Photostability of pyranine and suitability as a spray drift tracer

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Abstract Pyranine, a water soluble fluorescent tracer, has been used in spray drift studies, yet there is limited information regarding analytical methods, and conflicting information on the rapidity and extent to which the pyranine may degrade. This study assessed the suitability of pyranine as a tracer for drift studies. While pyranine did decompose when exposed to sunlight whilst in solution, spray droplets dry in minutes and photodecomposition appears to be significantly slower in the solid phase. Droplet residues showed minimal photodecomposition after exposure to sunlight for 2 h. Pyranine samples could be stored in cool, dark conditions, as dried deposits or in solution, for over 2 weeks without significant degradation. Pyranine is a suitable tracer for spray drift determinations as there is no significant degradation over the time required to collect, process and measure samples allowing residues to be accurately quantified, provided liquid extracts are suitably buffered.

Keywords photodecomposition, fluorescence, pH.

INTRODUCTION

In order to improve spray application techniques it is important to be able to measure and quantify spray deposition and distribution. Researchers use tracers, which must be detectable in minute quantities, as very fine droplets can drift a long way leaving very tiny deposits. Fluorescent compounds are considerably more detectable than coloured (absorbance) dyes. Pyranine is a strong candidate as a tracer, having a high extinction coefficient ($22,000 \text{ cm}^{-1} \text{ M}^{-1}$; Setareh 2015), as well as high water solubility (300 g/litre at 25°C; Anonymous 2011), low toxicity and its residues are easily recovered with water washes (Zhu et al. 2005). However, despite being used in spray trials (Koo et al. 1999; Farooq et al. 2003; Khot et al. 2011a, 2014; Al-Jumaili & Salyani 2014), the methodology is ill-defined and there is some concern with pyranine stability and potential

photodegradation (Aley 2002; Khot et al. 2011b). Many dyes, including pyranine, have highly conjugated chromophores allowing photons to be absorbed. This can be an Achilles heel if the absorbed energy causes bonds to break and/or rearrange into new non-conjugated moieties, so most fluorescent dyes are photosensitive and prone to bleaching (Cai & Stark 1997; Pergher 2001; Hakonen & Hulth 2008). This study sought to confirm the suitability of pyranine as a spray drift tracer and how to minimise any such degradation during application and analysis.

MATERIALS AND METHODS

Chemicals

Pyranine (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) (Ravenswoof, Bayswater VIC 3153 Australia) was made up with distilled

deionised (DDI) water (40–40000 µg/ml). Tris(hydroxymethyl)-methylamine (Tris) buffer (AnalaR, BDH Chemicals Ltd, England) was made by adding 0.1 M hydrochloric acid (36%, Ajax Chemicals NSW 2144 Australia) to 0.1 M Tris (until the desired pH was achieved, typically pH 8.5).

Photodecomposition

Photodecomposition of pyranine in water (40, 400, 4000 and 40000 µg/ml) was measured in direct sunlight by placing 5 ml in 20 ml glass vials sealed to prevent drying out and laid sideways to maximise the surface exposed. To mimic spray deposits, 50 × (for the most dilute) or 5 × 1 µl droplets of these pyranine solutions were applied onto steel plates (7.5 cm × 15 cm × 0.9 mm thick). These plates were either placed in direct sunlight or, in order to mimic very humid (78–98%) conditions, floated in tupperware containers in a paddling pool covered with clingfilm. Controls were prepared and processed similarly but kept indoors under fluorescent lighting (ca 15 µmol/m²/s light intensity). To ensure accurate comparison additional controls under a clingfilm covered cage were exposed to sunlight in parallel to the samples in the paddling pool. The plates were placed into plastic bags and washed with 50 ml distilled deionised water after predetermined intervals. Audits showed a single wash gave ~100% pyranine recovery after droplet dry-down.

Meteorological data

Meteorological data (temperature, humidity and wind speed) were gathered continuously via a LB150 weather station (AIRMAR Technology Corporation, Milford, NH USA). A hygro-thermometer (Metermaster, Papakura, Auckland, New Zealand) was used to measure the humidity within the paddling pool. Sunlight intensity was measured every 15–30 min using a LI-1776 solar monitor (Design Electronics Ltd, Palmerston North, New Zealand). Sunny, mostly cloudless, summer days were used for the outdoor experiments. Typical weather conditions were: ca 28°C, 55% humidity, < 1 m/s wind speed and ca 1600 µmol/m²/s light intensity on average (clouds could reduce light intensity to 900–250 µmol/m²/s).

