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## **Batch effect of the fluorescent tracer BSF on breakdown in sunlight and storage time**

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### **Summary**

Fluorescent dyes are convenient tracers in field studies on spray deposition and spray drift. However, such tracers are known to degrade when exposed to direct sunlight. Brilliant Sulpho Flavine (BSF) is one of the more stable tracers. Recent drift studies questioned the reliability of particular batches of this tracer. Therefore the rate of degradation of several batches of BSF was investigated. Passive collectors as used in field experiments were sprayed with aqueous solutions of BSF at various concentrations. The collectors were exposed to sunlight for times between 0 and 60 minutes. Afterwards, collectors were analysed directly or stored in a dark and cool place for up to 8 weeks before analysis of the deposits. The results indicated that all batches used showed degradation in sunlight, though differences between various batches can be large. Storage up to 8 weeks appeared to have no adverse effects on recovery. There seemed to be some effects of BSF concentration on degradation rate, though a clear relation between concentration and degradation was not found.

**Key words:** Tracer stability, spray drift, spray deposition, fluorescent tracer, BSF

### **Introduction**

In experiments concerning spray drift and spray deposition it is convenient to use a fluorescent tracer. A major advantage of fluorescent tracers is their high sensitivity, which means that spray deposits can be measured very accurately at low concentrations. Besides, the costs of analysis are low compared to those for deposits of active ingredients of plant protection products (PPP). However, a clear disadvantage of fluorescent tracers is their limited stability when exposed to direct sunlight as often is the case in field experiments on spray distribution and spray drift. Earlier research showed that the tracer Brilliant Sulpho Flavine (BSF) is one of the more stable tracers (Cai & Stark, 1997; Pergher 2001) which makes BSF a useful tracer in experiments involved with spraying. From earlier experience, about 95% of the sprayed BSF could be recovered when sampled within half an hour after spraying. However, several experiments in 2007 gave unexpectedly low recoveries for a specific batch of BSF. Similar problems with an apparently poor quality of BSF were noticed by Herbst (2006). Possibly, the degradation in sunlight for this specific batch was higher than that for other batches used before. Also, doubt was raised about the stability of BSF during storage of the loaded collectors and whether storage time and degradation were related to the amount of spray deposit on the collectors. Therefore, experiments were carried out to verify

the stability of BSF in sunlight and after storage in relation to the amount of deposits for three different batches of BSF used in field experiments for spray drift and canopy spray deposition. The setup and results of these verification experiments are reported. The potential of the described procedure for batch screening before use of the tracer under field conditions is addressed.

## Materials and Methods

To quantify the effect of BSF breakdown in sunlight in relation to storage time several experiments were setup in the WUR-PRI spray laboratory (Wageningen, The Netherlands). Three batches of Brilliant Sulpho Flavine (Chroma 1F 561, CI 56205) were used, indicated as BSF2007, BSF2009a and BSF2009b. Filter cloth collectors (Technofil TF-290; 50 cm × 10 cm) were sprayed at the spray track with a spray boom equipped with five nozzles moving at a speed of 6 km h<sup>-1</sup> and applying 315 L ha<sup>-1</sup>. The collectors were placed underneath the centre nozzle of the spray boom (boom height 50 cm) perpendicular to the driving direction. Collectors were with needles stucked to tempex plates (50 cm × 50 cm) placed next to each other in the driving direction to facilitate handling of the collectors during the experiments. The aim of the experiments was to evaluate the effect of BSF concentration on degradation in sunlight and storage time. On 16 and 17 March 2010 the BSF concentrations evaluated were 3, 0.3, 0.03 and 0.003 g L<sup>-1</sup>. On 19 April and 3 May 2011 additional measurements were done with concentrations 0.50, 0.25, 0.09, 0.050, 0.025, 0.010 and 0.005 g L<sup>-1</sup>. For each spraying occasion samples of the spray liquid were taken from below a spraying nozzle. After spraying, the tempex plates with the collectors were exposed to sunlight by placing them outdoors in the sun for 15, 30 and 60 minutes; a reference set of collectors was not exposed. Sunlight intensity during the exposure periods was on average 199, 243, 412 and 478 W m<sup>-2</sup> for the four measuring days. Measurements were performed in three repetitions. After exposure collectors were picked and put in coded plastic bags and were stored in a dark and cooled room at 10°C for a period of 2, 4, 6 and 8 weeks. A reference set of collectors was not stored but was analysed immediately. Finally, the collectors were analysed for amount of BSF by washing the collectors in 1.0 L demineralised water. Fluorescence was measured using a Perkin Elmer LS45 with optimised wavelengths for excitation and emission (BSF2007: λ<sub>ex</sub>=400; λ<sub>em</sub>=515; BSF2009a and BSF2009b: λ<sub>ex</sub>=450; λ<sub>em</sub>=500). Untreated collectors were analysed for background detection of fluorescence. Background fluorescence of the dilution water was determined also. The sprayed volume of BSF per unit surface area (μL cm<sup>-2</sup>) was computed by:

$$D_{collector} = \frac{(F_{collector} - F_{demi} - F_{blanc}) \times f_{cal} \times V_{dil}}{C_{tank} \times A_{collector}}$$

where D is spray deposition in μL cm<sup>-2</sup>; F<sub>collector</sub> is fluorescence value of the collector; F<sub>demi</sub> is fluorescence value of the demineralised water; F<sub>blanc</sub> is contribution of collector background; f<sub>cal</sub> is the calibration factor; V<sub>dil</sub> is the extraction (dilution) volume in litres; C<sub>tank</sub> is concentration of the used spray mix in g L<sup>-1</sup>; A<sub>collector</sub> is the surface area of the collector in cm<sup>2</sup>. If (F<sub>collector</sub> - F<sub>demi</sub> - F<sub>blanc</sub>) is smaller or equal to 0, a small positive value of 0.001 is used instead for further analyses. From this spray deposition, the percentage of spray deposition on a collector can be calculated, by relating the spray drift deposition to the amount applied in the field on the same unit of area:

$$P = \frac{D_{collector}}{O/100} \times 100\%$$

where P is the spray deposition percentage (%); D<sub>collector</sub> is the spray deposit in μL cm<sup>-2</sup> and Q is the applied dose L ha<sup>-1</sup>.

A statistical analysis (ANOVA, P < 0.05; Payne *et al.*, 2006) was performed on the measured spray deposition after different exposure times and storage periods for the different concentrations of BSF in the tank mix.

## Results

### *Breakdown in sunlight*

The results of the experiments on 16 and 17 March show that the BSF2007 batch has a significantly lower recovery than the batches BSF2009a and BSF2009b. Already at start ( $t=0$ ) there is a remarkably lower recovery for BSF2007 (44–80%) which decreases over time even further: after 30 minutes of sunlight exposure 26–68% of BSF is left and after 60 minutes only 11–59% (Fig. 1).

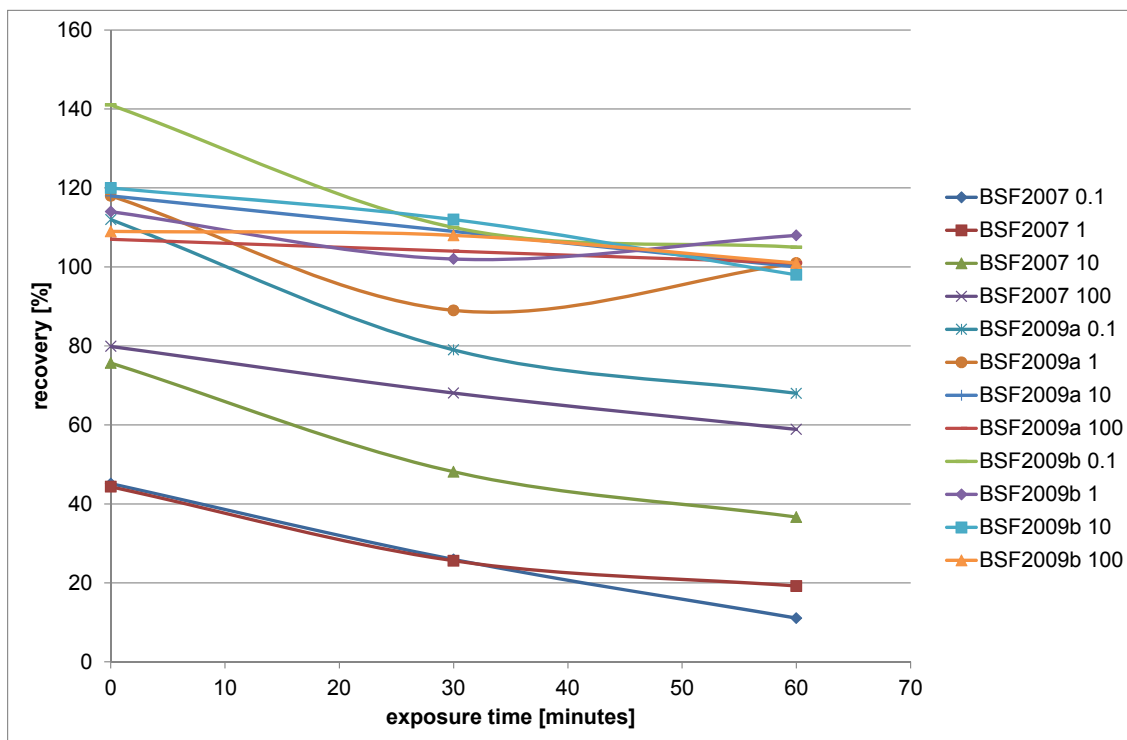


Fig. 1. Recovery (% of applied dose) of the fluorescent tracer Brilliant Sulpho Flavine (batches BSF2007, BSF2009a, BSF2009b) in sunlight sprayed on filter cloth collectors. For each batch the indices 100, 10, 1 and 0.1 represent the applied dose as a percentage of a  $3 \text{ g L}^{-1}$  spray solution directly after spraying and after 30 and 60 minutes of sunlight exposure.

