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TOXICITY OF STORM-WATER RUNOFF AFTER DORMANT SPRAY APPLICATION IN A FRENCH PRUNE ORCHARD, GLENN COUNTY, CALIFORNIA, USA: TEMPORAL PATTERNS AND THE EFFECT OF GROUND COVERS

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Abstract—Organophosphorous (OP) insecticides, especially diazinon, have been detected routinely in surface waters of the Sacramento and San Joaquin River watersheds, coincident with rainfall events following their application to dormant orchards during the winter months. Preventive best management practices (BMP) aim at reducing off-site movement of pesticides into surface waters. Two proposed BMPs are: The use of more hydrophobic pyrethroid insecticides believed to adsorb strongly to organic matter and soil and the use of various types of ground cover vegetation to increase the soil's capacity for water infiltration. To measure the effectiveness of these BMPs, storm water runoff was collected in a California prune orchard (Glenn County, CA, USA) during several rainstorms in the winter of 2001, after the organophosphate diazinon and the pyrethroid esfenvalerate were applied to different orchard sections. We tested and compared acute toxicity of orchard runoff from diazinon- and esfenvalerate-sprayed sections to two species of fish (*Pimephales promelas*, *Onchorhynchus mykiss*) and three aquatic invertebrates (*Ceriodaphnia dubia*, *Simocephalus vetelus*, *Chironomus riparius*), and determined the mitigating effect of three ground cover crops on toxicity and insecticide loading in diazinon-sprayed orchard rows. Runoff from the esfenvalerate-sprayed orchard section was less toxic to waterflea than runoff from the diazinon-sprayed section. However, runoff from the orchard section sprayed with esfenvalerate was highly toxic to fish larvae. Samples collected from both sections one month later were not toxic to fish, but remained highly toxic to invertebrates. The ground cover crops reduced total pesticide loading in runoff by approximately 50%. No differences were found between the types of vegetation used as ground covers.

Keywords—Diazinon Esfenvalerate Storm runoff Best management practices Toxicity

INTRODUCTION

Storm water runoff is a major water quality problem in agricultural and urban areas in California's Sacramento and San Joaquin River (USA) watersheds [1,2]. During the winter rainy season, when dormant sprays are applied to stonefruit and almond orchards, organophosphate insecticides (OPs) have been found in surface waters at concentrations that are toxic to the cladoceran *Ceriodaphnia dubia* [3,4]. Diazinon and chlorpyrifos, in particular, have been identified as primary aquatic toxicants [4,5]. In 1998, the state of California placed the Sacramento and San Joaquin Rivers and their delta on the Clean Water Act 303(d) list of impaired waterways due in part to elevated levels of diazinon and chlorpyrifos. State water quality plans have now been implemented by regulatory agencies to prevent movement of OPs into surface water, and growers greatly have reduced OP application [6].

With the use of OPs becoming more restricted, application of another class of highly effective insecticides (synthetic pyrethroids) is increasing in agricultural and urban areas [6,7]. Pyrethroid insecticides are photostable analogs of the natural pyrethrins of botanical origin [8]. They are potent neurotoxicants that interfere with neuronal membrane function by interacting with sodium-gated axon channels [9]. Among approximately 1,000 synthetic pyrethroids produced during the past 25 years [8], esfenvalerate, a stereoisomer of fenvalerate, has become widely used in orchard agriculture in the United States and many other countries. Esfenvalerate and other pyrethroids are moderately toxic to amphibians, mammals, and birds, but many invertebrates and fish are highly susceptible to these compounds [10,11]. For example, acute toxicity (96-h median lethal concentration [LC50]) of esfenvalerate to fathead minnow larvae has been reported at 0.25 μg/L [12]; the respective 96-h LC50 of diazinon is >10,000 times higher [13] (http://ace.orst.edu/info/extoxnet/).

Off-site movement of OPs from agricultural areas due to rainfall has been demonstrated, but off-site movement of pyrethroids is believed to be minimal due to their chemical properties. The high adsorption coefficient and low water solubility of esfenvalerate (K_d = 5,248 and log K_{ow} = 6.2) results in rapid sorption of this pyrethroid to soil and organic matter. However, a study by Werner et al. [12] reported toxic concentrations of both diazinon and esfenvalerate in orchard runoff. Runoff from diazinon-sprayed sections was toxic only to invertebrates, whereas runoff from esfenvalerate-sprayed sections was toxic to both invertebrates and fish larvae. As a continuation of that study, we investigated the persistence of toxicity in orchard runoff for several consecutive rainfall

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Table 1. Experimental design used for the French prune orchard at the Talbot–Vereschagin Ranch, Glenn County, California (USA)

