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Influence of 2,2′-dipyridyl on the toxicity of fluridone to hydrilla[1]

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ABSTRACT

In greenhouse experiments, hydrilla (*Hydrilla verticillata* (L.f.) Royle) was exposed to three levels (0, 15.6, or 31.2 mg/L) of 2,2′-dipyridyl (DPD) prior to treatment with four levels (0, 0.05, 0.25, or 0.50 mg/L) of fluridone (1-methyl-3-phenyl-5-[3-(trifluromethyl) phenyl]–4(1H)-pyridinone). Hydrilla with low or high internal iron levels were examined. Results showed that both DPD and fluridone significantly reduced plant growth, however pretreating the plants with DPD did not enhance the toxicity of fluridone. Measurement of internal iron concentrations demonstrated that DPD treatment did not alter the internal concentrations of iron in hydrilla plants.

Key words: Hydrilla verticillata, 2,2′-dipyridyl, fluridone, active iron.

INTRODUCTION

The response of a plant to herbicide treatment is partially a function of the plant's physiological status (i.e., age, nutritional status, and others) at the time of treatment (Ross and Lembi 1985). This relationship holds for terrestrial weeds, but there are few published results with aquatic species (Anderson 1981; Westerdahl and Hall 1987; Spencer *et al.* 1989). Iron is known to be an important nutrient in plant growth (Mengel and Kirkby 1982). The effects of iron on hydrilla depend upon the growth parameter measured (Reid *et al.* 1975; Basiouny *et al.* 1977). Spencer and Ksander (1989) demonstrated that the response of hydrilla (*Hydrilla verticillata* (L.f.) Royle) to treatment with different levels of the herbicide fluridone depended in part on the level of active iron (Fe^{2+}) within plant tissues at the time of herbicide treatment. Hydrilla plants with high levels of active iron recovered from treatments with up to 0.5 mg/L fluridone, whereas, plants with low tissue levels of active iron did not recover as quickly or as fully. One approach to enhancing fluridone's efficacy might be to reduce tissue levels of active iron. The mechanism of action of bidentate chelators has not been completely eludicated, but their effects are consistent with their ability to chelate transition metals, especially Fe^{2+} (Duggan and Gassman 1974; Hodgkins and van Huystee 1989). Ryan (1988) observed that treating hydrilla with the iron chelator, 2,2′-dipyridyl (DPD), for one week resulted in chlorotic plants. Mean weights of hydrilla measured three weeks after the plants were treated with 31.2 mg/L DPD were reduced to 40% of those of untreated plants. One explanation for these results was that DPD resulted in plant damage by interfering with the plant's internal iron reserves. This led us to hypothesize that treating plants with combinations of DPD and fluridone might lead to increased efficacy of fluridone. We report here the results of two experiments designed to test this hypothesis.

METHODS

Apical segments (15 cm long) of hydrilla, dioecious biotype, were planted in individual plastic containers (7 cm by 7 cm by 7 cm) filled with modified UC mix (Spencer and Anderson 1986). Plants were then placed in large tubs (52 cm by 52 cm by 34 cm; filled with 90 L of well water) for one week in a greenhouse. Plants were then transferred to 18 l glass jars filled with well water. The plants were allowed to grow an additional three weeks. After this period, individual jars were randomly assigned to the following treatments: 0, 0.05, 0.25, or 0.50 mg/L fluridone, or 0, 15.6, or 31.2 mg/L DPD, or combinations of DPD and fluridone. One jar containing three plants was the treatment unit (i.e., replicate) in these experiments. There were three replicates per treatment combination for a total of 36 jars containing three plants each. For treatments, the water in the jars was replaced then the chemicals were added. For jars receiving DPD or fluridone treatments, an appropriate volume of DPD (from a stock solution made with reagent grade DPD) or fluridone (from a stock solution made with fluridone 4AS) was added. The treatments lasted one week. Other greenhouse studies with fluridone have used seven to ten day treatment periods (Anderson 1981; Van and Steward 1985; Spencer *et al.* 1989). At this time the water in the jars was replaced by flushing with at least 90 l of well water, and new water added. Water in the jars was replaced weekly. The plants were allowed to grow two additional weeks. [The length of the post treament period was based on previous studies with DPD. Ryan (1988) reported that the effects of DPD were evident for plants harvested two weeks after a one-week treatment with DPD. Since we sought to determine if DPD treatment would enhance (i.e., speed up) fluridone's phytotoxic effect, it seemed reasonable to use an experimental protocol similar to that used by Ryan (1988). Anderson (1981) reported that sago pondweed treated with 1 mg/L fluridone for one to ten days exhibited reduced growth by 14 days after treatment.] The plants were harvested and dried at 80 C for 48 h (McCoombs *et al.* 1985). The mean dry

