both the dosages evaluated caused excessively low droplet surface tension as a result of the dosage being too high. This might have caused run-off from the more exposed leaf samples used for deposition assessment. Visual observations following spray application supported the latter explanation, as more run-off was seen from grapevines following spray application with Villa 51. Nonetheless, as only two dosages were tested, the optimal dosage for Villa 51 use in grapevine could not be determined in this study.

Similar to varying adjuvant dosage at a constant spray volume, varying spray volume at a constant dosage also had a significant effect on quantitative and qualitative deposition on grapevine leaves. Deposition on lower leaf surfaces was most often improved by a higher application volume. The smaller droplets in the spray plume resulting from the use of certain adjuvants (Spanoghe *et al.*, 2007; Holloway, 1994) might enter and penetrate the leaf canopy better and might therewith increase deposition on lower leaf surfaces and inner canopy leaves, especially in larger and more dense canopies. Maximum quantitative and qualitative deposition on upper leaf surfaces was achieved at 600 L/ha with an adjuvant in the spray mixture. However, with increased volume to 900 L/ha, decreased quantitative and qualitative

deposition was observed with these spreader adjuvants, which can be attributed to spray run-off. A similar observation was made by Gaskin *et al*. (2002) with a superspreader adjuvant evaluated at different spray volumes on Chardonnay grapevine foliage. Their results clearly showed decreased droplet retention at a higher spray volume, attributed to more spray run-off. This illustrates the risk of applying adjuvants at high spray volumes. Therefore, spray volumes should be optimised to prevent losses due to run-off. With regard to spray deposition, it is hypothesised that spray volume and adjuvant dosage are inversely correlated factors, which should be evaluated for each adjuvant product in a specific crop. Gaskin *et al*. (2002; 2004b) also highlights the need to match adjuvant dosages to application volumes on the spray target to achieve improved spray retention and distribution.

Another factor, which was not evaluated in this study, is the influence of sprayer technology on adjuvant use. The droplet size spectrum of sprayers can be influenced by various parameters such as nozzle type, orifice size, fan angle, discharge angle relative to airstream, spray pressure, and physical properties of the spray mixture like adjuvants (Spanoghe *et al.*, 2007; Hewitt, 2008). Air shear sprayer technology, such as used in the STIHL SR400 motorised backpack mistblower, generates a relatively small droplet spectrum, especially when used at lower volumes (Hoffmann *et al.*, 2007). As the relative surface tension in smaller droplets will be lower compared with bigger droplets (Tolman, 1949), the effect of adjuvant type and dosage on droplets from varying sizes might differ. Therefore, sprayer technology and specifically the droplet spectra generated should also be considered in development and recommendation of adjuvants. In the present study, exposed outer leaves were sampled for deposition assessment. In relative terms, the upper leaf surfaces on these leaves would have been impacted by the highest number of droplets, which at higher spray volumes would have led to a coalescence of droplets and a more complete film-wetting of the leaf, as opposed to individual droplet deposition as witnessed on lower leaf surfaces. In the latter case, adjuvant effect on deposition was less pronounced, which might be attributed to the smaller droplet spectra impacting on lower leaf surfaces.

The findings from this study clearly demonstrate the potential as well as some of the problems that are likely to be encountered when using adjuvants to improve spray application in grapevines. Adding an adjuvant at present-day high volume applications (1000-1500 L/ha) will have a detrimental effect on fungicide run-off, and may result in even less disease control than excluding an adjuvant in the spray mixture. This study demonstrated that with the

