Spray deposition assessment and benchmarks for control of Alternaria brown spot on mandarin leaves with copper oxychloride

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\textbf{Abstract}

Inadequate disease control on citrus foliage and fruit is often attributed to insufficient fungicide spray deposition on target surfaces. This study describes a novel spray deposition assessment protocol and determines deposition benchmarks indicative of the biological effectiveness for better interpretation of spray deposition results. Suitability of a yellow fluorescent pigment as tracer for copper oxychloride deposition was demonstrated through its similar particle concentration and size. Spray deposition assessment of spray targets, which were sprayed with a mixture that included the fluorescent pigment, involved photomacrography of whole leaf or fruit surfaces, followed by digital image analyses. This protocol proved to be very accurate in determining the quantity and quality of deposition. To determine deposition benchmarks, detached young ‘Nova’ mandarin leaves were sprayed with copper oxychloride and fluorescent pigment at different concentrations (0.1–2 times the recommended concentration) and spray deposition assessed. Subsequently, leaves were spray inoculated with a spore suspension of \textit{Alternaria alternata} (causal agent of Alternaria brown spot (ABS) of mandarins), moist-incubated for c. 48 h and symptom expression rated. A very good linear relationship was found between fungicide concentration, leaf area covered by fluorescent pigment particles (%FPC) ($r = 0.879$) and Cu residue analysis ($r = 0.992$). A von Bertalanffy growth curve best fitted the relation between ABS control and deposition quantity (%FPC) (91% of the percentage variance accounted for) with a good correlation between observed and predicted values ($r = 0.825$). Benchmarks for 50% and 75% disease control were calculated as $2.07$ %FPC and $4.14$ %FPC, respectively. These corresponded with Cu residue levels of 59.4 and 91.0 mg kg$^{-1}$, respectively. These FPC benchmarks can be used to evaluate spray technology research, specifically for control of ABS and similar citrus fruit and foliar diseases.

\textbf{1. Introduction}

In South Africa and most citrus-producing regions of the world, fruit and foliar diseases cause major economic losses, often due to the poor implementation of disease control measures. Diseases such as Alternaria brown spot (ABS) (\textit{Alternaria alternata} (Fr: Fr) Keissl., tangerine pathotype) (Schutte, 1996; Timmer, 2000; Timmer et al., 2000), citrus black spot (CBS) (\textit{Guignardia citricarpa} Kiely) (Schutte et al., 1997; Kotzé, 2000), and melanose (\textit{Phomopsis citri} H. Fawcett non (Sacc.) Traverso & Spessa) (\textit{Whiteside and Timmer, 2000}) are serious threats to production and market-ability of fresh market citrus.

Citrus trees are often large and dense. This complicates adequate deposition on difficult-to-reach inner canopy leaves and fruit. Hence, fruit and foliar diseases are currently being controlled by regular fungicide spray applications at spray volumes ranging from 9000 to 16,000 l ha$^{-1}$ (medium to full cover sprays, respectively) in citrus-producing areas of South Africa. These methods of application provide an acceptable balance between efficacy and efficiency based on existing economic considerations (Grout, 1997, 2003). Spray application is a complex procedure due to the large number of contributing factors influencing spray deposition. Major influences on spray deposition efficiency and efficacy include canopy geometry and density (jejic et al., 2011), environmental conditions (Salyani, 2005, 2006), the use of appropriate machinery (Cook and Hislop, 1993; Cunningham and Harden, 1998a, 1998b, 1999; Furness et al., 2006b; Salyani, 2005, 2006), spray technique (Furness et al., 1998; Salyani and Farooq, 2004; Salyani and Whitney, 1990), spray volume (Cunningham and Harden, 1999; Fourie et al., 2009; Salyani and Hoffmann, 1996), the fungicide or pesticide used (Sundaram...
and Sundaram, 1987; Zabkiewicz, 2007), the influence of adjuvants (Butler Ellis et al., 1997; Gent et al., 2003; Green and Beestman, 2007; van Zyl et al., 2010a, 2010b), and the complex interaction between these factors (Grout, 2003; Salyani, 1994, 2005, 2006; Stover et al., 2002b; Whitney et al., 1988, 1989). Effective deposition of the active ingredient on the target surface (citrus leaves, twigs or fruit) is needed for effective disease control since disease control and spray deposition are directly related (Holownicki et al., 2002).

