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Dissipation of Phloxine B and Uranine in Protein Bait Sprayed in a Coffee Field for the Suppression of Mediterranean Fruit Fly

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Tephritid fruit fly (Diptera: Tephritidae) infestations limit export of agricultural commodities from infested areas like Hawaii, thus control of these pests has a paramount importance to the growing diversified agricultural industries in this state. The xanthene dyes phloxine B and uranine showed promising results for fruit fly control as a replacement for the currently used insecticide malathion based on laboratory and field trials (Liquido et al. 1995, 1996 and 1997; Mangan and Moreno 1995 and 1997). The aerial bait spray of malathion in urban areas to suppress introduced fruit fly populations is facing a strong public opposition because of human health concerns. In addition, malathion being a neurotoxin is nonselective between target and nontarget insects. Phloxine B and uranine have a long history of human safety. In fact, phloxine B and uranine are registered for human use in coloring drugs and cosmetics in the U.S. Phloxine B is even used as a food dye in other countries. The selectivity of xanthene dyes as an insecticide stems from the fact that these dyes turn toxic only after ingestion and exposure to light. Appropriate bait mixture can make the dye selective to target species and safe to nontarget organisms (Heitz 1997). Applications of phloxine B and uranine are unlikely to cause significant impacts on the environment since these dyes undergo rapid photodegradation in sunlight and bond strongly to soil clay particles (Li et al. 1997 and 1998; Wang et al. 1998).

Research efforts currently are underway to develop and register phloxine B and uranine in protein baits for fruit fly control under the tradename “SureDye”. A large-scale experiment of SureDye was conducted in 41,270 acres of coffee plantation in Guatemala from December 1997 to February 1998 (Peck and McQuate 1998). Field tests have also been done in Hawaii, Mexico, and Texas to determine the field efficacy of these dyes to several fruit fly species (Liquido et al. 1997; Mangan and Moreno 1997).

Weather conditions such as high rainfall, temperature, and sunlight intensity at the Kauai experimental site offer an opportunity to study the fate of xanthene dyes in a unique environment. Phloxine B and uranine are quite water soluble, and this may have high leaching potential. However, based on the spill incident in a pond in Kauai, these dyes undergo rapid photodegradation and are highly adsorbed in soils

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and sediments, thus contamination of groundwater is highly unlikely (Li et al. 1998). Based on GLEAMS (Groundwater Loading Effects of Agricultural Management Systems) model, the maximum amount of phloxine B and uranine residues was predicted to be 0.0154 ± 0.004 and 0.00696 ± 0.002 µg/g, respectively, in surface soil at an application rate of 0.018 kg active ingredient (a.i.) per hectare (Bergsten 1995). In this study, an actual field experiment using 0.039 kg a.i./ha of phloxine B and uranine revealed that no uranine was found in the Kauai soils two weeks after the 10th weekly spray, but phloxine B concentration (> 0.182 ± 0.08 µg/g) was five times higher than those predicted. This is the first report on the residue of xanthene dyes resulting from a field experiment targeting Mediterranean fruit fly, Ceratitis *capitata* (Wiedemann), in coffee fields on the island of Kauai, Hawaii in 1996.

MATERIALS AND METHODS

Phloxine B and uranine standards were purchased from ICN Biochemicals (Cleveland, OH). Phloxine B was purified as previously reported (Alcantara-Licudine et al. 1997). Optima grade methanol (MeOH), acetonitrile (ACN), and acetone used in extracting coffee cherries were obtained from Fisher Scientific (Pittsburgh, PA). All reagents used in this study were of analytical or HPLC grade. SPE amino columns were from Mallinckrodt and Baker Inc. (Phillipsburg, NJ).

The experiment was conducted at the latter part of one coffee fruiting season (January 31- April 26, 1996) on 150 acres of semi-isolated coffee field in Kipu, Kauai (Figure 1). The coffee field was divided into four treatment sites and each site was further classified into four subsite replicates. The treatment field was located about 5 miles from the main coffee production fields on the island. A 1:1 molar ratio of phloxine B and uranine in protein bait was formulated as described in Table 1. The bait was applied weekly by a helicopter as an ultra-low volume spray for a total of 10 applications (Table 2). Traps were serviced on a weekly basis to assess the population of Mediterranean fruit flies and other tephritid fruit fly species in the fields. Fifteen trimedlure (a male attractant)-baited traps and 15 hydrolyzed protein-baited traps were set out in the treatment and control fields.