addition of the correct dosage of an adjuvant, spray volumes might be reduced without jeopardising the quantity or quality of spray deposition. A similar observation was made by Gaskin *et al*. (2004a) who demonstrated much better deposition with the addition of an adjuvant at lower volumes with improved disease control in vineyards. High volume spray application is less cost effective due to fewer hectares sprayed per load, more travel to and from the water supplies, higher fuel bills, greater labour inputs and less flexibility in spraying operations. Therefore, the economic benefits of lower-volume spray application should be well recognised, while adjuvants showed excellent potential to improve spray technology in grapevines. However, these results need to be confirmed on more grapevine trial sites, as well as with commercially used spray applicators, as it was clear that factors such as canopy size and applicator technology might influence the efficacy of spray deposition. It is also recommended to extend adjuvant research to other cultivars, as plant surface morphology may largely influence deposition diversity (Holloway, 1970; Gaskin *et al.*, 2005), even different plant parts may show great variability in deposits. Moreover, evaluations in this study were done using fenhexamid as model fungicide in the spray mixture and addition of other agrochemical formulations in the spray mixture might influence the adjuvant dosage and spray volume interaction. Composition differs between agrochemical formulations and will influence deposition properties differently for a particular adjuvant in the spray mixture. The correct adjuvant dosage for a specific application is therefore a relatively complex function between the specific agrochemical mixture applied, spray volume, plant surface morphology, canopy size and sprayer technology. Different dosage and volume combinations should be tested for spray adjuvants to optimise formulation and spray parameters, to ensure that this application technology is used to its full potential in pest and disease control.

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Table 1. Properties of selected adjuvants sourced in South Africa[1]

Common name	Registration holder	Main Components	Possible properties	Chemistry classification	Grams pure active ingredient	Recommended dosage
Agral 90	Kynoch Agrochemicals	Alkylated phenyl ethelene oxide condensate	Surfactants	Ethoxylated alkylphenols	940 g/L	18 mL/100L
BB5	Gouws and Scheepers	Nonyl phenol etoxylate + acid + colour indicator	Acid+Surfactant	Acidifiers	600 g/L	100 mL/100L
Break-thru S 240	Degussa Africa	Polyether- polymethylsiloxane-copolymer	Surfactants	Organosilicones	1000 g/L	50 mL/100L
Nu-film-P	Miller Chemical and Fertilizer	Poly-1-p menthene	Sticker	Terpene oils	875 g/L	30 mL/100L
Solitaire	Safagric	Polyether-polymethylsiloxane-copolymer/vegetable oil	Oil+Surfactants	Silicone/Plant oils	300/650 g/L	50 mL/100L
Villa 51	Villa Crop Protection	Isotridecanol	Surfactants	Alkylpolyethylene glycol ether	918 g/L	100 mL/100L

[1]Data collected from product label, http:/www/nda.agric.za/act36/AR/Adjuvants.htm

Table 2. Analyses of variance for effects of camera setting, sprayer setting and leaf side on median values for quantitative, qualitative deposition and Furness droplet ratings on upper and lower surfaces of Chardonnay grapevine leaves following spray application with an aqueous fluorescent pigment

Source	Quantitative deposition			Qualitative deposition			Furness droplet ratings		
	DF*	MS**	*P***	DF	MS	*P*	DF	MS	*P*
Camera	1	1198.88	<0.0001	1	56715.51	<0.0001			
Sprayer setting	5	513.50	<0.0001	5	150120.81	<0.0001	1	79.43	<0.0001
Camera*Sprayer setting	5	53.51	0.2812	5	8503.08	0.0080			
Error a	24	39.91		24	2083.31		24	1.83	
Leaf side	1	423.55	0.0372	1	194021.04	<0.0001	1	413.76	<0.0001
Camera*Leaf side	1	204.74	0.1382	1	13595.90	0.0788			
Sprayer setting*Leaf side	5	217.83	0.0585	5	1431.90	0.8739	5	41.19	<0.0001
Camera*Sprayer setting*Leaf side	5	126.31	0.2426	5	2236.89	0.7334			
Error b	24	87.05		24	4033.64		24	1.78	
Error Sample	283	14.17		284	466.66		287	0.58	
Corrected Total	354			355			358		