Inadequate spray deposition is the most common reason for treatment failures. Inadequate deposition is due to a number of factors of which run-off and the use of poor equipment and technique are amongst the most common (Fourie et al., 2009; Grout, 1997, 2003; Salyani, 1994; Stover et al., 2002b). Cunningham and Harden (1998a; 1998b; 1999) showed that spraying mature citrus trees with application volumes above 2000 l ha$^{-1}$ is inefficient since the amount retained by trees decreases rapidly at spray volumes above 2000 l ha$^{-1}$. It was estimated from laboratory experiments on navel leaves and confirmed in field trials on 5 × 5 m mandarin trees that these mature citrus trees can retain about 2300 l ha$^{-1}$ only (Cunningham and Harden, 1998b). Thus a large proportion of higher spray volumes are lost due to run-off and exo- and endo-drift (Salyani and Farooq, 2004). Off-target application of fungicides and/or pesticides is not only an economic loss, but also a potential environmental problem (Furness et al., 2006a, 2006b; Salyani, 1994; Salyani and Farooq, 2004; Stover et al., 2002a). Given the history of reliance on high-volume spray application in South African citrus production, research on the optimization of spray application is urgently needed. The use of lower volume spray applications (Cunningham and Harden, 1999) adding adjuvants to spray mixtures (van Zyl et al., 2010a, 2010b) and increasing treatment concentrations are possible means to optimize fungicide deposition and reduce fungicide losses through run-off and drift in citrus (Cunningham and Harden, 1999; Salyani and Farooq, 2004).

Various methodologies for the evaluation of spray deposition effectiveness have been developed for a range of crops. Methods of evaluation range from relatively simple to more advanced methods. These include qualitative visual assessment of spray deposition on sprayed targets through the use of fluorescent tracers (Furness et al., 2006a, 2006b; Holownicki et al., 2002; Salyani and McCoy, 1989) and the use of droplet rating charts to evaluate deposition on actual or artificial targets (Furness et al., 2006a; Holownicki et al., 2002). These methods are relatively simple but lack the ability to accurately measure deposition quantity and quality since it is dependent on human discretion (Jiang and Derksen, 1995; Salyani and Whitney, 1988). More advanced methods for determining deposition quantity include chemical residue recovery techniques such as gas chromatography or atomic absorption, spectrophotometry of metals and nutrients (Byers et al., 1984; Ware et al., 1969; Yates et al., 1974) and also recovering sprayed fluorescent tracers from artificial and plant surfaces through washing techniques and determining deposition through fluorometry and colorimetry (Lake, 1988; Salyani and Whitney, 1988, 1990). These methods lack the ability to quantify the quality of coverage, such as uniformity of spray coverage on the target surface (Juste et al., 1990). Spray deposition measurement, specifically in terms of quantity and quality, was greatly improved through the development of deposition assessment protocols that combines fluorometry, digital photomicrographic imaging and digital image analysis (Brink et al., 2004, 2006; Fourie et al., 2009; Salyani and Hoffmann, 1996; van Zyl et al., 2010a, 2010b).

The objectives of this study were first to describe a novel deposition assessment protocol for the assessment of spray deposition quantity, quality and uniformity, based on previously described methods (Brink et al., 2004, 2006; Fourie et al., 2009; van Zyl et al., 2010a, 2010b), specifically for use in citrus spray application research; and second, to determine deposition benchmarks indicative of biologically effective deposition quantities. These benchmarks should allow for better interpretation of spray deposition results. For the latter objective, control of ABS with copper oxycholoride was used as model system. ABS is an economically important disease of leaves, fruit and twigs of susceptible mandarin or tangerine (Citrus reticulata Blanco) and tangerine × grapefruit (C. reticulata × Citrus paraisd is Macfad,) hybrids in many citrus-producing regions of the world (Akimitsu et al., 2003; Elena, 2006; Kiely, 1964; Reis et al., 2006; Schuttle et al., 1992; Solel, 1991; Timmer, 2000; Timmer et al., 1998, 2003; Vicent et al., 2000; Whiteside, 1976). The causal agent of ABS is the necrotrophic tangerine pathotype of A. alternata (Fr: Fr) Keissl., which produces the host selective/specific ACT-toxin (Kohmoto et al., 1979, 1991, 1993; Lin et al., 2009; Solel, 1991; Timmer et al., 2003). The control of ABS relies mainly on preventative fungidal sprays (Swart et al., 1998). This is also the case for citrus diseases such as CBS (Schutte et al., 1997; Kotzé, 2000) and melanose (Whiteside and Timmer, 2000). Like A. alternata, causal agents of these diseases infect at average temperatures between 22 and 27°C and wetness periods of c. 12 h (Canhös et al., 1999; Timmer et al., 2000), making ABS a good model pathosystem for the purpose of this study.

