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Natural Products as Potential Herbicide Adjuvants:
Citric Acid Esters and Quercetin

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HEATHER ENID JOHNSON

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Natural Products as Potential Herbicide Adjuvants:

Citric Acid Esters and Quercetin

BY

HEATHER ENID JOHNSON

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Submitted to
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Natural Products as Potential Herbicide Adjuvants: Citric Acid Esters and Quercetin

HEATHER ENID JOHNSON

Abstract

With the addition of activator adjuvants, herbicides become more effective, whether the adjuvant increases absorption, prevents photodegradation or facilitates wetting. Citric acid esters have been shown to enhance herbicidal activity. Though the mode of action is not yet known, the structure of the citric acid ester is known to be related to their function. The structure-function relationship of citric acid esters, which vary in alkyl chain length, ethylene oxide number and number of chains, was examined. Ethylene oxide number, alkyl chain length and number of chains influenced adjuvant efficacy as well. Nineteen experimental adjuvants were evaluated in the greenhouse with five commercial herbicides on various weed species. Adjuvant efficacy was weed and herbicide specific for both the citric acid esters and the 19 experimental adjuvants.

Two naturally occurring, known UV absorbing compounds, carotenoids and quercetin were studied to determine if they could prevent photodegradation of cyclohexanediones herbicides. The herbicides BAS 620 and clethodim were determined to be photolabile. In addition, the UV absorbing compound quercetin was added to the spray solution, applied to the plants, and irradiated in the UV light chamber for 0, 1, and 3 hrs. When quercetin was added to the spray solution and subjected to UV irradiation, the rate of phototransformation of clethodim was decreased.

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CHAPTER 3

INTRODUCTION

Adjuvant research has been through many changes in the past 100 years. The focus went from concentrating on the spreadability of an adjuvant to studying action and searching for more effective adjuvants. Adjuvants are now screened in the greenhouse with many herbicides and on difficult to control weeds. Once potential adjuvants are found they are compared to those already on the market.

Another focus of adjuvant research has been to find natural materials that act as adjuvants. Natural products are more easily accepted by the public as well as by a plant. In this paper we looked at natural products in two ways; as activator adjuvants and secondly as an adjuvant to prevent photodegradation of an herbicide.

Citric acid is a natural product obtained from cornstarch. Citric acid esters are readily synthesized and have been found to have herbicidal activity.

Quercetin is also a natural product that is a yellow dye obtained from several different plants. Not only is quercetin naturally occurring in plants, its function in plants is to absorb UV light thereby preventing photodegradation. Therefore, since quercetin is naturally occurring and acts as a UV absorbing compound, adding it to a photolabile herbicide might prove to be beneficial.

The overall objectives of this study were; evaluate citric acid esters for adjuvant efficacy and relate structure to function, to screen 19 experimental adjuvants with five herbicides on several weed species for adjuvant efficacy and to confirm photolability of BAS 620 and clethodim, and determine the effect of quercetin as a UV protectant.

CHAPTER 1

Effect of Ethylene Oxide Number, Alkyl Chain Length and Number on the Efficacy of Citric Acid Esters with Four Herbicides[1]

HEATHER ENID JOHNSON

Abstract. Activator adjuvants may facilitate wetting, spreading, dispersal, decrease phototransformation and/or increase absorption. The objective of this research was to evaluate structure-function relationships of citric acid esters which vary in alkyl chain number (mono-, di- and tri-), ethylene oxide number (EO 4,7,9,25,35,52), and alkyl chain length (C_8, $C_{12/14}$, $C_{16/18}$). Adjuvant efficacy was evaluated on two weed species for each of four herbicides. The experimental adjuvants were applied with glyphosate and glufosinate on giant foxtail and common lambsquarters, imazamox on velvetleaf and common lambsquarters and nicosulfuron on giant foxtail and large crabgrass. Adjuvant efficacy was weed and herbicide specific. Ethylene oxide number, chain length and number influenced adjuvant efficacy with the effectiveness of various substitution combinations dependent on both herbicide and weed species.

NOMENCLATURE: Glufosinate, 2-amino-4-(hydroxymethylphosphinyl)butanoic acid; Glyphosate, *N*-(phosphonomethyl)glycine; Imazamox, 2-[4,5-dihydro-4-methyl-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid, ammonium salt; Nicosulfuron, 2-[[[[(4,6-dimethoxy-2-

[1] Received for publication on _______ and in revised form on ________.

pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide; Common Lambsquarters, *Chenopodium album* L. #CHEAL; Giant Foxtail, *Setaria faberi* Herrm. #SETFA; Large Crabgrass, *Digitaria sanguinalis* (L.) Scop. #DIGSA; Velvetleaf, *Abutilon theophrasti* Medicus. #ABUTH.

ABBREVIATIONS: ANOVA; Analysis of Variance, DAT; Days After Treatment, EO; Ethylene Oxide, LSD; Least Significant Difference

INTRODUCTION

Activator adjuvants increase the activity of a given herbicide. The most common activator adjuvants are surfactants, which facilitate wetting, spreading, dispersal, decrease phototransformation, and/or increase absorption (Penner 1999).

Specific surfactants can alter the solubility of a leaf surface, or perhaps the solubility of the herbicide, increasing absorption. This allows polar herbicides to penetrate the non-polar cuticle, as well as enhance penetration through the slightly polar pectin portion of the leaf (McWhorter 1982). The herbicide is available for cellular uptake, translocation, or action only after penetration through the cuticle, pectin, and cell wall (Koskinen 1982).

Citric acids (Figure 1) have been shown to enhance herbicidal activity by chelating various salts in hard water (Thelen 1995). They are natural products extracted from cornstarch and are considered to be easily absorbed by a plant. Since citric acid is a tri-carboxylic acid, esterification can occur with any or all of the carboxylic groups (Figure 1). Ethoxylated alkyl chains can be readily conjugated with citric acid to form these esters. The citric acid esters are potential spray adjuvants thereby adding value to existing natural products.

Structure-function relationships for adjuvants have been studied (Green 1999; Gaskin 1992). However the information on ethylene oxide (EO) number appears directly relevant to citric acid ester efficacy. The efficacy of citric acid esters with various herbicides on specific weed species is unknown. Therefore the objective of this study was to determine the relationship between structure of the adjuvants and their function. The specific objectives were to determine the effect of variation in alkyl chain length, ethylene oxide number and number of alkyl substitutions on efficacy of the citric acid esters as adjuvants to enhance the activity of four herbicides on several weed species. The adjuvant structure-function relationships to herbicide and weed specificity was also explored.

MATERIALS AND METHODS

General. The following studies were conducted in and between the greenhouses at Michigan State University from May 1999 to March 2000. Plants applied with accent and imazamox were placed outside between greenhouses from May to September of 1999, due to the occurrence of hot temperatures in the greenhouse. Plants were in an enclosed area outside, leaving the plants undisturbed, yet subject to environmental conditions such as rain or cloudy days. Plants applied with liberty and accord were kept in the greenhouse from September 1999 to March 2000 and were exposed to less variable conditions.

Weed Species. Four weed species, giant foxtail, common lambsquarters, velvetleaf, and large crabgrass were used in this study. Seeds were placed in 945 ml plastic black pots, using Baccto, a professional potting soil. Approximately 1 wk before herbicide application, excess plants were removed from the pots, leaving 3-5 plants per pot, each with a similar height and leaf stage to ensure a uniform stand for herbicide application. When the plants reached the 3-5 leaf stage, they were thinned down to 1 plant per pot for common lambsquarters, 3 plants per pot for giant foxtail, 1 plant per pot for velvetleaf, and 2 plants per pot for large crabgrass for herbicide application.

Herbicide Application. A link belt sprayer was used with a flat fan nozzle[5] that delivered 94 Lha^{-1} spray volume at 138 kPa pressure and a boom height of 33 cm. Distilled water was used for the herbicide carrier to avoid any interaction that might

[5] TeeJet Flat Fan Nozzle 80005EV model Spraying Systems Co., Wheaton, IL 60188

occur between the herbicide and hard water. Formulated glyphosate[6] was evaluated on giant foxtail (0.406 kg/ha) and common lambsquarters (0.11 kg/ha). Formulated glufosinate[7] was evaluated on giant foxtail (0.101 kg/ha) and common lambsquarters (0.067 kg/ha). Formulated imazamox[8] was evaluated on common lambsquarters (0.015Kg/ha) and velvetleaf (0.015 kg/ha). Formulated nicosulfuron[9] was evaluated on giant foxtail (0.0056 kg/ha) and large crabgrass (0.0336 kg/ha). Adjuvants were applied at 0.25% (v/v). There were a total of eight trials and each was repeated.

Statistics. Data presented are the means of two experiments with four replications each. Plants and treatment sequences were selected at random. Treatments were applied in a random sequence in a double blind study, which means that the chemistry of the adjuvants was not known and the treatment sequence was randomly selected. Data were subjected to ANOVA (SAS Institute, 1996) and means were separated using Fischer LSD with a P value of 0.05.

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[6] Accord® Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167
[7] Liberty® AgrEvo USA Co., 2711 Centreville Rd. Wilmington, DE 19808
[8] Raptor® American Cyanamid, One Campus Dr., Parsippany, NJ 07054
[9] Accent® DF, DuPont Co., Walker's Mill, Barley Plaza Wilmington, DE 19880-0038

RESULTS AND DISCUSSION

Structure-Function Relationship of Citric Acid Esters to Herbicide Efficacy

Ethylene Oxide (EO) Number. *Glyphosate.* The application of glyphosate to giant foxtail produced the same results as reported by Green (1999), as EO number increased, efficacy of glyphosate increased. The data showed that as EO number increased there was an overall increase in efficacy of the adjuvants with glyphosate (Table 1). With mono- and di- substitutions there was an increase in efficacy from EO4 to EO9 on common lambsquarters. However results with the tri- substituted molecule were variable (EO9>EO4>EO12).

Glufosinate. As EO number increased, efficacy of the adjuvants with glufosinate tended to increase for giant foxtail (Table 2). The greatest increase in efficacy was with EO25, EO35 and EO52. The EO number of seven was the most effective adjuvant with glufosinate on common lambsquarters(EO7>EO9>EO4). EO25, EO35 and EO52 showed potential as well to be effective EO chain lengths on common lambsquarters.

Imazamox. EO chain length of 4 was the most efficacious with imazamox on velvetleaf, with efficacy increasing from EO9 to EO4 (Table 3). The long EO chains were not as effective with imazamox, as was expected. The effectiveness of the adjuvants with imazamox on common lambsquarters, with regards to EO number was variable, although the long EO chains (25, 35, and 52) were very effective.

