EVALUATION OF 1, 3, 6, 8-PYRENE TETRA SULFONIC ACID TETRA SODIUM SALT (PTSA) AS AN AGRICULTURAL SPRAY TRACER DYE

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ABSTRACT. *The ability to measure spray deposition and movement with the use of tracer materials is a necessity for agricultural application research. Ideally, any tracer material used is highly soluble in the solution being sprayed, easily recoverable from both artificial and plant samples, stable in solution, and not easily or quickly degraded in sunlight. The objective of this work was to evaluate 1, 3, 6, 8-pyrene tetra sulfonic acid tetra sodium salt (PTSA) for these properties. Comparison of four solvents showed that a 10% isopropyl alcohol solution provided the maximum recovery of PTSA deposits. Once in solution, PTSA proved to be highly stable, with no significant degradation after a week. Exposed to sunlight, PTSA deposits degraded less than 5% to 6% in the first 20 min and less than 15% to 20% after an hour. Examining recoverability from a variety of plant samples averaged approximately 80% recovery of total PTSA deposits. In addition to being readily available and affordable, PTSA proved to be an excellent option for agricultural spray research.*

Keywords. *Agricultural sprays, Dye recovery, Dye degradation, PTSA, Tracer dyes.*

The tracking of agricultural sprays from a sprayer to the intended target can be accomplished by chemical analyses, visual assessment, and the use of colorimetric and fluorometric tracer dyes. The appeal of dyes is that typically they are not phytotoxic to treated plants, have low mammalian toxicity, and can be inexpensive. Researchers have used tracer dyes to quantify spray deposition on soils (Barber and Parkin, 2003), crops (Briand et al., 2002), workers and applicators (Fishel, 2012), and to measure spray drift (Bui et al. 1998; Majewski et al., 1998). A number of different dyes have been investigated and the pros and cons of using each dye have been reported (Cai and Stark, 1997; Pergher, 2001) with one of the most significant limiting factors when using fluorescent dyes being that they can degrade rapidly in ultraviolet light or with heat (Yates and Akesson, 1963). Cai and Stark (1997) reported that 85% of Tiponal and 79% of Eosine dyes were lost in the first 15 min of exposure to sunlight. In the pursuit of more options for application researchers and applicators, this work evaluated the use of 1, 3, 6, 8-pyrene tetra sulfonic acid tetra sodium salt (PTSA) as a tracer dye for agricultural sprays.

The appeal of the PTSA dye as a new tracer are many. It is readily available, inexpensive, and extremely water soluble. In field concentrations it is nearly colorless and odorless. Further, it is reported to be highly detectible by fluorometry down to 0.1 part per billion in solution with capable devices. It is less susceptible to quenching at increased concentrations than many other common fluorescent tracers, resulting in a higher than normal linearity in fluorometers of five orders of magnitude or more, as compared to three to four orders with common tracers such as Rhodamine WT and fluorescein (Turner Designs, 2013a). Its fluorescence is also reported to be relatively insensitive to pH and temperature changes, with a temperature coefficient of 0.00126/degree centigrade (Turner Designs, 2013b). This is useful since its native pH as a 10% solution is reported to be approximately 9.5 (Spectra Color Corp, 2013a), so buffering in solution may be required if used with certain actives. This broad useful range of temperature and pH will make it easier for users of this tracer to extract comparable data under varying conditions with less risk of data variability and corruption. Lastly, it is sold as a precursor in Drug and Cosmetic (D C) Yellow #8 dye (Spectra Colors Corp, 2013b) which the FDA lists as acceptable for use in external application to the human body (USDA, 2011). The MSDS lists it as nontoxic and Class Zero (0) Health rating. This is potentially a very low risk, high performance fluorescent tracer.

The objective of this work was to evaluate the use of PTSA as a tracer dye for agricultural sprays through the evaluation of solubility, UV exposure stability, and recovery. The first step in this evaluation was to determine which solvents were both compatible with the dye and

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could remove a deposited spray solution from either an artificial sampler (mylar) or plant surfaces. The second step involved determining how stable fluorometric readings were once the dye had been placed in different solutions since it is not always possible to read samples in a timely fashion. The next step investigated the stability of the fluorescent dye in sunlight, as many dyes are subject to extreme degradation when exposed to UV light. The final step was to look the recoverability of deposits sprayed on different plant species.

