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HERBICIDE COMBINATIONS FOR THE ENHANCEMENT OF DIQUAT PHYTOTOXICITY FOR HYDRILLA CONTROL

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ABSTRACT

Diquat, a fast-acting contact herbicide, has been used for weed control in the US for over 45 years. It was widely recommended for control of hydrilla, often in combination with copper. Restriction of copper use in public waters in Florida in the 1980s and recent discovery of fluridone resistant hydrilla has resulted in renewed interest in using diquat for hydrilla control. Therefore, a greenhouse study was conducted to evaluate the effect of five herbicides (carfentrazone, dipotassium and alkylamine salts of endothall, and flumioxazin) and acibenzolar, a plant elicitor or systemic acquired resistance inducer in vegetables and tobacco, when applied alone and in combination with diquat for hydrilla control as compared to copper. The efficacy of all tested compounds was determined using dry weight and total length reduction fourteen-days after treatment (14 DAT). Diquat was evaluated at the previously determined EC_{50} concentration of 10 µg L^{-1}, while all other compounds were tested at five different concentrations. The combination of copper, flumioxazin and endothal salts with diquat gave additive effect based on dry weight. The interaction of Actigard at 1000.0 and 2000.0 µg L^{-1} with diquat was synergistic based on hydrilla dry weight, as was carfentrazone at rates ranging from 10 to 200 µg L^{-1} based on hydrilla total length. These results indicate that all compounds tested can be used to replace copper where its use is restricted.

Keywords: hydrilla, EC_{50}, diquat, synergistic.

INTRODUCTION

Herbicide combinations for improved weed control in aquatic and terrestrial programs have been utilized for decades. Increased efficacy of combinations over the application of a compound alone is the primary reason to mix different materials (Baldwin and Oliver, 1985); however, other benefits can also be accrued from herbicide mixtures. A wider spectrum of weed species can be controlled when herbicides with different modes of action are combined in a single application (Norris *et al*., 2001). Combination of different herbicides can reduce the propensity of weeds to develop resistance (Marshall, 1998), but can also reduce crop damage (Webster *et al*., 2006; Brown *et al*., 2004; Omokawa *et al*., 1996; Hoffman, 1953), application cost (Norris *et al*., 2001) and reduce the phytotoxicity of herbicides to non-target organisms (Follak and Hurle, 2003), consequently reducing environmental concerns (Brimner *et al*., 2005).

One of the most common examples of increased herbicide efficacy used in aquatic weed control is the use of diquat-copper for hydrilla control when they are applied in combination, compared to the activity of each compound applied alone (Sutton *et al*., 1972; Sutton *et al*., 1971; Sutton and Bingham, 1970; Blackburn and Weldon, 1970; Mackenzie and Hall, 1967). Further studies involving the combination of diquat with endothall in aquatic (Poovey *et al*., 2002; Nelson *et al*., 2001; Pennington *et al*., 2001) and terrestrial (Ivany, 2004) settings also revealed that both compounds were more effective when applied together than singly. Improved performance of other herbicides when applied in combination with diquat for the control of terrestrial weeds has also been reported. Ivany (2005) found that pyraflufen-ethyl efficacy on potato leaf and vine desiccation was improved when diquat was applied after it in a follow up application or in combination as a single application.

Although increased herbicide efficacy has been observed in many studies involving diquat, antagonistic effects have also been reported. Sørensen *et al*. (2007) tested synergistic and antagonistic effects using a concentration addition model in binary mixture toxicity studies and reported that acifluorfen was antagonistic to diquat on duckweed (*Lemna minor* L.). Cedergreen *et al*. (2007) examined the toxicity of six binary herbicide combinations on pigment content and plant growth and subjected the data to concentration addition (CA) and independent action (IA) reference models; both models showed that acifluorfen combined with diquat were antagonistic. In a previous study, Cedergreen *et al*. (2006) examined the synergistic effects of prochloraz, an imidazole fungicide, when applied in combination with diquat, azoxystrobin, acifluorfen, dimethoate, chlorfenvinphos and pirimicarb on four aquatic species [bacteria (*Vibrio fischeri*), daphnia (*Daphnia magna* Straus), algae [*Pseudokirchneriella subcapitata* (Korshikov) Hindak)] and duckweed. They found that although the mixtures between prochloraz-azoxystrobin and diquat-esfenvalerat had shown a synergistic effect on daphnia, and diquat-prochloraz on algae, all combinations were antagonistic on duckweed. These results emphasize the need to test not only individual herbicides, but also mixtures of herbicides to identify possible adverse effects on non-target species and reduction in herbicide performance on target species. Furthermore, despite the knowledge of increased efficacy as the result of certain

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combinations, the type of interaction (synergy, additivity or antagonism) involved in most cases is not known. Therefore, this study was conducted to determine the interaction involved when diquat is mixed with acibenzolar, carfentrazone, copper, flumioxazin, and alkylamine and dipotassium salts of endothall.