The spray was applied at a time of seasonal decline of the Mediterranean fruit fly population coincident with the completion of the coffee fruiting season. However, after factoring out seasonal declines in trap catches in food bait traps in the control field, a rapid reduction in the population of Mediterranean fruit flies was still apparent soon after the start of the spraying cycle in the sprayed (Treatment) field (unpublished data). Subsequent field trials have shown good suppression of Mediterranean fruit fly population with only 0.5% phloxine B incorporated into the bait spray (Peck and McQuate 1998).

Soil samples are classified as Pohakupu silty clay loam with colors ranging from brown to reddish brown (USDA Soil Conservation Service 1972). The soil has

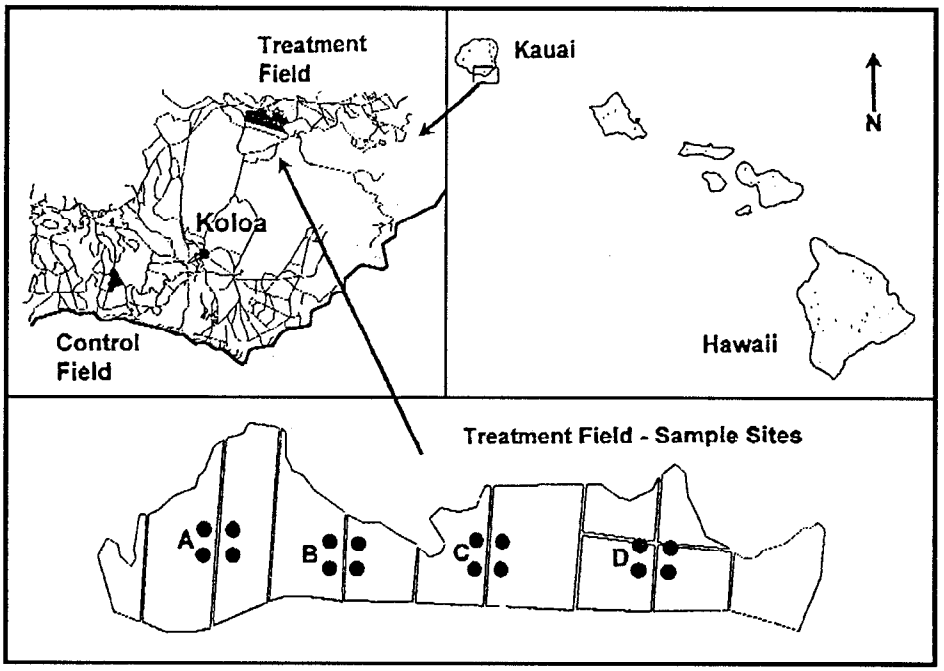

Figure 1. Map of phloxine B and uranine sampling site.

Table 1. SureDye bait formulation used in aerial sprays.

Ingredient	% (w/w)	Amount per Acre (g)
ICN Yeast Hydrolysate	30.00	476.3
Fructose	10.00	158.5
Phloxine B	0.68	11.1
Uranine	0.32	4.8
Water	59.00	936.7

slight to moderate acidity (pH <6). Soil samples (O-5 and 5-10 cm layers) were taken from four cores in each subsite using an auger at the following dates: before the 1st spray, right after the 1st spray; 6 days after the 3rd spray (i.e., one day before the 4th spray), right after the 4th spray, 6 days after the 9th spray (i.e., one day before the 10th spray), right after the 10th spray and 1, 4, 7 and 14 days after the 10th spray (Table 2). Samples were collected from control plots every four weeks. Coffee cherries were randomly collected from each subsite in the treatment field for the residue analyses before the first spray, six days after the ninth spray, and right after and 1, 4, 7 and 14 days after the 10th spray. Soil and coffee samples were transported to the laboratory by air and stored at - 15 °C until sample preparation and analysis.

Soil samples were brought to room temperature, air-dried, sieved through 20 mesh

and stored in mason jars in the dark at room temperature. Soil moisture was determined followed by extraction. Extraction and analysis of soil samples followed the procedure of Alcantara-Licudine et al. (1997). Soil (2g) was mixed with Na_4EDTA (100 mg) after adjusting soil moisture to about 10% and quantitatively transferred to a 2.5-mL extraction vessel. MeOH (1 mL) and *n*-butyl amine (0.05 mL) were added on top of the soil as modifiers. Analytes were extracted in an Isco model SFX 2-10 supercritical fluid extractor at 60 °C, and at 272 and 476 atm. The extracts were collected in MeOH, concentrated under a N_z stream to 1 mL, and filtered through a Gelman 0.45-µm acrodisc for HPLC analysis. Analysis of phloxine B and uranine in coffee was also done by HPLC after solid phase extraction (SPE) cleanup of the extracts (Alcantara-Licudine et al. 1998). Coffee cherries (25 g) that were ground with dry ice were extracted with 300 mL of MeOH/ACN/*n-BA* (1/1/0.05) using Sorvall Omni mixer. The extract was filtered through a Buchner funnel fitted with a no. 7 glass fiber filter, into a 500-mL suction flask. SPE cleanup of the extracts was done using a Supelco vacuum manifold. Sample extracts (40-50 mL) were applied on the amino SPE column (500 mg) after washing with MeOH and activating with aqueous HCl/MeOH. Hexane, acetone, and MeOH were passed successively through the column to remove interferences. The dyes were finally eluted with aqueous NaOH/MeOH, concentrated to 1 mL, and filtered in Gelman acrodisc for HPLC analysis.