Fluorescence

Water sample extracts were buffered with an equal volume of Tris buffer. The most concentrated samples required a 10-fold dilution prior to buffering. A Jenway 6285 fluorimeter (Bibby Scientific Limited, Stone, Staffordshire, UK) fitted with a sipper pump was used to measure the fluorescence at 514 nm with 425 nm excitation.

Pyranine stability

Pyranine samples (10–0.01 µg/ml concentration) were prepared by serial dilution with DDI water from a 1000 µg/ml stock solution. These samples were stored in a cool dark room for 0, 6 and 13 days before 2 ml aliquots were diluted 10–20 fold with water plus Tris buffer (pH 8.5) and fluorescence measurements taken.

Statistical analysis

Treatments were compared using analysis of variance (Statistix 9) and Fisher's Protected LSD tests.

RESULTS AND DISCUSSION

Fluorescence detection range

Pyranine can be detected at very low concentrations; the detection limit on the Jenway 6285 instrument is < 1×10⁻⁴ µg/ml. The linear response line started at ca 1×10⁻³ µg/ml, but above 1 µg/ml is too concentrated for measurement. Researchers should aim to keep their samples between these concentrations to allow accurate measurement of spray deposit concentration. This may require increasing spray tank concentrations, reducing washing volumes and/or increasing spray target catchment areas.

pH dependence

The pH dependence of pyranine fluorescence follows the classic sigmoidal form; 40% change from pH 6.6 to 8.5 where it begins to plateau out, with only a further 8% change up to pH 10.7. Loss of fluorescence is not due to decomposition but the reversible protonation of the acidic moieties of the fluorophore. This highlights the need that when using pyranine as a tracer the methodology must include buffering of

the samples prior to analysis. While some studies using pyranine as a spray tracer refer to the pH sensitivity, none mention the pH, or buffering, when making fluorescent measurements (Koo et al. 1999; Farooq et al. 2003; Khot et al. 2011a, 2014; Al-Jumaili & Salyani 2014). Water used for tank mixes is often sourced from ground water streams, bores, etc., which can be highly variable in pH. Moreover, natural targets such as leaves may leach and change the pH of the wash solution. Unbuffered samples and standards could be very different in pH, impacting sensitivity and making quantitative comparisons impossible. In order to be able to compare between pyranine measurements, the extract should be buffered to the same pH > 8.5.

Storage stability

Pyranine samples were stable when stored in a cool (15°C) dark room for over 3 weeks, either as dried deposits or in unbuffered or buffered solution, and with or without prior photo-irradiation (Figure 1, not all data presented).

Photodecomposition in solution

Aley (2002) observed that pyranine tracer in streams and rivers decomposed on exposure to direct sunlight. Here, photodecomposition

was observed within sealed vials. This is not due to photoacidification (Hakonen & Hulth 2008) as the process is not reversible upon buffering. While the rate of photodecomposition increases with pyranine concentration (Figure 2), due to more material available to intercept the photons, the relative percentage decomposition decreases with concentration (Figure 3) due to photon saturation (Pergher 2001). There is an obvious red shift in colour of the samples with longer sunlight exposure (Figure 4 insert, liquid becomes darker over time). This can be seen in the absorption spectrum as a drop in the absorption peaks at 370 and 410 nm and the evolution of shoulders at 320 and 450 nm (Figure 4). Pyranine photodecomposition is very concerning in relation to use of the compound as a tracer for agrichemical spray where the targets and the spray deposits are likely to be exposed to direct sunlight for some length of time. However, the behaviour of pyranine in solution may not be representative of dried spray deposits.

Photodecomposition on steel plates – “normal” conditions

In order to better replicate spray deposits, 1 µl droplets, containing varying pyranine concentrations, were placed on steel plates and

Figure 1 Fluorescence of recovered pyranine in unbuffered water solution (wet) or from dry deposits (dry) on steel plates after storage for 6 to 13 days under cool dark conditions.

