Journal of the Arkansas Academy of Science

Volume 41 Article 6

1987

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DIMILIN FOR CONTROL OF *LERNAEA* IN GOLDEN SHINER PONDS

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ABSTRACT

A single application of Dimilin (UNIROYAL), diflubenzuron, was tested in 9 (nine) ponds containing golden shiner minnows, *Notemigonus crysoleucas,* infested with the parasitic copepod *Lernaea cyprinacea.* The chemical was applied at a rate of 10 or 30 μg/l and compared to untreated controls. Fish were periodically sampled to determine levels of infestation, and zooplankton numbers were monitored for chemical effect. Dimilin treatment significantly reduced ($P < 0.05$) parasite infestation four to eight days after treatment. No significant difference ($P < 0.05$) was noted between the two levels of treatment. Zooplankton populations decreased in the Dimilin treated ponds following chemical application. Rotifer populations rebounded later during the period, but copepod populations in the ponds treated with 30 μg/L remained depressed from two days after treatment for one month until the study ended. Complete parasite control was not obtained with either chemical concentration using a single application. These results suggest that a single treatment is not effective for *Lernaea* control. Future research will test two applications 10 days apart at the 10 and 30 μg/L levels.

INTRODUCTION

The fish parasite, *Lernaea cyprinacea,* is an ectoparasite that lowers fish stamina, kills small fish and causes red sores that adversely effect the marketability of many fish (Gaines and Rogers, 1975). This crustacean lacks host specificity and probably infects all freshwater fish (Hoffman, 1970; Meyer, 1966). One *Lernaea* attached in the heart region can kill a golden shiner (Guidice, *et al.,* 1981). *Lernaea* control is particularly important to Arkansas fish producers because of the large acreage devoted to golden shiner and goldfish production. In 1985, 20,181 acres were devoted to golden shiner production with a value of $14,681,100, while goldfish were valued at $1,823,300 in 1,452 acres. (Anonymous, 1986). *Lernaea* infection is common in these fish. Fish producers are reluctant to admit that *Lernaea* exists on their farms. However, recent brisk sales of insecticides commonly used for *Lernaea* control indicate a high level of concern of baitfish producers that *Larnaea* is a problem in baitfish ponds. Cases documented at the Lonoke and Pine Bluff laboratories of the Arkansas Cooperative Extension Service show that *Lernaea* control is practiced by more than half of the baitfish producers.

Benzene hexachloride was used to control *Lernaea* in the 1950's and 1960's but, evidence of resistance to this chemical was determined by Meyer (1966). Compounds presently used to control *Lernaea* infections are trichlorfon and fenthion (Guidice *et al.,* 1981; Rogers, 1968; Dupree and Huner, 1984; Brown and Gratzek, 1980). Trichlorfon and fenthion become less effective at temperatures near and above 85 °C because of temperature enhanced degradation (Dupree and Huner, 1984). Dimilin, the trade name for diflubenzuran, is a chitin inhibitor used to control mosquitos, and insect pests of rice and cotton. By preventing chitin production, Dimilin may be effective at low concentrations, less than 30 μg/L, in preventing carapace formation in the copepod stages of *Lernaea* development. Water temperature does not significantly reduce Dimilin activity (Moody, 1986). Dimilin may have less toxic effects on fish than fenthion and trichlorfon. Dimilin is not toxic to fathead minnows at concentrations of 100 mg/liter (Julin and Sanders, 1978), while fenthion and trichlorfon are toxic at 2.44 mg/l and 7.90 mg/l, respectively (Johnson and Finley, 1980). The toxicity (48-h EC 50, 22 °C) of Dimilin to *Daphnia magna* is 16 μg/L. Fenthion is toxic (48-h EC 50 15 °C) to *Daphnia pulex* at 0.8 μg/L and trichlorfon kills *Daphnia pulex* at 0.18 μg/L (48-h EC 50, 16 °C) (Johnson and Finley, 1980). Even though fenthion and trichlorfon are more toxic than Dimilin, recommended treatment rates are higher than those proposed for Dimilin — 0.25 mg/l versus 0.010 or 0.030 mg/l (Dupree and Huner, 1984).