Nicosulfuron. There was a general increase in efficacy of the adjuvants from EO4 to EO9 with nicosulfuron on giant foxtail (Table 4). However adjuvants with an EO number of 7 were the least effective whereas adjuvants with long EO chains were very effective adjuvants with imazamox on giant foxtail. Efficacy trends for large crabgrass with nicosulfuron were dependent upon substitution of the adjuvant. For mono and di substitutions there was an increase in efficacy of imazamox as EO number increased from EO4 to EO9. However for the tri-substituted esters, the opposite occurred; efficacy of imazamox decreased from EO4 to EO9. Adjuvants with long EO chains enhanced overall efficacy of imazamox.

There have been several reports on the effect of EO number on herbicidal activity (Gaskin 1992; Kirkwood 1993; Riechers 1995; Green 1999). Much of this research was done with glyphosate. Riechers (1995) found that glyphosate efficacy increased with increasing EO number. We confirmed these results with glyphosate and found the same to be true with glufosinate and nicosulfuron but not with imazamox. Gaskin (1992) found that an EO content of 15-20 was the most effective number. Green (1999) looked at the effect of EO content on sulfonylureas and found that with an alkyl chain length of 16/18, the most effective EO number was 12-30. We also found that the most effective EO number with the chain length of 16/18 was between 9 and 12.

Alkyl Chain Length. Table 5 shows the alkyl chain length that had the greatest effect on increasing efficacy of the four herbicides, regardless of EO number. For all di-substituted molecules, the most effective alkyl chain length was C16.

Glyphosate. The mono-substituted ester, with an alkyl chain length of 12, the di-substituted ester with an alkyl chain length of 16, and the tri-substituted ester with an alkyl chain length of 16 were the alkyl chain lengths that, when added to glyphosate and applied to both common lambsquarters and giant foxtail were most effective.

Glufosinate. The mono-substituted ester with an alkyl chain length of 12 or 16, and the di-substituted ester with an alkyl chain length of 16 were the alkyl chain lengths that, when added to glufosinate and applied to both common lambsquarters and giant foxtail were most effective. There was no efficacy trends observed for the tri-substituted ester with glufosinate.

Imazamox. There were no efficacy trend observed for the mono-substituted ester for either weed species. The di-substituted ester with an alkyl chain length of 16 for both common lambsquarters and velvetleaf and the tri-substituted ester the alkyl chain length of 12 on common lambsquarters and both the alkyl chain length of 12 and 16 on velvetleaf were effective when added to imazamox.

Nicosulfuron. There were no efficacy trends observed on giant foxtail since alkyl chain length was dependent upon EO number and number of chains. However, the mono-substituted molecule with an alkyl chain length of 12, the di-substituted ester with an alkyl chain length of 16, and the tri-substituted molecule with an alkyl chain length of 8 or 12, when added to nicosulfuron were the most effective for large crabgrass.

Though little work has been directed towards alkyl chain, Green (1999) found that $C_{16/18}$ was the most effective alkyl chain length, which we confirmed. Tann (1995) also reported that C18 was the most effective alkyl chain length.

Number of Chains. Figures 2 through 9 illustrate the effectiveness of the number of chains for a given herbicide on a specific weed species. Results are summarized in Table 6. Effectiveness was dependent upon both weed species and herbicide. The tri-substituted ester was found to be the least effective adjuvant.

Glyphosate. Both giant foxtail and common lambsquarters responded similarly to glyphosate. The di-substituted ester increased efficacy of glyphosate, followed by the mono-ester and the tri-ester.

Glufosinate. The mono-ester was more effective than the di-ester for increasing the efficacy of glufosinate on giant foxtail and the di-ester was more effective than the mono ester for increasing the efficacy of glufosinate on common lambsquarters. The tri- ester was the least effective for increasing efficacy of glufosinate on both weed species.

Imazamox. The tri-ester was most effective for increasing efficacy of imazamox on velvetleaf, followed by the di-ester and the mono-ester. The di-ester was most effective for increasing the efficacy of imazamox on common lambsquarters, followed by the tri-ester and the mono-ester.

Nicosulfuron. Both large crabgrass and giant foxtail responded similarly to nicosulfuron. The mono-ester was the most effective with respect to the number of chains on the efficacy of nicosulfuron, followed by the di-ester. The least effective was the tri-ester.

We found that when high EO number was most effective, if EO number was decreased and alkyl chain length was increased, effectiveness remained the same. Green (1999) described similar observations. He found that when the effective EO number was 12 with an alkyl chain length of 16, by increasing the EO number and equally decreasing the alkyl chain length, efficacy remained the same. Therefore there may be an optimum molecular weight. There were adjuvant molecules evaluated that were larger than 4,500g mol^{-1} and were very effective on herbicide activity, however little is known on the mode of action of these large molecules. Can the plant even absorb molecules this large or do they simply act on the surface of the plant?

Efficacy of Citric Acid Esters as Herbicide Adjuvants.

No adjuvants increased the effectiveness of all four herbicides on all weed species. However there were two adjuvants that increased the effectiveness of three out of the four herbicides. Both the mono- and di- substituted molecules with an alkyl chain length of 8 and an EO chain length of 12 increased the effectiveness of glyphosate, glufosinate, and nicosulfuron compared to the herbicide alone treatment, on all weed species.

Glyphosate. The only adjuvant that increased the efficacy of glyphosate, compared to the herbicide alone treatment, on both common lambsquarters and giant foxtail was the di- substituted ester with an alkyl chain length of 16 and an EO chain length of 9 (Table 1). There were six adjuvants that increased the effectiveness of glyphosate, compared to the herbicide alone treatment, on common lambsquarters and not on giant foxtail, mono-, C12, EO4, mono-, C16, EO7; mono-, C16, EO9; di-, C8, EO4; tri-, C12, EO9; and tri-, C16, EO4. There was only one adjuvant that increased effectiveness of glyphosate, compared to the herbicide alone treatment, for giant foxtail and not for common lambsquarters, the di-, C16 EO52 (Table 1).

Glufosinate. There were two adjuvants that increased the efficacy of glufosinate, compared to the herbicide alone treatment, on common lambsquarters and giant foxtail, the mono- and di-substituted molecule with an alkyl chain length of 8 and EO chain length of 12 (Table 2). There was one adjuvant that increased effectiveness of glufosinate, compared to the herbicide alone treatment, for common lambsquarters and not giant foxtail, mono-, C16, EO35. There were three adjuvants that increased the effectiveness of glufosinate, compared to the herbicide alone treatment, for giant foxtail and not common lambsquarters, mono-, C12, EO9; mono-, C16, EO9 and di-, C12, EO4 (Table 2).

Imazamox. No adjuvants were evaluated that increased the effectiveness of imazamox, compared to the herbicide alone treatment, on both common lambsquarters and velvetleaf (Table 3). There was one adjuvant that increased effectiveness of imazamox, compared to the herbicide alone treatment, for common lambsquarters and not on velvetleaf, the di-, C8 EO7. There was one adjuvant that increased the effectiveness of imazamox, compared to the herbicide alone treatment, for velvetleaf and not for common lambsquarters, the tri-, C16, EO7 (Table 3).

Nicosulfuron. All of the adjuvants increased the effectiveness of nicosulfuron, compared to the herbicide alone treatment, (Table 4). There was one adjuvant that increased the effectiveness of nicosulfuron, compared to the herbicide alone treatment, for giant foxtail and not on large crabgrass, the di-, C13, EO25. There were two adjuvants that increased the effectiveness of nicosulfuron, compared to the herbicide alone treatment, for large crabgrass and not for giant foxtail: mono-, C8 EO4 and mono-, C16 EO4 (Table 4).

Table 7 is a list of the adjuvants that were most effective with a given herbicide on a specific weed species. From a commercial perspective it would be desirable to have one very effective adjuvant that would be effective with all herbicides for all weed species. The data presented indicates that this goal will remain elusive.

The mechanism that the spectrum of citric acid esters exert their efficacy may vary with the size of the adjuvant molecule. Since some of the citric acid esters had large numbers of EO units, thus having a large molecular weight it is appealing to conclude that they exert their action on the surface of the leaf.

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Table 1. Efficacy of various esters of citric acid applied at 0.25% with glyphosate on common lambsquarters (0.11 kg/ha) and giant foxtail (0.406 kg/ha).

Trt	R-Group number	Chain length	EO number	Common lambsquarters 7 DAT	Common lambsquarters 14 DAT	Giant foxtail 7 DAT	Giant foxtail 14 DAT
				(% Control)		(% Control)	
1	Mono	8	4	73	81	55	53
2	Mono	8	7	35	38	50	50
3	Mono	8	9	76*	93*	60	80*
4	Mono	8	12	85*	96*	60	81*
5	Mono	12	4	81*	91*	50	53
6	Mono	12	7	64	80	56	73*
7	Mono	12	9	81*	94*	63	74*
8	Mono	16	4	75*	84	53	50
9	Mono	16	7	75*	89*	45	49
10	Mono	16	9	85*	94*	51	58
11	Di	8	4	86*	95*	50	58
12	Di	8	7	68	78	51	63
13	Di	8	9	66	83	61	73*
14	Di	8	12	80*	89*	65	79*
15	Di	12	4	76*	84	41	44
16	Di	12	7	70	81	64	70
17	Di	12	9	79*	86	64	83*
18	Di	16	4	66	78	54	59
19	Di	16	7	90*	99*	69	86*
20	Di	16	9	89*	93*	71*	85*
21	Tri	8	4	69	78	44	39
22	Tri	8	12	68	73	29	38
23	Tri	12	4	59	74	40	31
24	Tri	12	7	56	70	45	41
25	Tri	12	9	91*	94*	58	63
26	Tri	16	4	85*	95*	44	55
27	Tri	16	7	73	85	55	64
28	Tri	16	9	76*	83	56	70
29	Di	13	25	64	83	66	83*
30	Di	16	52	71	86	68*	91*
31	Mono	16	52	69	85	65	89*
32	Mono	16	35	65	79	61	81*
33[a]				64	78	60	60
34[b]				0	0	0	0
$LSD_{0.05}$				11.3	9.8	8.9	11.2

[a]Herbicide alone
[b]Control
*Values that were significantly greater than herbicide alone

Table 2. Efficacy of various esters of citric acid applied at 0.25% with glufosinate on common lambsquarters (0.067 kg/ha) and giant foxtail (0.101 kg/ha).