Figure 2 Rate of photodecomposition (µg/min) of 40, 400, 4000 and 40000 µg/ml pyranine solutions in sealed vials on exposure to sunlight over time.

Figure 3 Photodecomposition (% pyranine lost) over time of 40, 400, 4000 and 40000 µg/ml pyranine solutions in sealed vials on exposure to sunlight.

exposed to direct sunlight for up to 120 min. These samples showed little photodecomposition with close to 100% recovery (Figure 5). The more susceptible (see Figure 3) dilute samples, 40 and 400 µg/ml, were not significantly different to the non-exposed control (0 min) even after 120 min of direct sunlight exposure (Figure 5). The more concentrated 4000 and 40000 µg/ml samples showed minor (5-6%) but statistically significant photodecomposition over 2 h ($P < 0.05$, Figure 5). The droplets used here are very large compared to typical spray droplets, yet were observed to dry within 3 min of application in direct sunlight. Finer spray droplets would be expected to evaporate very quickly. The rate of photodecomposition appears to be significantly slower in the solid phase, which would account for the difference in results observed in the liquid phase (Figures 2, 3 & 4). Other studies found sprayed dyes exhibited rapid initial photodecomposition before slowing significantly (Pergher 2001; Khot et al. 2011b). Pergher (2001) concluded faster evaporation times may explain the difference between spray and large droplet photodegradations of tartrazine deposits. In agreement with the current study, Khot et al. (2011b) found slight pyranine photodecomposition with spray droplets,

Figure 4 Absorption spectra of 400 µg/ml pyranine solutions exposed to sunlight for 0, 60, 120 and 180 min. Arrows indicate evolution and devolution of absorption peaks with exposure time. Insert: Vials showing shift in colour with exposure time.

reaching ca 10% after 180 min exposure, and prescribed collecting targets before 10 min exposure where photodecomposition was an acceptable 3-4%. Wind erosion, after dry-down, cannot be discounted as an additional cause of deposit reduction, especially with the more concentrated deposits.

Photodecomposition on steel plates – very high humidity condition

Pyranine photodecomposition of spray droplets may be more significant in humid conditions where droplet drying times are extended. The most extreme conditions were simulated using a small covered plastic pool, where the humidity approached 98%. Without any air flow, this increased droplet drying times to ca 30 min. Once again there was negligible photo-decomposition observed for the 400 µg/ml samples, $3.4 \pm 1.8\%$ after 120 min vs $2.5 \pm 1.1\%$ for the control samples under “normal weather” conditions. These values were slightly greater than for the samples measured immediately after the droplets were dry, i.e. $1.8 \pm 0.7\%$ after 2.5 min for the control and $2.7 \pm 1.1\%$ after 30 min for the samples inside the humid environment. Similar low photodecomposition ($3 \pm 4\%$) was observed for the more dilute 40 µg/ml

Figure 5 Percent recovery over time of dried pyranine residues of 1 µl droplets of 40, 400, 4000 and 40000 µg/ml solutions on steel plates after exposure to sunlight. Error bars indicate 95% deviation.

pyranine solutions but with more data variability. None of these values are significantly different due to the low rate of photodecomposition. If the photodecomposition rate was comparable to that of pyranine in solution, during the 30 min before the droplet was completely dry, approximately 10% decomposition would be expected. Lower photodecomposition rates could be due to condensation on the cling film cover of the pool reducing the light intensity underneath and/or that not all the pyranine remains in solution during the entire drying time but is crystallising as the droplet dries. Droplets deposited from sprays would be significantly smaller than these 1 µl droplets, with shorter drying times even at high humidity.

Conclusions

In conclusion, pyranine is suitable as a spray drift tracer for water based formulations.

Pyranine shows high detectability at very low concentrations. This coupled with high water solubility, allowing high concentrations in spray mixtures, enables minute drift deposits to be detected. While pyranine does slowly photodecompose it does not appear to decompose once in a dry deposit, which makes it stable for the practical application of a spray trial. Pyranine shows good stability under storage as a dried deposit or in solution removing the need for rapid processing of samples. In practice, tank mixes with pyranine should be of appropriate (high) concentration, spray deposits should be exposed to sunlight for the least time practicable, and samples should be stored in a cool dark room and buffered to pH > 8.5 before measuring.

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