The fact that Dimilin's effectiveness is not greatly diminished by heat or light and is less toxic to fish suggests it could be applied at a concentration that would be present in pond water for several days. During that time, *Lernaea* eggs in the pond would hatch and more developing *Lernaea* copepods would be exposed to Dimilin. This study was designed to test whether Dimilin toxicity to *Lernaea* is adequate with a single treatment to completely control the parasite. Also, because Dimilin remains in the water for several days at concentrations toxic to copepods, the toxic effect to nontarget copepods and other zooplankton was measured.

METHODS AND MATERIALS

Golden shiners infected with *Lernaea* were stocked in nine 1/12 acre ponds at a rate of 1,800 per pond on July 18, 1986. Ponds were randomly assigned to three treatments based on application rates of 0, 10 and 30 μg/l of Dimilin.

Dimilin wetable powder, 25% active, was suspended in three gallons of water and sprayed on the surface of each pond with a hand sprayer. Treatment quantities were calculated from depth and surface area measurements at the time of application on September 25.

Infestation rates were assessed on September 23 and 24. Because handling the *Lernaea* infected fish in July resulted in significant mortalities, the level of infection of the population was reduced. To allow the fish to recover from handling, application was postponed until September 25. At that time infection rates had increased and treatments were assigned to ponds after randomizing with random number tables. Each pond was seined on days 4, 8, 15, and 29 after treatment and approximately 500 fish were examined by visual inspection for adult *Lernaea.* Golden shiners with one or more entire, attached, adult *Lernaea* were counted as infected fish.

Zooplankton samples were taken on September 24 and on days 1, 5, 12, 19, and 27 after treatment. Six water column (2" diameter, from surface to near bottom) samples were taken with a column sampler (Boyd, 1979) from representative pond areas and pooled for each pond. Zooplankton was concentrated from a 10 liter volume of each pooled sample with a Wisconsin plankton net (80 micrometer mesh). Samples were preserved in 5% buffered formalin. The concentrated sample volume was recorded and a Sedgwick - Rafter cell count made for each sample (APHA, 1985).

All numerical analyses were performed on an IBM 3278 mainframe computer with SAS (SAS Institute, Inc., 1982). Data from counts of *Lernaea* infected fish were calculated as percentages transformed as arcsines to normalize the distribution of percentage values (Sokal and Rohlf, 1973) and means were compared by analysis of covariance (Steel and Torrie, 1960; Zar, 1974; SAS Institute, Inc., 1982). Mean copepod, rotifer and total zooplankton densities were also compared by analysis of covariance to address variation in population size and treatment effects over time.

RESULTS AND DISCUSSION

Lernaea infection rates (Table 1) varied from 1.0 to 4.5% at the time of treatment. These rates of infection were sufficient to cause observable mortalities, low tolerance to handling, and unmarketable golden shiners (because of visible sores and attached parasites).

Table 1. Lernaea infection rate in golden shiners, as percent of fish infected before and after treatment with Dimilin.

Treatment	Pond	9/24 Pretreatment	9/28	10/2	10/9	10/23
0	41	2.8	5.0	6.5	2.5	5.8
0	46	4.5	6.8	13.6	18.4	10.8
0	63	1.6	1.9	1.4	1.4	0.6
10 µg/L	43	1.9	0.8	0.6	0	0
10 µg/L	45	1.0	0.2	0.2	0	0
10 µg/L	65	2.0	0.4	0.6	0	0.2
30 µg/L	64	1.1	0.8	0.4	0	0
30 µg/L	67	4.2	4.2	2.2	0.6	0.2
30 µg/L	68	2.2	2.6	2.6	1.2	1.3
Mean infection rate per treatment.*						
0		3.0	4.6	7.2	7.4	5.7
10 µg/L		1.6	0.5	0.5	0.0	0.1
30 µg/L		2.5	2.5	1.7	0.6	0.5

*Analysis of covariance from 9/24 to 10/23 indicates a difference between 0 and 10 µg/L at $P < .0855$ and between 0 and 30 µg/L at $P < .0588$. Arcsine transformations of percent infection were used for the ACOVA.