Row no.	Ground cover	Insecticide sprayed
1–8	Resident vegetation	Nonsprayed
9	Nontillage clover	Diazinon
10	Perennial sod mix	Diazinon
11	Resident vegetation	Diazinon
12	Bare ground	Diazinon
13	Perennial sod mix	Diazinon
14	Bare ground	Diazinon
15	Resident vegetation	Diazinon
16	Nontillage clover	Diazinon
17	Bare ground	Diazinon
18	Nontillage clover	Diazinon
19	Resident vegetation	Diazinon
20	Perennial sod mix	Diazinon
21–25	Resident vegetation	Nonsprayed
26	Nontillage clover	Esfenvalerate
27	Perennial sod mix	Esfenvalerate
28	Resident vegetation	Esfenvalerate
29	Bare ground	Esfenvalerate
30	Perennial sod mix	Esfenvalerate
31	Bare ground	Esfenvalerate
32	Resident vegetation	Esfenvalerate
33	Nontillage clover	Esfenvalerate
34	Bare ground	Esfenvalerate
35	Nontillage clover	Esfenvalerate
36	Resident vegetation	Esfenvalerate
37	Perennial sod mix	Esfenvalerate
38–42	Resident vegetation	Nonsprayed

events in the winter of 2000/2001. We were interested in confirming results of our previous study and measuring the toxicity of runoff to species resident in California surface waters. In addition, we examined the influence of three types of ground cover vegetation on the volume, toxicity, and diazinon loading of runoff.

MATERIALS AND METHODS

Experimental design

Experiments were conducted in a French prune orchard at the Talbot-Vereschagin Ranch, Glenn County (CA, USA). Dormant sprays were applied to 42 orchard rows (Table 1) with similar slope. Ground cover treatments consisted of bare ground and three cover crops (nontillage clover, resident vegetation, and a perennial sod mix). The perennial sod mix and clover were planted in the fall of 1999. Resident vegetation consisted of annual and perennial broadleaf plants and grasses. Bare ground was obtained by spraying the orchard floor with glyphosate herbicide (Roundup®, Monsanto, St. Louis, MO, USA) in October 2000. These treatments were replicated in three orchard rows of each section. Rows 1 to 8, 21 to 25, and 38 to 42 were not sprayed. Rows 9 to 20 were sprayed with diazinon, and rows 26 to 37 were sprayed with esfenvalerate. The application rate of each insecticide was 0.1 L/m^2. Diazinon (Diazinon 4EC, emulsifiable concentrate with 49% active ingredient [a.i.]) was applied at 1,683 g (a.i.)/ha and esfenvalerate (Asana XL [Du Pont, Wilmington, DE, USA] emulsifiable concentrate with 8.4% a.i.) was applied at 68 g (a.i.)/ha. The grower sprayed the orchard on January 20, 2001, using a commercial air-blast orchard sprayer pulled by tractor.

Storm frequency and intensity

Rainfall was measured with a rain gauge located at the orchard site. Precipitation before the insecticide application was 6.1 mm in December 2000 and 71.2 mm during January 5 through 9, 2001. Following insecticide application, three rain events occurred on January 23 through 26, February 10 through 13, and February 17 through 22, 2001, and measured precipitation was 57.9 mm, 32.1 mm and 42.1 mm, respectively. Runoff volume was recorded and samples collected during January 23 through 27 and February 17 through 22, 2001.

Sample collection for comparison of diazinon versus esfenvalerate runoff toxicity

Before sample collection, 1.9-L acid-washed glass jars were recessed into the ground in each orchard row following the methods described by Werner et al. [12]. The use of glass jars minimizes adsorption of organic chemicals to collection containers. The jars remained capped until after the insecticides were sprayed. In each orchard section, diazinon- or esfenvalerate-sprayed runoff samples for toxicity testing were collected in three rows for each ground cover type (Table 1). Samples also were collected in the center row of each nonsprayed section, where the ground cover consisted of resident vegetation. The contents of the three jars from each treatment were combined to yield one sample per treatment used for toxicity testing. This resulted in four runoff samples from each orchard section (diazinon- and esfenvalerate-treated), and one sample from nonsprayed rows. Samples were collected on January 25 and February 20, 2001, following the first runoff-generating rainfall of two storm systems (January 23–29, February 17–25, 2001). Amounts of precipitation preceding sample collection were 45.68 mm and 26.90 mm, respectively. Samples were transported to the laboratory on ice, mixed, and stored at 4°C for a maximum of 10 d. Aliquots for chemical analysis were frozen immediately and stored at −20°C.

Sample collection for comparing ground covers

The effect of ground cover vegetation on runoff volume, insecticide loading, and toxicity was tested in the diazinon-sprayed orchard section. Samples were collected from three replicate rows per ground-cover type. The treatments were: No cover (bare); perennial sod mix (sod); nontillage clover (clover); and resident vegetation (RV). Autosamplers specifically designed for this purpose [14] were used to collect composite samples and measure runoff volume in each row. Earthen dams were built between two tree berms. The dams directed flow from a defined area (348 m^2) to a 19-L bucket buried in the soil. Water collecting in the bucket was pumped through a flow meter to a T-fitting, which diverted 2% of the total runoff water into a Nalgene® (Acros/Fisher Scientific, Pittsburgh, PA, USA) tub. Water samples for toxicity testing and chemical analyses were collected from this tub on January 27 and February 22, 2001, transported to the laboratory, and stored as described above.

Toxicity testing

Runoff samples collected in glass jars in esfenvalerate- and diazinon-sprayed orchard sections were tested for toxicity to larval fathead minnows (*Pimephales promelas*), larval rainbow trout (*Onchorhynchus mykiss*), midge larvae (*Chironomus riparius*), and the waterflea *C. dubia* according to U.S. Environmental Protection Agency (U.S. EPA) protocols [15]. The composite (2% of total runoff) water samples collected by autosampler for comparing the effect of ground cover crops were tested using two cladocerans, *C. dubia* and *Simocephalus* *vetelus*. Before initiating bioassays, the water samples were mixed rigorously in the original containers, filtered through a 60-μm screen, brought to test temperature, and aerated at a rate of 100 bubbles/min until the dissolved oxygen concentration was approximately 8.5 mg/L. The laboratory control water consisted of deionized water amended to U.S. EPA [15] moderately hard standards.