Phloxine B and uranine in soil or coffee extracts were determined by HPLC using a Perkin Elmer Model 250 binary pump equipped with an Applied Biosystems 10005 diode array detector (Alcantara-Licudine et al. 1997). HPLC column was Alltima C_{18} LL (Alltech, Deerfield, IL). Gradient elution was done with ACN-0.5 M NH_4OAc from 20 to 100% ACN in 40 min. Detection wavelengths were 493 and 546 nm for uranine and phloxine B, respectively.

RESULTS AND DISCUSSION

Table 2 shows the xanthene dye residues found in the soil samples. Samples collected a day before and immediately after a spray provide good estimates of deposition rates. Samples collected after the tenth spray provided information on dissipation. After the fourth spray, there was an average increase of 0.264 and 0.021 µg phloxine B in the 0-5 cm and 5-10 cm soil layers, respectively. Uranine was detected in top soil right after the fourth spray at 0.107 µg/g but was not found a day before the spray application. The average increase in phloxine B concentration during the tenth spray was 0.156 and 0.009 µg per g soil for the 0-5 cm and 5-10 cm soil layers, respectively. Maximum concentrations of 0.886 ± 0.73 and 0.094 ± 0.05 µg phloxine B/ g soil were found in O-5 cm and 5-10 cm soil layers, respectively, collected 1 day after the tenth spray. At this time, uranine concentration (0.235 ± 0.09 µg/g) was also the highest in top soil but was not found in any samples in lower soil layer. The increase in concentration of the dyes

Table 2. Phloxine B and uranine residues detected in soil samples collected during SureDye application in a coffee field.

Sampling Date	Days	Sampling Time	Concentration ± SD, µg/g[a]		
			0-5 cm		5-10 cm[b]
			Uranine	Phloxine B	Phloxine B
1/25/96	0	Before 1st spray	ND [c]	ND	ND
1/31/96	1	right after 1st spray	ND	0.080 ± 0.06	0.007 ± 0.01
2/28/96	27	6 d after 3rd spray	ND	0.361 ± 0.12	0.039 ± 0.07
2/29/96	28	right after 4th spray	0.107 ± 0.13	0.625 ± 0.38	0.060 ± 0.03
4/10/96	55	6 d after 9th spray	ND	0.320 ± 0.12	0.013 ± 0.03
4/12/96	57	right after 10th spray	0.193 ± 0.09	0.476 ± 0.06	0.022 ± 0.01
4/13/96	58	1 d after 10th spray	0.235 ± 0.09	0.886 ± 0.73	0.094 ± 0.05
4/16/96	61	4 d after 10th spray	0.062 ± 0.004	0.236 ± 0.13	0.017 ± 0.01
4/19/96	64	7 d after 10th spray	0.091 ± 0.01	0.394 ± 0.36	0.038 ± 0.05
4/26/96	71	14 d after 10th spray	ND	0.182 ± 0.08	0.017 ± 0.02

[a]Based on oven-dried soil. Sample detection limit is 7-10 ng/g for phloxine B and 35-40 ng/g for uranine.
[b]Uranine was not found in this soil layer.
[c]ND = not detected.
SD = standard deviation.

in the soil samples from right after to one day after the tenth spray is likely due to rain washing the bait spray off the leaves of vegetation in the field. By the 14th day after the tenth spray, these concentrations had decreased to 0.182 ± 0.08 and 0.017 ± 0.02 µg phloxine B/g soil in 0-5 and 5-10 cm layers, respectively. Uranine was no longer detected in 0-5 cm soil layer after 14 days. Although there was some rain in the course of the 14 days after the tenth spray (Figure 1), it is thought that leaching from those rain events was minimal, especially given that phloxine B and uranine adhere strongly to the soil particles (Alcantara-Licudine et al. 1997). Because of low application rate and strong adsorption of the dye molecules to soil particles, these dyes have little tendency to leach into groundwater. As minimal leaching is expected the data (Table 2) suggest an apparent steady breakdown of phloxine B and uranine in the soil over time with half-lives of roughly one week. Recoveries of phloxine B and uranine in control soil spiked at 0.5 µg/g are 89% ±4 and 104% ±11, respectively. None of the dyes were found in the control samples.