Trt	R-Group number	Chain length	EO number	C. lambsquarters 7 DAT	C. lambsquarters 14 DAT	Giant foxtail 7 DAT	Giant foxtail 14 DAT
				(% Control)		(% Control)	
1	Mono	8	4	40*	46	32*	14
2	Mono	8	7	35	39	36*	21
3	Mono	8	9	45*	49	44*	36*
4	Mono	8	12	56*	54*	46*	29*
5	Mono	12	4	24	35	28	13
6	Mono	12	7	45*	41	38*	26*
7	Mono	12	9	20	40	48*	41*
8	Mono	16	4	34	35	39*	21
9	Mono	16	7	48*	47	37*	29*
10	Mono	16	9	29	33	42*	29*
11	Di	8	4	25	34	32*	14
12	Di	8	7	20	40	19	21
13	Di	8	9	25	44	19	21
14	Di	8	12	59*	60*	46*	33*
15	Di	12	4	23	42	33*	26*
16	Di	12	7	29	40	25	23*
17	Di	12	9	43*	51	33*	20
18	Di	16	4	31	44	34*	13
19	Di	16	7	43*	46	29*	19
20	Di	16	9	40*	40	41*	22
21	Tri	8	4	13	28	19	14
22	Tri	8	12	39*	49	32*	22
23	Tri	12	4	14	33	23	11
24	Tri	12	7	35	46	14	19
25	Tri	12	9	24	33	17	11
26	Tri	16	4	21	36	19	17
27	Tri	16	7	26	38	18	14
28	Tri	16	9	24	36	26	15
29	Di	13	25	55*	58*	28	28*
30	Di	16	52	46*	51	26	25*
31	Mono	16	52	70*	63*	22	24*
32	Mono	16	35	49*	58*	21	18
33[a]				26	41	20	14
34[b]				0	39	18	12
$LSD_{0.05}$				11.8	11.1	9.1	8.5

[a]Herbicide alone

[b]Control

*Values that were significantly greater than herbicide alone

Table 3. Efficacy of various esters of citric acid applied at 0.25% with imazamox on common lambsquarters (0.015 kg/ha) and velvetleaf (0.015 kg/ha).

Trt	R-Group number	Chain length	EO number	C. lambsquarters 7 DAT	C. lambsquarters 14 DAT	Velvetleaf 7 DAT	Velvetleaf 14 DAT
				(% Control)		(% Control)	
1	Mono	8	4	46	64	8	31
2	Mono	8	7	48	68	15	23
3	Mono	8	9	51	70	11	26
4	Mono	8	12	38	63	13	20
5	Mono	12	4	53	68	30*	21
6	Mono	12	7	48	65	6	21
7	Mono	12	9	53	76*	10	33
8	Mono	16	4	50	71	10	28
9	Mono	16	7	58*	81*	11	20
10	Mono	16	9	50	71	33*	33
11	Di	8	4	53	79*	10	38
12	Di	8	7	56*	84*	5	28
13	Di	8	9	54	79*	9	36
14	Di	8	12	48	78*	4	21
15	Di	12	4	51	76*	13	35
16	Di	12	7	51	74*	13	34
17	Di	12	9	55	83*	0	24
18	Di	16	4	50	86*	29*	44*
19	Di	16	7	49	81*	28*	31
20	Di	16	9	65*	85*	29*	23
21	Tri	8	4	50	76*	29*	35
22	Tri	8	12	50	75*	26*	44*
23	Tri	12	4	54	78*	8	39*
24	Tri	12	7	56*	76*	26*	46
25	Tri	12	9	55	75*	0	6
26	Tri	16	4	50	75*	6	29
27	Tri	16	7	45	70	24*	39*
28	Tri	16	9	50	75*	10	41
29	Di	13	25	50	76*	20*	35
30	Di	16	52	53	76*	36*	65*
31	Mono	16	52	48	66	10	13
32	Mono	16	35	55	80*	11	9
33[a]				48	63	9	29
34[b]				0	0	0	0
$LSD_{0.05}$				7.4	10.1	9.5	10.2

[a]Herbicide alone

[b]Control

*Values that were significantly greater than herbicide alone

Table 4. Efficacy of various esters of citric acid applied at 0.25% with nicosulfuron on giant foxtail (0.0056 kg/ha) and large crabgrass 0.0336 kg/ha).

Trt.	R-Group number	Chain length	EO number	Giant foxtail 7 DAT	Giant foxtail 14 DAT	Large crabgrass 7 DAT	Large crabgrass 14 DAT
				(% Control)		(% Control)	
1	Mono	8	4	61*	78*	40*	23
2	Mono	8	7	53	70*	43*	56*
3	Mono	8	9	63*	79*	51*	60*
4	Mono	8	12	65*	91*	55*	75*
5	Mono	12	4	63*	74*	50*	48*
6	Mono	12	7	55*	76*	53*	63*
7	Mono	12	9	55*	73*	51*	70*
8	Mono	16	4	60*	78*	39*	35
9	Mono	16	7	59*	74*	43*	58*
10	Mono	16	9	59*	73*	48*	50*
11	Di	8	4	50	71*	54*	71*
12	Di	8	7	58*	70*	58*	71*
13	Di	8	9	66*	83*	53*	63*
14	Di	8	12	61*	78*	44*	56*
15	Di	12	4	56*	74*	58*	79*
16	Di	12	7	55*	74*	53*	73*
17	Di	12	9	59*	76*	58*	74*
18	Di	16	4	60*	78*	53*	79*
19	Di	16	7	50	69*	58*	79*
20	Di	16	9	65*	80*	58*	76*
21	Tri	8	4	63*	79*	60*	81*
22	Tri	8	12	69*	83*	53*	68*
23	Tri	12	4	60*	78*	55*	66*
24	Tri	12	7	61*	79*	59*	83*
25	Tri	12	9	55*	78*	63*	88*
26	Tri	16	4	68*	74*	65*	74*
27	Tri	16	7	55*	73*	58*	73*
28	Tri	16	9	58*	75*	55*	81*
29	Di	13	25	33	40	55*	70*
30	Di	16	52	68*	81*	56*	79*
31	Mono	16	52	65*	81*	54*	71*
32	Mono	16	35	60*	80*	56*	76*
33[a]				46	58	21	34
34[b]				0	0	0	0
$LSD_{0.05}$				8.7	7.4	7.2	11.7

[a]Herbicide alone

[b]Control

*Values that were significantly greater than herbicide alone

Table 5. Interpretation of statistically analyzed data by comparing the effectiveness of varying carbon chain length, regardless of EO number.

	Glyphosate (Carbon length)		Glufosinate (Carbon length)		Imazamox (Carbon length)		Nicosulfuron (Carbon length)	
	SETFA[a]	CHEAL[b]	SETFA	CHEAL	ABUTH[c]	CHEAL	DIGSA[d]	SETFA
Mono	12	12	12/16	8	NT[e]	NT	12	NT
Di	16	16	16	16	16	16	16	NT
Tri	16	16	NT	NT	12/16	12	8/12	NT

[a] Giant foxtail

[b] Common lambsquarters

[c] Velvetleaf

[d] Large crabgrass

[e] No trend

Table 6. Interpretation of statistically analyzed data from comparing the effectiveness of substitution number. Information obtained using Figures 2-9.

	Glyphosate		Glufosinate		Imazamox		Nicosulfuron	
	SETFA[a]	CHEAL[b]	SETFA	CHEAL	ABUTH[c]	CHEAL	DIGSA[d]	SETFA
Mono	2	2	1	2	3	3	1	1
Di	1	1	2	1	2	1	2	2
Tri	3	3	3	3	1	2	3	3

1 = Most effective number of chains

2 = Moderately effective number of chains

3 = Least effective number of chains

Table 7. Comparison of the chemistry of the adjuvants that most increased the efficacy of four herbicides on various weed species

Herbicide	Weed species	Alkyl chain length	EO number	Number of substitutions
Glyphosate	Common lambsquarters	16	7	di-
	Giant foxtail	16	52	di-
Glufosinate	Common lambsquarters	8	12	di-
	Giant foxtail	12	9	mono-
Imazamox	Common lambsquarters	16	9	di-
	Velvetleaf	16	52	di-
Nicosulfuron	Giant foxtail	8	12	mono-
	Large crabgrass	12	9	tri-

