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A Fluorescent Tracer Method for Evaluating Spray Transport and Fate of Field and Laboratory Spray Applications

ABSTRACT: Field and laboratory testing spray nozzles and application systems use spray collectors to assess where the spray deposits once it leaves the spray system. Tracer materials, such as oil and water soluble fluorescent dyes, can be mixed into spray solutions in small amounts with minimal impact on the solution physical properties and atomization characteristics of the nozzle or spray system. Depending on the dye and recovery methods used, detection levels may range from g/ha down to the parts per trillion making them practical for use in studies where these ranges may occur, such as drift studies that measure in-swath and downwind deposition. Exposed samples can be quickly processed, typically using non-toxic solvents such as purified water or ethyl alcohol. This quick, inexpensive processing allows users the flexibility to plan studies requiring large numbers of samples. Spray formulations using these dyes at specific rates are used as the basis for establishing a set of standard concentrations which are used to relate fluorescence to dye concentration. Laboratory evaluations of tracer deposition compare the fluorescence of processed samples with the fluorescence from a set of reference samples with known dye concentrations allowing users to calculate the actual amount of dye in the sample and thus concentration on the sample being processed. This work will present the step by step process of converting dye concentrations from known and unknown samples to deposition measurements. A downloadable Microsoft Excel® spreadsheet will be made available to allow users to replicate these techniques. This technique effectively allows these tracer materials to provide a direct measure of the amount of active ingredient and/or spray mixture that deposits on sampling surfaces of interest.

KEYWORDS: fluorescent tracer, spray deposition, tracer dye

Introduction

Fluorescent tracers have been used to measure both dry and wet application deposition rates of agrochemical products applied onto plant surfaces [1–3] for many years. Additionally, fluorescent dyes have been used extensively in spray drift trials [4,5] and provide a method for tracing spray movement at the low levels resulting from far field drift. They allow for both visual [6–8] and quantitative deposition measurement [9,10] in a quick, inexpensive method [2]. Typical tracer dyes also offer the advantage of not significantly altering the physical properties of the spray solution and thus do not affect the atomization process meaning that treatments are applied with droplets sizes and in spray patterns that are the same as what would result without the dye present [11]. With the ease of use and other advantages, there are also some disadvantages including sample recovery [12], fluorescent variation with solvent type [13] and solar degradation [14] that must be addressed when using these dyes.

The authors have used fluorescent tracers in studies examining spray drift and on crop deposits from aerially applied sprays [15–18], determining the collection efficiency of airborne spray collectors [19,20], assessment of the coverage and deposition from the application of barrier control treatments [21], and the assessment of insect bioassay cages [22]. The methods developed and used by the authors are a result of the dyes selected, the facilities used for processing sample media, and the equipment available for processing samples. The objective of this work is to document the fluorometric tracer methods used by the authors and provide guidance and tools for other users to adopt and replicate these methods.

Methods

Fluorescent Dyes

The two dyes predominately used by the authors are Caracid Brilliant Flavine (CBF) FFS (Carolina Color & Chemical Co., Charlotte, NC), a water soluble dye, and Tinopal OB (M F Cachat, Lakewood, OH), an

oil soluble dye. Both dyes are readily soluble and detectable at low concentration levels, which make them ideal for spray application studies. Once in solution, the fluorescence can be determined using a fluorophotometer. The authors use a Shimadzu (Kyoto, Japan) Model RF-5301PC spectrofluorophotometer that is interfaced through a computer using Hyper RF$^{©}$ software (Version 1.57, Shimadzu, Kyoto, Japan). The minimum detection level of the unit is 0.07 ng/cm^2. Each dye has a unique pairing of optimal excitation and emission frequencies that result in maximum detection. These frequencies can be determined a number of ways, but the Shimadzu RF-5301PC automates the process through a simple iterative scanning procedure. The RF-5301PC has workable excitation and emission wavelengths from 220 to 900 nm. Initially, users can scan through the entire frequency spectrum and determine an initial best guess for the optimal excitation and emission frequencies. Typically, on the second pass, one of the frequencies, the emission frequency for example, is set to a constant and the other, the excitation, is scanned through a narrower range of frequencies based on the initial first pass best guess. The new optimal excitation frequency is then set constant for the next iteration and a narrower range for the emission frequency scan is set. This process is repeated until an optimal pairing of excitation and emission frequency ranges is determined. The optimal excitation/emission frequency for the CBF dye in ethanol is 432/489 nm and is 372/427 nm for the Uvitex dye in hexane.