*DF = Degrees of freedom
**MS = Mean sum of squares
***P = Probability

Table 3. Pearson's correlation coefficients of median percentage quantitative and median qualitative spray values, and corresponding Furness droplet ratings values on upper and lower Chardonnay grape vine leaves

Protocol	Protocol	Upper leaf side	Lower leaf side
Quantitative	**Qualitative**	-0.594 (<0.0001)*	-0.520 (0.0001)*
Quantitative	**Furness droplet rating**	0.636 (<0.0001)*	0.207 (0.0056)*
Qualitative	**Furness droplet rating**	-0.669 (<0.0001)*	-0.375 (<0.0001)*

*Values are correlation coefficients and corresponding *P* values (in parenthesis) significant at $P = 0.05$

Table 4. Median values for quantitative (percentage fluorescent pigment coverage) and qualitative (distance between particles [*smaller values indicate a better quality spray cover*]) deposition on upper and lower surfaces of Chardonnay grape vine leaves following spray application at different spray volumes with an aqueous fluorescent pigment

Sprayer setting	Spray volume (L / ha)	Quantitative deposition	Qualitative deposition
A	40	0.85c	192.43a
B	225	2.61bc	91.86b
C	450	4.64c	74.26c
D	600	7.53a	66.22c
E	750	7.81a	58.09c
F	900	7.45a	74.29c
LSD ($P < 0.05$)*		2.397	17.298

*Least significant difference: values in each column followed by the same letter do not differ significantly

Table 5. Mean deposition values as determined by the Furness droplet rating chart on upper and lower surfaces of Chardonnay grape vine leaves following spray application at different spray volumes with a aqueous fluorescent pigment

Sprayer setting	Spray volume (L / ha)	Furness droplet ratings	
		Upper leaf surface	Lower leaf surface
A	40	0.00d	0.00c
B	225	1.03c	0.04c
C	450	1.58c	0.43bc
D	600	3.75b	0.51abc
E	750	4.91a	0.68ab
F	900	4.27ab	0.96a
LSD ($P < 0.05$)*		0.873	0.514

*Least significant difference: values in each column followed by the same letter do not differ significantly

Table 6. Analyses of variance for effects of experiment, leaf side and varying adjuvant treatments on median values for quantitative and qualitative deposition on upper and lower surfaces of Chardonnay grapevine leaves following spray application with a fluorescent pigment, fenhexamid with or without adjuvants at recommended dosages

Source	DF*	Quantitative deposition		Qualitative deposition	
		MS**	*P***	MS	*P*
Experiment (Exp)	1	1.4910	0.4031	4020.1971	0.1553
Block (Exp)	4	1.7087		1315.1961	
Treatment	7	14.3593	<0.0001	16641.2963	<0.0001
Error a	35	1.0572		1331.5592	
Leaf side	1	313.9084	<0.0001	56009.0793	<0.0001
Treatment*Leaf side	7	5.5294	0.0021	2054.5205	0.3949
Error b	40	1.3825		1904.9196	
Sample Error	863	0.601297			
Corrected Total	958				

*DF = Degrees of freedom
**MS = Mean sum of squares
****P*** = Probability

Table 7. Median values for quantitative (percentage fluorescent pigment coverage) and qualitative (pixel distance between particles [*smaller values indicate a better quality spray cover*]) deposition on upper and lower surfaces of Chardonnay grapevine leaves following spray application at 600 L/ha with a fluorescent pigment, fenhexamid and adjuvants at recommended dosages

Treatment	Upper leaf surface		Lower leaf surface	
	Quantitative deposition	**Qualitative deposition**	**Quantitative deposition**	**Qualitative deposition**
Agral 90	2.43a	41.85c	0.85a	76.28b
BB5	2.39a	43.79bc	0.77a	82.68b
Break-thru S 240	1.35b	50.18b	0.69ab	80.91b
Nu-film-P	2.13a	47.88bc	0.70a	88.24b
Solitaire	2.19a	44.05bc	0.83a	79.32b
Villa 51	1.12b	58.56a	0.67ab	87.77b
Fenhexamid (No adjuvant)	1.39b	64.66a	0.39bc	109.41a
Water sprayed control	1.36b	62.84a	0.30c	117.82a
LSD ($P < 0.05$)*	0.420	7.156	0.290	17.400