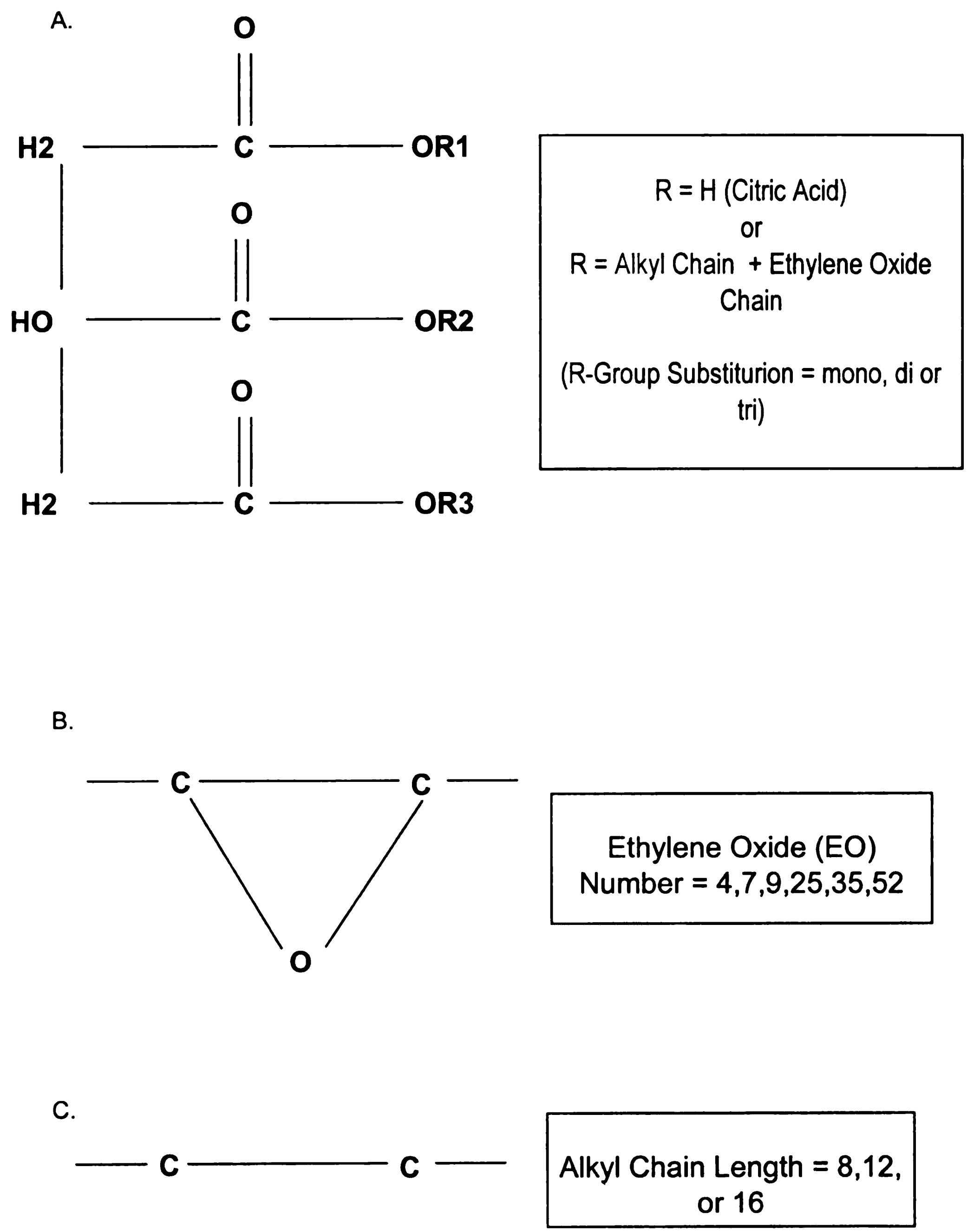

Figure 1. Structures a) Citric Acid Ester b) ethylene oxide and c)alkyl chain

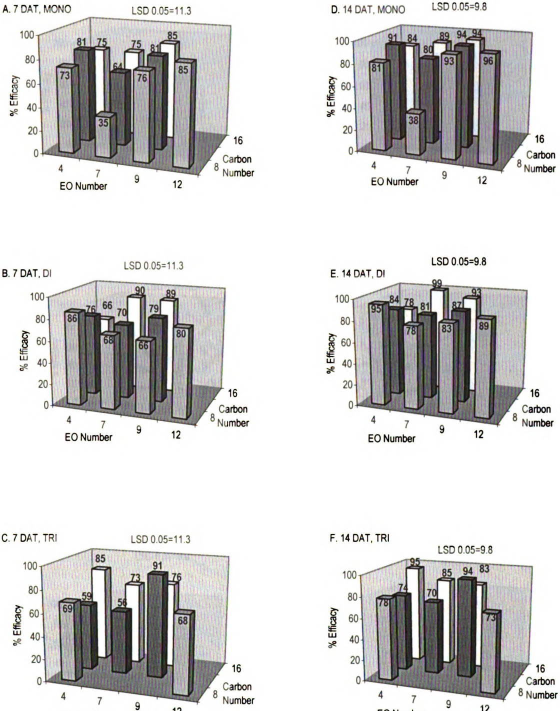

Figure 2. The effect of substitution number on the efficacy of glyphosate on common lambsquaters
In the absence of any adjuvant the weed control was 64% (7DAT) and 78% (14DAT)

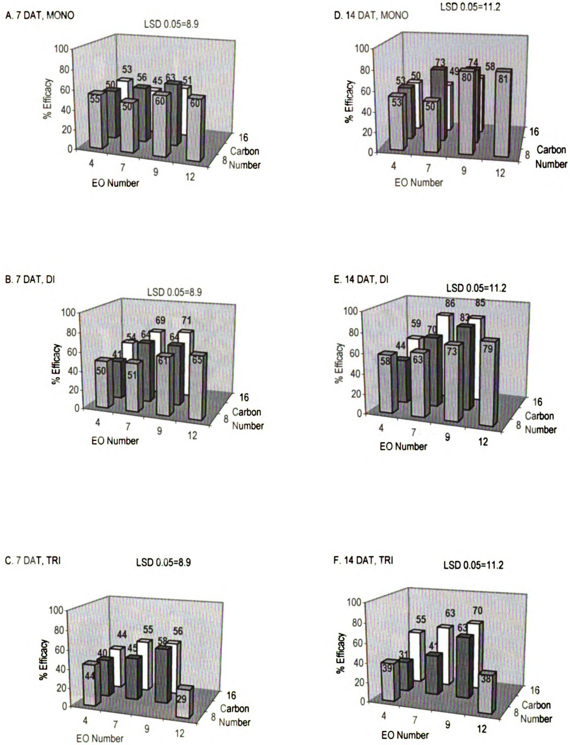

Figure 3. The effect of substitution number on the efficacy of glyphosate on giant foxtail
In the absence of any adjuvant the weed control was 64% (7DAT) and 78% (14DAT)

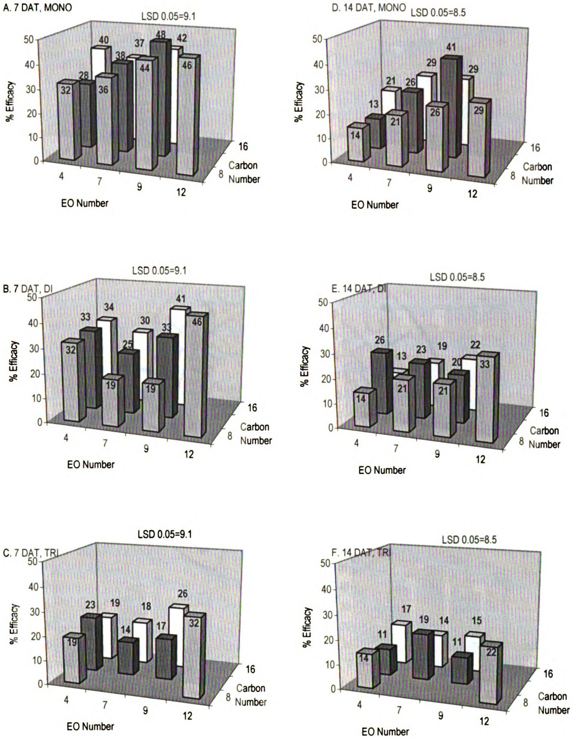

Figure 4. The effect of substitution number on the efficacy of glufosinate on giant foxtail
In the absence of any adjuvant the weed control was 20% (7DAT) and 14% (14DAT)

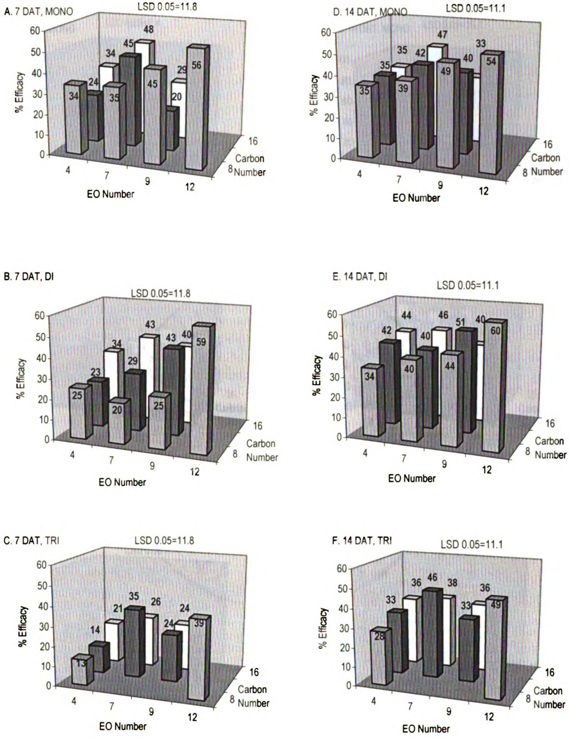

Figure 5. The effect of substitution number on the efficacy of glufosinate on common lambsquarters
In the absence of any adjuvant the weed control was 20% (7DAT) and 14% (14DAT)

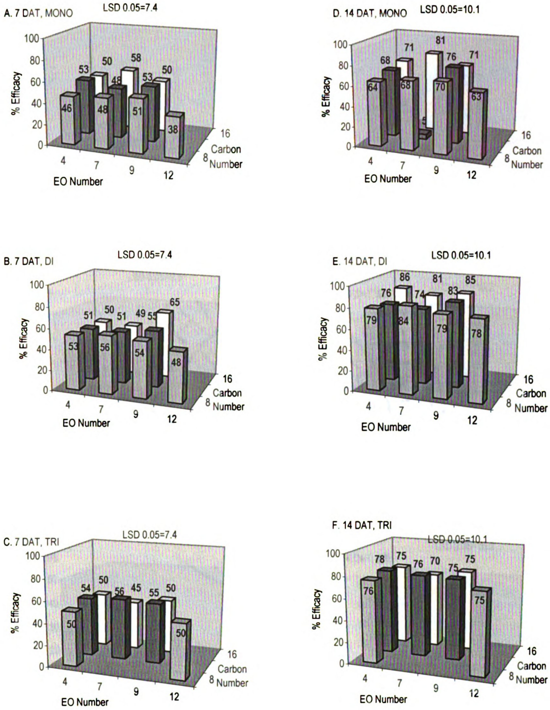

Figure 6. The effect of substitution number on the efficacy of imazamox on common lambsquarters
In the absence of any adjuvant the weed control was 48% (7DAT) and 63% (14DAT)

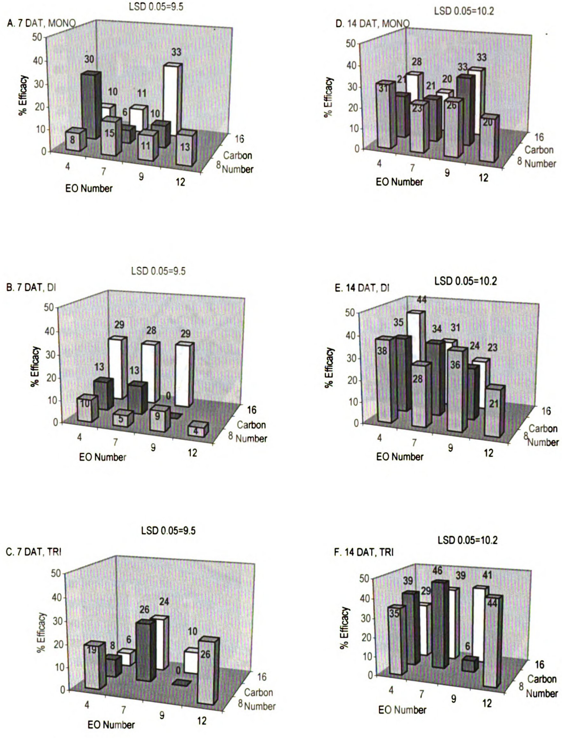

Figure 7. The effect of substitution number on the efficacy of imazamox on velvetleaf

In the absence of any adjuvant the weed control was 48% (7DAT) and 63% (14DAT)