Dye Concentration Standards and Determination of Known Sample Concentrations

Prior to processing and analyzing samples of unknown concentration, a set of references of known concentration must be generated. This process is a straightforward mixing of dye and solvent to generate an established range of concentrations. Our standard sets typically cover a range from 1 to 0.0001 parts per million (ppm) decreasing an order of magnitude with each standard (e.g., 1, 0.1, 0.01. 0.001, and 0.0001 ppm). The authors have generated an interactive Excel spreadsheet to aid users in calculation of the mixing and dilution volumes (Fig. 1).

When the user opens the worksheet, they will see 0s in all the user-entered fields and #DIV/0 in the calculated fields. The user-entered fields are highlighted in gray with blue lettering. The worksheet has room for the user to enter study name, date, and any notes. It is recommended that the user save the file since the interactive worksheet is a read-only file. To use the interactive worksheet, the user would follow 13 steps.
- Step 1. Enter the desired initial concentration. 600 ppm has been found to work well for both dyes.

FIG. 1—*Interactive worksheet to determine dilution rates from a starting solution of know concentration to standard reference concentrations as seen by the user with blanks in user fields.*

- Step 2. Enter the volume of solution one would like to make with 50 and 100 mL being suggested. 50 mL is used in this example.
- Step 3. Select the initial dilute. This will be water (specific gravity=1.0) for water soluble dyes or BVA Oil (specific gravity=0.8943) for oil soluble dyes. At this point, the user will be working with grams of dye, diluents, and solvents since much more accurate mixing can be done by weighing the components. Based on the information in Steps 1–3, the user is instructed to add 0.03 g of dye to 50 g of diluent.
- Step 4. After weighing the dye on the scale, enter the actual amount of dye added to the beaker or flask in which the solution will be mixed.
- Step 5. Enter the actual weight of the diluent added to the beaker or flask. The actual dye concentration in the diluent solution is then calculated. A magnetic stirrer is recommended to ensure that the dye fully goes into solution.
- Step 6. The next step is to select the type of solvent that the subsequent solutions will be made with. The authors highly recommend that this solvent be the same one used to wash off the dye from the deposition samplers. The specific gravity of the solvent selected is used to adjust volume dilutions to mass for more accurate mixing.
- Step 7. To make a 100 ppm solution, the user is told to mix 8.3897 g of the initial diluent/dye solution with 32.8306 g of solvent (ethyl alcohol in this example). To complete Step 7, the user enters the actual weights of solution and solvent measured out into another beaker or flask.
- Step 8. Since this is a serial dilution, the user will take the mixed solution from Step 7 and be instructed to mix 3.9191 g of this solution into 35.7400 g of solvent, then mix well. The user will notice that the recommended rates for the next step are not calculated until the user enters the actual weights for the current step into the spreadsheet.
- Steps 9–13. Repeat Step 8 sequentially for the remaining dilutions.

Given that the actual mixing masses typically vary by some fraction from the calculated masses, user recorded actual masses are used to calculated the actual dye concentrations of the generated standard concentrations (right side of Steps 7–13 in Fig. 1). These generated standards and associated known concentrations are then used in the analysis of unknown samples.

Processing and Analysis of Samples for Dye Concentration

Samples collected during a laboratory or field study that were used to measure spray deposits from spray systems are processed and analyzed by washing the samples with a solvent and measuring the fluorescent response from the collected wash solutions. The actual dye concentrations from the collected samples are then determined by comparison with the set of reference standard concentrations.

Samplers are typically collected in pre-labeled zip-top plastic bags and stored in light-proof containers (to shield samples from sunlight), which are transported to the laboratory for analysis. Samples exposed to aqueous solutions spiked with CBF tracer dye are washed in 100 % ethanol. The amount of ethanol used to wash the samples depends on the sampler type and the amount of spray deposited. Typically, samples are washed in 20 to 50 mL of ethanol. Ethanol is dispensed directly into the zip-top bag holding the individual samples after which the samples are agitated by hand for 15 to 60 s, depending on the sample type collected and the dye and solvent used. Following agitation, 5 mL of wash solution are decanted into a borosilicate glass culture tube (cuvette) (12×75 mm, 6 mL volume, Kimble Chase, Vineland, NJ) which is then topped with a plastic cap and then placed in a labeled rack.

