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Avian Exposure to Pesticides in Costa Rican Banana Plantations

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Currently, banana plantations occupy more than 50,000 ha of the total lowland area in Costa Rica and are confined almost exclusively east of the Cordillera mountain range. Banana cultivation on these plantations represents an intensely managed agroecosystem with high inputs of synthetic chemicals, generally in the form of pesticides and fertilizers. While much emphasis has been placed on estimating pesticide use and potential effects to humans in these plantations (von Düszeln, 1991, Forget, 1991, Lewis, 1992) little research has examined the distribution, fate, and potential wildlife exposure to agrochemicals used in banana plantations. The objective of this study was to develop methods to estimate pesticide distribution and avian exposure to selected chemicals in banana plantations. To that end, two plantations were compared: one treated with the insecticide carbofuran, the other was not treated with an insecticide. Both plantations received normal fungicide and herbicide applications. Chemical analyses were performed on avian foot wash samples to determine the relative frequency and magnitude of contact exposure and on plantation runoff samples to assess pesticide movement. Plasma cholinesterase activities were determined to establish the degree of carbamate exposure in birds. Our hypothesis was that birds from the plantation treated with carbofuran would have a higher incidence of cholinesterase depression relative to birds from the reference site.

MATERIALS AND METHODS

Solvents used in the US were pesticide residue grade or better (Burdick and Jackson, Muskegon, Ml; Mallinckrodt, Paris, KY; J.T. Baker, Phillipsburg, NJ). Solvents purchased or used in Costa Rica were assessed for interfering or contaminant residues once returned to the US.

All samples were collected in mature banana plantations (see Fig. 1) in the Province of Limón, Costa Rica. The treated site (340 ha) received a mixture of paraquat (0.5 L/ha) and ametryn (0.3 L/ha) every 28 days and either propiconazole (0.4 L/ha), tridemorph (0.6 L/ha), or mancozeb (3.5 L/ha) during this study. In addition, the treated site received an application of Furadan® 10G (carbofuran) at 30 g/plant or 55 kg/ha between February and April, 1994. The reference site received a mixture of paraquat (80 mL/ha) and diruon (43 g/ha) every 6 weeks and mancozeb (3.5-4 L/ha) during this study. The reference site received no insecticide applications during this study. The treated site was approximately 40 km NW of Puerto Limón and was the

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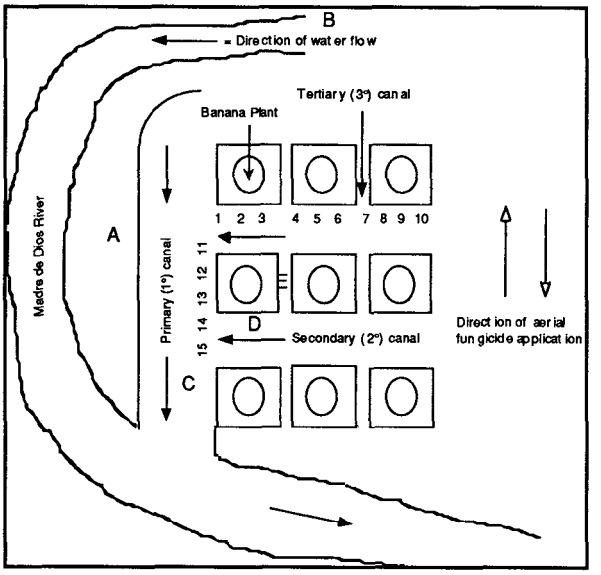

Figure 1. Schematic representation or a banana plantation. Numbers 1-15 and letters A-E represent drift card and water collection locations, respectively.

first banana plantation along the Madre de Dios river. The reference site was approximately 8 km downstream from the treated site.

Birds were mist-netted opportunistically within and adjacent to the banana plantations and were identified (Stiles *et al.* 1989) and weighed to the nearest 0.1 g. Blood for cholinesterase (ChE) analysis was stored in glass tubes containing EDTA and placed on ice until returned to the laboratory. There the blood was centrifuged and plasma was separated from RBCs and stored over dry ice until they arrived in the US, at which time they were transferred to a -80°C freezer until analysis. In an effort to collect blood non-lethally and at the same time have enough blood for analysis, blood was only taken from birds that weighed ≥ 25 g (McGuill and Rowan, 1989).

