Crop Protection 115 (2019) 92–98

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Due diligence required to quantify and visualise agrichemical spray deposits using dye tracers

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ARTICLE INFO

Keywords:
Spray
Retention
Coverage
Visualisation
Pyranine
Photodegradation

ABSTRACT

Dye tracers allow researchers to optimise spray application equipment and spray formulations by quantifying the amount of agrichemical spray retained and/or quantifying the area covered (including the size and number of spray deposits). This paper discusses the issues involved in selecting a dye fit-for-purpose, and outlines the experimental checks required to ensure the dye will provide accurate, reliable results of spray retention and spray coverage. This is illustrated using a pilot study to identify a dye, or combination of dyes, capable of quantifying both spray retention and spray coverage on the model species *Eucalyptus fastigata* H. Deane & Maiden. Due diligence in selecting a dye to quantify agrichemical spray retention requires checks to ensure the dye is quantifiable, extractable from the target surface, and stable not only in the spray formulation used, but also under the prevailing environmental conditions (sunshine, temperature and humidity), and after storage on the target surface prior to extraction and analysis. To visually quantify spray coverage and/or the number and size of deposits, tests are required to ensure the dye provides clear contrast between the dye deposits and non-covered surface. Tests are also required to ensure the dye selected does not alter the physical properties of the spray. There are numerous reasons why a dye may fail to provide accurate spray deposit or coverage results, or correct results. In this study, one dye was not representative of the formulation spread (and therefore coverage). Two others could be used to visualise spray deposits but, due to UV degradation, retention was not quantifiable. A fourth dye was too difficult to visualise on the target. A fifth dye (pyranine) was a suitable candidate; it was photo-stable in dried deposits, quantifiable and could be visualised with an intense UV light source. The plant target itself presented unexpected complications; the sampling method previously employed in retention trials enabled pyranine dye to penetrate the leaves, due to moisture from the dehydrating leaf solubilising and mobilising spray dye deposits during storage. A revised sampling method, allowing the leaves to dehydrate without condensation, was required to fully recover the dye. Using the identified methodology pyranine proved to be suitable as a dual-purpose dye capable of quantifying both spray retention and spray coverage under the anticipated experimental conditions. This paper highlights the requirement for due diligence in dye selection prior to investing in a full-scale field trial. Researchers must check every stage in the experimental process of selecting dyes as deposit tracers. If not, they may fail to get results or, more likely, unwittingly publish incorrect results.

1. Introduction

Key factors involved in achieving a high level of spray efficacy when using foliar applied agrochemicals are (1) maximising the quantity of spray retained by the target plant, (2) leaf coverage, and in the case of systemic pesticides (3) uptake and (4) translocation (Zabkiewicz, 2007). For many contact insecticides and fungicides the exact nature of the spray coverage is especially important, i.e. the size of and number of deposits delivered and the dose they contain (Ebert and Downer, 2006).

Researchers commonly use dye tracers to quantify spray retention and/or coverage in order to compare and optimise spray application equipment and spray formulations to enhance spray efficacy. Dyes suitable for determining spray retention (i.e. total mass retained per leaf area or plant area) must be 100% recoverable from the target, stable showing no loss in sensitivity under application and storage conditions, and quantifiable with sufficient sensitivity to be detected at concentrations of interest. Dyes suitable for visualising and quantifying spray coverage, and/or the size of the deposits and the number of deposits, must provide clear contrast between dye deposits and non-covered background. The dyes must be sensitive (intense) enough to allow measurements of small or thin spray deposits at concentrations relevant to the efficacy of the spray formulation of interest. For either application, the dyes must not

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https://doi.org/10.1016/j.cropro.2018.09.009
Received 29 May 2018; Received in revised form 11 September 2018; Accepted 14 September 2018
Available online 01 October 2018

alter the physical properties of the agrichemical spray formulation responsible for spray retention and coverage. Tartrazine is commonly used to quantify spray retention (Cross et al., 2001; Dorr et al., 2016; Gaskin et al., 2000). The fluorophore pyranine has been used to quantify spray retention (Gaskin et al., 2013; Khot et al., 2012) but is more commonly used to measure off-target spray drift due to its high sensitivity (Nairn and Forster, 2015; Richardson et al., 1995). Brilliant Blue and fluorescent dyes (e.g. SARDI or Leaf Check) have been used to visualise spray retention and coverage to demonstrate differences among sprays (formulations and/or application techniques), for instance to growers in the field or as pictorial evidence in publications (Gaskin, 2016; van Zyl et al., 2010). Both Blankophor and UVITEX fluorophores are routinely used in laboratory-based experiments to quantify and visualise the spread areas of individual droplets of known volume applied to leaves (Gaskin et al., 2000; Holloway et al., 2000; Nairn et al., 2016), which provides a gross indication of the differences in leaf coverage that might be expected when plants are sprayed with different formulations (Forster et al., 2014).

Natural targets such as plants are very complex with heterogeneous leaf surface properties, variable leaf area and leaf angle profiles, and canopy structure. This variability can cause different plant species to either retain high quantities of a spray formulation or retain almost no spray at all (Forster et al., 2014). Even within plant species large differences in wettability can be found with the age of the leaf and the season/life cycle of the plant (Forster and Van Leeuwen, 2010). Only by sampling the actual plant surfaces will direct information be obtained about the spray behaviour of each treatment which is directly relatable to spray efficacy on that species. Artificial targets are poor indicators of spray behaviour on natural surfaces (Forster et al., 2014) due to significant differences in surface properties, giving markedly different spray retention and coverage (Faers and Pontzen, 2008; Forster and Kimberley, 2015; Nairn et al., 2016). The mechanisms occurring once the spray contacts the actual plant (i.e. bouncing, shattering, adhering, spreading, uptake, etc.) are too dependent on the properties of the leaf surface to be accurately mimicked by an artificial target. Artificial targets, however, do provide consistent target properties (surface wettability, area, angle and placement within the spray swath). They may be used for spray accountancy in drift trials (e.g. Rotorods and artificial foliage, Richardson et al., 2017) or to determine the volume of spray available to the plant (e.g. plastic collection pottles, Dorr et al., 2016) and assist growers in sprayer setup to confirm that the spray is delivered to (intercepts) the target plant (e.g. water sensitive papers, Gaskin et al., 2011), thus allowing information to be garnered about formulation and application effects. The best choice of target depends on the scientific question being investigated.