MATERIALS AND METHODS

SPRAY SOLUTIONS

All of the following tests were conducted using PTSA obtained from Spectra Colors Corporation (2013a, Item Spectra Trace SH-P, www.SpectraColors.com, Kearny, N.J.). The dye was dissolved in distilled water at a rate of 0.03 g of dye in 49.97 g of distilled water or 0.6 mg/mL. Based on previous testing, a spray solution that has proven difficult to rinse off of spray target is a spray mixture of Roundup PowerMax® (EPA Reg. No. 524-549, Monsanto, St. Louis, Mo.) and Sylgard 309® (CA Reg. No. 2935-50161, Wilbur-Ellis Co., Fresno, Calif.). The PowerMax® was mixed at the rate of 67 g per L of water (32 oz/5gal of water) and the Sylgard® as the rate of 0.93 g per L of water (12 fl oz/100 gal of water). For these tests, 50 mL of mixed solution were made up containing 0.03 g of PTSA, 3.4 g of PowerMax (SG – 1.357 g/mL), 0.05 g of Sylgard, and 46.52 g of distilled water.

SAMPLE PROCESSING

For all the tests, samples (mylar or plant leaves) were placed in individually labeled plastic bags. Forty mL of the appropriate solvent (as described below) was pipetted into each bag, the bags were agitated by hand for ~15 s, and 6 mL of the effluent was decanted into a borosilicate glass culture tube cuvette (12×75 mm, 6 mL volume, Kimble Chase, Vineland, N.J.), which was then topped with a plastic cap and placed in a labeled rack. The cuvettes were then placed into a spectrofluorophotometer (Shimadzu, Model RF5000U, Kyoto, Japan) with an excitation wavelength of 375 nm and an emission at 405 nm. The fluorometric readings were converted to µg of dye. The conversion of a reading to dye concentration followed the method described by Fritz et al. (2011).

DYE RECOVERY IN DIFFERENT SOLVENTS

Four solvents (distilled water, ethyl alcohol, 10%v/v ethyl alcohol in distilled water, and 10%v/v isopropyl alcohol in distilled water) were evaluated in these tests. To select the most appropriate solvent for the PTSA dye, 10 µL of the water and dye solution was pipetted directly into a plastic bag or onto 12 mylar cards when testing each solvent. Six of the cards were immediately placed into individually-labeled plastic bags and 40 mL of the solvent was dispensed into the bag. This represented a scenario where the pipetted droplet was not allowed to dry and termed a wet sample. The other six cards were left for 15 min to allow the water in the pipetted droplet to evaporate and were termed dry samples.

DYE STABILITY IN VIALS OVER TIME

The next step was to evaluate the storability (i.e., stability) of the dye readings from vials that were stored up to 1 week. Readings were taken at 5, 15, 30, and 60 min, as well as, 1, 2, 3, and 7 days. The four solutions with 6 replications tested were distilled water, ethyl alcohol, 10%v/v ethyl alcohol in distilled water, and 10%v/v isopropyl alcohol in distilled water.

DEGRADATION OF PTSA DYE IN SUNLIGHT (NOON) OVER TIME

Two dye degradation test solutions (Water only solution and PowerMax and Sylgard solution) were evaluated for these tests with 10 replications for each test. At the start of each tests, 10 µL of each solution were pipetted onto mylar cards (10 × 10 cm). Holders were constructed that could securely hold 10 mylar cards (i.e., replications) for each of the times (0, 1, 5, 10, 15, 20, 30, and 60 min of sunlight exposure). After droplets had been pipetted onto each of the cards inside the laboratory, the cards were placed in direct sunlight around 12:00 p.m. The 0 min cards were not exposed to any sunlight. After each of the specified degradation time had passed, 10 cards were returned to the laboratory and placed in individually-labeled plastic bags. Dye deposition was determined using the methods described in the Sample Processing section using the 10% isopropyl solution as the solvent.

RECOVERY OF DYE FROM PLANT SURFACES

To evaluate the recovery of dye from plant surfaces, 10 µL of the simulated tank mix containing PowerMax and Sylgard was pipetted directly in the Ziploc bags (bag samples), onto mylar sheets (mylar samples), and onto leaves from seven different species (*Geranium sanguineum, Solanum lycopersicum, Cucurbita pepo, Solanum melongena, Capsium annum, Mora quachita,* and *Ixora coccinea)* with six replications of each test. The mylar and plant samples were allowed to dry for 15 min before washing each sample with 40 mL of a 10% isopropyl alcohol solution.