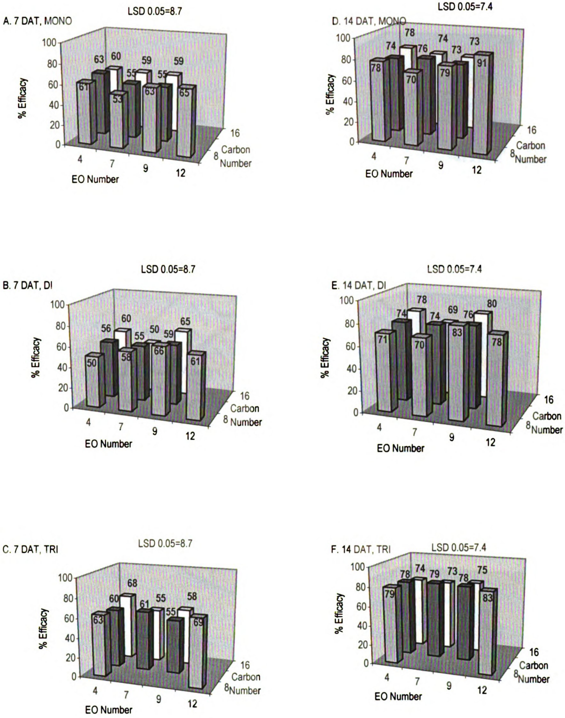

Figure 8. The effect of substitution number on the efficacy of nicosulfuron on giant foxtail
In the absence of any adjuvant the weed control was 46% (7DAT) and 58% (14DAT)

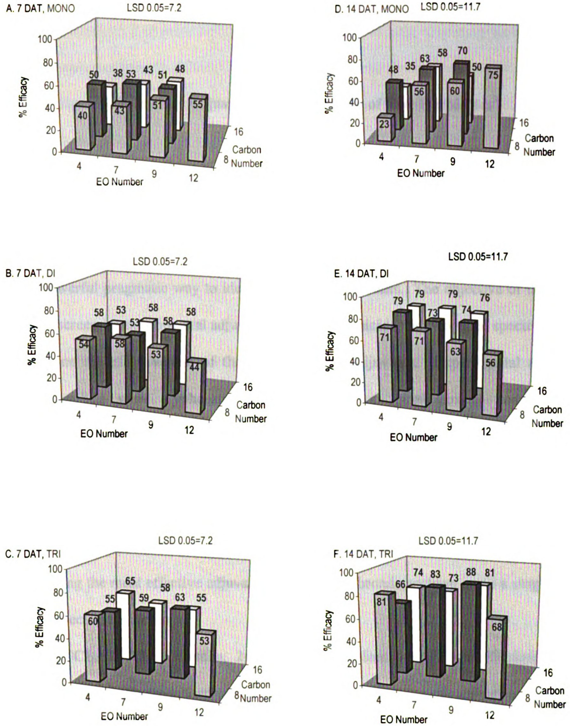

Figure 9. The effect of substitution number on the efficacy of nicosulfuron on large crabgrass
In the absence of any adjuvant the weed control was 46% (7DAT) and 58% (14DAT)

CHAPTER 2

Effect of 19 Adjuvants on the Efficacy of Five Herbicides[1]

HEATHER ENID JOHNSON

Abstract. Adjuvant screening with popular herbicides against difficult to control weeds is a powerful pragmatic way to identify effective adjuvants. The objective of this study was to screen 19 experimental adjuvants with five herbicides on two weed species each to determine the effectiveness of these experimental adjuvants. Experimental adjuvants were applied with glyphosate and glufosinate on giant foxtail and common lambsquarters, with imazamox and dicamba on common lambsquarters and velvetleaf and with nicosulfuron on giant foxtail and large crabgrass. Herbicide applications were made when weeds reached the 3 to 5 leaf stage. Adjuvant efficacy was dependent both on herbicide and weed species. Therefore screening adjuvants is very important in identifying the most effective adjuvant, whether it be broad spectrum or on a single weed with a specific herbicide.

NOMENCLATURE: Dicamba, 2-methoxy-3,6-dichlorobenzoic acid; Glufosinate, 2-amino-4-(hydroxymethylphosphinyl)butanoic acid; Glyphosate, *N*-(phosphonomethyl)glycine; Nicosulfuron, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide;

[1] Received for publication on Date, and in revised form on Date

Imazamox, 2-[4,5-dihydro-4-methyl-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid, ammonium salt; Common Lambsquarters, *Chenopodium album* L. #CHEAL; Giant Foxtail, *Setaria faberi* Herrm. #SETFA; Large Crabgrass, *Digitaria sanguinalis* (L.) Scop. #DIGSA; Velvetleaf, *Abutilon theophrasti* Medicus. #ABUTH.

ABBREVIATIONS: ANOVA; analysis of variance, DAT; days after treatment, LSD; least significant difference.

INTRODUCTION

Adjuvant research had reached an all time high by the 1960's. There were thousands of adjuvants being discovered and used, although very little research was being directed towards the efficacy of the adjuvants (Grondin 1985). Up until very recently, adjuvant research was directed towards spray droplet contact angle, surface tension and spreadability (McWhorter 1982).

It was believed that decreasing the surface tension of a spray solution increases spreadability and therefore increasing activity of an herbicide (Penner 1984). Under this assumption, the method of evaluating adjuvants consisted of screening materials that increased spreadability of a spray solution on artificial surfaces. They soon found out that their method of evaluation was not only flawed, but it had little value in identifying effective adjuvants. They found that spreadability depended completely upon the type of artificial surface that was used for testing (Penner 1984). There were adjuvants that showed great potential on a glass surface, yet when tested on a leaf surface they did poorly.

Today, adjuvant research has a very different focus. Screening of adjuvants by testing with herbicides on plants is used to identify the most effective adjuvants. Effective adjuvants are now found by screening in the greenhouse and field studies with herbicides that need adjuvants on weeds that are difficult to control.

The objective of this study was to screen 19 experimental adjuvants with five herbicides on various weed species to identify the most effective herbicide for a given situation.

MATERIALS AND METHODS

General. The following studies were carried done between the greenhouses at Michigan State University from May 1999 to March 2000. Plants applied with imazamox, nicosulfuron and dicamba, were placed outside between greenhouses from May to September of 1999, due to the very hot temperatures of the greenhouse. The area was enclosed thereby leaving the plants undisturbed, however they were subject to environmental conditions such as rain and cloudy days. Plants applied with glyphosate and glufosinate were kept in the greenhouse from September 1999 to March 2000 and were exposed to less variable conditions.

Weed Species. Four weed species, giant foxtail, common lambsquarters, velvetleaf, and large crabgrass were used in this study. Seeds were placed in 945 ml plastic black pots, using Baccto professional potting soil. Approximately 1 wk before herbicide application, excess plants were removed from the pots, leaving 3-5 plants per pot, each with a similar height and leaf stage to ensure a uniform stand for herbicide application. When plants reached the 3-5 leaf stage, they were thinned down to 1 plant per pot for common lambsquarters, 3 plants per pot for giant foxtail, 1 plant per pot for velvetleaf and 2 plants per pot for large crabgrass.

Herbicide Application. A link belt sprayer was used with a flat fan nozzle[5] that delivered 94 Lha^{-1} spray volume at 138 kPa pressure and a boom height of 33 cm. We used distilled water for the herbicide carrier to avoid any interaction that might occur between the herbicide and hard water. Formulated glyphosate[6] was tested on giant foxtail

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[5] TeeJet Flat Fan Nozzle 80005EV model Spraying Systems Co., Wheaton, IL 60188
[6] Accord® Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167

(0.406 kg/ha) and common lambsquarters (0.11 kg/ha). Formulated glufosinate[7] was tested on giant foxtail (0.101 kg/ha) and common lambsquarters (0.067 kg/ha). Formulated imazamox[8] was tested on common lambsquarters (0.015 kg/ha) and velvetleaf (0.015 kg/ha). Formulated nicosulfuron[9] was tested on giant foxtail (0.0056 kg/ha) and large crabgrass (0.0336 kg/ha). Formulated dicamba[10] was tested on velvetleaf and common lambsquarters. Adjuvants were added to the spray solution at 0.25% (v/v). There were a total of ten trials and each trial was repeated.

Statistics. Data presented are the means of two experiments with four replications each. Plants and treatment sequences were selected at random. Treatments were applied in a random sequence in a double blind study, which means that the chemistry of the adjuvant was not known nor was the sequence of treatment application known. Data were subjected to ANOVA (SAS Institute 1996) and means were separated using Fisher LSD with a P value of 0.05.

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[7] Liberty® AgrEvo USA Co., 2711 Centreville Rd. Wilmington, DE 19808
[8] Raptor® American Cyanamid, One Campus Dr., Parsippany, NJ 07054
[9] Accent® DF, DuPont Co., Walker's Mill, Barley Plaza Wilmington, DE 19898
[10] Banvel® Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167

RESULTS AND DISCUSSION

Efficacy of Citric Acid Esters as Herbicide Adjuvants

There were no adjuvants that increased the effectiveness of all five herbicides on all weed species, which is consistent with adjuvant research today. However there was one adjuvant that increased the effectiveness of three out of the five herbicides: adjuvant 18 increased the herbicidal activity of glufosinate, imazamox and nicosulfuron on all weed species. Adjuvant 8 increased efficacy of imazamox, nicosulfuron and dicamba but not of glyphosate or glufosinate on all weed species tested.

Glyphosate. The only adjuvant that increased the efficacy of glyphosate on both common lambsquarters and giant foxtail was adjuvant 7 (Table 1). However treatment number 8 was most effective for increasing glyphosate efficacy on common lambsquarters and treatment number 10 for giant foxtail. There were no adjuvants that increased the effectiveness of glyphosate compared to the herbicide alone treatment for common lambsquarters and not on giant foxtail. There were two adjuvants that increased the effectiveness of glyphosate compared to the herbicide alone for giant foxtail but not for common lambsquarters, treatments 3 and 17 (Table 1).

Glufosinate. There were three adjuvants that increased efficacy of glufosinate on both common lambsquarters and giant foxtail, adjuvant 4, 10 and 18(Table 2). However adjuvant number 4 was the most effective for both weed species. There was only one adjuvant that increased the effectiveness of glufosinate significantly compared to the herbicide alone for common lambsquarters and not on giant foxtail, adjuvant 19. There were four adjuvants that increased the effectiveness of glufosinate significantly compared to herbicide alone for giant foxtail and not for common lambsquarters, adjuvants 11, 12, 13 and 15. However adjuvant number 11 was the most effective (Table 2).

