Avoidance Chamber Responses of Mayfly Nymphs Exposed to Eight Herbicides

L. C. Folmar[1]

U. S. Fish and Wildlife Service
Fish-Pesticide Research Laboratory
Denver Federal Center
Denver, Colo. 80225

[1]Present address:
U. S. National Marine Fisheries Service
Northwest Fisheries Center
2725 Montlake Boulevard East
Seattle, Wash. 98112

Avoidance reactions of rainbow trout fry exposed to nine herbicides that may be used in or near irrigation systems were described by FOLMAR (1976). Irrigation canals that contain viable sport fisheries also support populations of aquatic insects, which serve as a primary source of forage for the fish. In a survey of the Colorado Big Thompson Irrigation Project, OTTO (1975) reported that six orders of aquatic insects were collected at several sampling stations. Because movement (stream drift) or death of the insects may have a deleterious influence on the nutrition of a resident fishery, it was important to determine whether a representative insect (*Ephemerella walkeri*) would, like fry of rainbow trout (*Salmo gairdneri*), avoid some of the most commonly used herbicides. This information could be applied to natural waters, since fractions of the treated water may be returned to receiving streams, either as return flow from irrigated fields or as unused irrigation water.

Methods and Materials

Immature mayflies collected from Boulder Creek near Nederland, Colorado, as late-instar nymphs were acclimated for 72 h to experimental water before avoidance testing began. No mortality occurred during the acclimation period. Denver city water, charcoal filtered and sterilized by ultraviolet light was used in all experiments. Characteristics of the experimental water were as follows: temperature 11 C; pH, 8.0; hardness, 90 mg/l as $CaCO_3$; conductivity 355 mohms/cm; chlorine and chloramines, <0.01 mg/l; and dissolved oxygen, 7.0 mg/l.

The eight herbicides tested, all of which are used or are being considered for use in the control of aquatic or ditchbank weeds by the U.S. Bureau of Reclamation and various independent irrigation organizations, were as follows: 2,4-D DMA (dimethylamine salt of 2,4 -dichlorophenoxyacetic acid); Aquathol K®[1/] (dipotassium salt of 7-oxabicyclo (2.2.1) heptane - 2,3 - dicarboxylic acid); copper sulfate; dalapon (2,-dichloropropionic acid); Roundup® (isopropylamine salt of n-phosphonomethyl glycine plus surfactant); diquat (6,7-dihydrodipyridol 1,2-*a*:2',1'-*c* pyrazidinium); emulsified xylene (*p*-1,4-dimethyl benzene); and acrolein (2-propenal). The amount of active ingredient in each herbicide and the expected concentration in water after routine field applications are given in Table 2.

[1/]The use of registered trade names does not imply Government endorsement.

Bulletin of Environmental Contamination & Toxicology,
0007-4861/78/0019-0312 $1.40

TABLE 1

Target plants and normal rates of application for eight experimental herbicides

Common name	International Union of Pure and Applied Chemists name	Plants to be controlled[1/]	Normal rate of application
Copper sulfate	Copper sulfate	A	0.05-0.1 lb/cfs per day
2,4-D DMA	Dimethylamine salt of 2,4-dichlorophenoxyacetic acid	DW	1.5-2.5 lb/ditchbank acre
Aquathol K	Dipotassium salt of 7-oxa bicyclo (2.2.1) heptane-2,3-dicarboxylic acid	AM	1-2 mg/l in static treatments
Dalapon	2,-Dichloropropionic acid	DW	1.0 lb/ditchbank acre
Roundup	Isopropylamine salt of n-phosphonomethylglycine plus surfactant	DW	2.0 lb/ditchbank acre
Diquat	6,7-Dihydrodipyridol 1,2-*a*: 2', 1'-*c* pyrazidinium	AM	0.2-0.5 mg/l in static treatment
Xylene (emulsified)	p-1,4-Dimethyl benzene	AM	10 gal/cfs for 30 min
Acrolein	2,-Propenal	A;AM	0.1 mg/l for 48 h

1/A=algae; AM=aquatic macrophytes; DW=ditchbank weeds

TABLE 2

Avoidance reactions of mayfly nymphs to three concentrations of eight herbicides

Herbicide	Expected water concentration at application site when applied at recommended rates (mg/l)	Experimental concentration (mg/l)	Insects in untreated water after 1 h (%)
$CuSO_4 \cdot 5H_2O$	0.005-0.01	0.001 2/	60
		0.01 2/	50
		0.1 2/	73*
2,4-D DMA (4 lb/gal acid equivalent)	0.1 3/	1.0	38
		10	48
		100	0 4/
Acrolein (technical grade)	0.1	0.001	68
		0.01	50
		0.1	70
Xylene + 2% AD-410	740 5/	0.1	62
		1.0	53
		10	48
Diquat (2 lb/gal acid	0.2-0.5	0.01	44
		0.1	58
		1.0	76**
Dalapon (powder 74% active ingredient)	0.2 3/	0.1	50
		1.0	23***
		10	50

TABLE 2 (continued)

Avoidance reactions of mayfly nymphs to three concentrations of eight herbicides

Herbicide	Expected water concentration at application site when applied at recommended rates (mg/1)	Experimental concentration (mg/1)	Insects in untreated water after 1 h (%)
Aquathol K (3 lb/gal acid equivalent)	1-2	0.1	50
		1.0	48
		10	60
Roundup (3 lb/gal acid equivalent)	0.02	0.1	41
		1.0	58
		10	74*