MATERIAL AND METHODS

Three series of greenhouse studies were conducted to determine the effects of five herbicides and acibenzolar-s-methyl (1, 2, 3-benzothiadiazole-7-thiocarboxylic acid-s-methyl-ester), a plant elicitor or systemic acquired resistance inducer in vegetables and tobacco (Syngenta, 2006), on the enhancement of diquat phytotoxicity on hydrilla. All experiments were conducted with hydrilla tips collected from Rainbow River, FL. In the first series of experiments, hydrilla tips were exposed for 24 h in 0.5 L plastic cups containing DI water plus acibenzolar-s-methyl (hereafter referred as acibenzolar) and copper at different concentrations. Acibenzolar was applied at concentrations of 125, 250, 500, 1000 and 2000 μg L^{-1}, and copper at concentrations of 31.3, 62.5, 125, 250 and 500 μg L^{-1}. The second series of experiments included carfentrazone-ethyl (hereafter referred as carfentrazone) and flumioxazin. Carfentrazone was applied at 10, 25, 50, 100 and 200 μg L^{-1} and flumioxazin at 12.5, 25, 50, 100 and 200 μg L^{-1}. The last series of phytotoxicity experiments on hydrilla tips compared the dipotassium and alkylamine salts of endothall at 5, 10, 25, 50, 100, 250 and 500 μg L^{-1}. All compounds were applied alone and in combination with diquat at the previously determined diquat EC_{50} concentration of 10 μg L^{-1}. Treatments in each series of studies were assigned in a completely randomized design, repeated and replicated five times. Two weeks following a 24 h exposure to the compounds, hydrilla was harvested and total length (main stem plus any lateral shoots) was measured. Plants were then dried at 90°C for 72 h and dry weight determined.

Statistical analysis

The interaction between each compound and diquat was determined following the method described by Colby (1967). Data were converted to percent dry weight reduction for evaluation in this analysis by the formula: DWR = 100 - [(treated plant dry weight/nontreated plant dry weight) x 100]. Based on this method, the expected value of dry mass reduction for the herbicide-diquat combination is determined using the formula: $E = [(X + Y) - (XY/100)]$, where E is the percent of expected dry mass reduction, X the observed percent of dry mass reduction with compound 1 at rate x and Y the observed percent of dry mass reduction with compound 2 at rate y.

Expected and observed values were separated using Mixed Procedures (SAS Institute, 2002) with experiment, replication (nested within experiment), and all interactions considered as random effects. Experiments and their repeats were also considered random effects, and each treatment was considered a fixed effect to allow inferences about treatments at different levels (Hager *et al*., 2003; Carmer *et al*., 1989). Type III statistics were used to test all possible effects of fixed factors. Least square means were used for mean separation at $p \leq 0.05$. If the observed value of the combination of a compound with diquat was significantly less than the expected value, the interaction was considered antagonistic. In contrast, if the value was significantly higher than the expected value, the combination was considered synergistic. The interaction was considered additive when there was not a significant difference between the two values. Data from all repeated sets of experiments were not different; therefore, they were pooled for analysis.

RESULTS AND DISCUSSIONS

The height of hydrilla plants following the 14 d grow out period was highly variable due to the random sprouting of adventitious buds in the leaf axils, which often occurred if the growing tip was damaged or destroyed by the 24 h herbicide exposure period. The total length of the main stem and all lateral shoots was a more accurate measure of the response to these herbicides.

Diquat applied alone at the pre-determined EC_{50} concentration (10 μg L^{-1}) in the first experimental series resulted in a 44% reduction in hydrilla dry weight compared to nontreated control plants (Table-1). The reduction of dry weight with the addition of acibenzolar ranged from 50 to 69%, and the copper addition ranged from 64 to 96%. Copper applied at concentrations of 125 μL^{-1} and greater produced greater dry weight reductions compared to acibenzolar, which at its highest concentration (2000 μg L^{-1}) was not different from copper at 31.3 μmg L^{-1}. Although copper produced the highest dry mass reduction, its response was additive, whereas acibenzolar had two synergistic responses at 1000 and 2000 μg L^{-1}. Synergistic effects between two or more compounds occur whenever their combined effects are greater than the sum of the effects of each compound applied alone (Kim *et al*., 2002). High copper toxicity to hydrilla when applied alone (21 to 89%) compared to acibenzolar (4 to 17%) and average efficacy increases of 21% and 49% when combined with diquat, respectively, contributed to the toxicity of the combination, but Colby's analysis determined this to be an additive response.

