INVESTIGATIONS ON THE POTENTIAL USE OF IMIDAZOLINONES FOR FIELD BINDWEED CONTROL IN WINTER WHEAT AND *IN VITRO* SELECTION FOR IMAZAPYR RESISTANCE IN WHEAT

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IMAZAPYR RESISTANCE

IN WHEAT

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TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Each chapter of this thesis is a manuscript to be submitted for publication in Weed Technology, a Weed Science Society of America publication.

CHAPTER I

RESPONSE OF FIELD BINDWEED (*Convolvulus arvensis*) AND WINTER WHEAT (*Triticum aestivum*) TO IMIDAZOLINONE HERBICIDES

Response of Field Bindweed (*Convolvulus arvensis*) and Winter Wheat (*Triticum aestivum*) to Imidazolinone Herbicides.[1]

DAVID C. HEERING and THOMAS F. PEEPER[2]

Abstract. Field experiments were conducted in Oklahoma to evaluate the effect of three imidazolinone herbicides on field bindweed and hard red winter wheat. Imazapyr at 280 g ha^{-1} and imazethapyr at 560 g ai ha^{-1} controlled field bindweed from 78 to 100% for 48 weeks, but imazaquin at 560 g ha^{-1}, metsulfuron at 17.5 g ha^{-1}, and 2,4-D plus picloram at 1120 plus 280 g ae ha^{-1} did not. Imidazolinone herbicides reduced forage and grain yield of wheat seeded the fall after herbicide application. Only imazapyr reduced grain yield of wheat seeded 15 months after treatment. Nomenclature: Imazapyr, (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid; imazaquin, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-

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5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid; imazethapyr, (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid; metsulfuron, 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl] amino] sulfonyl] benzoic acid; picloram, 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid; 2,4-D, (2,4-dichlorophenoxy)acetic acid; field bindweed *Convolvulus arvensis* L. #[3] CONAR; wheat *Triticum aestivum* L.

Additional index words. imazapyr, imazaquin, imazethapyr, metsulfuron, picloram, 2,4-D.

INTRODUCTION

Field bindweed is a deep rooted perennial with a twining growth habit that reproduces both vegetatively and by seed (18, 19). In addition to competition with the crop for water and nutrients, its twining growth habit can cause lodging of small grains and interfere with harvest (19). The first report of field bindweed in the midwest was in Kansas in the 1870's (11). Now approximately 2.9 x 10^6 hectares in the western half of the United States are infested (3). Several researchers have investigated both chemical and cultural methods of field bindweed control (6, 12, 15, 16, 17, 21). In the mid-1960's researchers reported

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[3]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 309 W. Clark St., Champaign, IL 61820.

effective field bindweed control with picloram, but wheat injury can occur from residual picloram in the soil (1, 7). Glyphosate and 2,4-D have both been reported to control field bindweed, but control with these herbicides has been variable in the midwest (13, 15, 16, 21). Wiese and Lavake (21) reported that in 22 experiments field bindweed control with 3.3 kg ha^{-1} of glyphosate varied from 0 to 100% with a mean of 71%. The variability of field bindweed control reported with 2,4-D and glyphosate may be attributable to varying susceptibility of field bindweed to the herbicides (5, 20). Because these herbicides have not consistently selectively controlled established field bindweed, new control methods are needed.

Because imazapyr effectively controls field bindweed on non-cropland (2, 14), and other imidazolinones effectively control other Convolvulaceae family members (14) in cropland situations, field experiments were initiated to evaluate three imidazolinone herbicides for field bindweed control and to determine the response of winter wheat seeded after herbicide application.