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weight for the three plants per jar was used as the response variable in the statistical analysis. Subsamples of shoot material collected at the end of the experiment and just prior to DPD—fluridone treatment were ground to a fine powder and analyzed for active iron using the procedure described by Pierson and Clark (1984). This procedure measures Fe^{2+} which is the internal iron fraction believed to be available for use by the plant (Katyal and Sharma 1980). We used the RSREG procedure in SAS (SAS Institute, 1988) to test for treatment effects and examine trends (i.e., linear or quadratic) in the data collected at the end of the experiment (Kirk 1968).

Two experiments were conducted. They were as described above except that in one experiment the plants were treated with 5 mg/L Fe (as Fe-EDTA) during the two week period prior to the application of the DPD-fluridone treatments. [The background levels of Fe for this water source are quite low (0.046 ± 0.027 mg/L; mean ± standard deviation; N = 5)]. This yielded plants with high internal levels of active iron (Experiment 1). In the second experiment we did not treat the plants with extra iron during the two-week period. These plants had low internal levels of active iron (Experiment 2). Because of space limitations experiment 1 was conducted March 28 to May 9, 1988 and experiment 2 from July 5 to August 16, 1988. The light intensity at the level of the plants was 26% of full sunlight. Based on related measurements, for experiment 1 the estimated daily minimum air temperature in the greenhouse varies from 20 to 22 C and the daily maximum from 22 to 31 C. For experiment 2, the daily minimum air temperature varies from 22 to 25 C and the maximum daily air temperature from 26 to 36 C.

RESULTS AND DISCUSSION

In the first experiment, mean plant weight ranged from 386 to 580 mg (Table 1). Plants treated with fluridone, but not DPD, weighed between 15 and 22% less than the controls. Van and Steward (1985) reported 17 to 20% injury for hydrilla treated with 0.25 or 0.5 mg/L fluridone for seven days and evaluated six weeks later. Weights of DPD-treated plants were reduced by 28 to 33% relative to the control plants. This agrees with Ryan (1988) who reported a 40% reduction for DPD treated plants. The statistical interaction between DPD and fluridone was significant ($P < 0.05$) suggesting that the effect of DPD was not the same across all fluridone concentrations. Examination of the means (Table 1) shows that contrary to expectations DPD did not enhance the phytotoxic effects of fluridone. It appears that DPD resulted in decreased plant weight when fluridone concentrations were 0.05 mg/L or less. At fluridone concentrations of 0.25 mg/L or greater the effects of DPD were neutral or a slight enhancement of growth. The levels of available iron were comparable to those reported for hydrilla by Spencer and Ksander (1989) and were unaffected by treatment with DPD ($P > 0.05$; Table 2). This does not support the hypothesis that DPD reduced growth by interfering with iron nutrition.

TABLE 1. TOTAL DRY WEIGHT FOR HYDRILLA GROWN WITH COMBINATIONS OF DPD AND FLURIDONE. VALUES ARE THE MEAN AND STANDARD ERROR OF THE MEAN (SEM) FOR THREE REPLICATES.