2. Materials and methods

2.1. Spray deposition assessment protocol

2.1.1. Fluorescent pigment

The physical suitability of a yellow fluorescent pigment [South Australian Research and Development Institute (SARDI) Yellow Fluorescent Pigment, 40% EC (SARDI, Loxton, South Australia); 1 ml 1$^{-1}$] as tracer for a contact copper fungicide [Villa Copper Oxycholoride, 85% WP (Villa Crop Protection SA, Kempton Park, South Africa); copper oxycholoride with 50% metallic copper equivalent; 2 g 1$^{-1}$] was studied by comparing particle size. This was done together and separately at ×400 and ×1000 magnification (Nikon Eclipse E600 microscope; www.Nikon.com). Digital photographs (15 photographs for each particle type) were taken at both magnifications (Nikon DXYM1200C; www.Nikon.com) mounted on the microscope using image capturing software (Nikon NIS elements imaging software F version 3.00SP7; www.nis-elements.com). The photos were stored in Exif-TIFF file format (image size 1372 × 1024 pixels) for subsequent image analysis (Image Pro Plus software version 6.2; www.mediacy.com). In total, 176 particles of each particle type were measured to determine mean diameter (average length of diameters measured at 2 intervals passing through measured particle’s centroid; μm), each image calibrated accurately to scale of magnification. The concentration (ml$^{-1}$) of fluorescent or copper oxycholoride particles was determined using a haemocytometer. Six haemocytometer counts were done for each particle type separately from three yellow fluorescent pigment and three copper oxycholoride agitated suspensions (1 l); two counts from each solution as replications. All counts were done manually at ×400 magnification (Zeiss Axioskop; www.Zeiss.com). An ultraviolet light source (UV-A at ≈ 365 nm: Labino Mid-Light; www.labino.com) was used to illuminate the fluorescent particles.

2.2. Deposition benchmarks indicating effective disease control

2.2.1. Leaves

ABS-susceptible ‘Nova’ mandarin hybrid (C. reticulata Blanco; hybrid of clementine ‘Fina’ and tangelo ‘Orlando’) trees were grown in 10-l plastic pots in a glasshouse at 27°C. Drip irrigation and a monthly application of slow release fertilizer (3:1:2 of N:P:K)
were used to maintain the plants. The trees were regularly pruned to stimulate young growth (flush) production for use in experiments and too keep the trees small.

2.2.2. Inoculum

An isolate of *A. alternata* was recovered from symptomatic mandarin leaves in Nelspruit (Mpumalanga province, South Africa). It was single-spored and thereafter identified using conidium morphology as *A. alternata*. Pathogenicity tests on susceptible ‘Nova’ mandarin leaves confirmed it to be the tangerine pathotype of *A. alternata* (Whiteside, 1976). It was stored in the Stellenbosch University culture collection (STE-U no. 6592-6593). Single spore isolates were placed on potato dextrose agar (PDA; MERCK Biolab, USA) with a *A. alternata* leaf was positioned on a wire mesh tray (angled at 30°) and deionized water was sprayed at different concentrations (10 ml). After the incubation period, the leaves were removed from the spray chamber. Upper leaf surfaces of sprayed leaves were spray inoculated with pre-run-off volumes (0.3 ml) of 1 × 10^5 spores ml^{-1} suspension of *A. alternata*. Spraying was done in the same manner described for spray application. The spray-inoculated leaves were placed on moistened paper towels and inoculated in the plastic containers at high relative humidity (>95%) at 27 °C in the dark for 48 h until pin-point necrotic lesions (<2 mm in diameter) developed on the control treatment leaves.

