Acute Toxicity Of A Herbicidal Combination Of Diquat Plus Copper Ion To Eggs, Alevins, And Fry Of Rainbow Trout And Two Aquatic Macroinvertebrates[1]

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ABSTRACT

A combination of diquat (6,7-dihydrodipyrido (1,2-:2′, 1′-c) pyrazinediium ion) plus copper ion was used to control algae in artificial spawning channels. The rate of application recommended for algae control was 100 plus 150 ppbw applied for 3 hr. The toxicity of different concentrations of the herbicidal combination was determined for newly fertilized eggs, alevins, and fry of steelhead rainbow trout (*Salmo gairdneri* Richardson) and for mayfly nymphs (*Baetis bicaudatus*) and immature water scavenger beetles (*Tropisternus lateralis*). Concentrations of up to 800 ppbw diquat plus 1,200 ppbw copper ion did not reduce the number of viable eggs or the number of hatched eggs, nor harm alevins. Alevins were killed at 4,000 plus 6,000 ppbw. A significant number of fry and mayfly larvae were killed when exposed to 400 ppbw diquat combined with 600 ppbw copper ion, but not at levels recommended for algal control. Water scavenger beetles were unharmed by concentrations of 800 plus 1,200 ppbw. But concentrations five times the latter caused a 17% reduction of beetles.

INTRODUCTION

The Tehama-Colusa Fish Facility at Red Bluff, California, had a problem with *Cladophora glomerata* (L.) Kutz infesting artificial spawning channels for chinook salmon (*Onchorynchus tshawytscha* Wal.) in 1972. The filamentous algae clogged fish retention screens, reduced water flows, and caused inaccurate counts of released juveniles in electronic fish counters unless periodic adjustments were made in the counters.

Spawning operations began in mid-October and continued through November. Eggs hatched through January, and the alevin stage of development was generally complete by the end of February. Juvenile salmon were 4 to 5 cm long when the algae became a problem in mid-March. Algae usually persisted through June, when the rearing operation was completed and the spawning channels were emptied. Several 3-hr applications of a mixture of diquat at 100 ppbw and copper ion at 150 ppbw were made, seven applications at the beginning of the channel and four at 0.5 km downstream to control the algae in the channels in 1973. Singly, diquat or copper ion will control algae and weeds; however, smaller amounts of each chemical are needed to achieve equivalent control if they are combined (23). Channel applications did not harm juvenile salmon. If a change in climatic conditions enhanced earlier development of the *Cladophora*, the use of the herbicide combination at this time might have adverse effects on the incubation eggs or alevins. In addition to having direct effects on the salmon, the herbicidal treatments might affect them indirectly by reducing the populations of aquatic invertebrates they feed on.

The effects of different concentrations and exposure periods of copper ion on trout and salmon have been thoroughly investigated (4, 12, 14, 16, 20, 21). Other factors affect toxicity Jones (9) showed that carbonate was important in determining copper toxicity. He found 60 ppbw of copper ion was toxic in water with 12 ppm carbonate, and 600 ppbw was toxic in water with 320 ppmw carbonate. Short exposures to copper ion were not harmful to hatchery trout dipped in concentrations as high as 500 ppmw for 1 to 2 min for disease control (11). Some trout build up a resistance to the effects of copper. A population of rainbow trout survives in the copper-ladened waters of the upper Sacramento River, but hatchery-reared trout planted into these waters die (17). Lloyd (4) found copper toxicity increased as dissolved oxygen concentration decreased.

The purpose of this study, therefore, was to determine the effects of diquat plus copper ion on fertilized eggs, alevins, and young fry of a salmoid fish and on two species of aquatic invertebrates.

METHODS AND MATERIALS

The study was started on 15 February and completed 1 May 1973. Experiments were conducted in a small (3 m by 3 m), portable building placed at the downstream end of the fish access channel at the Tehama-Colusa Fish Facility. Three stacks of incubation trays were placed inside the building, and a large-capacity electric water pump was positioned to deliver water from the canal to the trays at 38 liters per min per set of eight trays. The water flowed down through the trays and returned to the channel by gravity flow. The weather during the tests

[1]Cooperative research of the Agricultural Research Service, U.S. Department of Agriculture: California Agricultural Experiment Station; Mid-Pacific Region, Bureau of Reclamation, U.S. Department of Interior; and California Department of Water Resources.

TABLE 1. WATER QUALITY FACTORS OF SACRAMENTO RIVER WATER USED IN THE SPAWNING CHANNELS DURING TREATMENT OF STEELHEAD RAINBOW TROUT EGGS WITH MIXTURES OF DIQUAT AND COPPER SULFATE.

Factor	Factor values
pH	7.2
Calcium hardness	62.0 ppmv
Total hardness	77.0 ppmv
Phenolphthalein alkalinity	0.0 ppmv
Total alkalinity	75.0 ppmv
Nitrate	0.9 ppmv
Orthophasphate	0.2 ppmv
Total phosphate	0.4 ppmv
Turbidity	70.0 ppmv
Total colloids	189.0 ppmv
Oxygen	12.0 ppmv
Diquat (background)	0.0 ppbw
Copper ion (background)	5.0 ppbw

was cold, so the water temperature in the trays did not increase above the 10.4 C channel temperature. Water quality factors were constant throughout the trial (Table 1).