Cuvettes containing the samples are then placed one at a time on the spectrofluorometer and the fluorescence reading recorded. The fluorescence value of the sample is compared to fluorescent measurements of the standard concentrations and the concentration of the dye in the sample is determined using Eqs 1 and 2. The actual volume of spray material deposited can then be calculated by dividing C_{sample} (mass of dye per area of sampler) by the mixing rate of the dye in the tank mix (Eq 3)

$$C_{wash} = \frac{F_{wash} \times C_{standard}}{F_{standard}} \qquad (1)$$

where:

C_{wash}=concentration of dye in the wash solution in the cuvette ($\mu g/mL$),
F_{wash}=fluorescent reading from the wash solution in the sample cuvette,

FIG. 2—*Fluorescence of samples of dye to ethanol concentrations ranging from 0.001 to 100 ppm with linear fit of fluorescence versus dye concentration.*

$C_{standard}$=known concentration of dye in standard solution (μg/mL), and
$F_{standard}$=flourescent reading from $C_{standard}$.

$$C_{sample} = \frac{C_{wash} \times V_{wash}}{A_{collector}} \qquad (2)$$

where:
C_{sample}=concentration of dye per area of the sampler (μg/cm^2),
V_{wash}=volume of solvent used to wash the sampler during processing (mL), and
$A_{collector}$=area of the collector used (cm^2).

$$C_{spray\ material} = \frac{C_{sample}}{R_{tank}} \qquad (3)$$

where:
$C_{spray\ material}$=concentration of spray material deposited per area of the sampler (mL/cm^2), and
R_{tank}=mixing rate of tracer dye into spray tank solution (μg/mL).

The relationships above are based on the assumption of a linear response between fluorescence and dye concentration which require that samples tested fall within concentration ranges under which the standard concentrations follow a linear response. Standard concentrations of 0.001, 0.01, 0.1, 1, 5, 10, 25, 50, 75, and 100 ppm (dye to ethanol) were generated and the relative fluorescence plotted to determine the range of linearity. The CBF and ethanol solution was not linear over the full range (R^2=0.917 for a linear fit, Fig. 2) but was linear over the range of 0.001 to 1 ppm (R^2=1.0 for a linear fit, Fig. 3). Based on this, the authors typically design field study treatment mixes and sample processing procedures such that the sample depositions of dye per area of sampler fall in the 0.001–1 ppm range.

Determination of Mix Rates for Optimal Detection Under Field Conditions

When conducting a field or laboratory study, it is critical to mix dye concentrations in a manner such that the deposited values fall into the range of linear response as established above. Controlled laboratory studies can be easily designed to meet this requirement as the majority of application conditions are known. However, for a field study, especially one measuring both in-swath and downwind drift, determin-
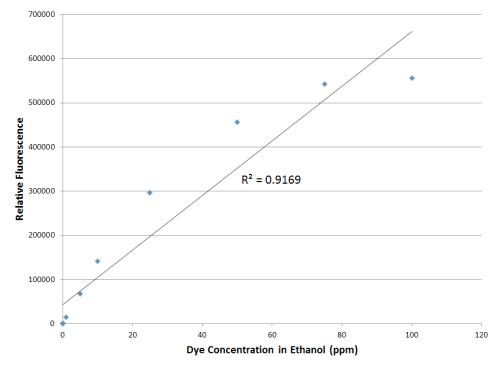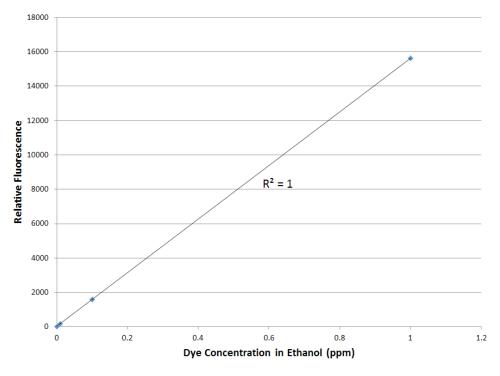
FIG. 3—*Fluorescence of samples of dye to ethanol concentrations ranging from 0.001 to 1 ppm with linear fit of fluorescence versus dye concentration.*

ing the dye mixing rates for spray treatments of varying spray droplet size and rate requires some level of prior experience or quantitative assessment. The authors' research generally focuses on aerial application of crop production and protection materials using a wide variety of treatment parameters including spray rate, spray droplet size and nozzle type. The use of a model such as AGDISP can help to determine appropriate mixing rates to meet the targeted dye deposition rates.

An initial maximum deposition value for a specific sampler is set based on the linear ranges defined earlier. For this example, a maximum deposition concentration on a 10×10 cm ($A_{collector} = 100$ cm^2) mylar fallout plate must be established. The maximum concentration of a sample in the cuvette, or C_{wash}, of 1 ppm (1 μg/mL) was established earlier. Given that the maximum concentration during a field study would typically occur in-swath, the sample would be washed with 40 mL (a volume selected by the authors based on prior experience and testing of sample recovery) of solvent, V_{wash}, during the processing stage. Using Eq 2 our maximum field deposit, C_{sample}, is determined to be 0.4 μg/cm^2. This provides a targeted dye deposition rate that can be used to determine an optimal dye mixing rate based on AGDISP modeling results as described below.