2.2.5. Inoculation with *A. alternata*

Following deposition analysis, sprayed leaves were placed back into the containers and transported back to the spray chamber. Upper leaf surfaces of sprayed leaves were spray inoculated with pre-run-off volumes (0.3 ml) of 1 × 10^5 spores ml^{-1} suspension of *A. alternata*. The spray inoculation was done in the same manner described for spray application. The spray-inoculated leaves were placed on moistened paper towels and inoculated in the plastic containers at high relative humidity (>95%) at 27 °C in the dark for 48 h until pin-point necrotic lesions (<2 mm in diameter) developed on the control treatment leaves.

2.2.6. Disease severity evaluation

After the inoculation period, the leaves were removed from the plastic containers and the midrib of the leaves excised by means of a scalpel, splitting the leaves into two pieces. Leaf infection was photographed before these lesions expanded and connected. Each piece was digitally photographed under white light on a white Perspex covered light box in exactly the same order as the leaves had been photographed previously for deposition analysis. Digital photographs of the symptomatic leaves were taken in JPEG (JPG) format. Each photograph was manually analysed with Image Pro Plus software version 6.2 to determine the percentage symptomatic area per leaf. This was subsequently expressed as the relative percent disease control per leaf compared to the nontreated control treatment. After the leaves were photographed, they were stored by treatment in plastic bags at −20 °C for copper residue analysis. The experiment was repeated 18 times.

2.2.7. Copper residue analysis

The 18 repetitions were grouped into three batches to allow sufficient biomass for copper residue analysis, which was done on each batch separately by an accredited analytical laboratory (SGS Analytical Laboratory, Somerset-West, South Africa). Briefly, analysis involved dry ashing of 1 g of plant material in a crucible, and digested (ashed) by heating in a muffle furnace (500 °C for 4 h). The ash residue was then dissolved in 5 ml 6 N HCl and 6 N HNO_3 mixture, diluted to 100 ml with distilled water, filtered and copper ionic particle residue determined from 25 ml (each sample) as mg kg^{-1} by inductively coupled plasma (ICP) spectrometer (Perkin—Elmer A Analyst 400; www.perkinelmer.com).

2.3. Statistical analyses

Particle size, number of particles ml^{-1}, deposition quantity (% FPC) and quality (%CV per leaf), copper residue and percentage disease control (actual and predicted) data were subjected to appropriate analysis of variance (ANOVA). Fisher's LSD was calculated to identify significant differences between treatments at a 95% confidence interval. Data were also subjected to Pearson's
correlation was used to demonstrate the linear relationship between treatment (concentrations) and copper residue, deposition quantity and quality measurements. All statistical analyses were done using software Addinsoft XLSTAT Version 2011.2.06 (www.xlstat.com).

2.4. Benchmark modelling

For benchmark modelling, various functions (natural growth functions, allometric, Gompertz and Hoerl) were evaluated to model the relationship between deposition quantity (%FPC) and ABS control by fitting the selected data to the functions through iterative non-linear least square regression using the NLIN procedure-modified Gauss-Newton method of SAS Version 8.2 statistical analysis software. Pearson’s correlation between actual and predicted values was used to evaluate goodness of fit, from which the best model was selected. Proportion of Variance Explained (%PVE) was used to evaluate goodness of fit. Two benchmarks, FPC50 and FPC75, were selected, which indicate deposition quantity levels that would result in a predicted 50% and 75% disease control, respectively.

Protected leaf area was determined by expressing the number of blocks (as obtained in the deposition quality analysis) that had a % FPC value above that of the FPC50 benchmark as a percentage of the total number of blocks per leaf. This was also indicative of the quality of coverage per leaf.