Prior to the first spray, phloxine B was not detectable on the coffee cherries but right after the first and fourth sprays, 0.015 and 0.012 µg of the dye were deposited per g of oven-dried coffee cherries. Six days after the ninth spray, phloxine B was detected at 0.006 µg/g coffee cherry. Phloxine B concentration

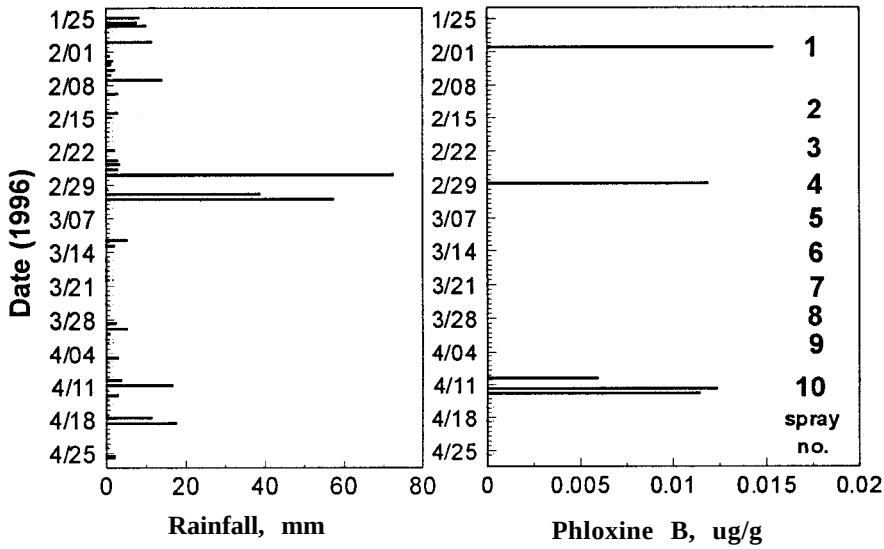

Figure 1. Correlation between the daily rainfall in coffee field in Kauai during the experimental period. Phloxine B concentration was computed based on oven-dried weight of coffee cherries. Sample detection limit for phloxine B is 5-7 ng/g..

increased to 0.0125 µg /g coffee cherry right after the tenth spray, then decreased slightly to 0.0115 µg/g , one day after the tenth spray. Coffee cherries collected 4, 7, and 14 days after the tenth spray had no detectable level of phloxine B. Uranine was not found in any of the samples at a minimum detection limit of 32-37 ng/g.

A maximum amount of coffee cherries produced per week was estimated to be less than 1.1 kg per 10-foot section of a row. As coffee rows are spaced about twelve feet apart, there are about 3600 linear feet of coffee cherries per acre. Using the maximum average coffee cherry production recorded for one week in a 10-foot transect, the maximum production in the treated field would be about 396 kg/acre. If the average moisture content is about 70%, the equivalent dry weight of coffee cherries is 119 kg. If these coffee cherries all had residue levels equal to the maximum measured residue on coffee cherries, then there would be a total of only 1.78 mg per acre of phloxine B residue on the harvested coffee cherries. With delays in harvesting after spraying and further breakdown of phloxine B during processing of the coffee cherries, this small quantity should be further reduced. Considering the low total quantities, it is unlikely that workers in the processing plant could be exposed to phloxine B levels in excess of 1.25 mg/kg body weight which is the established maximum allowable daily intake (FDA 1993) especially given the degree of automation found in the processing plant.

Some dye residue on the coffee cherries would be washed off in the course of processing. The rinsate used for the washing process is ultimately transferred to settling ponds next to the coffee mill where it is expected that phloxine B will

either break down while in the water column or will gradually break down in the sediment over time. Negligible quantities of dyes should be found in the final product, because the dyes which adhered to the coffee cherries should be carried away in the water used in processing coffee cherry to green beans. Roasting the green beans may further reduce or decompose the dye residue.

In conclusion, an equimolar mixture of phloxine B and uranine, when applied at an application rate of 0.039 kg. a.i. per hectare, degraded rapidly in coffee field samples with a half-life of approximately one week in soil and coffee cherries. Phloxine B concentration in the top soil was about five times higher than that predicted by GLEAMS model. Very low concentration of phloxine B was detected at 5-10 cm soil depth. The slow movement of the dye and the nature of the soil on the island indicate that phloxine B is unlikely to contaminate groundwater. Further studies are being done to assess the effects of phloxine B and uranine in the environment.

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