Imazamox. There were five adjuvants that increased the effectiveness of imazamox on both common lambsquarters and velvet leaf, 2, 6, 8, 11, and 18, however adjuvant number 6 was the most effective for both weed species (Table 3). There were two adjuvants that increased the effectiveness of imazamox significantly compared to the herbicide alone for common lambsquarters and not on velvetleaf, adjuvant 5 and 13. There were no adjuvants that increased the effectiveness of imazamox significantly compared to herbicide alone for velvetleaf and not for common lambsquarters (Table 3).

Nicosulfuron. There were several adjuvants that increased the effectiveness of nicosulfuron compared to the herbicide alone treatment, 3, 7 through 10, 14 and 18 (Table 4). However the adjuvant that was the most significant was number 10. Adjuvant number 5 was most effective for glyphosate control on giant foxtail and adjuvant number 10 on large crabgrass. There were several adjuvants that increased the effectiveness of nicosulfuron significantly compared to the herbicide alone for giant foxtail and not on large crabgrass, 2, 4, 6, 11, 12, 13, 15, 16, 17, and 19, however adjuvant number 11 was the most effective. There were no adjuvants that increased the effectiveness of nicosulfuron significantly compared to herbicide alone for large crabgrass and not for giant foxtail; however the most effective adjuvant compared to herbicide alone was adjuvant number 10 (Table 4).

Dicamba. There was only one adjuvant that increased the effectiveness on both common lambsquarters and velvetleaf, adjuvant 8 (Table 5). However, adjuvant number

11 was the most effective for control with dicamba on common lambsquarters and treatment number 7 on velvetleaf. There were two adjuvants that increased the effectiveness of dicamba significantly compared to herbicide alone for common lambsquarters and not giant foxtail, adjuvant 11 and 14. There were no adjuvants that that increased the effectiveness of dicamba significantly compared to the herbicide alone only on giant foxtail (Table 5).

Adjuvant screening can take on many different goals. For example, adjuvants can be screened against several different herbicides to determine which herbicide with which the adjuvants are most effective. We evaluated whether the adjuvants worked with all herbicides on all weed species. Some adjuvants are weed species specific whereas others are simply herbicide specific and some are both. All this information is very important when identifying the most effective adjuvant to use for a specific situation. Information on weed specific adjuvants may prove to be very useful in today's market, especially with the invention of glyphosate resistant crops. For example those adjuvants that are effective only on monocots may prove to be very useful on glyphosate resistant corn. Whereas those adjuvants that were effective only on dicots would be useful for glyphosate resistant soybeans. Although conclusions on the efficacy of the adjuvants can be drawn, all potentially effective adjuvants need to be compared with those already on the market.

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Penner, D., K. VanFleteren, and F. Roggenback. 1984. Evaluation of soybean oil concentrates as a substitute for petroleum oil based crop oil concentrates. Proceedings: Ag-Chem Uses of Soybean Oil. Workshop: American Soybean Association. pp. 26-29.

Table 1. Efficacy of experimental adjuvants with glyphosate on common lambsquarters and giant foxtail.

Adjuvant	Common lambsquarters		Giant foxtail	
	7 DAT	14 DAT	7 DAT	14 DAT
	(% Control)		(% Control)	
1	54	69	71	5
2	61	71	71	71*
3	59	60	75*	74*
4	78*	73	70	64*
5	68*	83*	70	78*
6	59	89*	71	69*
7	68*	93*	74*	75*
8	83*	95*	71	66*
9	55	64	74*	70*
10	68*	90*	71	85*
11	60	71	71	70*
12	55	78*	70	64*
13	51	79*	70	65*
14	68*	93*	73	75*
15	66*	74	71	66*
16	54	84*	71	66*
17	61	84	74*	66*
18	53	74	71	69*
19	60	76*	71	63*
20[a]	56	64	70	26
21[b]	0	0	0	0
$LSD_{0.05}$	8.0	12.8	3.7	10.5

[a]Herbicide alone
[b]Control
*Values that were significantly greater from the herbicide alone treatment

Table 2. Efficacy of experimental adjuvants with glufosinate on common lambsquarters and giant foxtail

Adjuvant	Common lambsquarters		Giant foxtail	
	7 DAT	14 DAT	7 DAT	14 DAT
	(% Control)		(% Control)	
1	22	16	33*	25
2	98*	95*	36*	21
3	81*	68	51*	33*
4	86*	78*	58*	48*
5	84*	58	35*	25
6	84*	68	28*	19
7	65	49	25*	21
8	81*	66	21*	11
9	53	21	25*	15
10	95*	90*	43*	31*
11	15	6	41*	35*
12	64	30	29*	30*
13	54	30	41*	29*
14	74*	43	26*	24
15	19	8	43*	38*
16	35	29	25*	23
17	56	26	20*	23
18	90*	78*	26*	30*
19	88*	76*	8	20
20[a]	59	50	6	15
21[b]	0	0	0	0
$LSD_{0.05}$	14.1	18.5	11.9	11.9

[a]Herbicide alone
[b]Control
*Values that were significantly greater from the herbicide alone treatment

Table 3. Efficacy of experimental adjuvants with imazamox on common lambsquarters and velvetleaf.

Adjuvant	Common lambsquarters		Velvetleaf	
	7 DAT	14 DAT	7 DAT	14 DAT
	(% Control)		(% Control)	
1	15	5	15	4
2	26*	70*	26*	50*
3	31*	69*	19*	36
4	23*	61	13	46
5	30*	76*	16	43
6	34*	78*	25*	56*
7	33*	68*	16	55*
8	31*	79*	20*	55*
9	28*	71*	13	50*
10	20*	61	16	41
11	24*	73*	21*	55*
12	25*	65	11	20
13	20*	70*	11	36
14	16	63	9	40
15	26*	61	10	40
16	19*	64	6	9
17	21*	63	10	35
18	24*	69*	19*	51*
19	19*	64	15	50*
20[a]	13	55	10	38
21[b]	0	0	0	0
$LSD_{0.05}$	5.6	10.5	6.6	11.2

[a]Herbicide alone
[b]Control
[*]Values that were significantly greater from the herbicide alone treatment

Table 4. Efficacy of experimental adjuvants with nicosulfuron on large crabgrass and giant foxtail.

Adjuvants	Large crabgrass		Giant foxtail	
	7 DAT	14 DAT	7 DAT	14 DAT
	(% Control)		(% Control)	
1	-	21	25	5
2	-	58	50*	71*
3	-	70*	51*	74*
4	-	53	50*	64*
5	-	58	51*	78*
6	-	58	56*	69*
7	-	80*	51*	75*
8	-	61*	50*	66*
9	-	69*	53*	70*
10	-	84*	54*	75*
11	-	48	51*	70*
12	-	49	53*	64*
13	-	48	55*	65*
14	-	64*	54*	75*
15	-	58	50*	66*
16	-	39	53*	66*
17	-	55	53*	66*
18	-	69*	54*	69*
19	-	48	53*	63*
20[a]	-	48	30	26
21[b]	-	0	0	0
$LSD_{0.05}$	-	10.7	3.4	10.5

[a]Herbicide alone
[b]Control
*Values that were significantly greater from the herbicide alone treatment

Table 5. Efficacy of experimental adjuvants with dicamba on common lambsquarters and velvetleaf.

Adjuvant	Common lambsquarters		Velvetleaf	
	7 DAT	14 DAT	7 DAT	14 DAT
	(% Control)		(% Control)	
1	43	75*	14	30
2	59	74*	20	30
3	59	74*	30	40*
4	44	85*	26	39
5	55	85*	31	49*
6	56	69*	35*	51*
7	55	76*	34*	54*
8	66*	81*	34*	44*
9	60	78*	29	30
10	59	83*	29	36
11	69*	85*	16	28
12	51	70*	25	36
13	60	79*	30	35
14	68*	76*	24	29
15	51	76*	23	28
16	50	76*	25	21
17	51	74*	25	25
18	51	78*	28	39
19	55	70*	18	29
20[a]	45	53	26	31
21[b]	0	0	0	0
$LSD_{0.05}$	17.2	10.9	8.0	9.4

[a]Herbicide alone

[b]Control

*Values that were significantly greater from the herbicide alone treatment

CHAPTER 3

The Effect of UV Light on Bas 620 and Clethodim Photodegradation and the Efficacy of Querciten as a Potential UV Protectant [1]

HEATHER ENID JOHNSON

Abstract. Various cyclohexanediones are known to be vulnerable to phototransformation. Plants produce compounds that protect themselves from phototransformation; two such compounds are carotenoids and quercetin. Upon confirmation of the photolability of BAS620 (spectrophotometrically and in the greenhouse) and clethodim (in the greenhouse), carotenoids and quercetin were evaluated for their ability to prevent phototransformation of the two herbicides. Giant foxtail plants were grown in the greenhouse and thinned down to 2 to 3 plants per pot. The herbicides were applied when plants reached the 3 to 5 leaf stage. Formulated clethodim (Select 2EC) was applied at 0.007 kg/ha. Following herbicide application, plants were exposed to UV light in a UV chamber where plants were continually rotated to assure uniform exposure to the UV light. Plants were exposed for 0, 1, and 3 hours and returned to the greenhouse where they were evaluated at 7 and 14 DAT. Tests showed that BAS 620 was labile when exposed to UV light, and evaluated spectrophotometrically. Greenhouse studies also

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confirmed photolability of BAS 620. Upon confirmation of the photolability of BAS 620, both carotenoids and quercetin were evaluated for their potential as UV protectants. At the carotenoid concentrations tested, they were ineffective at preventing photodegradation. However quercetin appeared to be effective both spectrophotometrically and in greenhouse evaluations.

KEY WORDS: Quercetin, Carotenoids, UV Protectant, and Clethodim

NOMENCLATURE: BAS 620 $C_{17}H_{24}ClNO_4$; Clethodim (±)-2-[(E)-1-[(E)-3-Chloro-allyloxyimino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; Quercetin, 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benxopyran-4-one; Giant Foxtail, *Setaria faberi* Herrm. #SETFA;

ABBREVIATIONS: DAT, days after treatment; EC, emulsifiable concentrate

INTRODUCTION

Various cyclohexanediones are known to be vulnerable to phototransformation such as clethodim (Figure 1a) (McMullan 1996; Falb 1990) and sethoxydim (Matysiak 1999; Campbell 1985). As a result, herbicides may either lose or gain activity, depending on the activity of the breakdown product. For those compounds that lose activity upon phototransformation, a material that could prevent transformation would prove to be beneficial.

There are many known compounds that naturally protect a plant from phototransformation. Plant systems contain natural defense mechanisms, which absorb UV light thereby preventing photodegradation. Two such mechanisms involve carotenoids and/or quercetin.