Plasma samples were analyzed for total ChE and acetylcholinesterase (EC 3.1.1.7; a portion of the plasma sample was pre-incubated for 5 min with 10^{-4} M iso-OMPA, a specific butyrylcholinesterase [EC 3.1.1.8] inhibitor) activities using a modification of Ellman et al. (1961) which tested specifically for carbamate reactivation (Hunt and Hooper, 1993). Briefly, 350 µL diluted plasma (1:20 v/v) was mixed for 10 min with 20 mg of activated C_8 sorbent in the upper chamber of a 0.65-µm microcentrifuge filter unit. The unit was then centrifuged to retrieve the sample volume. An aliquot of this sample was immediately assayed for ChE activity following centrifugation and again after a 4- and 24-h 37°C incubation. A sample was considered reactivatable if its total (ChE) post-incubation activity was ≥ 110% of its pre-incubation ChE activity and was determined to be statistically significant using a one-tailed Student t-test ($P < 0.05$).

Surface water samples were collected opportunistically with an emphasis on collection immediately following rain events. Approximately 500 mL of water was collected per sample in clean, brown glass bottles from five locations within or adjacent to plantation drainage canals (Figure 1; A-E). Less than two hours after collection, the samples were extracted with a pretreated solid phase extraction (SPE) cartridge filled

with a silica based octadecyl sorbent (C_{18}, 6 mL/1 g cartridge, Fisher Scientific, Pittsburgh, PA).

Under vacuum, the C_{18} SPE cartridges were pretreated sequentially with 5 mL each of ethyl acetate, methanol and water, and followed by 250 mL of the sample. The cartridges were stored in a -20°C freezer until they were returned to the US, where they were eluted with 3 mL of methanol followed by 3 mL hexane, yielding two samples per cartridge. Samples were stored in 2 mL amber autosampler vials at -20°C freezer until the time of analysis.

Foot wash samples from birds were collected by rinsing the feet with approximately 15 mL of ethanol. The samples were collected in 30 mL glass vials, wrapped in aluminum foil, and stored in a -20°C freezer until they were returned to the US. The samples were then quantitatively transferred, brought to final volume of 2 mL under a stream of nitrogen gas, filtered through a 0.45 µm filter, and stored in a 2 mL amber vial at -20°C until the time of analysis.

During this study, two birds died due to heat stress. Their carcasses were collected and stored in dry ice until they were returned to the US. The carcasses were rinsed with approximately 50 mL hexane which was filtered through sodium sulfate, reduced to a final volume of 2 mL under a stream of nitrogen gas and stored at -20°C in amber vials until the time of analysis.

Drift cards (11 cm diameter, Whatman #41 filter paper; Whatman, Maidstone, England) were placed next to the canals (Figure 1) in both the treated and reference site for at least one aerial fungicide (mancozeb) application. The drift cards were collected within 24 h after application, individually wrapped in aluminum foil, placed in plastic bags, and stored at -20°C (Brown et al., 1993).

Upon arrival in the US, the samples were cut into 2.5 cm squares, acid digested with concentrated hydrochloric acid (5 mL acid:1 g sample) for 4 hr at 180°C, and subsequently analyzed for zinc.

The water, foot wash, and carcass samples were screened for 14 organochlorines (OCs), 9 organophosphates (OPs), 23 herbicides, 12 carbamates and 4 fungicides. The OC, OP, herbicide and the fungicide screens (except for mancozeb) were on Hewlett-Packard Model 5890 Series II gas chromatographs (GCs) equipped with electron capture (EC), flame photometric (FP) or nitrogen-phosphorus (NP) detectors, respectively. The GCs were operated with a 1:10 split injection and a purge time of 0.75 min. The columns (J & W Scientific, Pancho Cordova, CA) were 30 m x 0.25 mm id. fused-silica capillary coated with either a DB-1701 (EC and NP) or a DB-5 (FP) stationary phase (film thickness of 1.0 mm). All injector and detector temperatures were 250 and 300°C, respectively.

The carbamates were screened on a Hewlett-Packard 1090 liquid chromatograph (LC) coupled with a post-column derivatizer (Pickering Laboratories PCX-5000, Mountain View, CA) and a programmable fluorescence detector (Hewlett-Packard 1046A). The column was a 10.0 cm C_{18} carbamate column (Pickering Laboratories) operated at a flow rate of 1.0 mL/min. The system operating conditions were similar to McDonald *et. al.* (1990). Briefly, the LC was operated under isocratic conditions with water:methanol at 75:25 for 0.5 min, followed by a linear gradient to 25:75 water:methanol over 26.5 min, up to 100% methanol with a final hold of 2 min and then back to 75:25 water:methanol. The detector was operated at excitation and emission wavelengths of 340 and 455 nm, respectively.

The drift cards were analyzed for mancozeb, which was quantified based on zinc content (2.5% by weight) on a Varian SpectrAA-20 Plus atomic absorption spectro-

meter (Sugar Land, TX). The samples were analyzed in an airacetylene flame which was monitored at a wavelength of 214 nm (1.0 nm slit width). Samples were corrected for background levels of zinc in the drift cards.