Fertilized salmon eggs were not available; therefore, eggs of a salmonid with similar spawning requirements (11), steelhead rainbow trout, were obtained and used in the study. The Coleman National Fish Hatchery at Anderson, California supplied newly fertilized eggs. Eggs were divided into lots of 500 or more. Lots were determined by counting 500 water-hardened eggs in a 100 ml graduated cylinder and measuring the water displacement. This procedure was repeated three times, and the average amount of displaced water was calculated. Subsequent lots of 500 eggs were obtained by adding eggs to the cylinder until the calculated amount of water was displaced. This rapid method of counting was necessary to complete handling the eggs during the 24-hr period after fertilization. Twenty-one lots of 500 eggs were placed in shallow 15.2 by 15.2 by 7.6 cm trays made of a 100-mesh plastic screen. Groups of three trays were exposed by submersing them 5 cm deep in each of seven large plastic tubs containing 75 liters of herbicide-treated channel water.

Six concentrations of diquat plus copper ion were used, 25 ppbw plus 35 ppbw, 50 plus 70, 100 plus 150, 200 plus 300, 400 plus 600, 800 plus 1,200 respectively, and an untreated control. The eggs were exposed for 3 hr in each treatment, rinsed, and placed in the incubation trays. The eggs were kept in the dark and not examined until they were shocked at 21 days. Shocking was done by gently shaking the egg trays. This treatment did not cause mortalities but made dead eggs turn white (11). Dead eggs were counted and removed from the trays.

Alevins hatched from a portion of the eggs unused for the above experiment were separated into 21 lots of 100 to 200. One lot of alevins was put in each of the screened trays used in the previous egg study. Three trays were submersed 5 cm deep in tubs containing 75 liters of channel water treated with the following concentrations of diquat plus copper ion: 100 ppbw plus 150 ppbw, 200 plus 300, 400 plus 600, and 800 plus 1,200, respectively, and an untreated control. Aerators were placed in each tank and the alevins were exposed for 3 hr, rinsed gently, and placed in incubation trays. The number of dead alevins was determined after 24 hr, and the average percent mortality was calculated.

The remaining untreated fish were used to determine the toxicity of the herbicide combination to fry. Fry were treated about one week after the yolk sack had been absorbed. Three replicates of 25 fry each were placed in the screened trays and submersed, in the manner previously described, in channel water containing diquat plus copper at concentrations of 100 ppbw plus 150 ppbw, 200 plus 300, 400 plus 600, 800 plus 1,200, 4,000 plus 6,000, and 8,000 plus 12,000, respectively and an untreated control. Aerators were placed in each tank and the fry were exposed to the chemicals for 3 hr. After exposure, the fry were rinsed gently and placed in incubation trays for 48 hr. The average percent mortality of treated fry was then calculated.

A survey of the various kinds of aquatic macro-invertebrates was made in one of the spawning channels in December, 1972. The rock substrate was stirred and the disturbed organisms were caught in a fine mesh screen as they floated downstream. Two of the most numerous organisms were mayfly nymphs and the larvae of water scavenger beetles. The toxicity of diquat plus copper ion to these aquatic insects was tested on 15 April 1973.

The organisms and a large container of channel water were transported to the Aquatic Weed Laboratory at Davis, California for the trials. Three replicates of 15 to 33 mayfly nymphs each and 10 to 15 young water scavenger beetles, 1 to 3 mm long, were placed in separate polyethylene containers filled with 4 liters of channel water. The insects were exposed to combinations of diquat plus copper ion at concentrations of 100 ppbw plus 150 ppbw, 200 plus 300, 400 plus 600, 800 plus 1,200, 4,000 plus 6,000, 8,000 plus 12,000, and an untreated control. Each container was aerated, and water temperatures were held at 15.7 C. Insects were exposed for 3 hr, rinsed, and placed in fresh-aerated channel water for 24 hr. At the end of 24 hr dead insects were counted and the average percent mortality for each species was calculated.

Duncan's Multiple Range Analysis at the 5% confidence level was used in each of the studies to determine the difference between mortalities of controls and treatments (22).

RESULTS AND DISCUSSION

Egg counts made after shocking showed the number of eggs in each replicate varied from 506 to 694. Hatching was not affected by the herbicide (Table 2). The average mortalities for all treatments at shocking varied from 6 to 9%, and at hatching ranged from 8 to 12% which falls within the normal range for untreated steelhead rainbow trout eggs during incubation. The differences between the percents of mortality of control and treatments were not significant. The resistance of the eggs to the chemicals may be due to the natural characteristics of the eggs. Smith (19) described the released eggs of salmon and trout as swelling until the outer membrane, the chorion, stretched to form a hard, rigid structure with considerable hydrostatic pressure established within it. The egg is relatively impermeable to salts, but permits the passage of ammonia, hydrogen, and hydroxyl ions. Shaw and Brown (18) found that fertilized rainbow trout eggs exposed for 30 min to 100 ppbw of copper ion did not reduce hatching or kill the fry. However, Hazel and Meith (8) concluded that exposure to copper ion concentrations greater than 100 to 300 ppbw for 10 days was acutely toxic to chinook salmon eggs. The latter exposure periods were in excess of the 3 hr used in the present experiment with diquat plus copper ion.