Reductions in infection rates (Table 1) occurred after treatment with both 10 and 30 µg/L Dimilin while increases were observed in control ponds. The decrease observed in the treated ponds was significant, but not significant between treatment concentrations ($P < 0.05$). The treatments did not cause all of the adult *Lernaea* to drop off their hosts. Attachment after treatment in both levels of treatment shows that Dimilin does not immediately eliminate all mature *Lernaea* at these concentrations. Dimilin apparently kills copepods as they regenerate carapaces and prevents reinfection of fish until eggs released by adults hatch and start the four-week life cycle again. Reinfection at 29 days was apparent in ponds at both treatment levels. The study was terminated after 29 days because cooling temperature contributed to a general decline in zooplankton numbers.

Copepod populations were markedly reduced ($P < 0.05$ within 5 days after treatment at both levels of application and remained depressed in the 30 µg/L treatment. Considerable repopulation had occurred by 28 days after treatment with 10 µg/L. Cooling temperatures were presumed responsible for the gradual decline of the control populations (Table 2).

Rotifer populations also showed reduction within 6 days after treatment but, the populations appeared to recover 11 days after treatment. No significant differences between treatment mean rotifer numbers over time were indicated by analysis of covariance ($P < 0.05$). No significant ($P < 0.05$) decline over time in total zooplankton density was detected between treated or control ponds (Table 2). When copepod populations remained low, rotifer populations increased, presumably because of reduced consumption by copepods. By 19 days after treatment, copepod and rotifer populations had begun to rebound in the 10 µg/L treatment. However, the 30 µg/L treatment showed suppressed copepod populations and high rotifer populations as a percentage of total zooplankton until the end of observation.

Table 2. Zooplankton population means after golden shiner ponds were treated with Dimilin* (Organisms/ml).

DATE	TREATMENT	COPEPODS	ROTIFERS	TOTAL ZOOPLANKTON
9/24	0	368^a	548^a	1014^a
	10	563^a	970^a	1699^a
	30	302^a	2217^b	2778^a
9/26	0	378^a	497^a	932^a
	10	105^{ab}	273^a	392^a
	30	23^b	592^a	689^a
9/30	0	290^a	641^a	1073^a
	10	16^b	286^{ab}	313^a
	30	0^b	97^b	195^a
10/7	0	151^a	236^a	424^a
	10	48^{ab}	1006^b	1093^b
	30	0^b	264^a	348^a
10/14	0	175^a	145^a	334^a
	10	198^a	436^{ab}	660^a
	30	20^a	1460^b	1474^a
10/22	0	174^a	328^a	502^a
	10	224^a	480^{ab}	664^a
	30	0^b	1364^b	1363^b

*Means in columns by date followed by the same letter are not significantly different $P < .05$) after arcsine transformations of percent total zooplankton.

CONCLUSIONS

Dimilin kills *Lernaea* and other copepods in ponds. Although complete, lasting control of *Lernaea* was not achieved with one application, other application frequencies for Dimilin may provide long term control.

The low level of 10 µg/L was effective when temperatures were declining. However, a higher treatment concentration may be required in the spring or summer if copepod populations recover more rapidly in warmer weather.

ACKNOWLEDGEMENTS

This study was supported by a grant from UNIROYAL Chemical Company, Inc., Raleigh, North Carolina and the USDA Cooperative States Research Service project ARX.1253.

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