3. Results

3.1. Spray deposition assessment protocol

3.1.1. Fluorescent pigment

Microscopic observation of the fluorescent and copper oxychloride particles indicated distinct differences in particle shape and colour. The more abundant, smaller, solid, sharp edged, light green fluorescent particles could easily be differentiated from the larger, rigid, granular, dark grey copper oxychloride particles in suspension. Analysis of variance (ANOVA) indicated a significant difference (P = 0.004) in the mean diameter (μm) between the two particle types with copper oxychloride particles having a larger average mean diameter (3.89 μm) than the fluorescent pigment particles (3.31 μm; Table 1). Likewise, there was significantly (P = 0.007) fewer particles ml⁻¹ counted for the copper oxychloride (3.73 × 10⁶ particles ml⁻¹) than for the pigment particles (4.26 × 10⁶ particles ml⁻¹; Table 1).

3.2. Deposition benchmarks indicating effective disease control

3.2.1. Spray application

A distinct droplet pattern could be observed on leaves following the addition of yellow fluorescent pigment to the spray mixture when illuminated with black light. Droplets formed separately in a distinct deposition pattern that occasionally connected, forming larger elongated droplets on leaf surfaces (Fig. 1). The presence and aggregation of pigment particles inside the spray deposit varied with different treatment concentrations. The fluorescent pigment particle residue in the dried spray deposit made droplet formation visible on the leaf surface. The illumination of formed spray deposit on sprayed leaf surfaces varied in intensity as the concentration of fluorescent pigment varied per spray treatment. Light reflectance of formed spray deposit were most intense and visible at the highest spray treatment concentration of 2 × copper oxychloride and fluorescent pigment with intensity decreasing linearly to a point of least visible observation at the lowest spray treatment concentration of 0.1 × copper oxychloride and fluorescent pigment. On the control treatments, no fluorescent pigment was observed as no fluorescent pigment was added.

3.2.2. Deposition analysis

ANOVA of deposition quantity (%FPC) and deposition quality (CV%) data indicated significant effects for copper oxychloride concentration (treatments) (P < 0.0001). From Pearson’s correlation it was shown that deposition quantity increased linearly with increase of treatment concentrations (r = 0.879). Deposition quantity (%FPC) values ranged from 0.86% at 0.1 × to 12.39% at 2 × concentration (Table 2). There was a decrease in CV% values (i.e. improved deposition quality) as the treatment concentrations increased (36.40% at 0.1 × to 24.24% at 2 × concentration), but deposition quality did not differ significantly for concentrations from 0.4 to 2 (Table 2).

3.2.3. Disease severity evaluation

Very small brown to black lesions (0.5–2 mm) with white to yellow halos were observed on the spray-inoculated leaf surfaces after ≈48-h incubation. Leaf infection was photographed before these lesions expanded and connected. From visual observation, it was clear that as copper oxychloride concentration increased, the number and size of infection points decreased. ANOVA of disease control data (%) indicated significant treatment effects (P < 0.0001). The percentage disease control improved linearly (r = 0.743) from 37.22% to 92.94% as the treatment concentration increased (Table 3).

3.2.4. Copper residue analysis

ANOVA of Cu-residue data (mg kg⁻¹) indicated significant treatment effects (P < 0.0001). As the treatment concentrations increased, the Cu residue levels increased with a very good linear fit (r = 0.992). The highest residue level was obtained following the highest treatment concentration 2 × (239.512 mg kg⁻¹) and the lowest level on the control treatment (7.80 mg kg⁻¹), which is indicative of the inherent Cu content of the sprayed leaves (Table 3). Pearson’s correlation indicated a very good linear relationship between Cu residue levels and deposition quantity (%FPC) (r = 0.851) and between Cu residue levels and the disease control achieved (r = 0.748).

3.3. Benchmark modelling

Various models were fitted for deposition quantity and disease control data with a ‘von Bertalanffy growth function’ with the asymptote set at 100 (i.e. maximum disease control that can theoretically be achieved) fitting the data best: EIControl[%] = 100 [1 − exp(−0.3346(%FPC))] (91.04 %PVE) with a good correlation between observed and predicted values (r = 0.825) (Table 3). The 95% confidence limits for the b-value, −0.3346, were −0.3741 to −0.2951.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Differences in mean particle diameter (μm) and number of particles ml⁻¹ of suspensions of yellow fluorescent pigment (1 ml⁻¹) and copper oxychloride (2 g l⁻¹).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Particle type</strong></td>
<td><strong>Measurements</strong></td>
</tr>
<tr>
<td>Pigment</td>
<td>Minimum</td>
</tr>
<tr>
<td>Copper oxychloride</td>
<td>Maximum</td>
</tr>
<tr>
<td>Pigment</td>
<td>Mean</td>
</tr>
<tr>
<td>Copper oxychloride</td>
<td>Standard Deviation</td>
</tr>
</tbody>
</table>

a Average length of diameters measured at 2 intervals passing through the centroid of the measured objects.