Experiment	Fluridone (mg/L)	DPD (mg/L) 0 MEAN	0 SEM	15.6 MEAN	15.6 SEM	31.2 MEAN	31.2 SEM
1	0	578	14	417	29	386	26
	0.05	491	53	416	36	408	40
	0.25	474	91	468	60	457	22
	0.50	450	77	580	68	516	53
2	0	1504	221	1427	223	1224	227
	0.05	1430	81	765	107	1021	96
	0.25	1044	81	977	146	811	27
	0.50	1213	191	644	116	1131	202

TABLE 2. AVAILABLE IRON (% DRY WEIGHT) FOR HYDRILLA GROWN WITH COMBINATIONS OF DPD AND FLURIDONE. VALUES ARE THE MEAN AND STANDARD ERROR OF THE MEAN (SEM) FOR THREE REPLICATES. THE AVAILABLE IRON LEVEL FOR HYDRILLA AT 3 WEEKS (I.E., JUST PRIOR TO THE DPD-FLURIDONE TREATMENTS) WAS 0.0139 ± 0.0024; MEAN ± SEM; N = 7, FOR EXPERIMENT 1 AND 0.0020 ± 0.0002; mean ± SEM; N = 10, FOR EXPERIMENT 2.

Experiment	Fluridone (mg/L)	DPD (mg/L) 0 MEAN	0 SEM	15.6 MEAN	15.6 SEM	31.2 MEAN	31.2 SEM
1	0	0.0115	0.0028	0.0151	0.0033	0.0244	0.0035
	0.05	0.029	0.0018	0.0105	0.0032	0.0132	0.0027
	0.25	0.0116	0.0056	0.0118	0.0038	0.0182	0.0005
	0.50	0.0106	0.0009	0.0104	0.0018	0.0137	0.0034
2	0	0.0010	0.0002	0.0011	0.0003	0.0017	0.0004
	0.05	0.0017	0.0004	0.0028	0.0006	0.0016	0.0006
	0.25	0.0015	0.0004	0.0011	0.0002	0.0027	0.0006
	0.50	0.0013	0.0002	0.0008	0.0001	0.0015	0.0000

In experiment 2, mean hydrilla weight ranged from 644 to 1504 mg and was greater than in experiment 1, probably reflecting the growing conditions. In this experiment fluridone treatment resulted in plant weights that were reduced by 6 to 31% compared to controls. In this experiment DPD-treated plant weights were reduced by 5 to 19% relative to control plants on average. Both DPD and fluridone resulted in reduced mean plant weight ($P < 0.05$; Table 1). For this experiment the statistical interaction between DPD and fluridone was not significant suggesting that DPD neither enhanced or inhibited the effect of fluridone. However, the significant quadratic trend ($P < 0.05$) indicates that mean plant weight responded to DPD and fluridone treatment in a nonlinear fashion. Overall levels of tissue available iron were lower in this experiment than in experiment 1. This was expected since these plants were not exposed to elevated iron levels prior to DPD and fluridone treatments. However, as in Experiment 1, there was no evidence that tissue available iron levels were influenced by treatment with DPD (Table 2).

The data do not support the idea that treatment with DPD resulted in a long lasting deficit of iron in the tissue. DPD may have caused a temporary decrease in tissue levels

of Fe^{2+}, which was alleviated by replenishment of iron through the roots of the plant, or it may have simply allowed the Fe^{2+} to move throughout the system. There was a detectable interaction between treatment with DPD and the toxicity of fluridone in experiment 1, but not in experiment 2. The nature of the interaction in experiment 1 did not suggest that DPD-treated plants were more susceptible to fluridone treatment, however. There was enough DPD to chelate a significant amount of the Fe^{2+} in the plant tissue. For instance, 18 l of DPD solution contained 1.8 mmoles of DPD. In experiment 2, the plant material after treatment with fluridone and 15.6 mg/L DPD had a dry weight of 1250 mg and an iron content of 0.001%, to give a total weight of 12.5 ug Fe^{2+}, or 2.24×10^{-7} moles of iron in the sample. The molar ratio of DPD to iron is thus 8000:1. Treatment with DPD did not make hydrilla plants more susceptible to fluridone. The previously observed phytotoxic effect of DPD on hydrilla (Ryan 1988) does not appear to be related to disruption of internal mechanisms related to iron accumulation.

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