MATERIALS AND METHODS

Field bindweed control. Two field experiments were conducted from 1987 to 1989 to determine the efficacy of three imidazolinone herbicides and metsulfuron applied postemergence for control of established field bindweed in a wheat field near Gould, in southwestern Oklahoma. Imazapyr, imazaquin, and imazethapyr were each applied at 140, 280, and 560 g ai ha^{-1}. Other treatments included metsulfuron at 4.4, 8.7, and 17.5 g ai ha^{-1}, a tank-mix of 2,4-D[4] plus picloram at 1120 plus 280 g ae ha^{-1}, respectively, and an untreated. All herbicides were applied in water to 3.5- by 7.6-m plots in a spray volume of 187 l ha^{-1} with 0.25% v v^{-1} X-77[5] nonionic surfactant added. In one experiment treatments were applied July 20, 1987 in a field seeded to a sorghum by sudan hybrid (Sorghum bicolor L. x Sorghum sudanese Piper) in May after the wheat crop failed. The established field bindweed plants had 6 to 10 inch runners. In the other experiment treatments were applied July 20, 1988 in wheat stubble when the bindweed was blooming. Plots were tilled about 10 cm deep 17 DAT[6] $\pm$1 day. 'TAM W-101' wheat was seeded at 70 kg ha^{-1} October 28, 1987 or September 28, 1988. Prior to seeding, the plots were disked 10 cm deep. Field bindweed control was estimated visually 2, 8, and 48 WAT, and wheat injury was visually estimated as the

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[4]Weedar 64 a dimethylamine salt of 2,4-D marketed by Union Carbide Agriculture Products Co. Inc., P.O. Box 12014, T.W. Alexander Drive, Research Triangle Park, NC 27709.

[5]X-77 contains alkylarylpolyoxyethylene glycols, free fatty acids, and isopropanol and is marketed by Chevron Chemical Co., 940 Hensley St., Richmond, CA 94120.

[6]Abbreviations: DAT = days after treatment; WAT = weeks after treatment.

crop matured. Dates of major field operations, soil information, and rainfall data are in Table 1.

In three other experiments, treatments included only imazapyr at 140, 280, and/or 210, 420, and 560 g ha^{-1} plus an untreated. Treatments were applied as described above on June 2, 1987, July 6, 1987, and August 5, 1988 in wheat fields near Alva, Stillwater, and Mangum, Oklahoma, respectively, when the field bindweed had 8 to 16 inch runners. At Stillwater and Alva the field bindweed was in good growing condition, and at Stillwater was blooming. At Mangum the field bindweed was under some moisture stress and the leaves were wilted the afternoon of treatment. Plots were disked 37 DAT at Alva, 22 DAT at Stillwater, and 13 DAT at Mangum, and were tilled again 10 to 12 cm deep immediately prior to seeding (Table 1). At Alva, TAM W-105 wheat was seeded 176 DAT at 70 kg ha^{-1}. At Stillwater 'Pioneer 2157' wheat was seeded 67 DAT at 80 kg ha^{-1}, and at Mangum TAM W-101 wheat was seeded at 80 kg ha^{-1} 91 DAT.

The experimental design was a randomized complete block with 4, 3, and 2 replications at Mangum, Stillwater and Alva, respectively. Field bindweed control was estimated visually at 5 to 7, 11 to 14, and 45 to 56 WAT. Wheat injury was estimated visually as the crop matured. Plots were harvested with a small plot combine in June. Data were analyzed using analysis of variance procedure and means were separated using protected LSD's.

Wheat tolerance. Two field experiments were conducted from

1987 to 1989 under weed free conditions at the Agronomy Research stations near Altus and Chickasha, Oklahoma, to determine the effect of herbicide treatments applied in mid-summer on wheat seeded the fall after treatment and the following fall. Treatments included imazapyr, imazaquin, and imazethapyr, each applied at 140, 280, and 560 g ha^{-1}, metsulfuron at 4.4, 8.7, and 17.5 g ha^{-1}, 2,4-D plus picloram at 1120 plus 280 g ha^{-1}, respectively, and an untreated check.

Herbicides were applied as previously described to tilled 3.5- by 7.6-m plots on July 20, 1987 at Altus, and August 7, 1987 at Chickasha. Plots were tilled once 10 to 13 cm deep 15 and 17 DAT, respectively. The experimental design for each experiment was a randomized complete block with a factorial arrangement of treatments plus an added standard treatment (2,4-D plus picloram) and an added untreated. Factors included herbicide, herbicide rate, and planting date.

'Chisholm' wheat was seeded at 70 kg ha^{-1} 68 and 102 DAT at Altus, and at 100 kg ha^{-1} 55 and 96 DAT at Chickasha. The second growing season Chisholm wheat was seeded at 67 kg ha^{-1} October 15 and November 3, 1988 at Altus, and October 14 and November 4, 1988 at Chickasha.