RESULTS AND DISCUSSION

DYE RECOVERY IN DIFFERENT SOLVENTS

The dye recovery was significantly different for the bag, wet mylar, and dry mylar samples for the ethyl alcohol and 10% ethyl alcohol solvents (table 1). The fact that the dry mylar recovery was significantly lower, at one-third the recovery rate, than both the bag and wet mylar, indicates that the PTSA does not go into solution well with the ethyl alcohol. While the difference with the 10% ethyl alcohol was small, numerically, given the poor performance of the ethyl alcohol only, this solution is also not recommended with the PTSA dye. The distilled water and 10% isopropyl solvents had no significant differences between the three samples. However, the distilled water only sample showed a synergistic effect resulting in a greater than 100%

Table 1. Dye recovery in µg dye per 40 mL of solvent used to wash 10 µL of spiked sample solution.

Sample	Solvents			
	Distilled Water (µg dye ± SEM)	10% Ethyl Alcohol (µg dye ± SEM)	Ethyl Alcohol (µg dye ± SEM)	10% Isopropyl Alcohol (µg dye ± SEM)
Bag	7.241 ± 0.065a[a]	6.773 ± 0.077a	6.633 ± 0.089a	6.620 ± 0.071a
Mylar (wet)[b]	7.188 ± 0.079a	6.535 ± 0.079b	6.122 ± 0.072b	6.587 ± 0.071a
Mylar (dry)[b]	7.317 ± 0.065a	6.785 ± 0.074a	2.534 ± 0.057c	6.716 ± 0.073a
DF (2, 131)	F=0.86 ns	F=3.40**	F=938.83***	F=0.98ns

[a] Means within each column followed by the same lower case letter are not significantly different.

[b] Wet refers to rinsing the mylar plate before the 10 µL droplet was allowed to dry, while dry refers to rinsing the mylar plate 15 minutes after the 10 µL droplet was pipetted onto the mylar plate.

recovery. Based on these results, the 10% Isopropyl alcohol solution was selected as the primary solvent when using PTSA spiked spray solutions.

Dye Stability in Vials over Time

The only solution with significant ($P<0.039$) degradation of dye concentration in the vials over time was the ethyl alcohol solution. Subsequent unreported testing revealed that the dye was not soluble in ethyl alcohol; therefore, ethyl alcohol should not be used as a rinsate with this dye.

Degradation of PTSA Dye in Sunlight (Noon) over Time

For the degradation tests with the water only and PowerMax and Sylgard solutions, the PTSA was stable with only moderate degradation over time (figs 1 and 2). For the water solution, the dye degraded approximately 1.1% for every 10 min (fig. 1), while the PowerMax and Sylgard solution had a dye degradation rate of 3.4% for every 10 min of sunlight exposure. The higher rate with the second solution could have been confounded by the adhesive behavior of the spray solution to the mylar surface as it dried.

Recovery of Dye from Plant Surfaces

The recovery for each of the different plant species is shown in table 2. Six additional replications were done for the tomato and pepper plants where the samples were left in the isopropyl alcohol solution for 10 min before being vigorously shaken and an aliquot of the rinsate was poured into the glass vials. This soaking time increased the recovery from 72.1% to 82.8% for the tomato plants and from 59.4% to 63.2% for the peppers. These two plants typically have significantly less wax on the leaf surface compared to the other species tested and it is hypothesized that there may have been uptake of the solution into the plant during these tested, which decreased recovery.

Conclusions

From the evaluations and tests conducted, 1, 3, 6, 8-pyrene tetra sulfonic acide sulfonic acid tetra sodium salt (PTSA) proved to be a stable, recoverable dye for water-based agricultural sprays. The dye is highly water soluble, has good photostability, and has a high recoverability off of most artificial and plant surfaces. The methods described will serve as a guideline for researchers who need to establish basic recovery analyses from collectors they use to assess spray deposition and drift. At the current time, the dye is available for approximately \$90/kg (\$40/lb), combined with the use of distilled water and isopropyl alcohol solution, make PTSA tracer dye a very affordable option for agricultural spray studies.