*Least significant difference: values in each column followed by the same letter do not differ significantly

Table 8. Analyses of variance for effects of experiment (repetition), leaf side and with varying adjuvant treatments on median values for quantitative and qualitative deposition on upper and lower surfaces of Chardonnay grapevine leaves following spray application with a fluorescent pigment, fenhexamid, and with or without adjuvants at varying spray dosages

Source	DF*	Quantitative deposition		Qualitative deposition	
		MS**	*P***	MS	*P*
Experiment (Exp)	1	0.105	0.8435	9251.517	0.0957
Block (Exp)	4	2.360		1963.653	
Treatment	4	14.496	<0.0001	5892.773	0.0269
Dosage	1	11.435	0.0014	5013.031	0.1138
Treatment*Dosage	3	17.292	<0.0001	13756.291	0.0006
Error a	40	0.972		1918.114	
Leaf Side	1	909.829	<0.0001	1626700.189	<0.0001
Treatment*Leaf Side	4	11.270	<0.0001	1164.770	0.7734
Dosage*Leaf Side	1	9.968	0.0052	83.016	0.8590
Treatment*Dosage*Leaf Side	3	12.701	<0.0001	2970.968	0.3423
Error b	45	1.156		2600.726	
Sample Error	1329	0.417		741.923	
Corrected Total	1436				

*DF = Degrees of freedom
**MS = Mean sum of squares
****P* = Probability

Table 9. Median values for quantitative (percentage fluorescent pigment coverage) and qualitative (pixel distance between particles [*smaller values indicate a better quality spray cover*]) deposition on upper and lower surfaces of Chardonnay grapevine leaves following spray application at 600 L/ha with a fluorescent pigment, fenhexamid and adjuvants at varying application dosages

Treatment	Rate applied (Per 100 L water)	Upper leaf side deposition parameters		Lower leaf side deposition parameters	
		Quantitative %	Qualitative	Quantitative %	Qualitative
Agral 90	**6 mL**	1.58de	53.41a	0.20c	135.73a
	18 mL	2.44b	39.87ef	0.34ab	105.40b
Nu-film-P	**20 mL**	1.99bcd	46.99cd	0.40a	108.72b
	50 mL	3.41a	39.48f	0.36ab	110.44b
Solitaire	**50 mL**	2.11bc	43.36def	0.40a	101.38b
	100 mL	1.22e	51.98ab	0.23bc	122.12ab
Villa 51	**50 mL**	1.51de	48.17bc	0.27abc	115.53ab
	100 mL	1.82cd	44.40cde	0.40a	102.54b
No adjuvant	**75 mL**	1.69cde	52.46ab	0.29abc	120.78ab
LSD ($P < 0.05$)*		0.491	4.687	0.137	23.213

*Least significant difference: values in each column followed by the same letter do not differ significantly

Table 10. Analyses of variance for effects of experiment (repetition), leaf side and with varying adjuvant treatments on median values for quantitative and qualitative deposition on upper and lower surfaces of Chardonnay grapevine leaves following spray application with a fluorescent pigment, fenhexamid, and with or without adjuvants (recommended dosages per 100L water) at different spray volumes