While dyes are routinely used to audit spray behaviour, researchers still need to do due diligence to ensure the chosen dye provides meaningful results on the chosen target. This paper demonstrates the range of experimental tests required to ensure a chosen dye will provide accurate, reliable results for the intended purpose. The dye vetting process is illustrated through a pilot study which sought to identify a dye, or combination of dyes, capable of quantifying both spray retention and spray coverage on the model target species *Eucalyptus fastigata* H. Deane & Maiden (brown barrel; the target plant). The pilot study was for a planned field trial to test aerial spot spraying technologies. Commonly used dyes, as referenced above, were tested in this study. The photo-stability and percentage wash recoveries of dye formulations were also audited on the target foliage. A substantial field trial is likely to produce more samples than can be feasibly processed in a day, hence dye formulations were also tested for their storage stability over time.

2. Materials and methods

2.1. Plant material

E. fastigata (brown barrel) leaves were taken from the lower canopy of outdoor grown 10–12m tall plantation trees in the Scion nursery, Rotorua, NZ.

2.2. Chemicals

The following dyes were investigated: 189 Leaf Check (“Leaf Check”; Topline paint PTY Ltd.); Blankophor P 167% (Bayer AG); pyranine (Ravenswoof Australia); UVITEX (Tinopal NFW 450%, CIBA-GEIGY New Zealand Ltd.); tartrazine and Brilliant Blue (both Hawkins Watts Ltd.). The surfactants Li-1000 and Bond Xtra (both Etec Crop Solutions Ltd.) were added to some treatments. Tris buffer (required to quantify pyranine) was made by adding 0.1M hydrochloric acid (36%, Ajax Chemicals) to 0.1 M Tris(hydroxymethyl)-methylamine (AnalaR Biochemical, BDH Chemicals Ltd,) until a pH $>$ 8.5 was achieved (see Nairn and Forster, 2015). Concentrations (%w/v) of dyes and surfactants used are given in Tables 2 and 3.

2.3. sensitivity test (dye quantification limit of detection)

A Jenway 6285 fluorimeter (Cole-Parmer Ltd., Beacon Road, Stone, Staffordshire, ST15 OSA, UK) was used to measure the fluorescence of pyranine samples at 514 nm from 425 nm excitation. Fluorescence from samples containing UVITEX, Blankophor or Leaf Check dye were measured using a Polar Star Galaxy fluorimeter (Alphatech Systems Ltd., 630a Great South Rd, Green Lane, Auckland, New Zealand) at 460 nm from 320 nm excitation. A UV mini 1240 spectrophotometer (Shimadzu Corp., 1 Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604–8511, Japan) was used to measure dye absorbance of tartrazine (λ = 427 nm) and Brilliant Blue (λ = 630 nm) samples. The concentration of dye in each sample was determined using standard curves of concentration vs. fluorescence or absorbance for each dye.

2.4. Spreading test (spread area measurement)

For spread area analysis, droplets (0.25 μL; 24 droplets per treatment as six droplets on each of four different leaves) of each treatment (Table 2) were applied to the adaxial surfaces of freshly excised leaves (harvested July/August 2016). Once the droplets had dried, they were photographed under UV light, using an ordinary digital camera (Panasonic Lumix DMC-TZ20) mounted above the leaves. Images were converted to black and white binary images using Adobe Photoshop® (CS3 extended version 10.0, Adobe Systems Inc.) and the areas measured by Digital Optics V++™ image processing software. The treatment without fluorescent dye added was photographed under strong fluorescent lighting (Tri-lite 3 × 30W bulbs) before droplet dry-down. The droplet spread boundaries were defined by hand using Adobe Photoshop® and the area measured using Digital Optics V++.

2.5. Visibility test and spray coverage quantification

To test visibility, dye solutions were sprayed onto *E fastigata* leaves using a spray bottle producing a spectrum of fine droplets. These sprayed leaves were photographed, under appropriate illumination, and processed to determine suitability of the dye visibility for the coverage quantification as described above.

To test the methodology for visualising and analysing spray coverage produced from a commercial sprayer setup, a calibrated belt tracksprayer was used to apply spray onto *E. fastigata* cuttings using either a flat fan TT11003VP nozzle (Spraying Systems Co., Wheaton, USA. @100kpa, flow 0.68L/min, speed 0.19 m/s, rate 450L/ha, giving approx. 560 μm volume median diameter (VMD) droplets) or a flat fan XR11001VP nozzle (Spraying Systems Co., Wheaton, USA. @ 400kpa, 0.45L/min, speed 0.13 m/s, rate 450L/ha, giving approx. 160 μm VMD droplets) mounted 0.5 m above mean foliage height. Sprayed leaves were photographed as above for fluorescent dyes (for pyranine, two high intensity Spectroline Maxima™ 3500/FA lights were required), or under laboratory fluorescent lighting, for coloured dyes. The images were individually cropped using Adobe Photoshop® to eliminate the background while at the same time maximising the leaf area within the cropped image, and then converted into a black and white binary image. A