Fathead minnow larvae were obtained from Aquatox (Hot Springs, AK, USA). Upon arrival, fish were acclimated to laboratory control water for 6 h. Ten 48-h-old larvae were selected randomly and placed into each of three replicate 500-ml glass beakers containing 250 ml of test solution. Tests were conducted at 25 ± 1°C and a 16:8-h light:dark cycle. Minnows were fed *Artemia* nauplii three times daily. Eighty percent of water was removed from each beaker and renewed with freshly aerated test or control water each day. Dead organisms were removed before each renewal. Mortality was recorded after 96 h.

Rainbow trout larvae were obtained from Mount Lassen Trout Farms (Red Bluff, CA, USA). Upon arrival, 14-d-old fish were acclimated to laboratory control water for a minimum of 6 h. Organisms were maintained and tests conducted at 12 ± 1°C and a 16:8-h light:dark cycle. Larvae were selected randomly and placed in each of four replicate 1,000-ml glass beakers containing 500 ml of sample water. The first set of tests (samples collected January 25, 2001) used 5 fish per replicate; the second set of tests (samples collected February 20, 2001) used 10 fish per replicate. Water was renewed each day and trout fed as described for fathead minnow larvae. Mortality was recorded after 96 h.

Ceriodaphnia dubia were from an in-house culture maintained at the Aquatic Toxicology Laboratory, University of California (Davis, CA, USA). The daphnids were maintained at 25 ± 1°C and a 16:8-h light:dark cycle. Tests were set up according to U.S. EPA [15] protocol using 6- to 18-h-old *C. dubia* with the endpoint being mortality within 48 h. One *C. dubia* was placed into each of 10 borosilicate glass vials containing 15 ml of sample water. Trout chow and algae (*Selenastrum capricornutum*) were added according to the guidelines. Every 24 h, each daphnid was transferred to a new vial containing 15 ml of sample water. When 100% mortality occurred within 24 h, dilutions of the respective water sample were tested to determine the lowest-observed-effect dilution (LOED) and the no-observed-effect dilution (NOED). Each dilution tested contained half the amount of runoff than the preceding test solution. Results are presented as dilution factors.

The indigenous cladoceran species *S. vetelus* was collected from the Sacramento–San Joaquin Delta, and maintained in culture at the Aquatic Toxicology Laboratory, University of California. Test procedures followed the 48-h bioassay protocol described for *C. dubia*.

Midge larvae (*C. riparius*, 3rd instar) were obtained from Aquatic Research Organisms (Hampton, NH, USA). Upon arrival, the midge larvae were transferred to a 1,000-ml crystallizing dish in a chamber maintained at 25 ± 1°C. Before the tests, organisms were fed 17 mg Tetramin® (Tetra Werke, Melle, Germany), and acclimated by changing approximately 80% of the control water three times. Five organisms were added to each of four replicate 250-ml glass beakers containing 200 ml of sample water and 0.25 g of silica sand. Tests were conducted at a temperature of 25 ± 1°C and 16:8-h light:dark cycle. Approximately 75% of the sample water was renewed, and midges were fed 17 mg Tetramin daily before water renewal. Mortality was recorded after 96 h.

Chemical analysis

Diazinon, esfenvalerate, parathion, and decachlorobiphenyl (DCBP) analytical standards were obtained from ChemService (West Chester, PA, USA). Liquid–liquid extraction was used for the analyses of esfenvalerate and esfenvalerate/diazinon. Water samples were thawed at room temperature and suspended sediment was allowed to settle. A 100-ml aliquot was decanted and put into a 250-ml separatory funnel, and 10 g of NaCl was added. The mixture was shaken vigorously until the salt was dissolved. Fifty milliliters of ethyl acetate was added to the funnel and the mixture was shaken for 1 min, and then allowed to stand for 60 min for phase separation. The organic phase was transferred to a 50-ml conical test tube and evaporated to approximately 3 ml under a stream of N_2 at 60°C. The extract was dried with anhydrous sodium sulfate and transferred to a 5-ml volumetric flask. The test tube was washed with 1 ml of ethyl acetate, and the wash was added to the flask. Decachlorobiphenyl and parathion internal standards were added and the volume adjusted to 5 ml.

Solid phase extraction (SPE) was used for the analysis of diazinon. Water samples were treated as described above. A 100-ml aliquot was decanted and run through a SPE C18 column (Bond Elut, 500 mg, 3 ml; Varian, Harbor City, CA, USA) at a slow drip rate under vacuum. The column was eluted twice with a 2-ml volume of ethyl acetate into a test tube. The eluate was transferred to a 5-ml volumetric flask, and the test tube was washed with 0.5 ml of ethyl acetate, which was added to the flask. Parathion internal standard was added and the volume adjusted to 5 ml for analysis by gas chromatography (GC). Aliquots of 1 μl were injected into the GC (Hewlett-Packard, model 5890, series II Plus, Avondale, PA, USA). Diazinon was analyzed using a DB-35MS column (J&W Scientific, Agilent Technologies, Palo Alto, CA, USA) and a nitrogen–phosphorus detector; esfenvalerate was analyzed using a DB-608 column (J&W Scientific) and an electron-capture detector. The amount of insecticide was determined by comparison with measurements of analytical standards. Detection limits were 0.070 μg/L for diazinon, and 0.126 μg/L for esfenvalerate. Recoveries from the liquid–liquid extraction were 71.4% and 79.3% for diazinon and esfenvalerate, respectively. Recovery of diazinon by SPE was 69.1%.