The effects of these treatments on the total length of hydrilla are presented in Table-2. The response to all acibenzolar treatments with diquat was additive. Copper at 62.6 μg L^{-1} antagonized diquat efficacy; however, at other concentrations, it was considered additive.

In the second series of studies, diquat applied alone at the expected EC_{50} concentration reduced dry weight by 46% (Table-3). Carfentrazone applied alone reduced dry weight by 12 to 27%, while flumioxazin reductions similarly ranged from 7 to 28%. Combinations of carfentrazone with diquat further increased hydrilla dry weight reduction from 49 to 66%, while flumioxazin addition reduced dry weight similarly by 49 to 58%. Both carfentrazone and flumioxazin had very similar rate responses above 25 mg L^{-1} and there were few differences from the application of diquat alone. This similarity is likely due to their common mechanism of action (PPO inhibitors) (Iverson and Vandiver, 2005; WSSA, 2002) and rapid hydrolytic degradation in high pH water (Elmarakby *et al*., 2001). The pH of the deionized water used in these studies was 7.1.

Conversely, when interaction responses were analyzed based on total length of hydrilla, carfentrazone growth inhibition varied from 5 to 24% and was synergistic with diquat at all concentrations. Flumioxazin addition to diquat antagonized diquat activity at 12.5 μg L^{-1} and reduced growth by 18 to 26%. At flumioxazin concentrations >12.5 μg L^{-1} all responses were additive (Table-4).

The dipotassium and alkylamine salts of endothall applied for 24 h alone followed by 14 d grow out reduced hydrilla dry weight by 4 to 14% (Table-5) and 8 to 31%, respectively. Addition of diquat at the EC_{50} concentration to these two endothall salts increased dry weight reductions similarly from 45 to 71% and 47 to 78%, respectively. However, the observed response for both compounds at all concentrations was additive. Total length of hydrilla yielded results very similar to dry weight data (Table-6).

Higher dry weight reductions and complete control of hydrilla when endothall salts or copper were combined with diquat have been reported (Pennington *et al*., 2001; Netherland *et al*., 1991; Blackburn and Weldon, 1970), and increased efficacy of these combinations on other aquatic weeds has also been observed in other studies (Nelson *et al*., 2001; Netherland *et al*., 2000). However, the concentrations of copper, endothall and diquat have always been higher than those evaluated in this study. The type of response from these combinations, including others involving diquat, has never been determined, nor modeled based upon exposure to sublethal doses. These results, with the exception of the carfentrazone and diquat results on total hydrilla length indicate that these combinations are all additive.

The EC_{50} of a 24 h exposure to diquat, followed by a 14 d grow out period, was pre-determined in several studies conducted prior to these experiments. The EC_{50} of diquat (10 μg L^{-1}) applied alone had dry weight reductions of 44, 46 and 53%, and reduced total length by 62, 69 and 70% compared to the growth of nontreated plants. The evaluation of herbicide combinations to determine additive or synergistic responses can only be conducted at sublethal herbicide concentrations, otherwise valid comparisons are impossible.

The variability of the growth reduction at 10 μg L^{-1} of diquat by the response in dry weight was 9% (44-53%), and for total plant length was 8% (62-70%), which suggests that this method was very effective at providing meaningful results on the interaction (additive, antagonistic or synergistic) effects of these chemical combinations. These results also suggest that this method would not likely determine if the addition of a herbicide, surfactant or other additive to diquat provides an improvement in efficacy of as little as 5 to 10% as claimed by some field applicators.

CONCLUSIONS

Reports by applicators which suggest improved efficacy in the field appear to be due largely to additive effects, with the exception of the synergistic diquat/carfentrazone effects on total plant length and diquat/acibenzolar effects on dry weight at 1000 and 2000 μg L^{-1}. Field or large scale studies with carfentrazone and acibenzolar are warranted to confirm these greenhouse studies, but variance in field applications will also increase. Potentially, a shorter exposure period, e. g. 12 h, might be used with higher diquat and additive concentrations to determine the effects of concentrations more typical of field use.

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Table-1. Effect of diquat, acibenzolar and copper exposed for 24 h alone or in combination on the dry weight (% reduction) of hydrilla. Hydrilla was harvested 14 DAT.