b For each parameter separately, values in each column followed by the same letter do not differ significantly (P > 0.05) according to Fisher’s least significant difference test.
Following the curvature of the model (Fig. 2), predicted disease control increased as the deposition quantity (%FPC) increased. The slope of line in the initial stage of the graph (0–2 %FPC) was very steep, indicating a very high proportional contribution to disease control (c. 50%). The slope declined as the deposition quantity increased (2–4 %FPC), indicating a more moderate proportional contribution to disease control (c. 25%), and declined further towards the asymptote, with the effect of increasing deposition quantity on disease control declining proportionally (4–6 %FPC added c. 12.5% disease control and 6–8 %FPC added c. 6.5% disease control). The FPC50 and FPC75 benchmarks indicating a predicted 50% or 75% disease control were calculated from the model as 2.07 %FPC and 4.14 %FPC, respectively.

ANOVA of percent protected leaf area indicated significant effects for treatments (P < 0.0001). Protected leaf area was positively correlated with treatment concentration (r = 0.879). Means of actual deposition quantity (%FPC) data were used in the FPC benchmark model to calculate the predicted disease control following the various treatments (Table 3). Pearson’s correlation between the actual and predicted disease control (%FPC) was very good (r = 0.826).

4. Discussion

This study describes an improvement on a previously described spray deposition assessment protocols (Brink et al., 2006; Fourie et al., 2009; van Zyl et al., 2010a, 2010b) and provides new information on the suitability of a fluorescent pigment that has been used as tracer in these and other studies (Furness et al., 2006b). Additionally, by using control of ABS of mandarins with copper oxychloride as a model system, this study models fluorescent pigment deposition benchmarks indicative of effective disease control. These benchmarks are important in the biological interpretation of spray deposition.

### Table 2

Deposition quantity (%FPC) and deposition quality [CV% and protected leaf area (%)] on young 'Nova' leaves sprayed with various concentrations of yellow fluorescent pigment (0–2× of 1 ml l⁻¹) and copper oxychloride (0–2× of 2 g l⁻¹).

<table>
<thead>
<tr>
<th>Treatment⁴</th>
<th>Deposition Quantity (%FPC)⁵</th>
<th>Quality CV%</th>
<th>Predicted (%) c</th>
<th>Protected leaf area (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>12.39 a</td>
<td>24.24 c</td>
<td>97.63 a</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>9.63 b</td>
<td>25.24 c</td>
<td>94.99 a</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>5.45 c</td>
<td>23.79 c</td>
<td>89.02 a</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>4.70 c</td>
<td>25.76 c</td>
<td>75.87 b</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>3.47 d</td>
<td>25.85 c</td>
<td>59.11 c</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>2.72 d</td>
<td>26.31 c</td>
<td>40.94 d</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>1.42 e</td>
<td>31.52 b</td>
<td>18.74 e</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.86 ef</td>
<td>36.40 a</td>
<td>3.97 f</td>
<td></td>
</tr>
<tr>
<td>0 (control)</td>
<td>0.00 f</td>
<td></td>
<td>0 f</td>
<td></td>
</tr>
</tbody>
</table>

⁴ Factor of recommended application rate of copper oxychloride (2 g l⁻¹) and yellow fluorescent pigment (1 ml l⁻¹).

⁵ For each parameter separately, values in each column followed by the same letter do not differ significantly (P > 0.05) according to Fisher’s least significant difference test.

⁶ Deposition quantity as expressed by percentage leaf area covered by fluorescent particles.

### Table 3

Mean Alternaria brown spot control (%), predicted disease control (%), and copper residue (mg kg⁻¹) determined on young 'Nova' mandarin leaves sprayed with various concentrations of yellow fluorescent pigment (0–2× of 1 ml l⁻¹) and copper oxychloride (0–2× of 2 g l⁻¹) and subsequently spray-inoculated with Alternaria alternata.