Statistical analysis

Toxicity was defined as a statistically significant reduction in survival ($p < 0.05$) for animals tested in runoff water versus control water. Bartlett's test for homogeneity of variances was performed on all fish and chironomid mortality data. Toxicity, pesticide concentration, and pesticide loads were analyzed using analysis of variance and Fisher's least-significant-difference method or Dunnett's mean separation test when variance was homogeneous. If variance was not homogeneous, data were transformed to relative ranks and analyzed using analysis of variance and Dunnett's mean separation tests. Mortality data for *C. dubia* and *S. vetelus* were compared to controls using Fisher's exact test. The *t* test was used for pairwise comparison of diazinon concentrations, runoff volume, and runoff toxicity between rows containing ground cover crops and bare ground treatments [16].

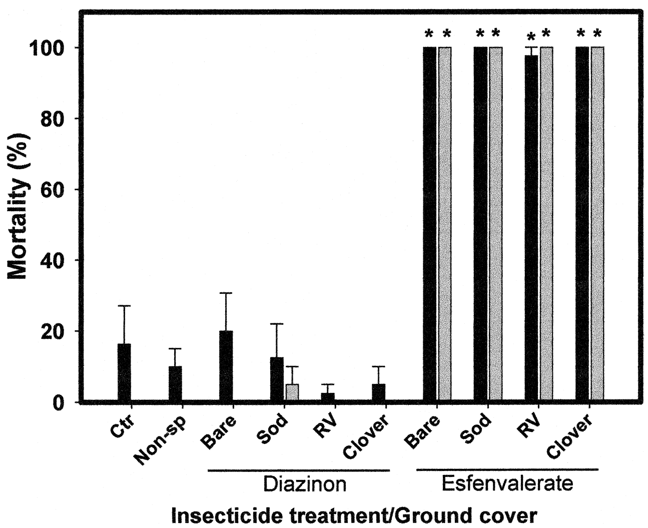

Fig. 1. Percent mortality of fathead minnow (dark bars) and rainbow trout (grey bars) larvae after a 96-h exposure to orchard runoff collected in diazinon- and esfenvalerate-treated sections on January 25, 2001; shown are means ± standard error ($n = 4$). * = significant ($p < 0.05$) increase in mortality compared to laboratory controls; Ctr = control; Non-sp = nonsprayed; Bare = no ground cover vegetation; Sod = perennial sod; RV = resident vegetation; Clover = nontillage clover.

Quality control

Because conductivity was lower in storm water runoff samples (68–121 μmhos/cm) than in standard laboratory control water (301–323 μmhos/cm), a soft water control was included in the toxicity test design. Conductivity of the soft water control was 34 to 51 μmhos/cm and, therefore, two to three times lower than conductivity measured in runoff samples. Mortality in soft control water was elevated slightly for fathead minnows (39.5 ± 16.4% and 15 ± 6.5% in January and February storm water testing, respectively), but not for rainbow trout. The pH and dissolved oxygen concentrations of orchard runoff samples and controls were within the normal physiological ranges of the test organisms, 7.11 to 8.31 and 8.2 to 10.9 mg/L, respectively. Toxicity tests were performed according to the recommended guidelines and met the test acceptability criteria [15]. Standardized procedures were followed in all aspects of research. For each set of bioassays, randomly chosen test samples were split and tested in duplicate. Monthly reference toxicant tests, consisting of five to six known concentrations of NaCl in laboratory control water, were conducted for each standard bioassay species to monitor changes in animal sensitivity over time. Blind duplicate, blank, and spiked samples were tested on a regular basis for quality control. Chemical analysis of water samples by GC was performed in duplicate. Gas chromatography response was linear over the range of the standards.

RESULTS

Toxicity of storm water runoff: First rainfall after insecticide application

The orchard runoff samples collected 5 d after dormant spray application, on January 25, 2001, from sections treated with esfenvalerate, were highly toxic to fathead minnows and rainbow trout (Fig. 1). Complete mortality of fish occurred in all runoff samples collected in esfenvalerate-sprayed orchard rows within 96 h of exposure, but no significant mortality occurred in runoff samples from diazinon-treated rows or from nonsprayed rows. Chemical analysis revealed that esfenvalerate was present at concentrations potentially toxic to fish larvae (Table 2). Measured esfenvalerate concentrations were at or above the reported 96-h LC50 values for fathead minnow and rainbow trout, the species used in this study (Table 3). Measured esfenvalerate concentrations must be regarded as minimum possible concentrations. Recovery for this compound generally is low, and loss due to sorption to container and filter surfaces during sampling, storage, and analysis can be significant [17]. These factors could account for the discrepancy between measured esfenvalerate concentrations and toxicity test results. For example, esfenvalerate concentrations measured in samples collected from rows with bare ground and resident vegetation as cover crop were approximately equal to the 96-h LC50s of the test species, but exposure to