Following along the previous example, consider a field drift study using an AirTractor 402B (Air Tractor, Inc., Olney, TX) applying 18.8 L/ha (2 gal/ac) with a 20 m (65 ft) swath at a height of 4.5 m (15 ft) over bare ground under moderate stability with 2.2 m/s (5 miles per hour) wind speeds and 21°C (70°F) with 70 % humidity and wind directions perpendicular to the swath. Also assume that a fine to medium spray droplet size (based on ASABE Standard S572, Spray nozzle classification by droplet size [1]) is being applied. After entering all of the appropriate information into AGDISP and assuming that 100 % of the spray mixture is active and nonvolatile that model is executed and returns a maximum in-swath deposition of 22.5 L/ha which is equivalent to 0.000 225 mL/cm^2. Based on the previous discussion, our maximum dye deposition rate, C_{sample} is 0.4 ug/cm^2 and based on the results of the AGDISP modeling the concentration of spray material per area of the sampler, $C_{spray\ material}$, is 0.000 225 mL/cm^2. The mixing rate of dye in the tank can be solved for using Eq 3; for this example we find that R_{tank} is equal to 1778 μg/mL. This rate is typical of previous studies conducted by the authors under these application conditions.

This worksheet will guide users through the process of analyzing a collected tank sample for the actual tracer dye concentration			

Step 1	Enter the intended dye mix rate per application area	2	Application Rate (gpa)
		10	Dye Rate (g dye/acre)

Step 2	Select tank solution carrier.	Water
Step 3	Select solvent to be used to dilute tank solution	Ethyl Alcohol

Using the values in red below, mix solvent and tank sample to create a sample with dye at a concentration of 100 ppm

Add	0.9463	g of the tank solution
Plus	38.7034	g of solvent

Record the actual amounts added below:

Step 4	USER INPUT	0.96 g of the tank solution
		39.2 g of solvent

To prepare the 0.01 ppm dilution,

Add	0.3945	g of the mixed solution
Plus	39.0555	g of solvent

Record the actual amounts added below:

Step 5.	USER INPUT	0.394 g of the solution from Step 4
		39 g of solvent

Fluorometric Analysis of 0.1 ppm sample and Reference Standard					
		Readings			Average
		Rep 1	Rep 2	Rep 3	
Step 6	0.1 PPM Sample	184	186	186	185.33
Step 7	Standard 0.1 ppm Solution	119	123	126	122.67

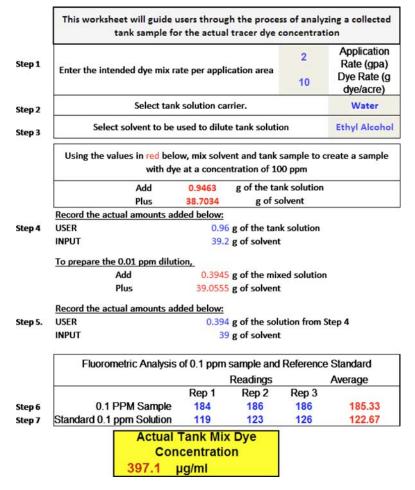**Actual Tank Mix Dye Concentration**
397.1 µg/ml

FIG. 4—*Determination of actual tank mix dye concentration by comparison to 0.1 ppm reference standard.*

Determination of Actual Tank Mix Concentrations

For studies where mass balance or spray accountability is important, the actual mixing rate of the dye in the tank mix is needed for accurate calculations of the deposition using the described methods. Once a dye mixing rate is calculated using the process described above, the required amount of dye is determined for the volume of spray solution to be used in a particular study. While this mixing process is usually very accurate, there are occasions where measurement errors, loss of weighed dye prior to addition to spray solution, or other losses result in a dye to spray solution rate that is different from that specified. The authors suggest that the actual spray solution dye concentration be determined for every application trial tank mix used. User need only collected 100 mL of tank mix to have adequate supply for analysis. An interactive spreadsheet (Fig. 4) was developed to provide dilution ratios for the spray solution tank sample and selected solvent to result in a sample with a dye concentration of 10 and 0.1 ppm (based on the theoretical mixing rate). The worksheet walks the user through seven steps to determine actual tank mix concentration of the dye. The numbers used in the following steps are only valid for the example detailed in Fig. 4.