<table>
<thead>
<tr>
<th>Treatment⁴</th>
<th>Disease control⁵</th>
<th>Predicted (%) c</th>
<th>Cu residue (mg kg⁻¹)⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>92.94 a</td>
<td>86.36 a</td>
<td>239.51 a</td>
</tr>
<tr>
<td>1.5</td>
<td>90.39 a</td>
<td>83.24 ab</td>
<td>189.94 b</td>
</tr>
<tr>
<td>1.0</td>
<td>75.49 b</td>
<td>76.26 abc</td>
<td>146.44 c</td>
</tr>
<tr>
<td>0.8</td>
<td>70.13 bc</td>
<td>70.36 bc</td>
<td>109.03 d</td>
</tr>
<tr>
<td>0.6</td>
<td>63.34 cd</td>
<td>64.56 cd</td>
<td>87.33 e</td>
</tr>
<tr>
<td>0.4</td>
<td>56.45 d</td>
<td>56.41 d</td>
<td>60.54 f</td>
</tr>
<tr>
<td>0.2</td>
<td>44.28 e</td>
<td>36.91 e</td>
<td>31.07 g</td>
</tr>
<tr>
<td>0.1</td>
<td>37.22 e</td>
<td>23.93 f</td>
<td>16.07 h</td>
</tr>
<tr>
<td>0 (control)</td>
<td>0.00 f</td>
<td>0.00 g</td>
<td>7.80 h</td>
</tr>
</tbody>
</table>

⁴ Factor of recommended application rate of copper oxychloride (2 g l⁻¹) and yellow fluorescent pigment (1 ml l⁻¹).

⁵ For each parameter separately, values in each column followed by the same letter do not differ significantly (P > 0.05) according to Fisher’s least significant difference test.

⁶ Predicted control calculated by subjecting deposition quantity data to fluorescent particle coverage benchmark function: [Control – 100*(1 – e⁻(-0.3346*%FPC))].
The yellow fluorescent pigment was shown to be an ideal tracer based on its particle physical characteristics in comparison with the contact fungicide copper oxychloride. Although not statistically similar, the particle types of these compounds are closely related in mean diameter and in formulation and recommended concentrations for use had similar amounts of particles. Prior unpublished studies have also shown that the suspension concentrate formulation of the yellow fluorescent pigment had minimal effects on water droplet characteristics on leaf surfaces (unpublished results). Cooke and Hislop (1993) and Palladini et al. (2005) showed the importance of choice of fluorescent tracer, specifically that it must be visualized when dry on a target surface and be photo-stable. Previous studies with the yellow fluorescent pigment did not investigate photo-stability but did show the effectiveness of using the tracer under various conditions, its adherence to the target surfaces and ease of visualization once the pigment has dried. Recently, Schutte et al. (2012) clearly showed the effectiveness, photo-stability and persistence of the yellow fluorescent pigment over a period of 6 weeks. Other commonly used water-soluble fluorescent tracers (sodium salt of fluorescein) degraded by 20% after 30 min if exposed to direct sunlight on artificial surfaces (e.g. watersensitive paper). Thus sampling must occur as soon as possible after application and stored in light proof containers. This reduces the size of trial layouts and amount of sample material that can be used since sampling must be done as soon as possible (Holownicki et al., 2002).

Previously, Brink et al. (2004, 2006), Fourie et al. (2009) and van Zyl et al. (2010a; 2010b) used high magnification photomicrography of specific areas of sprayed target surfaces. This method required specialized light sources and stereomicroscopes and was extremely time consuming. Criticism was also levelled at this technique in that it focussed on deposition on small target surfaces, and to some extent ignored the general deposition trends in the canopy since only smaller number of samples could be analysed. Image capturing methodology was changed to photomacrography of whole target surfaces, i.e. leaves or fruit. This enables spray deposition research on biological targets in natural environments. This is more accurate than the use of artificial targets that are sometimes used to simulate deposition on natural targets (Koch and Knewitz, 2008). The high quality digital images (8-bit Exif-TIFF (.TIF = 30 MB)) allowed for clear visualization, measurement and calculation of the quantity and quality of particle deposition on the target surfaces, even very small pigment particles on the target surface that could not be observed through the use of the image analysis software in previous protocols. For example, earlier comparisons with this technology and water sensitive paper showed pigment particles deposited on the paper where the amount of liquid in the droplet was not enough to induce colour change (results not shown). Additional changes to digital image analysis included improved image binarization, contrast and colour enhancement, and proprietary scripting of macros for quantity and quality analyses in Image Pro-Plus, which further improved the ease, accuracy and sensitivity of the deposition quantity and quality measurements.