Carotenoids are known to absorb UV light in both plants (Cen 1990; DeChazal 1994; Gerber 1994; Middleton 1993) and animals (Black 1998; Cuadra 1997; Gotz 1999; Harborne 1984; Krinsky 1989; and Savoure 1995). Quercetin has also been shown to absorb UV light in plants (Harborne 1984; Mohle 1985; Olsson 1998; Steerenberg 1998; Takahama 1984; Zhestkova 1984). Since carotenoids and quercetin are naturally occurring compounds and easy to obtain both were tested for their ability to protect herbicides from UV light, or phototransformation.

The first objective of this study was to determine the photolability of BAS 620 (Figure 1b) spectrophotometrically and in the greenhouse, and to evaluate carotenoids as a UV protectant for BAS 620. The second objective was to confirm the photolability of formulated clethodim (Select 2EC) and evaluate quercetin (Figure 2) as a UV protectant.

MATERIALS AND METHODS

BAS 620

Spectrophotometry. A spectral analysis of BAS 620 was obtained using the Spectronic Genesys 5. Solutions of BAS 620 diluted with methanol were prepared at four concentrations 0.06, 0.25, 0.5 and 1 mg ml^{-1}. An absorption spectrum was created using a program for spectral scans on the spectrophotometer from 200 nm to 700 nm. The peak absorbencies were labeled and printouts were obtained.

Exposure to UV Light. Photolability of BAS 620 was confirmed by exposing the herbicide to UV light followed by a spectral analysis. The same four concentrations were used to determine the effect of UV light on BAS 620. A system was devised to expose an herbicide to UV light in a consistent manner. The UV light source was an enclosed system with bulbs emitting 300 nm surrounding the opening in the center of the system. A rotating test tube rack in the center of the chamber allowed each test tube to be exposed to the same intensity of light. In replicates of four, the herbicide was monitored at its peak absorbency for degradation using the spectrophotometer. Recordings of the peak absorbance were monitored for change (increase or decrease in peak absorbance). A spectral scan was performed before UV exposure and after 6 hr to determine if photodegradation had occurred.

UV Protectants. Breakdown of an herbicide molecule may result in loss of activity, therefore, if a compound could prevent phototransformation, no activity would be lost. Both carotenoids and quercetin are known to absorb UV light in plant systems, thereby preventing negative effects from the UV light

Carotenoids were extracted from carrots (baby carrots from Mann's Sunny Shores). Several methods of carotenoid extraction were attempted but only one method was compatible with the spectrophotometer.

One gram of freeze-dried carrots was added to 75-ml acetone and blended for approximately 3-5 min supernatant was collected and centrifuged for 10 minutes. The supernatant was again collected and the volume was reduced under vacuum, leaving behind only the carotenoids which were then re-dissolved in hexane (1 mg ml^{-1}) (hexane was the only solvent that could be used other than acetonitrile).

Once the carotenoids were extracted, they were added to BAS 620 and exposed to UV light for 0, 2, 8, 10 and 12 hr to determine if the carotenoids prevented phototransformation of BAS 620. There were five concentrations of carotenoids tested; 0, 0.625, 0.25, 0.5 and 1mg ml^{-1}. BAS 620 concentration was 0.00025 mg ml^{-1} with hexane as the solvent. In order to determine the effect of BAS 620 alone, a control was included which contained hexane and carotenoids at each carotenoid concentration. Evaluations were made by taking absorbency readings at the peak absorbance for BAS 620of 274 nm at each time interval.

Quercetin is easily dissolved by methanol and acetone (unfortunately acetone could not be used due to interference with the spectrophotometer readings). BAS 620 was diluted with methanol to 0.000625 mg ml^{-1} and quercetin was added to BAS 620 at a concentration of 0, 0.2 and 0. 4 mg ml^{-1}. Methanol plus quercetin, at each concentration, was used as a blank, thereby analyzing the effects of UV light on BAS 620 in the presence of quercetin.

Greenhouse Studies. Giant foxtail plants were grown in 945-ml black pots in the greenhouse, using Baccto (Professional potting soil) potting soil. Approximately 1 wk before herbicide application, excess plants were removed from the pots, leaving 3-5 plants per pot each with a similar height and leaf stage. When the plants reached the 3-5 leaf stage, the plants were thinned to 3 plants per pot for herbicide application.

BAS 620 was subjected to UV irradiation in the UV light chamber (previously described), for 0, 1, and 3 hours. Upon completion of UV irradiation, BAS 620 was applied to giant foxtail at a rate of 0.007 kg ha^{-1} using a link belt sprayer with a flat fan nozzle[4] that delivered 94 Lha^{-1} spray volume at 138 kPa pressure and a boom height of 33 cm. The commercial adjuvant, Scoil, was included at 0.5% (v/v). Data presented are the means of the two experiments with three replications each.

CLETHODIM

Clethodim is known to be a photolabile compound (Falb 1990). To confirm photolability of formulated clethodim[5], the herbicide was subjected to UV irradiation and sprayed on giant foxtail.

Irradiation→Herbicide Application. Plants were grown in 945-ml black pots as previously described. Select 2EC was placed in test tubes and into the UV chamber, previously described, for 0, 1, and 3 hr.

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[4] TeeJet Flat Fan Nozzle 80005EV model Spraying Systems Co., Wheaton, IL 60188

[5] Formulated as Select 2EC

Herbicide Application. Irradiated and non-irradiated clethodim was sprayed on giant foxtail at a rate of 0.007kg ha^{-1} using a link belt sprayer with a flat fan nozzle[6] at 94 Lha^{-1} spray volume, 138 kPa pressure and a boom height of 33 cm. The adjuvant Scoil was applied with clethodim at 0.5% (v/v). Data presented are the means of two experiments with three replicates of each treatment.

Herbicide Application→Irradiation. For the second part of the greenhouse study, giant foxtail seeds were planted in yellow cone pots, 15 cm tall and 2.5 cm wide. Plants were kept in the greenhouse and watered daily and as needed. When the plants reached the 3-5 leaf stage, plants were thinned down so that only one giant foxtail plant remained for herbicide application.

A holder was made to carry the yellow cone pots that fit inside the UV chamber on the rotating stand. The holder was constructed out of Styrofoam and covered with aluminum foil to prevent melting by the UV light. Nine slots were made on the perimeter of the holder. The holder was used both in the herbicide application and in the UV chamber.

Herbicide Application. In the first application, clethodim was applied at a rate of 0.007 kg ha^{-1} using a link belt sprayer with a flat fan nozzle[7] delivered at 94 Lha^{-1} spray volume at 138 kPa pressure and a boom height of 33 cm. Tap water was the spray solution carrier. In addition to the tap water, 5% acetone was included since it was included in the spray solution including quercetin. In the second application, quercetin was added to the spray solution at 0.4 mg ml^{-1} (quercetin was dissolved in 5% acetone).

[5] TeeJet Flat Fan Nozzle 80005EV model Spraying Systems Co., Wheaton, IL 60188
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UV Exposure. Plants were then placed into a dark box and transported to the UV chamber for irradiation. Each yellow cone pot holder contained 3 pots that were sprayed with herbicide alone, 3 pots that were sprayed with herbicide plus quercetin and 3 pots that were controls. The holder was placed on the rotating rack in the UV chamber and exposed for 0, 1 and 3 hr (at 4 hr the control plants suffered damage from UV light) and returned to the greenhouse. The experiment was repeated.

RESULTS AND DISCUSSION

BAS 620

Spectrophotometry and Exposure to UV Light. A spectral scan was obtained ranging from 200 nm-700 nm. The spectral scan of BAS 620 had two peaks, one that tended to go off the scale at extremely low concentrations and another peak at 274 nm. (Therefore all readings done on the spectrophotometer with BAS 620 were read at 274 nm). Another clue to the phototransformation of BAS 620 was the reduction of absorbance at 274 nm, an indication that the parent product increased (Figure 3a). When BAS 620 was exposed to UV light, a third peak appeared on the spectral scan, suggesting that UV light had indeed phototransformed BAS 620 (Figure 3b).

UV Protectants.

Carotenoids. Table 1 shows that BAS 620 behaved similarly with and without carotenoids. An increase in the absorbance at 274 nm occurred regardless of the presence of carotenoids. Since the absorbance increased regardless of the presence of carotenoids, they did not act to protect BAS 620 from the effects of UV light. Carotenoids should be effective at preventing herbicidal breakdown since they absorb light in the UV portion of the light spectrum. It may be that the carotenoids were not pure enough or the concentration was too low.

Quercetin. Table 2 compares the effects of UV light on BAS 620 absorbance spectrum with and without quercetin. Unlike the results found using carotenoids, the absorbance for BAS 620 plus quercetin remained constant. The effect of UV light on BAS 620, as seen in both Tables 1 and 2, was a steady increase in absorbance at the peak absorbance (274 nm) suggesting that was preventing phototransformation.

Greenhouse Studies. Greenhouse studies confirmed that after exposure to UV light there was a decrease in biological activity of BAS 620 at 7 DAT (Figure 4). However since there were no statistical differences at 14 DAT between 0, 1 and 3 hr of UV irradiation, an herbicide known to be photolabile, clethodim, was used for the remainder of the research.

Clethodim

Irradiation→Herbicide Application.

Photolability of commercially formulated clethodim was confirmed in the greenhouse (Figure 4). Figure 3b showed that non-irradiated clethodim was more active than if it were irradiated for 1 and 3hrs. Therefore, since photolability was confirmed, a protectant would be a useful addition to clethodim.

Herbicide Application→Irradiation.

After quercetin was added to the spray solution and plants were irradiated in the UV chamber for 0, 1, and 3 hr (Table 3), there was a significant difference between those with quercetin added to the spray solution and those without quercetin at 3 hours after irradiation (Figure 5). Spraying the plants with the herbicide plus quercetin first and then irradiating the plants in the UV chamber proved to be an effective way to determine both photolability and the efficacy of a given UV protectant under for more realistic conditions than irradiated spray droplets on a glass slide or spray solution in a test tube.

Interestingly, the formulated clethodim still appears to be subject to phototransformation and increased herbicide efficiency should be possible with more effective UV protectants.

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Table 1. The evaluation of carotenoids as a UV protectant for BAS 620. Absorbency readings were taken at the peak wavelength of 274nm.