Source		**Quantitative deposition**		**Qualitative deposition**	
	DF*	**MS****	***P****	**MS**	***P***
Experiment (Exp)	1	27.1742	0.0034	11745.3547	0.0025
Block (Exp)	4	2.8157		257.6023	
Treatment	2	11.1925	0.0371	20245.4902	<0.0001
Volume	2	150.9423	<0.0001	122011.4646	<0.0001
Treatment*Volume	4	37.6684	0.0007	6584.5100	0.0018
Error a	40	62.5389		1267.3553	
Leaf side	1	475.3752	<0.0001	842318.7769	<0.0001
Treatment*Leaf side	2	0.9948	0.7482	1560.9051	0.1129
Volume*Leaf side	2	45.9605	<0.0001	17876.8317	<0.0001
Treatment*Volume*Leaf side	4	27.9400	0.0064	2337.2732	0.0157
Error b	45	76.6757		681.4687	
Sample Error	972	0.5145		614.1700	
Corrected Total	1079				

*DF = Degrees of freedom
**MS = Mean sum of squares
****P* = Probability

Table 11. Analyses of variance for effects of experiment (repetition), leaf side and with varying adjuvant treatments on median values for quantitative and qualitative deposition on upper and lower surfaces of Chardonnay grapevine leaves following spray application with a fluorescent pigment, fenhexamid and with or without adjuvants (recommended dosages per 100 L water) at different spray volumes

Source	DF*	Quantitative deposition		Qualitative deposition	
		MS**	*P***	MS**	*P***
Upper leaf surface					
Experiment (Exp)	1	50.4919	0.0025	8356.99	0.0018
Block (Exp)	4	1.1134		153.9012	
Treatment	2	4.6847	0.1441	6778.07	<0.0001
Volume	2	89.0263	<0.0001	25022.84	<0.0001
Treatment*Volume	4	15.6684	0.0003	5583.26	<0.0001
Error	40	2.3027		432.86	
Sample Error	486	0.6970		218.50	
Corrected Total	539				
Lower leaf surface					
Experiment (Exp)	1	0.0709	0.6890	3825.45	0.0526
Block (Exp)	4	0.3827		514.5566	
Treatment	2	1.4089	0.0765	15028.32	0.0004
Volume	2	9.4250	<0.0001	114865.46	<0.0001
Treatment*Volume	4	0.7337	0.2424	3338.52	0.0917
Error	40	0.5138		1549.13	
Sample Error	486	0.3321		1009.84	
Corrected Total	539				

*DF = Degrees of freedom; **MS = Mean sum of squares; ****P* = Probability

Table 12. Median values for quantitative (percentage fluorescent pigment coverage) and qualitative (pixel distance between particles [*smaller values indicate a better quality spray cover*]) deposition on upper and lower surfaces of Chardonnay grapevine leaves following spray application at different volumes with a fluorescent pigment, fenhexamid and adjuvants at recommended rates per 100 L water

Treatment	Volume applied (L/ha)	Upper leaf side coverage parameters		Lower leaf side coverage parameters	
		Quantitative %	Qualitative	Quantitative %	Qualitative
Agral 90	**225**	1.10c	56.37b	0.14 c	137.95a
	600	2.63a	39.56d	0.55ab	91.68cd
	900	1.77b	45.68cd	0.73a	82.73d
Solitaire	**225**	1.28bc	56.92b	0.21c	120.63b
	600	2.61a	40.87d	0.65a	93.03cd
	900	1.63bc	52.35bc	0.51ab	90.27cd
No adjuvant	**225**	0.50f	83.38a	0.11c	152.25a
	600	1.74b	50.69bc	0.32bc	103.29c
	900	2.42a	42.83d	0.51ab	99.52c
LSD ($P < 0.05$)*		0.560	7.677	0.265	14.523

*Least significant difference: values in each column followed by the same letter do not differ significantly