Table 2. Results of chemical analyses of runoff samples collected in glass jars recessed into the ground in diazinon- or esfenvalerate-sprayed orchard rows during rainfall events in January and February 2001. Detection limits for diazinon and esfenvalerate were 0.070 and 0.126 μg/L, respectively. Bare = No ground cover vegetation; Sod = Perennial sod; RV = Resident vegetation; Clover = Nontillage clover; ND = Not detected; — = Not measured

Sampling date	Insecticide applied	Cover crop	Diazinon (μg/L)	Esfenvalerate (μg/L)
Jan. 25, 2001	Nonsprayed	RV	10.20	ND
	Diazinon	Bare	339.70	—
	Diazinon	Sod	207.20	—
	Diazinon	RV	290.20	—
	Diazinon	Clover	277.10	—
	Esfenvalerate	Bare	1.96	0.37
	Esfenvalerate	Sod	2.25	0.64
	Esfenvalerate	RV	2.04	0.28
	Esfenvalerate	Clover	3.47	0.72
Feb. 20, 2001	Nonsprayed	RV	1.13	ND
	Diazinon	Bare	11.10	—
	Diazinon	Sod	10.70	—
	Diazinon	RV	19.50	—
	Diazinon	Clover	13.60	—
	Esfenvalerate	Bare	0.81	ND
	Esfenvalerate	Sod	0.79	ND
	Esfenvalerate	RV	0.73	ND
	Esfenvalerate	Clover	1.20	0.18

Table 3. Median lethal concentrations (LC50s) of diazinon and esfenvalerate for aquatic organisms used as test species in this study. Values are for 96-h tests unless stated otherwise

Species name	LC50 Diazinon (measured, μg/L)	LC50 Esfenvalerate (nominal, μg/L)
Waterflea (*Ceriodaphnia dubia*)	0.64[a] (48 h)/0.40[a]	0.36[a] (48 h)/0.28[a]
Waterflea (*Simocephalus vetelus*)	4.92[a] (48 h)/2.50[a]	1.2[a] (48 h)/0.4–0.8[a]
Midge (3rd instar) (*Chirononimus riparius*)	66.4[a]	0.41[a]
Fathead minnow (*Pimephales promelas*)	6,000[a]	0.25[b]
Rainbow trout (*Oncorhynchus mykiss*)	400–1,800[c]	0.30[d]

[a] University of California, Davis Aquatic Toxicology Laboratory (Davis, CA, USA).
[b] University of California, Davis Aquatic Toxicology Laboratory; these are LC50 values for 21-d-old fish.
[c] Environmental Effects Database; U.S. EPA, (Washington, DC).
[d] Extension Toxicology Network, Oregon State University (Corvallis, OR, USA).

these runoff samples resulted in 100% mortality of fish within the 96-h test period.

Runoff from both orchard sections was extremely toxic to waterflea (*C. dubia*). All water samples tested caused 100% mortality of the daphnids within 24 h. Water samples were then tested as dilutions until the NOED was determined. Table 4 shows the dilution factors needed to reach the LOED and NOED for mortality after 48 h. Runoff samples from rows treated with diazinon were 40 to 80 times more toxic to the cladoceran species than runoff from the esfenvalerate-treated orchard sections. Runoff from esfenvalerate-treated rows still was toxic following a 12.5-fold dilution with laboratory control water, whereas 500- to 1,000-fold dilutions of runoff from diazinon-sprayed rows were required to reach the *C. dubia* LOED. Chemical analyses generally confirmed the results of

Table 4. Toxicity of runoff samples from diazinon- and esfenvalerate-sprayed orchard sections to the cladoceran *Ceriodaphnia dubia.* Shown are 48-h lowest-observed-effect dilution factor (LOED) and 48-h no-observed-effect dilution factor (NOED). Bare = No ground cover vegetation; Sod = Perennial sod; RV = Resident vegetation; Clover = Nontillage clover

Sampling date	Insecticide applied	Cover crop	NOED/LOED
Jan. 25, 2001	Nonsprayed	RV	40/20
	Diazinon	Bare	1,000/500
	Diazinon	Sod	2,000/1,000
	Diazinon	RV	1,000/500
	Diazinon	Clover	2,000/1,000
	Esfenvalerate	Bare	25/12.5
	Esfenvalerate	Sod	25/12.5
	Esfenvalerate	RV	25/12.5
	Esfenvalerate	Clover	25/12.5
Feb. 20, 2001	Nonsprayed	RV	10/5
	Diazinon	Bare	400/200
	Diazinon	Sod	200/100
	Diazinon	RV	200/100
	Diazinon	Clover	200/100
	Esfenvalerate	Bare	5/2.5
	Esfenvalerate	Sod	5/2.5
	Esfenvalerate	RV	5/2.5
	Esfenvalerate	Clover	5/2.5