The recovery of the batches BSF2009a and BSF2009b is around 100%, which indicates absence of breakdown. The BSF2009b 0.1% concentration at  $t=0$  shows a remarkably high recovery of 141%, whereas for the other concentrations of both 2009 batches initial recovery was within the range of 107–120%. No explanation could be found for this exception. Another strange result is the recovery of 89% for the BSF2009a at 1% concentration after 30 minutes exposure whereas at  $t=0$  and after 60 minutes the recovery was 118% and 101%, respectively. The lowest recoveries for the 2009 batches were found for the 0.1% concentration of BSF2009a after 30 minutes and 60 minutes of exposure in sunlight, yielding 79% and 68%, respectively. This may indicate that at lower concentrations of BSF the degradation in sunlight is faster.

With boom spraying, usually spray drift deposits next to a treated field decrease rapidly with distance and typically a few metres downwind from the field edge deposits below 1% of the applied dose are obtained (van de Zande *et al.*, 2010; Rautmann *et al.*, 2001). For orchard spraying spray deposits typically are about 10% at surface water distance (5 m) in the Netherlands (Wenneker *et al.*, 2005). Therefore it was considered useful to investigate degradation effects at relatively low concentrations of BSF. The 2011 experiments were focussed on concentrations below 20% of a typical dose of  $3 \text{ g L}^{-1}$  involving the batches BSF2007 and BSF2009a. The results of these experiments are presented in Table 1 and Table 2 for the BSF2007 batch and the BSF2009a batch, respectively. An extra time step in sunlight exposure was added at 15 minutes.

Table 1. Recovery (% of applied dose) of Brilliant Sulpho Flavine batch BSF2007 from filter cloth collectors at different concentrations and exposure times to sunlight

Concentration [%]	Exposure time to sunlight								Average	
	0 min	15 min	30 min	60 min						
0.1	71	c	29	def	29	def	17	h	36	b
0.3	87	ab	34	d	23	fgh	19	h	41	a
0.8	70	c	25	efgh	21	gh	24	fgh	35	b
1.6	80	b	24	fgh	18	h	19	h	35	b
3.1	83	ab	28	defg	25	efgh	22	fgh	39	a
8	90	a	28	defg	23	fgh	22	fgh	41	a
16.8	87	ab	32	de	27	defg	25	efgh	42	a
Average	81	a	29	b	24	c	21	d		

100% = 3 g L<sup>-1</sup>; different letters mean significant differences ( $\alpha < 0.05$ ).

Table 2. Recovery (% of applied dose) of Brilliant Sulpho Flavine batch BSF2009a from filter cloth collectors at different concentrations and exposure times to sunlight

Concentration [%]	Exposure time to sunlight								Average	
	0 min	15 min	30 min	60 min						
0.2	99	abcde	95	bcde	97	abcde	85	fgh	94	a
0.9	98	abcde	90	efg	82	gh	68	ij	84	b
1.7	94	cdef	92	def	79	gh	64	ij	82	b
3.4	102	ab	105	a	82	gh	69	i	89	a
8.5	97	abcde	99	abcde	82	gh	59	j	84	b
16.2	101	ab	104	ab	93	cdef	68	ij	92	a
Average	98	a	98	a	86	b	69	c		

100% = 3 g L<sup>-1</sup>; different letters mean significant differences ( $\alpha < 0.05$ ).

As in the 2010 experiments (Fig. 1), for the BSF2007 batch at t=0 the recovery is low with on average 81% recovery (Table 1). After 15 minutes of exposure to sunlight the average recovery decreased to 29%, after 30 minutes to 24% and after 60 minutes to 21%. At lower concentrations of BSF the exposure to sunlight leads to slightly lower recoveries although differences are hardly significant. For the 0.1% concentration and after 60 minutes of exposure the lowest recovery was observed (17%).

The exposure to sunlight of the BSF2009a batch (Table 2) shows that there is no significant breakdown (recovery 98%) for t=0 and 15 minutes exposure time. After 30 mins of sunlight exposure the recovery is slightly lower at 86% and decreases significantly to 69% after 60 mins of exposure time. Contrary to the findings for batch BSF2007, after 60 minutes of sunlight exposure the highest recovery is found at the lowest BSF concentration (0.2%). However, there is no clear relation between concentration and recovery after 60 mins of exposure to sunlight. Clearly, the results for the BSF2009a batch are completely different from those for the BSF2007 batch.