TABLE 2. AVERAGE PERCENT MORTALITY OF FERTILIZED EGGS, ALEVINS, AND FRY OF STEELHEAD TROUT TREATED WITH DIFFERENT CONCENTRATIONS OF DIQUAT AND COPPER ION.

Concentrations (ppbw) of herbicides tested			Average percent mortality[a]	
Diquat		Copper ion	Eggs at shocking	Eggs at hatching
0	+	0	6 a	8 a
25	+	35	9 a	12 a
50	+	70	8 a	11 a
100	+	150	8 a	11 a
200	+	300	8 a	10 a
400	+	600	6 a	11 a
800	+	1,200	7 a	9 a
			Alevins 24 hr after exposure	
0	+	0	0 a	
100	+	150	0 a	
200	+	300	0 a	
400	+	600	0 a	
800	+	1,200	0 a	
4,000	+	6,000	100 b	
8,000	+	12,000	100 b	
			Fry 48 hr after exposure	
0	+	0	4 a	
100	+	150	0 a	
200	+	300	4 a	
400	+	600	20 b	
800	+	1,200	88 c	
4,000	+	6,000	100 c	
8,000	+	12,000	100 c	

[a]Means within a column followed by the same letter are not significantly different at the 5% confidence level, as determined by Duncan's Multiple Range Analysis.

No acute toxicity to alevins was evident at 800 ppbw of diquat plus 1,200 ppbw of copper ion (Table 2), a rate eight times greater than that applied in the channels to control *Cladophora*. Concentrations five times greater than this were toxic, and 100% of the alevins were killed 24 hr after a 3-hr exposure. Copper toxicity is a function of dosage (concentration and exposure time). Hazel and Meith (8) reported alevins mortalities of 12, 93, and 100% with 10-day exposure of 20, 40, and 80 ppbw, respectively.

Concentrations of diquat plus copper ion up to 200 ppbw plus 300 ppbw did not kill fry within 24 hr of a single 3-hr exposure (Table 2). This was twice the amount of diquat plus copper ion used to control *Cladophora* in the spawning channel during each treatment. A significant number of mortalities occurred at concentrations of 400 ppbw plus 600 ppbw and greater. All fry were killed when exposed to 4,000 ppbw plus 6,000 ppbw. The toxic action of copper is described as the reaction of copper on the mucus secreted by the gills. At high concentrations the mucus precipitates, causing respiratory failure. Low concentrations of copper still cause the precipitate to form; however, mucus is replaced rapidly enough to protect the fish (6). Concentrations of diquat used to control aquatic weeds do not produce acute toxicity to salmon and trout (2, 3, 9, 10).

The aquatic insects were more sensitive to the herbicide combination than were the trout, and mayfly nymphs were more susceptible than immature water scavenger beetles (Table 3). Mayfly nymphs were sensitive to handling, and 5% of the untreated individuals died during the trial. Significant mortality occurred with diquat plus copper ion at 400 ppbw plus 600 ppbw and toxicity was severe in the treatment with 800 ppbw plus 1,200 ppbw. Mortality of young beetles in the test with 4,000 ppbw of diquat plus 6,000 ppbw of copper ion was 73% and was significant.

TABLE 3. AVERAGE PERCENT MORTALITY OF MAYFLY NYMPHS AND WATER SCAVENGER BEETLES AFTER 24 HR EXPOSURE TO COMBINATIONS OF DIQUAT PLUS COPPER ION.

Concentrations (ppbw) of herbicides applied			Average percent of mortality[a]	
Diquat		Copper ion	Mayfly nymphs	Water scavenger beetles
0	+	0	5 a	0 a
100	+	150	11 a	0 a
200	+	300	15 a	0 a
400	+	600	34 b	0 a
800	+	1,200	70 c	0 a
4,000	+	6,000	95 c	17 b
8,000	+	12,000	100 c	73 c

[a]Means within a column followed by the same letter are not significantly different at the 5% confidence level as determined by Duncan's Multiple Range Analysis.

Macroinvertebrates responded differently to the combination of diquat plus copper ion than they reportedly do to each chemical alone. May and coworkers (15) showed that a combination of diquat plus copper ion at 1,000 plus 1,000 ppbw rapidly reduced populations of caddisfly and chironomid larvae. Generally, diquat is less toxic to aquatic invertebrates than is copper ion (1, 5, 7, 24, 25). The recommended rate of diquat plus copper ion is 100 ppbw plus 150 ppbw, respectively. Although this concentration is probably safe to mayfly nymphs, caution should be taken not to exceed it.

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