the toxicity tests (Table 2), but again, the observed mortality was higher than would be expected based on the measured insecticide concentrations and LC50 data. Diazinon was present at concentrations of 207 to 340 μg/L, which is 323 to 531 times the 48-h LC50 of *C. dubia* (0.64 μg/L, Table 3). We cannot exclude the possibility that insecticide was lost during sampling, testing, and storage. It also is possible that crosscontamination between orchard sections occurred via spray drift during the application of insecticides. Because *C. dubia* is sensitive to both compounds, trace amounts of esfenvalerate present in these samples could add to or enhance the toxicity of diazinon. Spray drift is the likely reason for diazinon detected in runoff samples from nonsprayed and esfenvalerate-sprayed rows. Concentrations measured were 10.2 μg/L and 2 to 3.5 μg/L diazinon, respectively, corresponding to approximately 16 and 3 to 5.5 toxic units (TU). In this case, TU are defined as the ratio of measured concentration to the 48-h LC50 for *C. dubia*. The combined effects of diazinon and esfenvalerate also may be responsible for the *C. dubia* toxicity observed in runoff from esfenvalerate-sprayed orchard sections. In these samples, esfenvalerate was detected at concentrations of 0.28–0.72 μg/L. These concentrations correspond to approximately 0.8 to 2 TU for *C. dubia*. However, even the sum of TU (2.8–5.5) for diazinon and esfenvalerate does not explain the 12.5-fold dilution needed to reach the LOED for *C. dubia*. Again, loss of insecticide during storage and analysis and other factors discussed below may have contributed to the discrepancy between measured concentrations and toxicity to the test organisms.

Toxicity of storm-water runoff: Third rainfall after insecticide application

All runoff samples collected from the orchard on February 20, 2001, were less toxic than samples collected one month earlier. No fish mortality was observed within the 96-h test period. However, runoff samples from esfenvalerate-sprayed sections still caused 100% mortality in 3rd instar larvae of the midge *C. riparius* (Fig. 2). Samples collected in January from the diazinon- and esfenvalerate-sprayed sections were 2.5 to 10 and 5 times, respectively, more toxic to *C. dubia* than samples collected on February 20 (Table 4). Toxicity of samples from nonsprayed rows was four times higher in January than in February. Chemical analyses largely confirmed the results of toxicity testing, although measured concentrations of the two insecticides were always lower than would be expected based on the results of the toxicity tests. Runoff from diazinon-treated rows contained 10.7 to 19.5 μg/L diazinon, while diazinon concentrations in runoff from nonsprayed and esfenvalerate-treated rows were 1.13 μg/L and 0.73 to 1.2 μg/L, respectively. This corresponds to approximately 17 to 31, 2, and 1 to 2 TU for *C. dubia*, respectively. Esfenvalerate was above detection limits in only one sample from esfenvalerate-sprayed orchard sections (clover ground cover, see Table 2).

The effect of ground cover type on the toxicity, volume, and diazinon loading of runoff

A significant influence of ground covers on the toxicity of runoff samples to *C. dubia* and *S. vetelus* was not evident (Table 5), although runoff samples from rows covered with clover or resident vegetation generally were less toxic than runoff from rows with bare ground and sod. This trend was observed for both cladoceran species. Significantly lower diazinon concentrations were measured in January composite

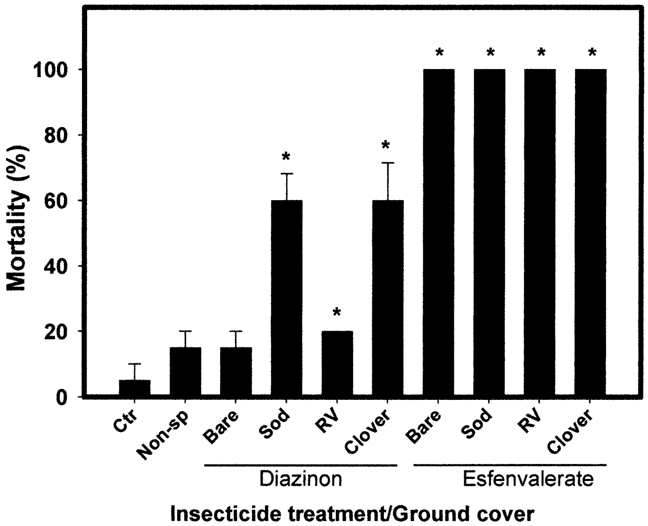

Fig. 2. Percent mortality of midge (*Chrionomus riparius*) larvae after a 96-h exposure to orchard runoff collected in diazinon- and esfenvalerate-treated sections on February 20, 2001; shown are means ± standard error ($n = 4$). * = significant ($p < 0.05$) increase in mortality compared to laboratory controls; Ctr = control; Non-sp = nonsprayed; Bare = no ground cover vegetation; Sod = perennial sod; RV = resident vegetation; Clover = nontillage clover.