Detection limits in water (µg/L) and foot wash and carcass samples (µg/bird), respectively: Organochlorines, 0.1 µg/L (water only); OPs, 20.0 and 1.0; herbicides, 0.4-8.0 and 0.1-2.0; carbamates, 1.6 and 1.0; fungicides, 4.0-8.0 and 1.0-2.0. Since it was known prior to analysis that carbofuran was applied to the treatment site and mancozeb was applied to both the reference and treatment sites, recovery percentages from fortified samples were obtained for those compounds. Recoveries were 95 ± 6% and 90 ± 8% (mean ± SE) for carbofuran in water (n=4) and mancozeb on drift cards (n=4), respectively. This information is not available for the other pesticides and matrices.

Quality assurance and quality control procedures were followed in the field and the laboratory and included following standard operating procedures, data tracking and data verification.

RESULTS AND DISCUSSION

From February 16 to March 31, 1994, 43 bird species were identified on or near the reference or treated banana plantations, 30 of which were caught in mist nets and sampled. Table 1 summarizes the birds which were caught and from which a blood sample was successfully collected. Also summarized, are the results from the carbamate reactivation analysis. Reactivation of carbamate-inhibited total ChE, indicating carbamate exposure, was successful in 7 of the 18 birds captured on the treated site compared to 1 of 8 from the reference site.

Due to logistical problems, we were unable to begin netting birds on the reference site until 3 weeks after the treatment site, therefore, the lower number of plasma samples collected from the reference site is due primarily to a decrease in total net hours. Despite the difference in the number of plasma samples collected from the treated or reference site, one would expect the number of carbamate exposed birds would be higher on the site treated with carbofuran. It should be noted that while there were more reactivated plasma samples from the treated site compared to the reference site, the number of plasma samples collected and the number of reference and treated sites is too small to draw any statistical conclusions. It does appear, however, that the plasma reactivation method is promising for the evaluation of birds exposed to carbamate pesticides in tropical environments.

This study was conducted during the two months of the year with the least precipitation, February and March (Stiles *et al.* 1989), and the composition of birds and other organisms which inhabit banana plantations may change dramatically during the wet season. Therefore, studies should be extended to monitor exposure during both the dry and wet season to better understand potential fluctuations in exposure patterns throughout the year. This could lead to important information regarding pesticide management practices.

Carbofuran was detected in water samples (Table 2) collected during or soon after rain events on the treated site and in one sample collected approximately 1 km upstream from the reference site (downstream from the treated site). Levels ranged from 2.89-41.69 µg/L. Ametryn and propiconazole were detected on the treated site with levels ranging from 0.45-2.15 and 6.10-24.20 µg/L, respectively. Propiconazole was also detected in one sample collected from a standing puddle of water within 24 hr following a rainfall event on the reference site.

Table 1. Summary of birds captured in banana plantations, their plasma ChE activities and responsiveness to carbamate reactivation condition.

Family name	Common name	Scientific name	Number of blood samples	Plantation [a]	Absolute ChE activity (units/mL)			Total ChE reactivation
					Total ChE	AChE	BChE	
Scolopacidae	Solitary Sandpiper	*Tringa solitaria*	1	T	1.10	0.06	1.04	-
	Spotted Sandpiper	*Actitis macularia*	1	T	0.89	0.27	0.63	+
Columbidae	Ruddy Ground Dove	*Columbina talpacoti*	2	T	0.95	0.10	0.85	+
				T	0.39	0.01	0.39	-
Cuculidae	Groove-billed Ani	*Crotophaga sulcirostris*	2	T	1.42	0.15	1.26	+
				R	2.60	0.15	2.45	-
Tyrannidae	Gray-capped Flycatcher	*Myiozetetes granadensis*	1	T	1.52	0.16	1.35	-
	Great Kiskadee	*Pitangus sulfuratus*	3	R	1.55	0.37	1.18	-
				R	0.86	0.57	0.30	+
				R	0.93	0.49	0.43	-
	Social Flycatcher	*Myiozetetes similis*	2	T	4.39	0.26	4.13	-
				T	4.31	0.07	4.24	-
Turdidae	Clay-colored Robin	*Turdus grayi*	1	T	1.73	0.69	1.04	-
Icteridae	Bronzed Cowbird	*Molothrus aeneus*	3	T	1.73	0.10	1.63	+
				T	1.25	0.07	1.17	-
				R	1.19	0.06	1.13	-
	Great-tailed Grackle	*Quiscalus mexicanus*	5	T	2.01	0.10	1.91	+
				T	2.18	0.10	2.07	-
				T	2.45	0.12	2.34	+
				T	0.95	0.03	0.92	+
				R	1.97	0.07	1.90	-
Thraupidae	Blue-Gray Tanager	*Thraupis episcopus*	1	T	1.75	0.10	1.65	-
Emberizidae	Black-headed Saltator	*Saltator atriceps*	1	R	1.52	0.01	1.50	-
	Blue-black Grosbeak	*Cyanocompsa cyanoides*	1	R	1.66	0.07	1.59	-
	Buff-throated Saltator	*Saltator maximus*	2	T	1.32	-	-	-
				T	1.48	0.13	1.35	-
		Totals:	**26**	**R=8; T=18**				**R=1/8; T=7/18**

[a] R = Reference site: T = Treatment site.

