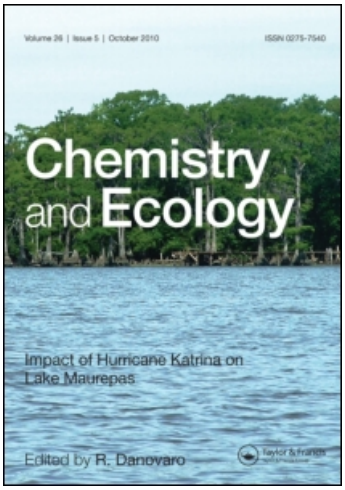

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Diazinon Residues in Insects from Sprayed Tobacco

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Pooled samples of tobacco hornworms collected from a field sprayed with 0.84 kg/ha of diazinon were analyzed for residues at various intervals after application. No residues of the toxic metabolite diazoxon were detected (sensitivity 0.5 ppm) in any sample. Only one sample exceeded 1.0 ppm of the parent compound and was collected 4 hours after spraying. Residues declined over time ($P<0.01$) and none were detected (sensitivity 0.1 ppm) 18 days after spraying. The potential hazard to birds eating these insects appeared to be minimal.

Concern for non-target effects of organophosphate pesticides has produced speculation about the possibility of secondary poisoning of birds that eat poisoned insects (Mills, 1973; Stickel, 1975). Only one fully documented instance of this phenomenon has been reported (White *et al.*, 1979). This report concerned mortality of laughing gull (*Larus atricilla*) chicks that had been fed parathion-poisoned insects by adult birds which had foraged in a sprayed cotton field. These authors included cholinesterase measurements diagnostic of organophosphate poisoning as well as residue analyses of alimentary tracts indicative of potentially lethal exposure. The paucity of information on the amounts of biologically active anti-cholinesterase pesticides (organophosphates and carbamates) and toxic metabolites present in insect tissue under field conditions makes it extremely difficult to evaluate laboratory studies of the avian toxicity of these pesticides. The only organized body of data published on cholinesterase-inhibitors in insects dealt with honey bees that had died from exposure to insecticide-treated crops. Results of analyses for the carbamate insecticide carbaryl were uniformly below 1.0 ppm (Morse *et al.*, 1963; Gutenmann and Lisk, 1965; Morse and Gunnison, 1967; Strang *et al.*, 1968; Butler and McDonough, 1970), assuming eight bees/g (Argauer *et al.*, 1970:691) for data expressed as g/bee. Levin *et al.* (1968) reported similar results for bees

exposed to malathion. Neither of these pesticides was particularly hazardous to birds in laboratory studies (e.g. Hill *et al.*, 1975), and the reported residues did not appear to represent a hazard to birds. Other insecticides and insect species might be quite different, however. This report describes an experimental approach to the problem of estimating toxic residues in insects.

METHODS

An organophosphate insecticide of intermediate toxicity to birds, diazinon (Hill *et al.*, 1975), was chosen for study to complement ongoing laboratory studies of its avian toxicity (Stromborg, 1981). For ease of collection, mixed instars of tobacco hornworms, *Manduca sexta* (L.), were chosen as a target. A few tomato hornworms, *M. quinquemaculata* (Haworth), were present and included in the collections. The study was conducted on the University of Maryland's Tobacco Experimental Farm in Upper Marlboro, Maryland. The outer three rows of a seed tobacco field were sprayed with 1½ pints of Diazinon AG500[R] in 60 gallons of water per acre (0.84 kg active ingredient per ha) using a standard highboy spraying rig. Hornworms were collected by hand before spraying to provide uncontaminated material for verification of chemical methodology, and at 4 h, 1, 2, 4, 8, and 18 days after spraying. Postspray samples consisted of at least 5 g of larvae collected by random search within the sprayed rows of tobacco. When larvae were abundant enough, duplicate samples were collected. Samples were frozen on dry ice in the field and kept frozen until analysis.

Analytical methods

Each individual sample was placed in a stainless steel cup and homogenized with a Virtis 45 high-speed blender. A 5-g portion, or the entire sample if the weight was less than 5 g, was thoroughly mixed with 200 g of anhydrous sodium sulphate using an Osterizer food blender. This mixture was placed in a soxhlet apparatus, covered with methylene chloride, stored overnight, and extracted the next day for 7 h. The liquid extract was concentrated to a volume of 1 ml using a rotary evaporator with a 30°C water bath.

Samples were analyzed with a Hewlett-Packard 5840 gas chromatograph equipped with a flame photometric detector in the phosphorus mode and a 1.5% OV-17/1.95% QF-1 column. The carrier gas was nitrogen at 60 ml/min flow; the flame gasses were a mixture of hydrogen at 150 ml/min, air at 50 ml/min, and oxygen at 20 ml/min. The temperature was 200°C for the oven and 225°C for the detector.

Recoveries were determined by spiking 5 g of control tissues mixed with sodium sulphate at 20 ppm each of diazinon and its toxic metabolite, diazoxon. The average percentage recoveries were 100 and 98.6 for diazinon and diazoxon, respectively. The lower limits of quantifiable residues were 0.1 ppm diazinon and 0.5 ppm diazoxon. Reported residues were not corrected for recovery.

RESULTS

On the first three postspray sampling dates, hornworms were found on both the tobacco plants and the ground. Because no hornworms were found on the ground during prespray collections, those on the ground were probably strongly affected by the insecticide. A large percentage of the hornworms collected had externally visible parasite cocoons (*Apanteles* sp., Braconidae) regardless of the substrata from which they were collected. Data are reported separately for each of the four substratum-parasite categories where adequate samples were obtained (Table I). Residues exceeded 1.0 ppm only on the day of spray in unparasitized insects found on the ground. By 18 days postspray, no residues were detected in any sample. No diazoxon was detected in any of the samples. Residues decreased by about 50% within 4 days. For unparasitized insects collected on plants, a linear regression of residues versus time was significant ($P<0.01$, $r^2=0.77$) when the first five sampling dates were included and zero substituted for no detectable residues. Although insufficient samples and inconsistent patterns of residues precluded similar analyses of the other insect categories, there was a relationship between parasitism and residue occurrence. Within those insects healthy enough to remain on plants, the frequency of residue occurrence was higher in unparasitized than parasitized hornworms ($P<0.05$, Fisher's exact test). Whether or not this was a result of reduced activity and consequently lower pesticide exposure because of the burden of parasites is unknown.

It is unlikely that these levels of diazinon could adversely affect birds feeding on these insects because reported effective concentrations for avian effects are much higher than those found in these insects (Stromborg, 1981; Hill *et al.*, 1975). Insecticides other than diazinon are recommended for hornworm control (Harding and Harrison, 1977); had they been used, results might have been different. Our purpose was, however, to evaluate this technique for obtaining environmental data on insecticides and we feel that the utility of this procedure has been demonstrated. Further studies should be undertaken with insects chosen for their value as avian food items and using insecticides designed to control these insects.

TABLE I

Diazinon residues (ppm wet weight) in replicate samples (> 5g) of hornworms from a sprayed tobacco field

Days after spray	Plants		Ground	
	Parasitized	Not parasitized	Parasitized	Not parasitized
0.17	0.68	0.32	0.15	2.5
	nd	0.17		
1	0.26	0.20	0.28	
	nd	0.15		
2	nd	0.19	0.17	
	nd	0.13		
4	nd	0.10		
	nd	0.15		
8	0.19	nd		
	nd	nd		
18	nd	nd		
	nd	nd		

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