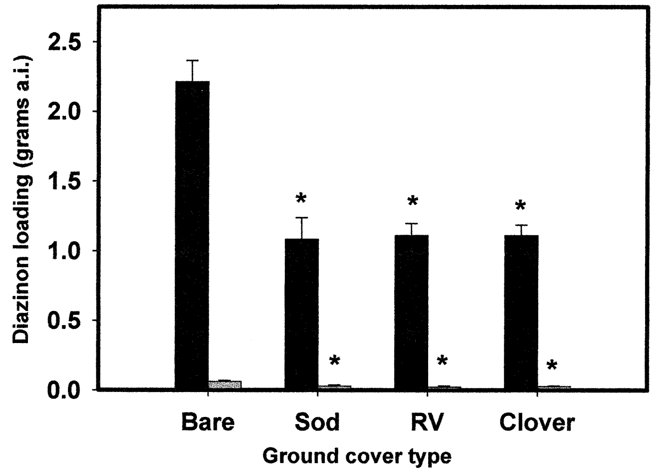

Fig. 3. Total diazinon-loading (insecticide concentration *x* total runoff volume) in runoff collected during precipitation events on January 27 (dark bars) and February 22, 2001 (grey bars) from defined areas (348 m^2) in orchard rows with different ground cover types; shown are means ± standard error ($n = 3$). * = significantly ($p \leq 0.006$) different compared to rows with no ground cover vegetation; Bare = no ground cover vegetation; Sod = perennial sod; RV = resident vegetation; Clover = nontillage clover.

samples from rows with two types of ground covers, perennial sod and resident vegetation (Table 5). Concentrations of diazinon also were lower in runoff from rows planted with clover, but variability was high and results not significant statistically. Although runoff volume was not significantly different between ground cover types in January, orchard rows with ground cover crops had significantly less runoff than rows with bare ground in February (Table 5). Total pesticide loadings were calculated for each ground cover type by multiplying measured runoff volumes (number of liters) and diazinon concentrations (Table 5). Results showed a highly significant ($p \leq 0.006$) reduction of diazinon loads in rows where ground covers were used (Fig. 3). Pesticide loads were reduced by approximately 50%, compared to bare soil, by all three ground covers. This was evident in both the January and the February rainfall events.

DISCUSSION

The study presented here demonstrates the potential for off-site movement of dormant spray insecticides, both for the OP diazinon and the pyrethroid esfenvalerate, from orchards during winter rainfall events. Storm water runoff samples contained both insecticides at concentrations high enough to be acutely toxic to aquatic organisms even one month after sprays were applied, and after several intervening rainstorms had occurred. However, it is important to note that the sampling design of our study was aimed at examining a worst-case scenario. Runoff samples were collected directly in the orchard to measure pesticide concentration, toxicity, and runoff volume moving from a small section of the orchard. Soil type and topography was similar across all experimental plots in our study, but such factors must be considered when extrapolating our results to other sites. Volume of storm water runoff leaving the orchard site, type of surface and soil runoff travels over before entering waterways, distance of the orchard from surface waters of concern, and dilution after leaving the orchard also should be taken into consideration. Clearly, quantification of hydrological parameters is needed to assess realistically proportional contributions of toxic runoff from specific sites to surface water bodies.

Acute toxicity of orchard runoff from esfenvalerate-treated sections was very high for fish larvae (100% mortality), but declined to 0 about one month after application. This finding

Table 5. The effect of ground cover vegetation on diazinon loading and toxicity of orchard runoff: Shown are means and standard errors ($n=3$) of runoff volume, diazinon concentrations, and lowest-observed-effect dilution factor (LOED) values. (*Ceriodaphina dubia, S. vetelus,* 48-h tests) of composite orchard runoff samples (2% of total runoff) collected using autosamplers. Bare=no ground cover vegetation; Sod=perennial sod; RV=resident vetgetation; Clover=nontillage clover; * significantly different ($p<0.05$) from bare ground treatment

Sampling date	Cover crop	Runoff volume (L/348 m^2)	Diazinon concentration (μg/L)	*C. dubia* LOED (dilution factor)	*S. vetelus* LOED (dilution factor)
Jan. 27, 2001	Bare	7,848 ± 815	284.2 ± 10.0	667 ± 167	1,000 ± 500
	Sod	5,656 ± 1,172	197.5 ± 15.9*	750 ± 250	1,000 ± 500
	RV	5,715 ± 623	195.8 ± 7.2*	517 ± 159	250 ± 0
	Clover	5,299 ± 897	226.3 ± 49.5	417 ± 83	583 ± 220
Feb. 22, 2001	Bare	3,157 ± 147	19.9 ± 1.8	58 ± 22	17 ± 4
	Sod	1,792 ± 457*	17.3 ± 1.3	50 ± 0	12.5 ± 0
	RV	1,466 ± 529*	19.3 ± 4.5	58 ± 22	27 ± 13
	Clover	1,380 ± 330*	24.4 ± 7.6	58 ± 22	27 ± 13

indicates that much of the pyrethroid had either degraded or became bound firmly to soil and organic matter. Degradation rates in soil for fenvalerate, the parent compound of esfenvalerate and chemically almost identical, depend on soil type, moisture, temperature, and microbial activity. The $t_{1/2}$, the time it takes to break down 50% of the chemical, ranges from 2 d to three months [8]. In aquatic environments, (es)fenvalerate sorbs rapidly to organic matter and sediments, where it can be relatively stable. For example, in estuarine sediments 50% of the compound still was present after six weeks [7]. Persistence of esfenvalerate increases under conditions of reduced light, low microbial activity, low oxygen, and high organic carbon content [7].