- Step 1. Enter the application rate (gal/acre) and the intended dye rate (g of dye/acre).
- Step 2. Select the tank solution carrier (water or oil).
- Step 3. Select the solvent used to dilute the tank sample. This must be the same solvent used in the preparation of the dye concentration standards (Fig. 1). The user is then instructed to mix 0.9463 g of the tank solution with 38.7034 g of the solvent (ethyl alcohol in this case).
- Step 4. Enter the actual weights of tank solution and solvent poured into the beaker or flask used in the mixing. These values are then used to instruct the user to add 0.3945 g of the mixed solution (Step 3) with 39.0555 g of solvent.
- Step 5. Enter the actual weights of solution and solvent added from Step 4. The resulting solution
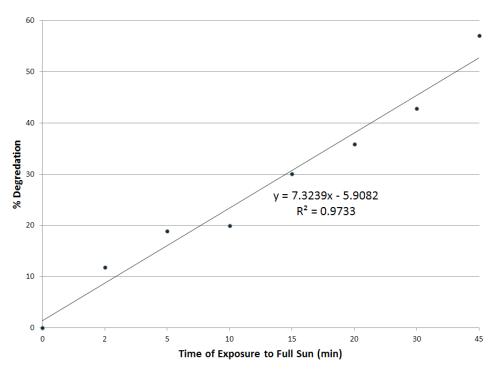
FIG. 5—*Dye degradation with exposure to full sunlight.*

should in theory be a 0.1 ppm solution which can be compared to the 0.1 ppm standard concentration prepared earlier to determine the actual tank mix dye concentration.

- Step 6. Measure the fluorescence of three subsamples (i.e., replications) of the solution prepared in Step 5 and record the reading as Reps 1–3 under 0.1 ppm sample. The average will be calculated.
- Step 7. Measure the fluorescence of three subsamples (i.e., replications) of the solution prepared in Step 10 of the Standard Preparation procedure and record the reading as Reps 1–3 under the standard 0.1 ppm solution. The average will be calculated.
- Step 8. The actual tank mix dye concentration will be calculated at the bottom of the worksheet.

Recovery and Degradation of Dyes

Prior to analysis of sampled data, especially data collected under field conditions, it may be critical to adjust the data for degradation of the dye's fluorescence due to sunlight and for sample recovery values less than 100 %. Dye degradation rate with solar exposure for the CBF dye was determined by spiking a number of samplers with a volume of solution with a known dye concentration and exposing them to full sunlight for a specified period of time prior to collection and processing following the method discussed previously. Prior to the solar exposure 10 mylar plate samplers were spiked with 10 μL of a solution of CBF and water (1500 μg CBF dye/mL water) and processed using ethanol to determine the recovery rate. The average recovery rate was 89 %, which was accounted for in the degradation tests. To account for recovery loss, for any set of collectors processed following these methods, the dye per area concentration determined through the fluorescent analysis procedure is divided by the fractional recovery loss. For samples with a recovery rate of 90 %, the final concentration determined would be divided by 0.9 to correct for recovery losses.

Five replicated samples per time period were spiked with 10 μL of the CBF/water solution and exposed for ten different time periods; 0, 2, 5, 10, 15, 20, 30, and 45 min. After each time period expired, the five designated samples were collected and processed for deposition. This process was repeated on two different days and the results averaged. The average incoming solar radiation, as measured by a CS300-L pyranometer (Campbell Scientific, Logan, UT) and recorded every minute to a CR23X datalogger (Campbell Scientific, Logan, UT), was 879 and 915 W/m^2 for days one and two, respectively. Degradation was less than 20 % at up to 10 min of exposure, however after that degradation occurs rapidly up to almost 60 % at 45 min exposure (Fig. 5). Similar to the recovery loss corrections, for field collected samples

processed following these methods, the final determined dye per area concentration is divided by the fractional degradation losses.

Discussions and Conclusions

With growing concerns and necessity for greater understanding of the input pathways that contribute to climate change [23], there is an increased need for proven methods to study these pathways, which include environmental inputs resulting from agrochemical application. A methodology for using fluorescent tracer dyes for spray application research was documented. The use of these tracer dyes is not a new technique, but the methods used may vary from user to user, depending on research needs and available resources and equipment. This work describes, in detail, a proven procedure for incorporating the use of these fluorescent tracers. Use of the interactive worksheets will guide users through the generation of a set of reference standard concentrations and the determination of actual tank mix dye concentrations. These worksheets are readily available for use and can be obtained by contacting the authors. Additional guidance and recommended practices discussed should allow users to obtain the highest quality data allowing for a greater understanding of environmental spray transport and fate.

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