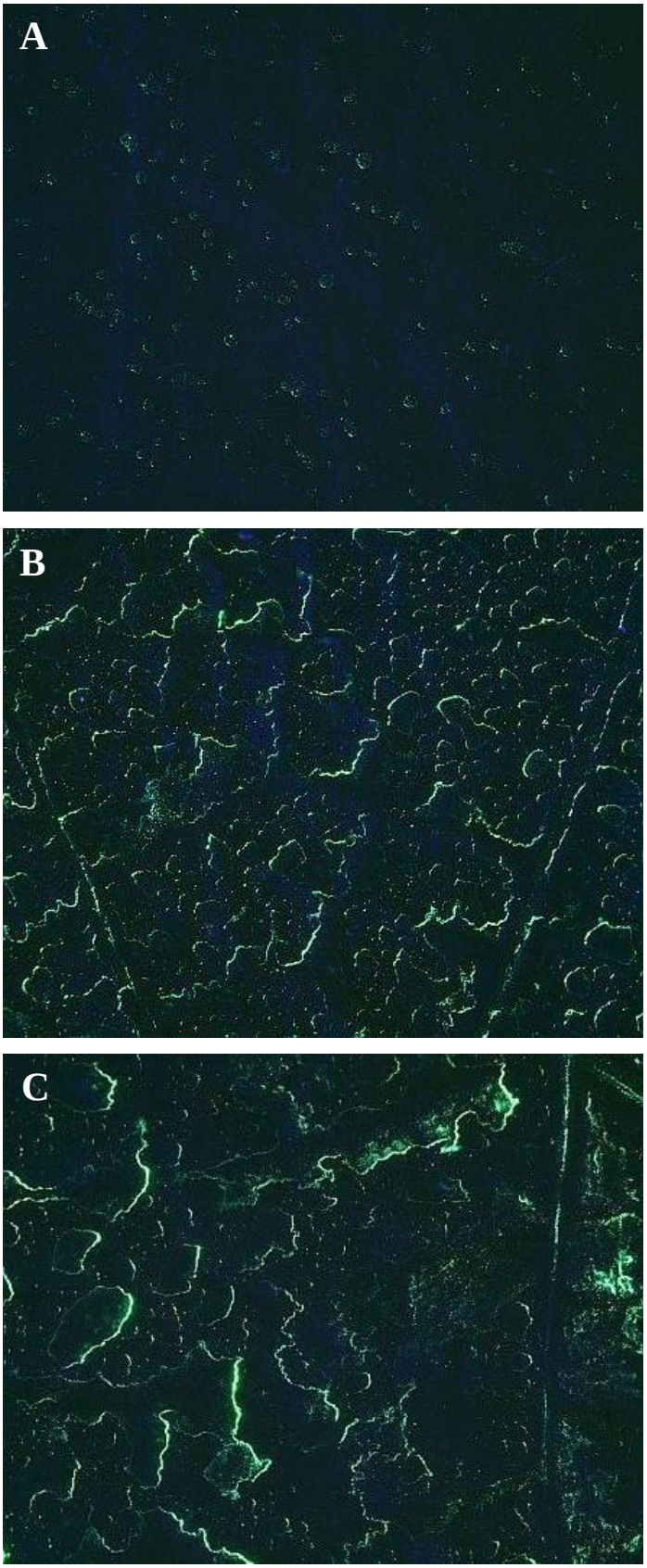

Figure 1. Digital images of upper surfaces of mature Chardonnay leaves sprayed at 225 L/ha (**A**); 600 L/ha (**B**) and 900 L/ha (**C**) with a SARDI Yellow Fluorescent Pigment solution and visualised under black light illumination at 10× magnification.