	Diquat (10 μg L^{-1})					
Compound	**Rate**	**X[1]**	**Y[2,3]**	**Expected**	**P value**	**Response**
	μg L^{-1}	**---- % dry weight reduction ----**			**P > | t | [4]**	
None	0	0	44 d	0		
Acibenzolar	125	4	50 cd	46	0.5753	Additive
Acibenzolar	250	10	56 bcd	50	0.4194	Additive
Acibenzolar	500	17	64 bc	54	0.1630	Additive
Acibenzolar	1000	14	69 b	46	0.0028	Synergistic
Acibenzolar	2000	16	65 bc	48	0.0188	Synergistic
Copper	31.3	21	65 bc	55	0.2086	Additive
Copper	62.5	54	69 b	75	0.4743	Additive
Copper	125	66	89 a	83	0.4128	Additive
Copper	250	78	96 a	88	0.3185	Additive
Copper	500	89	95 a	94	0.7974	Additive

[1] Observed percent dry weight reduction when compound applied alone.
[2] Observed percent dry weight reduction when compound combined with diquat.
[3] Means within a column followed by the same letter are not significantly different according to the t-test on difference of least square means at $P \leq 0.05$.
[4] P values used to compare the differences between the observed and the expected values at $P \leq 0.05$.

Table-2. Effect of diquat, acibenzolar and copper exposure for 24 h alone or in combination on the total length (% reduction) of hydrilla. Hydrilla was harvested 14 DAT.

	Diquat (10 μg L^{-1})					
Compound	**Rate**	**X[1]**	**Y[2,3]**	**Expected**	**P value**	**Response**
	μg L^{-1}	**---- % total length reduction ----**			**P > | t | [4]**	
None	10	0	69 cd	0	0	
Acibenzolar	125	11	71 bcd	72	0.8628	Additive
Acibenzolar	250	18	64 d	73	0.3012	Additive
Acibenzolar	500	10	86 abc	71	0.1070	Additive
Acibenzolar	1000	20	74 abcd	75	0.9291	Additive
Acibenzolar	2000	22	76 abcd	75	0.9255	Additive
Copper	31.3	16	88 ab	74	0.1183	Additive
Copper	65.5	50	67 d	85	0.0101	Antagonistic
Copper	125	44	80 abcd	83	0.7386	Additive
Copper	250	56	92 a	86	0.5118	Additive
Copper	500	76	91 a	92	0.9517	Additive

[1] Observed percent dry weight reduction when compound applied alone.
[2] Observed percent dry weight reduction when compound combined with diquat.
[3] Means within a column followed by the same letter are not significantly different according to the t-test on difference of least square means at $P \leq 0.05$.
[4] P values used to compare the differences between the observed and the expected values at $P \leq 0.05$.

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Table-3. Effect of diquat, carfentrazone and flumioxazin exposure for 24 h alone or in combination on the dry weight (% reduction) of hydrilla. Hydrilla was harvested 14 DAT.

	Diquat (10 μg L[-1])					
Compound	**Rate**	**X[1]**	**Y[2,3]**	**Expected**	**P value**	**Response**
	μg L[-1]	**---- % dry weight reduction ----**			**P > | t | [4]**	
None	0	0	46 c	0		
Carfentrazone	10	12	49 abc	52	0.7028	Additive
Carfentrazone	25	16	66 a	55	0.1455	Additive
Carfentrazone	50	17	61 abc	55	0.4618	Additive
Carfentrazone	100	18	58 abc	56	0.7443	Additive
Carfentrazone	200	27	66 a	60	0.4415	Additive
Flumioxazin	12.5	7	49 abc	50	0.8539	Additive
Flumioxazin	25	14	54 abc	54	0.9828	Additive
Flumioxazin	50	17	50 abc	56	0.3890	Additive
Flumioxazin	100	16	54 abc	54	0.9926	Additive
Flumioxazin	200	28	58 abc	59	0.9290	Additive

[1] Observed percent dry weight reduction when compound applied alone.
[2] Observed percent dry weight reduction when compound combined with diquat.
[3] Means within a column followed by the same letter are not significantly different according to the t-test on difference of least square means at $P \leq 0.05$.
[4] P values used to compare the differences between the observed and the expected values at $P \leq 0.05$.

Table-4. Effect of diquat, carfentrazone and flumioxazin applied for 24 h alone or in combination on the total length (% reduction) of hydrilla. Hydrilla was harvested 14 DAT.