Wheat forage yield was determined by clipping forage from one meter of row per plot on March 10 $\pm$ 1 day each year at both locations and drying the forage in a forced air drier for seven days. Grain yields were determined as previously described. Operation dates, soil information, and rainfall data are in Table 1.

RESULTS AND DISCUSSION

Field bindweed control. At Gould, both imazapyr and imazethapyr at all three rates killed 94% or more of the field bindweed foliage by two WAT in 1987 (Table 2). Imazaquin, metsulfuron, and 2,4-D plus picloram killed less than 40%. In 1988 foliage kill at 2 WAT was essentially opposite of the previous year. Metsulfuron at 17.5 g ha^{-1} and 2,4-D plus picloram killed 83 to 90% of the field bindweed top growth by two WAT, and other treatments controlled 45% or less. The different response may have been associated with a difference in rainfall. In 1988, 1 cm of rain fell 5 DAT, and in 1987 it did not rain for over 2 WAT. Daily high temperatures averaged 36 C during the 2 WAT each year.

Control of regrowth after the first post treatment tillage (8 WAT evaluations) was more consistent from year to year. Imazapyr and imazethapyr at all rates, and 2,4-D + picloram controlled field bindweed regrowth from 98 to 100% both years. Except at the high rate in 1987, imazaquin was less effective than imazethapyr or imazapyr. Metsulfuron controlled from 80 to 98 percent of the field bindweed in 1987 and from 58 to 94 percent in 1988.

Control with imazapyr at 280 or 560 g ha^{-1} remained at 88 to 100% for 46 weeks in both experiments. At 140 or 280 g ha^{-1} imazethapyr was less effective than imazapyr. At all rates imazaquin was less effective than imazethapyr.

Both years imazapyr at 280 or 560 g ha^{-1} and imazethapyr at 560 g ha-1 killed over 90% of the wheat seeded the fall after treatment. Imazaquin at 560 g ha^{-1} did not affect the wheat stand either year, nor did metsulfuron or 2,4-D plus picloram. Both years, imazethapyr at 280 g ha^{-1} injured wheat less than imazapyr at the same rate. Thus the tolerance of fall seeded wheat to the imidazolinones decreased in the order imazaquin > imazethapyr > imazapyr. Drought each winter caused severe crop damage with yield of check plots as low as 200 and 180 kg ha^{-1} in 1987 and 1988, respectively. Thus yield data was not indicative of treatment effects (data not shown).

At Alva and Mangum all imazapyr treatments controlled field bindweed 90 to 100% for 14 weeks, even though the field bindweed was under some moisture stress at Mangum at the time of application (Table 3). At Stillwater, after 14 weeks, there was less control with imazapyr at 140 and 210 g ha^{-1} than at Mangum. The better control at Mangum than at Stillwater with imazapyr at 140- and 210 g ha^{-1} was still apparent in early May, 1988 (data not shown) and in mid summer (Table 3.) Control at Alva was very similar to control at Mangum. Although the reason for the differences in control are not readily apparent, it could be related to the dates of final tillage prior to seeding wheat. At Stillwater, the plots were tilled and seeded in mid-

September in contrast to November final tillage and seeding at the other sites. Field bindweed foliage is not usually killed by frost until late November or December in central and southern Oklahoma. Thus, cessation of tillage in September at Stillwater may have permitted some recovery of field bindweed prior to winter.

Wheat stands were reduced at all locations by imazapyr at 420, and/or 560 g ha^{-1} and at Mangum by all treatments. The stand reductions were indicative of yield reductions. Time from treatment to seeding did not seem to affect wheat response.

Wheat tolerance. There were no location or planting date interactions except for a location interaction in the 1989 grain yield data. Thus, all data are pooled across locations and planting dates except the 1989 grain yield.

Dry forage yields of wheat seeded 2 and 3 months after herbicide application were reduced by imazapyr and imazethapyr at all three rates, and by imazaquin at the two higher rates (Table 4). When the imidazolinone herbicides were applied at 140 g ha^{-1}, forage yields were reduced by imazapyr > imazethapyr > imazaquin = untreated. This same order of response occurred in plots treated with 560 g ha^{-1} when they were replanted the next fall (about 15 months after treatment). Forage yields were not reduced by metsulfuron or 2,4-D plus picloram either year.