Table 2. Summary of water samples collected with detectable levels of pesticide.

Plantation[a]	Location[b]	Pesticide detected	Concentration (µg/L)
R	A*	Propiconazole	8.65
R	B	Carbofuran	2.89
T	C	Carbofuran	41.7
T	C	Ametryn	0.71
T	C	Propiconazole	6.10
T	C	Carbofuran	12.9
T	C	Ametryn	0.45
T	C	Ametryn	0.58
T	D	Carbofuran	7.36
T	E	Ametryn	2.15
T	E	Propiconazole	24.2
T	E	Ametryn	1.43
T	E	Propiconazole	10.9

[a]R=reference site; T=treatment site.
[b]Refer to Figure 1.
*Sample taken along roadside (adjacent to plantation) after a rain event.

Water samples collected from the treated site (5 hr post rain event; PRE) showed the highest concentrations of carbofuran in the primary (41.69 µg/L) and secondary (7.36 µg/L) canals, respectively. After another rain event, samples were collected approximately 14 hr PRE and carbofuran was detected in the primary canal (12.91 µg/L) but not in the secondary canal The next day, approximately 20 hr after the samples from the treated site were collected, a relatively small but detectable amount of carbofuran (2.89 µg/L) was detected nearly 7 km downstream of the treated site, approximately 1 km upstream from the reference site. It could not be determined, however, whether this source of carbofuran came from the treated site or another plantation further downstream.

Water samples were collected during the dry season when water flowing through canals and rivers is reduced. Such low-flow conditions could lead to relatively high pesticide concentrations. Since water samples from this study had highest concentrations of pesticides after a rain event, however, it is possible that the wet season may produce concentrations of pesticide equal to or greater than those during the dry season. Additional water samples, and a sampling scheme that includes both dry and wet seasons, are needed to show the effect of seasonal fluctuations in plantation runoff, and the water flow in streams and rivers, on ambient pesticide concentrations. Regardless of the seasonal changes in runoff, a feature of banana culture that has important consequences for pesticide movement in agricultural watersheds in the tropics is the direct connection of in-plantation drainage canals to streams and rivers.

Mancozeb residues from drift card analysis ranged from 0.77 ± 0.11 to 2.38 ± 0.52 µg/cm^2 in the secondary canals and 0.50 ± 0.20 to 4.00 ± 2.28 µg/cm^2 in the primary canals. The higher variablitiy between mean mancozeb residues from the primary canals compared to the secondary canals is likely a function of the number of drift cards placed (5 along the primary and 10 along the secondary canals) and times sampled in each location (3 on the primary and 4 on the secondary canals).
Only one foot wash and one carcass wash had detectable amounts of pesticides, both from the treated site. The foot wash, from a Great-tailed Grackle, had a total of 0.12 µg of ametryn. The whole carcass wash produced 0.12 µg of ametryn and 3.03 µg of propiconazole from a Buff-throated Saltator.

During this study, fungicides were applied aerially, herbicides were applied manually in a flowable formulation, and carbofuran was applied manually in a granular formulation. Because carbofuran is acutely toxic, extra precautions were taken during those applications to ensure the protection of the applicators. Since few birds were observed foraging on the ground within the banana plantations, but several birds were perched on or foraging in the banana plants, it is not surprising that the foot and carcass wash had detectable levels of fungicide and herbicide but not carbofuran. This may not be the case during the wet season when insecticides could be more readily dissolved and dispersed or in, "puddles" that could make them more accessible to birds and other organisms.

As only one reference and one treatment site were evaluated, conclusions drawn from this study should be considered tentative and may be best used to plan future work. The results suggest, however, that: (1) Birds can be used to monitor wildlife exposure to pesticides in banana plantations. (2) Water and drift card samples are indicators of pesticides present in water ways and pesticide migration during application, respectively. (3) Water sampling should be increased during and after rain events. (4) It is imperative that sample collection occur during both the dry and wet season. This could aid in identifying seasons of maximum pesticide concentrations and times of the year that wildlife populations are at greatest risk to pesticide exposure.

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