1/*=significant at $P \leq 0.05$; **=significant at $P \leq 0.01$; ***=significant at $P \leq 0.001$
2/Cu ion
3/Ditchbank application
4/No avoidance, 70% mortality
5/Reduced to 0.1 in return flows

TABLE 3

Comparison between avoidance concentrations of mayfly nymphs and rainbow trout fry

Herbicide	Lowest observed avoidance concentration (mg/l)	
	Mayfly nymphs	Rainbow trout1/
$CuSO_4 \cdot 5H_2O$	0.1	0.0001
2,4-D DMA	>10	1.0
Acrolein	>0.1	0.1
Xylene	>10	0.01
Diquat	1.0	>10
Dalapon	>10	1.0
Aquathol K	>10	>10

1/From FOLMAR (1976)

The Y-shaped avoidance maze was similar to that used by FOLMAR (1976), except that the holding area was reduced to one-tenth of that used by him. Fresh water flow rates into each arm of the maze were maintained at 400 ml/min for all toxicants. Herbicides were administered by Mariotte bottle at the inlet of one arm while fresh water was similarly administered at an equivalent rate at the inlet of the other arm. All herbicide concentrations were calculated rather than measured.

The mayfly nymphs were placed in the holding area of the maze for 15 min before the toxicant was administered and were then allowed equal access to the treated or untreated water. The numbers of nymphs in each portion of the maze were recorded 1 hr. later. The nymphs were used only once and then discarded. Tests at each concentration were repeated five times with 10 nymphs per trial. Mariotte bottles containing toxicant and water were reversed after each trial to eliminate preference for either arm of the maze.

Avoidance of herbicides was evaluated statistically by the chi-square goodness of fit test on the assumption that if the mayfly nymphs could not discriminate between treated or untreated water, they would leave the holding area and enter each section of the maze with equal frequency. Avoidance was considered statistically significant if the probability that observed distributions would occur by chance was 5% or less.

Results and Discussion

Mayfly nymphs avoided the highest test concentration of copper sulfate, diquat, and Roundup, but did not avoid the other herbicides at the experimental concentrations tested (Table 2). No avoidance of 2,4-D DMA was observed at 10 mg/l; however, when the concentration was increased to 100 mg/l, a 70% mortality occurred still with no avoidance. The nymphs displayed a marked attraction to 1 mg/l dalapon. Attraction of aquatic organisms to one concentration of a toxicant and avoidance of another was demonstrated with rainbow trout and xylene (FOLMAR 1976), and with whitefish (Coregonus clupeaformis) and drilling fluids (LAWRENCE and SCHERER 1974). The nature of this biphasic response is difficult to interpret when a limited range of test concentrations are used; if the range were enlarged, perhaps an attraction as well as an avoidance concentration could be determined for each of the test chemicals.

The lowest concentration avoided by the test organisms presumably approximates the lowest level of toxicant perception, at least among the concentrations tested. No avoidance was observed for any of the chemicals present at or below concentrations that might be expected in receiving waters or return flows after a normal application at recommended rates.

With the exception of diquat and Aquathol K, the mayfly nymphs appeared to be less sensitive to the eight herbicides than did the rainbow trout fry previously tested (Table 3). However, due to the inherent simplicity of the testing apparatus and the absence of acute toxicity information, it was not clear whether the nymphs were less sensitive than fish (higher LC50 values) or their chemoreceptive systems were less efficient. Morphological and experimental evidence presented by HODGSON (1953) on the amphibious beetle, Laccophilius maculosus, indicated that the sensilla basiconia on the tips of the labial and maxillary palpi function as chemoreceptors for stimuli in solution while the distal ends of the antennae functioned as a chemoreceptor for stimuli in both the gaseous and aqueous phase. Furthermore, he found no evidence to suggest specialization of any morphologically distinguishable receptor sites sensitive only to a particular modality of stimulus. This lack of specialization may relate to the inability of insects to discriminate between different classes of chemical stimuli and may partly explain the higher avoidance thresholds observed in the mayflies than in rainbow trout.

The relatively low avoidance sensitivity of mayflies to the eight herbicides tested suggests that concentrations expected in irrigation return flows should not cause avoidance or otherwise dislodge benthic insects from their habitats in natural receiving streams. Also, anticipated herbicide residues in irrigation canals downstream from ditchbank spraying, or from points of direct application for aquatic weed control, should not adversely affect mayflies. However, the inability of mayflies to perceive and avoid various concentrations of a herbicide could be detrimental because they may receive a lethal exposure before attempting to avoid the chemical. For example, the mayflies did not avoid 10 mg/l of 2,4-D DMA, but 70% were killed by a concentration of 100 mg/l. It is conjectured that such a situation could potentially exist at, or immediately downstream from the point where an herbicide is introduced into a canal.

ACKNOWLEDGMENT

The author wishes to thank Ms. C. LeFever for her technical assistance.

REFERENCES

FOLMAR, L.C., Bull. Environ. Contam. Toxicol. 15(5):509. (1976).

HODGSON, E.S., Biol. Bull. 105:115. (1953).

LAWRENCE, M. and SCHERER, E., Dep. Environ., Res. Dev. Dir., Can. Fish. Mar. Serv. Tech. Rep. 502. 46 p. (1974).

OTTO, N.E., U.S. Bur. Reclam. Rep. REC-ERC-75-9. 59 p. (1975).