Grain yield the first year, pooled across locations and seeding dates at each location, was reduced by imazapyr at

140 g ha^{-1}, imazethapyr at 280 g ha^{-1} and imazaquin at 560 g ha^{-1}. Neither metsulfuron nor 2,4-D plus picloram reduced grain yield. Thus, grain yields were reduced when forage yields were reduced except when imazethapyr was applied at the lowest rate and imazaquin was applied at 140, and 280 g ha^{-1}.

Imazapyr at 280 g ha^{-1} severely reduced grain yield of wheat seeded about 15 months after treatment at Altus but did not reduce yield at Chickasha. Also, imazapyr at 560 g ha^{-1} reduced grain yield more at Altus than at Chickasha. The greater yield reductions at Altus may be attributable to the higher pH, finer soil texture, and lower rainfall at Altus which could influence imazapyr availability and persistence as reported for imazaquin and imazethapyr (4, 8, 9).

Thus the imidazolinone herbicides can be effective for field bindweed control. However, the potential for wheat injury could limit development for use in wheat except for spot treatment or for field bindweed control on land temporarily out of crop production.

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Table 1. Dates of major operations, soil descriptions, and rainfall data for the five field bindweed control experiments and the wheat response experiments at Altus and Chickasha.

	Locations						
Parameter	Gould 87	Gould 88	Alva	Stillwater	Mangum	Altus	Chickasha
Treatment	July 20,87	July20,88	June 2,87	July 6,87	Aug.5,88	July 20,87	Aug.7,87
Tillage	Aug.7,87	Aug.4,88	July 9,87	July 28,87	Aug.18,88	Aug.4,87	Aug.24,87
			Aug.20,87				
Tilled	Oct.28,87	Sept.28,88	Nov.25,87	Sept.11,87	Nov.4,88	Sept.26,87	Oct.1,87
and	-	-	-	-	-	Oct.30,87	Nov.6,87
seeded	-	-	-	-	-	Oct.15,88	Oct.14,88
	-	-	-	-	-	Nov.3,88	Nov.4,88
Soil							
Texture	Sandy loam	Sandy clay	Clay loam	Loam	Silt loam	Clay loam	Loam
pH	7.6	8.1	7.4	5.8	6.6	7.5	6.2
	%						
Organic matter	0.4	1.2	0.9	2.0	0.8	1.3	1.4

Table 1. (Continued.)

Rainfall WAT[a]				cm			
-4 to 0	6	9	19	21	3	12	3
0 to 1	0	1	4	2	1	0	4
1 to 2	0	0	2	2	1	0	0
2 to 3	2	0	3	0	0	5	4
3 to 4	2	0	3	0	0	2	0
4 to 12	5	19	14	17	15	10	18
12 to 24	8	3	12	20	8	18	17
24 to 36	9	9	9	18	6	12	21
36 to 48	21	30	30	21	27	17	11
48 to 60	-[b]	-	-	-	-	19	29
60 to 72	-	-	-	-	-	5	11
72 to 84	-	-	-	-	-	13	12
84 to 96	-	-	-	-	-	29	43

[a]WAT = Weeks after treatment.

[b]Experiments were completed in 12 months.

Table 2. Field bindweed control from herbicides applied in July and stand reduction of fall seeded wheat in experiments initiated near Gould, OK, in 1987 and 1988.

		Field bindweed control						Wheat injury	
		2 WAT		8 WAT		48 WAT			
Treatment	Rate	1987	1988	1987	1988	1987	1988	1987	1988
	$g\ ha^{-1}$	%							
Imazapyr	140	97	10	99	98	78	67	78	30
	280	98	43	99	100	88	98	92	99
	560	99	30	99	100	100	99	100	99
Imazethapyr	140	94	10	99	89	20	23	5	10
	280	97	20	99	95	15	33	53	17
	560	98	33	99	99	78	95	91	99
Imazaquin	140	28	0	30	43	20	0	24	0
	280	20	13	58	57	0	0	0	17
	560	35	13	87	57	0	0	0	17
Metsulfuron	4.4	18	23	80	58	10	0	0	0
	8.7	35	63	96	88	20	0	0	0

Table 2. (Continued.)