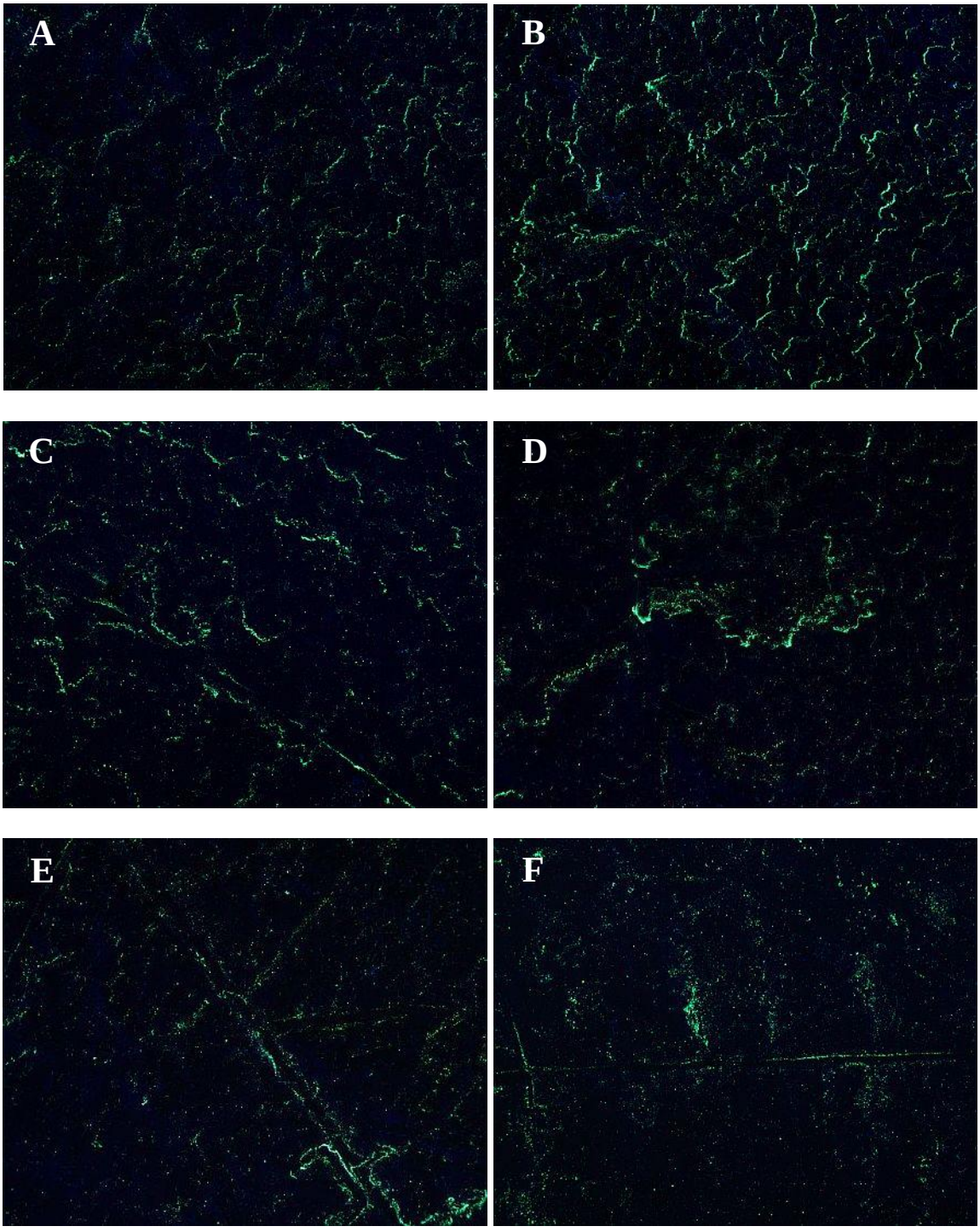

Figure 2. Digital images of upper surfaces of mature Chardonnay leaves sprayed with a lower and a higher dosage of Nu film P (**A** and **B**, respectively), Solitaire (**C** and **D**, respectively) and Villa 51 (**E** and **F**, respectively) in a fenhexamid and SARDI Yellow Fluorescent Pigment solution at 600 L/ha and visualised under black light illumination at 10× magnification.