The aforementioned changes improved the efficiency and 'user-friendliness' of the deposition assessment protocol allowed for considerably improved throughput of sample analysis. Hence, spray deposition analysis of effects such as deposition quantity and quality on multiple target surfaces (for example, upper and lower leaf surfaces), as well as uniformity between target surfaces and spatial deposition in canopies could accurately be determined. van Zyl et al. (2010a; 2010b) demonstrated the superior sensitivity of the photomicrography and fluorometry deposition assessment protocol over that of Furness et al. (2006a). The photomacrography and fluorometry deposition assessment protocol developed in the present study was not evaluated against other relevant deposition assessment protocols, but the excellent correlation between deposition quantity measurements and copper residues and control of ABS should bear sufficient testament of its efficacy and robustness.

In the benchmark experiments, we observed very good positive linear correlation between copper oxychloride concentration, deposition quantity (%FPC), Cu residue and ABS control measured on the leaf surfaces. This supports the accuracy of the benchmark model as well as the suitability of yellow fluorescent pigment as tracer for copper oxychloride, and most probably other contact fungicides.
The FPC₅₀ and FPC₇₅ benchmarks were obtained from a ‘von Bertalanffy growth function’ fitted to 2592 deposition quantity vs. disease control data points, making the model sufficiently robust. The model predicts the deposition quantities needed for 50% and 75% disease control as 2.07 and 4.14 %FPC, respectively. Thus, the predicted copper oxide concentration needed for 50% and 75% disease control would be equivalent to 0.34× and 0.68× of the current registered concentration (200 g 100⁻¹, respectively). However, these benchmarks are built on efficacy data generated following inoculation on the day of fungicide application, and do not account for weathering, wash-off and residue breakdown. Vincent et al. (2009) evaluated rain fastness and protectant activity of copper fungicides against ABS at lower concentrations and found effective and similar disease control with copper oxide (50% metallic copper) at 0.25× and 0.5× of the recommended registered concentration (200 g 100⁻¹) over a 28-day period. Based on FPC benchmark model predictions, these concentrations would have realized 39.90% and 63.89% disease control, respectively. This indicates that field deposition quantities higher than the FPC₅₀ value might be sufficient for ABS control under normal disease pressure conditions, and that deposition quantities circa the FPC₇₅ value might be needed under high disease pressure conditions.

The benchmark model further indicates that very high deposition quantities (above FPC₇₅ value) appear not to contribute significantly to decay disease control and future use of these benchmarks in orchard spray deposition assessment might indicate cases of over application and potentially reduced agrochemical use. This in turn will reduce the risk of phytotoxicity (Albrigo et al., 1997), stippling burn (Schutte et al., 1997) and environmental pollution (Alva et al., 1993) induced by excessively high spray volumes or high concentrations of contact copper fungicides. Further field validation of the benchmark values would, however, be required to support such recommendations.

Deposition quality data were not used in the construction of the model, as the spray methods used (pre-run-off sprays at set spray volumes) attempted and succeeded to minimize deposition quality differences between treatments. Hence, the deposition quality dataset did not allow sufficient variation between treatments to be incorporated in the model. As deposition quality undoubtedly influence efficacy (Fourie et al., 2009; Koch and Kneuwentz, 2008, 2011; van Zyl et al., 2010b), future research will attempt to include deposition quality measurements in the FPC benchmark model. However, the model was effective in determining the percentage protected leaf area above the FPC₅₀ benchmark. This parameter can therefore also used as a deposition quality indicator.

The FPC benchmark model can be an effective tool to evaluate deposition of varying spray volumes, spray machines and technique for the control of ABS and similar fruit and foliar diseases. Fungicide dosage/concentration can also be evaluated leading to implementation of effective but environmentally sound application rates.

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