	17.5	3	83	98	94	18	33	0	0
2,4-D + picloram	1120 + 280	5	90	99	99	25	17	0	0
Check		5	0	0	0	0	0	0	0
LSD (0.05)		30	23	29	32	33	23	20	30

Table 3. Effects of imazapyr on field bindweed and hard red winter wheat seeded the fall after treatment at three locations[a].

		Field bindweed control									Wheat injury			Wheat yield		
		5 to 7 WAT			11 to 14 WAT			45 to 56 WAT								
Treatment	Rate	Alva[b]	Stw	Mag	Alva	Stw	Mag	Alva	Stw	Mag	Alva	Stw	Mag	Alva	Stw	Mag
	g ha^{-1}	%												kg ha^{-1}		
Imazapyr	140	90	62	100	100	58	100	0	0	53	0	7	30	934	1900	300
Imazapyr	210	-	68	100	-	72	100	-	0	94	-	8	45	-	2140	110
Imazapyr	280	100	95	100	100	95	100	85	65	83	0	48	78	1150	1200	60
Imazapyr	420	-	91	100	-	97	100	-	63	98	-	75	84	-	770	40
Imazapyr	560	100	-	100	100	-	100	98	-	99	70	70	97	340	-	30
Check		0	0	0	0	0	0	0	0	0	0	0	0	1280	1880	720
LSD (0.05)		23	50	1	1	23	1	11	12	30	1	8	16	340	580	330

[a]Stw = Stillwater; Mag = Mangum.

[b]Imazapyr was not applied at 210 or 420 g ha^{-1} at Alva or at 560 g ha^{-1} at Stillwater

Table 4. Forage and grain yields of wheat seeded in the fall of 1987 and the fall of 1988 following herbicide application in the summer of 1987 at Altus and Chickasha, pooled over the two seeding dates each fall.

Treatment	Rate	Forage yield[a]		Grain yield		
		1988	1989	1988[a]	CK89[b]	AL89[b]
	$g\ ha^{-1}$	$kg\ ha^{-1}$				
Imazapyr	140	450	1480	490	3070	2090
	280	10	830	10	3030	450
	560	0	280	0	940	60
Imazethapyr	140	920	1160	3230	2660	3050
	280	360	1020	1900	3390	3130
	560	60	640	310	3150	2730
Imazaquin	140	1870	1170	3740	2930	2820
	280	1270	1120	3460	2960	2880
	560	930	1290	2500	3340	3010
Metsulfuron	4.4	2080	1100	3830	3020	2610
	8.7	1990	1150	3730	2900	2630
	17.5	1970	1020	3420	2630	2760

Table 4. (Continued.)

2,4-D + picloram	1120 + 280	2050	1100	3510	2980	2740
Check		2110	990	3610	3040	2520
LSD (0.05)		370	280	410	—— 470 ——	

[a]Data are pooled across locations.

[b]CK89 = Chickasha 1989; AL89 = Altus 1989.

CHAPTER II

TOLERANCE OF HARD RED WINTER WHEAT CULTIVARS TO IMAZAPYR

Tolerance of Hard Red Winter Wheat Cultivars to Imazapyr[1]

DAVID C. HEERING, THOMAS F. PEEPER and ARRON C. GUENZI[2]

Abstract. Laboratory experiments were conducted to determine whether 20 hard red winter wheat cultivars differed in their response to imazapyr. Imazapyr at 63 ppb inhibited plant height of 'Chisholm' wheat 53%. Comparison of the plant height of all cultivars at 0 and 63 ppb indicated that 'TAM 200' was the most susceptible cultivar and 'Karl' was the most tolerant of those evaluated. The differences in tolerance did not appear sufficient to eliminate the potential for wheat injury with imazapyr.

Nomenclature: Imazapyr, (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid; wheat, *Triticum aestivum* L.