#### Storage time

The 2010 experiments comprised an investigation of the effect of storage time on recovery. The batches BSF2007, BSF2009a and BSF2009b were sprayed onto filter cloth collectors at relative doses of 100%, 10%, 1% and 0.1% and collectors were stored for 2, 4, 6 and 8 weeks in a dark refrigerator cell at 10°C. Reference collectors were analysed directly without storing. Results of these experiments are presented in Table 3.

Table 3. Recovery (% of applied dose) of Brilliant Sulpho Flavine batches BSF2007, BSF2009a and BSF2009b from filter cloth collectors at different concentrations after storage times of 2–8 weeks in a dark and cooled room)

BSF2007		Storage time								
Conc [%]	0 week	2 weeks		4 weeks		6 weeks		8 weeks		
0.1	30	a	25	ab	28	ab	30	a	23	b
1	33	a	29	a	29	a	28	a	30	a
10	56	a	53	a	53	a	51	a	54	a
100	72	a	67	a	68	a	67	a	70	a
BSF2009a		Storage time								
Conc [%]	0 week	2 weeks		4 weeks		6 weeks		8 weeks		
0.1	104	a	91	b	92	b	76	c	70	c
1	110	a	103	ab	104	ab	100	b	97	b
10	111	a	110	a	107	a	110	a	107	a
100	103	a	106	a	103	a	103	a	105	a
BSF2009b		Storage time								
Conc [%]	0 week	2 weeks		4 weeks		6 weeks		8 weeks		
0.1	129	a	148	a	113	ab	102	ab	100	b
1	117	a	114	a	110	a	99	a	100	a
10	111	a	109	a	116	a	104	a	111	a
100	107	a	105	a	111	a	100	a	107	a

100% = 3 g L<sup>-1</sup> (at t=0); different letters mean significant differences ( $\alpha < 0.05$ ) within batch.

For the BSF2007 batch there is no significant loss of recovery with increasing storage times. Recovery is low at the start of the experiment and remains low at the same level even after 8 weeks of storage. For the BSF2009a batch recovery remains constant over time for the concentration 100%, 10% and 1%. However, at 0.1% concentration a significant decrease with time can be observed, down to 70% after 8 weeks of storage. The BSF2009b batch shows no difference in recovery over storage time at all, although some small variations do occur. Due to the high recovery at the begin periods of storage the 0.1% concentration of the BSF2009b batch does show a significant decrease in recovery after 8 weeks.

## Discussion

Results presented show that large differences in sunlight stability can occur between individual production batches of one fluorescent tracer. Particularly batch BSF2007 shows a fast initial degradation compared to batches BSF2009a and BSF2009b which appear to be stable for at least 30 minutes. Clearly, these batches must contain chemically different substances. This is supported by the differences in excitation and emission wavelengths for these batches. At present it is unknown why BSF2007 started with such a low recovery at t=0. Such a batch is better not used in field experiments. For well-performing batches (like BSF 2009a and BSF2009b), in field experiments it is advised to secure the sprayed collectors from exposure to sunlight by collecting them within 15 mins after spraying. This puts stress on the speed of collecting and therefore on workload and number of people required in the field during spray experiments.

Some tests show that degradation may be dependent on BSF concentration, particular when concentrations are low. However, accuracy of fluorimetric analyses at low concentration obviously is reduced and this may well affect the observed effects. Further analysis is needed here. As



degradation is caused by exposure to solar radiation, a relation between cumulative radiation exposure and the level of degradation can be expected. This has not been investigated yet and needs further attention.

The experiments indicate that after picking the spray deposition collectors in the field the collectors can be stored for at least 8 weeks in a dark and cool storage room without significant loss of recovery.

The experiments carried out in this study are based on an even distribution sprayed directly over the collector surfaces for different batches of BSF at different concentrations. This is a good representation of spray deposition experiments over crop canopy or a bare soil surface. However, with spray drift measurements small spray drops are carried away downwind and deposit on soil surface next to the field at relatively low levels. During flight the drops decrease in size due to evaporation of the water solvent and subsequently concentration of BSF increases. Thus, considering drift, spray deposits consist of low deposition levels of small drops with high BSF content. It can be discussed whether direct spraying at low concentrations is representative for such drift deposits, though in both cases deposition levels are low. A better representation of drift deposits may be obtained by using spray nozzles that produce a very fine spray such as controlled droplet applicators, rotary atomisers or fumigators. Further research in this field is needed.

With the described methodology it is possible to evaluate batches of fluorescent tracers before they are used in field experiments for spray deposition or spray drift measurements. In fact, the present study showed that effects of exposure to sunlight on recovery can be large for particular batches of tracer. Therefore it is advised to always test a new tracer batch on degradation after exposure to sunlight before usage in field studies.

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