	Diquat (10 μg L[-1])					
Compound	**Rate**	**X[1]**	**Y[2,3]**	**Expected**	**P value**	**Response**
	μg L[-1]	**---- % total length reduction ----**			**P > | t | [4]**	
None	10	0	70 abc	0	0	
Carfentrazone	10	5	62 bc	34	0.0003	Synergistic
Carfentrazone	25	16	82 a	51	0.0008	Synergistic
Carfentrazone	50	14	77 abc	39	<0.0001	Synergistic
Carfentrazone	100	11	71 abc	39	0.0006	Synergistic
Carfentrazone	200	24	79 ab	48	0.0012	Synergistic
Flumioxazin	12.5	26	60 c	79	0.0456	Antagonistic
Flumioxazin	25	22	74 abc	78	0.7193	Additive
Flumioxazin	50	21	62 bc	77	0.3588	Additive
Flumioxazin	100	18	77 abc	76	0.4826	Additive
Flumioxazin	200	24	76 abc	77	0.4103	Additive

[1] Observed percent dry weight reduction when compound applied alone.
[2] Observed percent dry weight reduction when compound combined with diquat.
[3] Means within a column followed by the same letter are not significantly different according to the t-test on difference of least square means at $P \leq 0.05$.
[4] P values used to compare the differences between the observed and the expected values at $P \leq 0.05$.

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Table-5. Effect of diquat, dipotassium and alkylamine salts of endothall exposure for 24 h alone or in combination on the dry weight (% reduction) of hydrilla. Hydrilla was harvested 14 DAT.

	Diquat (10 µg L^{-1})					
Compound	**Rate**	**X**[1]	**Y**[2,3]	**Expected**	**P value**	**Response**
	µg L^{-1}	**---- % dry weight reduction ----**			**P > |t|** [4]	
None	0	0	53 bcde	0		
Dipotassium	5	4	49 cde	55	0.5727	Additive
Dipotassium	10	4	47 e	55	0.3615	Additive
Dipotassium	25	8	52 bcde	58	0.5517	Additive
Dipotassium	50	5	45 e	56	0.2361	Additive
Dipotassium	100	13	49 cde	60	0.2576	Additive
Dipotassium	250	11	60 abcde	57	0.6728	Additive
Dipotassium	500	14	71 ab	56	0.0934	Additive
Alkylamine	5	8	48 de	56	0.3622	Additive
Alkylamine	10	11	47 e	60	0.1330	Additive
Alkylamine	25	11	53 bcde	60	0.4690	Additive
Alkylamine	50	14	70 abc	60	0.2573	Additive
Alkylamine	100	9	69 abcd	58	0.2355	Additive
Alkylamine	250	14	59 abcde	57	0.8133	Additive
Alkylamine	500	31	78 a	67	0.2222	Additive

[1] Observed percent dry weight reduction when compound applied alone.
[2] Observed percent dry weight reduction when compound combined with diquat.
[3] Means within a column followed by the same letter are not significantly different according to the t-test on difference of least square means at $P \leq 0.05$.
[4] P values used to compare the differences between the observed and the expected values at $P \leq 0.05$.

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Table-6. Effect of diquat, dipotassium and alkylamine salts of endothall exposure for 24 h alone or in combination on the total length (% reduction) of hydrilla. Hydrilla was harvested 14 DAT.

	Diquat (10 μg L^{-1})					
Compound	**Rate**	**X[1]**	**Y[2,3]**	**Expected**	**P value**	**Response**
	μg L^{-1}	**---- % total length reduction ----**			**P >∣t∣[4]**	
None	0	0	62 cd	0		
Dipotassium	5	14	67 bcd	68	0.9228	Additive
Dipotassium	10	14	59 d	70	0.2513	Additive
Dipotassium	25	17	82 ab	70	0.2472	Additive
Dipotassium	50	14	66 bcd	67	0.9435	Additive
Dipotassium	100	23	75 abc	74	0.9229	Additive
Dipotassium	250	16	73 abc	69	0.7001	Additive
Dipotassium	500	16	85 a	67	0.0600	Additive
Alkylamine	5	11	69 bcd	66	0.7641	Additive
Alkylamine	10	23	63 cd	72	0.3554	Additive
Alkylamine	25	16	65 cd	71	0.5162	Additive
Alkylamine	50	13	86 a	67	0.0565	Additive
Alkylamine	100	13	86 a	68	0.0638	Additive
Alkylamine	250	27	76 abc	70	0.5183	Additive
Alkylamine	500	46	85 a	81	0.7036	Additive

[1] Observed percent dry weight reduction when compound applied alone.
[2] Observed percent dry weight reduction when compound combined with diquat.
[3] Means within a column followed by the same letter are not significantly different according to the t-test on difference of least square means at $P \leq 0.05$.
[4] P values used to compare the differences between the observed and the expected values at $P \leq 0.05$.

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