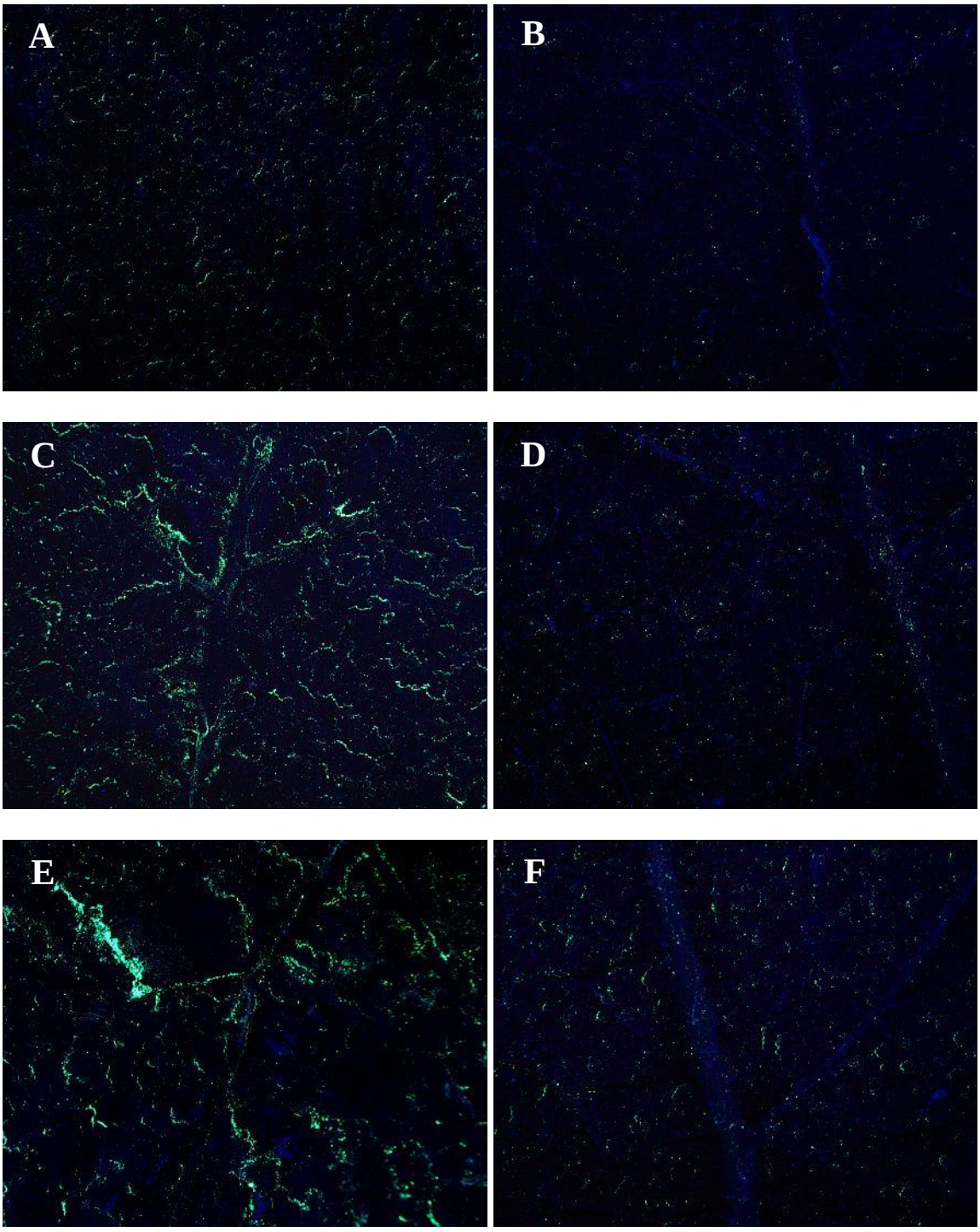

Figure 3. Digital images of upper and lower surfaces of mature Chardonnay leaves sprayed at 225 L/ha (**A** and **B**, respectively), at 600 L/ha (**C** and **D**) and 900 L/ha (**E** and **F**, respectively) with Agral 90, fenhexamid and SARDI Yellow Fluorescent Pigment solution and visualised under black light illumination at 10× magnification.