		Length of Irradiation				
Herbicide + Carotenoids	Calculations	O hr (O.D.)[a]	2 hr (O.D.)	8 hr (O.D.)	10 hr (O.D.)	12 hr (O.D)
BAS 620 + 0 mg ml^{-1}	Mean	2.079	2.001	2.408	2.566	2.808
	Difference (After-before)		-0.078	0.329	0.487	0.729
BAS 620 + 1 mg ml^{-1}	Mean	1.719	1.661	1.988	2.126	2.298
	Difference (After-before)		-0.058	0.269	0.407	0.579
BAS 620 + 0.5 mg ml^{-1}	Mean	1.753	1.681	1.998	2.146	2.331
	Difference (After-before)		-0.072	0.245	0.393	0.578
BAS 620 + 0.25 mg ml^{-1}	Mean	1.774	1.739	2.062	2.224	2.416
	Difference (After-before)		-0.035	0.288	0.466	0.642
BAS 620 + 0.06 mg ml^{-1}	Mean	1.749	1.682	1.983	2.187	2.350
	Difference (After-before)		-0.067	0.234	0.438	0.601

[a] Optical Density

*Herbicide concentration = 0.00625 mg ml^{-1}

Table 2. Evaluation of quercetin as a UV protectant for BAS 620. Absorbency readings were taking at the peak wavelength of 274 nm.

	Length of Irradiation		
Treatment	O hr (O.D.)	2 hr (O.D.)	5 hr (O.D.)
BAS 620 + 0 mg ml^{-1}	2.358	2.836	2.947
BAS 620 + 0.2 mg ml^{-1}	2.241	2.274	2.402
BAS 620 + 0.4 mg ml^{-1}	2.363	2.388	2.277

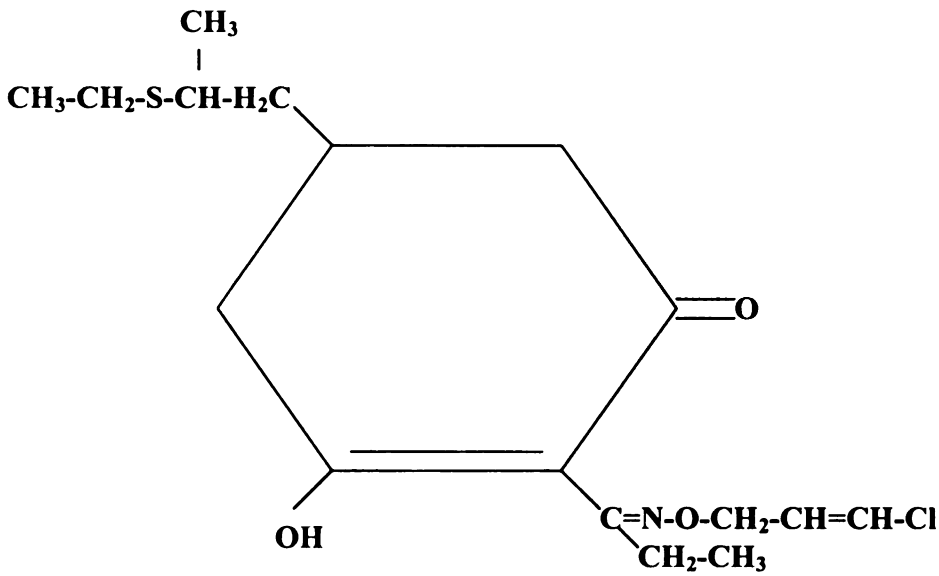
*Herbicide concentration = 0.00625 mg ml^{-1}

Table 3. Evaluation of quercetin (Q) as a UV protectant with clethodim for control of giant foxtail.

Treatment	Time Irradiated	7 DAT	14 DAT
	(hr)	(% Control)	
Clethodim + Q	0	52d	53c
Clethodim - Q	0	55c	55c
Control	0	0e	0d
Clethodim + Q	1	58b	53c
Clethodim - Q	1	53cd	56c
Control	1	0e	0d
Clethodim + Q	3	78a	75a
Clethodim - Q	3	60b	68b
Control	3	0e	0d

*Herbicide Rate = 0.007 kg/ha

A.



B.

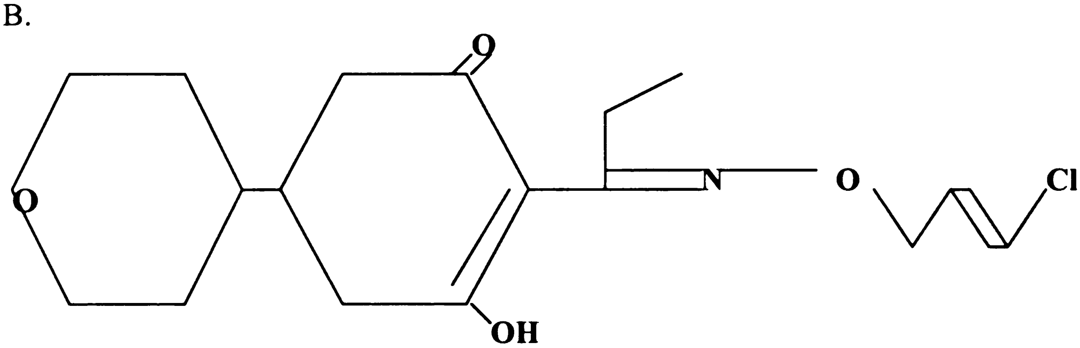


Figure 1. A) Structure of clethodim B) Structure of BAS 620

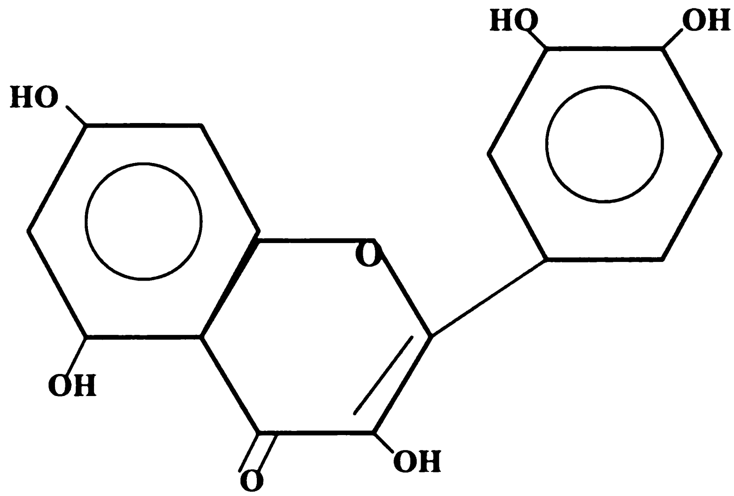
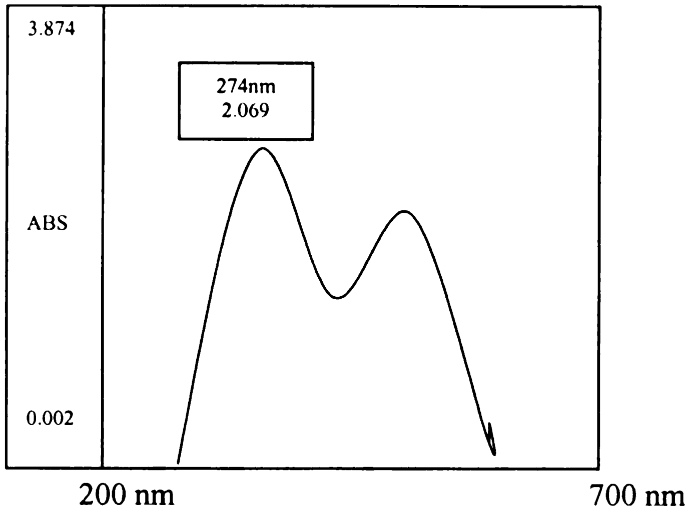


Figure 2. Structure of quercetin

A.



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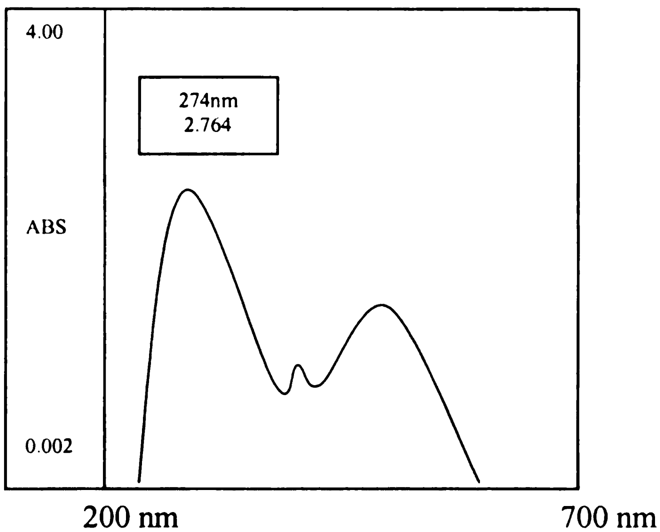
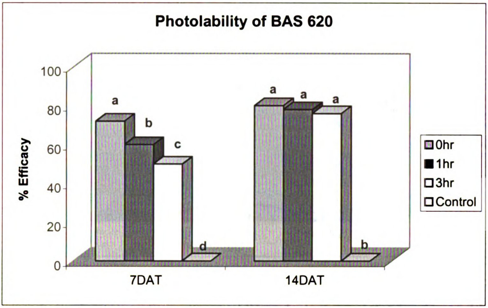


Figure 3. Spectral scans for BAS 620 from 200nm to 700nm a) Before exposure to UV light b) After exposure to UV light

A.



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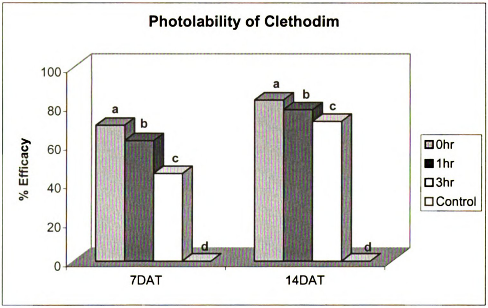

Figure 4. Confirmation of photolability for BAS 620 and clethodim 0, 1, and 3 hr after exposure to UV light

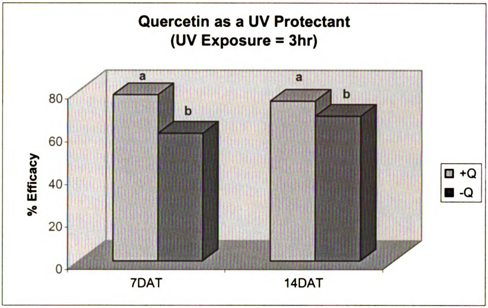

Figure 5. Evaluation of quercetin as a UV protectant for clethodim O and 3 hr after exposure to UV light.