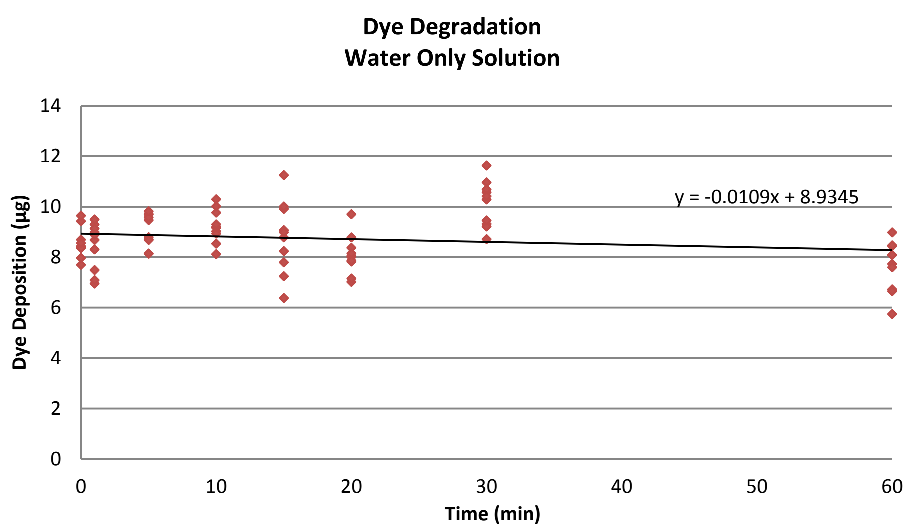

Figure 1. Dye degradation over time in direct sunlight for the water and dye only solution.

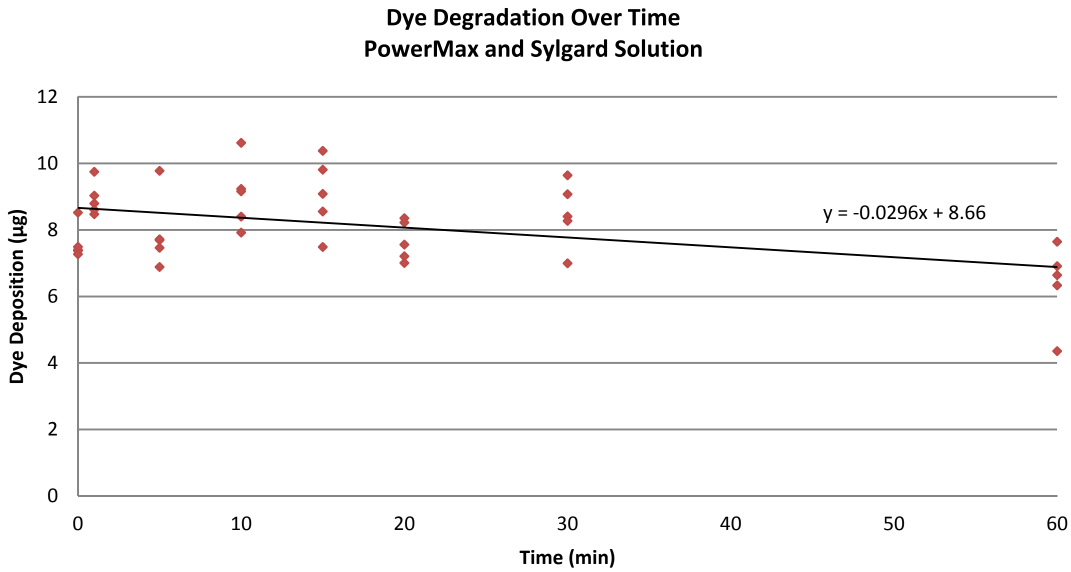

Figure 2. Dye degradation over time in direct sunlight for the PowerMax, Sylgard, and dye solution.

Table 2. Recovery of dye from different plant species.

Plant	Species	Percent Recovery
Geranium	*Geranium sanguineum*	90.6
Tomato	*Solanum lycopersicum*	72.1
Squash	*Cucurbita pepo*	78.7
Eggplant	*Solanum melongena*	81.6
Jalapeno Pepper	*Capsium annuum*	59.4
Blackberry	*Mora quachita*	84.3
Ixora	*Ixora coccinea* "Maui"	84.5

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