Runoff from esfenvalerate-treated sections was significantly less toxic to waterflea than runoff from diazinon-treated rows, but toxicity persisted throughout the study in samples from both orchard sections. Although samples from esfenvalerate-sprayed rows also contained diazinon due to spray drift during insecticide application, concentrations of diazinon accounted for only part of the toxicity to *C. dubia*. Because fish and invertebrates are about equally sensitive to esfenvalerate, but not to diazinon, toxicity of February runoff samples to invertebrates, but not to fish, suggested a contribution of diazinon. Convincing indication of the continued presence of esfenvalerate at toxic concentrations, although mostly at concentrations below our detection limits (0.126 μg/L), was indicated by the complete mortality of midge larvae in samples from esfenvalerate-sprayed orchard sections. Only two samples from diazinon-sprayed rows containing 10.7 to 19.5 μg/L diazinon caused some mortality ($<$60%) in *C. riparius*. The LC50s listed in Table 3 confirm that this species is more sensitive to esfenvalerate than to diazinon.

Toxicity of runoff water generally was greater than suggested by TUs calculated using data on chemical concentrations. Loss of insecticide, especially esfenvalerate, during sampling and storage is one possible cause for the difference seen, but the greater-than-additive toxicity also could be due to synergistic effects of the two pesticides applied. Spray drift probably was responsible for diazinon detected in samples collected in esfenvalerate-sprayed orchard rows, and it is possible that esfenvalerate was present in water samples from diazinon-sprayed orchard sections, albeit at concentrations below our detection limits. The toxic effects of diazinon and esfenvalerate have been shown to be additive or synergistic [18–20]. Additionally, the so-called inert ingredients in pesticide formulations may in some cases contribute to the toxicity of the active ingredient [21]. Formulated products, therefore, may be more toxic than the purified active ingredient alone [22].

The use of ground cover vegetation in orchard rows, a proposed best-management practice to reduce nonpoint-source pollution from agricultural areas, reduced total diazinon loading, the product of measured diazinon concentration, and total runoff volume in orchard runoff by about 50%, irrespective of the type of vegetation (Fig. 3). Ground covers, therefore, can diminish the impact that pesticides applied to orchards may have on aquatic systems. We did not detect a statistically significant reduction in toxicity of runoff due to type of ground cover vegetation, but toxicity generally was greater in rows with bare ground. A significant reduction was seen in both runoff volume (February samples) and diazinon concentration (January samples) depending on ground cover type (Table 5). This likely is due to enhanced water infiltration into the soil, which becomes modified by the root system of ground cover vegetation. A study by Angermann et al. [23] on the hydrology of orchard runoff showed that infiltration of rainwater into soil planted with resident vegetation, then ripped, was approximately ten times greater than that for bare soil. We conclude that ground cover vegetation can help reduce off-site movement of diazinon and possibly other pesticides by substantially reducing the total volume and pesticide loading of runoff.

Our study demonstrates that both the OP diazinon and the pyrethroid esfenvalerate can move off-site in orchard storm water runoff following major rainfall events. Although a considerable body of data exists on environmental concentrations and toxicity of diazinon to nontarget aquatic species [24,25], much information is needed still to determine the risk of pyrethroid pesticides to biotic receptors in aquatic environments. Because of the chemical properties of pyrethroid pesticides, it is important to note that our laboratory test results probably underestimate the concentrations present in and toxicity of actual field runoff, as adsorption of esfenvalerate to surfaces during sampling, transport, storage, and filtration will reduce concentrations of dissolved pyrethroid [17]. In addition, toxicity of pyrethroids increases with decreasing temperature [7], suggesting that laboratory tests with fathead minnows and cladocerans conducted at 25°C may underestimate ambient toxicity due to pyrethroids during winter months, when water temperatures in the Sacramento–San Joaquin watershed typically are $<$15°C (Bureau of Reclamation, Central Valley Operations, Sacramento, CA, USA). On the other hand, due to its low water solubility, a large proportion of esfenvalerate is likely to sorb to soil particles and organic matter before being transported into surface waters during heavy rainfall [26]. If dissolved esfenvalerate is transported into surface waters, it probably remains in the dissolved phase for only a short time [27,28] before adhering to suspended particulates and being deposited in the sediment [29]. This could, for example, explain why long-term mesocosm-derived chronic effect concentrations of esfenvalerate, which were $<$50 to 860 ng/L for crustaceans, insects, and fish, were at or above the 96-h LC50 values for these groups [30].

CONCLUSION

Our study demonstrates that the use of ground cover vegetation leads to a considerable reduction of insecticide loading in orchard storm water runoff. We also showed that the application of hydrophobic insecticides such as the pyrethroid esfenvalerate lowers acute toxicity of runoff to some invertebrate species, but increases toxicity to fish and chironomid species. In order to assess the risk of orchard runoff to aquatic ecosystems, it is essential now to identify site characteristics that allow runoff to reach surface water bodies, study the environmental fate of dormant-spray insecticides, and aim to better understand the consequences of exposure to environmentally realistic doses of insecticide(s) for the health and survival of aquatic organisms. For example, toxic effects of pyrethroid as well as OP insecticides at concentrations far below those considered to be acutely toxic have been reported for Atlantic salmon [31,32], Chinook salmon [33,34], and invertebrate species [35], but the long-term consequences of these are poorly understood. Given the complex mixtures of pesticides and other chemical contaminants that aquatic organisms can be exposed to, and the potential for synergistic and additive effects of multiple chemicals [18,19,36], the importance of minimizing the input of pollutants into aquatic systems cannot be overstated. As demonstrated in this study, one way to achieve this goal is to use ground cover vegetation in orchard agriculture.

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