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INTRODUCTION

Applications of imazapyr at rates up to 560 g ai ha^{-1} effectively control field bindweed (Convolvulus arvensis) (1, 4, 5). However hard red winter wheat seeded up to several months after imazapyr application is not tolerant to residual herbicide (4). Response of corn cultivars to imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid), a herbicide in the same family as imazapyr, varied when imazaquin was applied at 35 to 280 g ai ha^{-1}. Height reductions ranged from 38 to 59% of the control height (6). The differential response of corn hybrids to imazaquin was confirmed by other researchers but could not be related to differences in seed size, seedling growth, acetohydroxyacid synthase (AHAS) activity, AHAS sensitivity, uptake, translocation, or imazaquin metabolism (8).

Wheat cultivars differ in their tolerance to metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one) (7), cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl] amino]-2-methylpropanenitrile) (3), and atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) (2). Whether wheat cultivars differ in tolerance to imidazolinone herbicides is not known.

The objective of this research was to determine whether 20 hard red winter wheat cultivars differed in imazapyr tolerance.

MATERIALS AND METHODS

Laboratory experiments were conducted to determine the tolerance of 20 hard red winter wheat cultivars to imazapyr. The cultivars selected are grown on 90 to 95% of the wheat hectarage in Colorado, Kansas, Nebraska, Oklahoma, and Texas[3].

Standard curve: A standard curve was established to determine the concentration of imazapyr required to inhibit wheat shoot growth 50%. A loam soil with a pH of 6.4 and 1.5% organic matter was air dried, sieved through a 2 mm sieve, and treated with imazapyr at 0, 4, 8, 16, 31, 63, 125, 250, 500, and 1000 ppb. After imazapyr application, the soil was thoroughly mixed using a powder blender with internal beater bars, and 220 g of soil placed into 7 cm diameter styrofoam pots.

Four pregerminated 'Chisholm' wheat seed were planted per pot 1.3 cm deep. Pots were placed under fluorescent light providing 235 uE m^{-2} s^{-1} of photosynthetically active radiation with a 14 h photoperiod and subirrigated as needed. Plant height from the soil to the tip of the longest leaf were measured 14 days after planting. The experimental design was a randomized complete block with five replications. The experiment was repeated.

Cultivar bioassay: Based on data from the growth response

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[3]Smith, E. L. 1990. Personal communication. Agron. Dep., Okla. State Univ., Stillwater, OK 74078.

curve, the tolerance of 20 cultivars was determined by planting them in soil treated with imazapyr at 0 and 63 ppb. Soil was treated with imazapyr and pots prepared as described previously. Four pregerminated seed of each cultivar were planted per pot 1.3 cm deep. Pots were placed on the light table and treated as described previously. Shoot lengths were measured 14 days after planting. The experiments were replicated four times and repeated in time. Data were subjected to analysis of variance and means were separated using protected LSD's.

RESULTS AND DISCUSSION

Standard curve: There were no significant shoot length reductions with up to 31 ppb of imazapyr. As imazapyr concentration increased from 4 to 1000 ppb wheat shoot height decreased (Table 1). With imazapyr at 31, 63, and 125 ppb shoot height was reduced approximately 37, 47, and 65%, respectively. Imazapyr at rates above 125 ppb did not further reduce shoot height.

Cultivar bioassay: There were no differences between trials, therefore data were pooled. The 20 cultivars evaluated varied in tolerance to imazapyr. Plant height reductions ranged from 61.6% with 'TAM 200' to 44.0% with 'Karl' (Table 2). Although there were distinct differences between cultivars, the level of tolerance may not be sufficient to prevent damage from imazapyr at rates required to control field bindweed (1, 4, 5). Also, Chisholm, used to develop the growth response curve, was one of the most susceptible cultivars evaluated. Because the level of tolerance was not sufficient, experiments using _in vitro_ selection, seed mutagenesis, or transformation will be required to improve wheat tolerance to imazapyr to permit its use for selective control of field bindweed without reducing wheat grain yield.

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Table 1. Effects of imazapyr concentration on shoot height of Chisholm hard red winter wheat.

Imazapyr concentration	Height
ppb	cm
0	25.5
4	24.5
8	22.5
16	23.3
31	16.0
63	13.6
125	8.8
250	8.3
500	7.3
1000	6.8
LSD (0.05)	2.6

Table 2. Shoot length of 14 day old seedlings of 20 cultivars and % height reduction of plants grown in soil treated with imazapyr at 63 ppb.

Cultivar	Height of untreated	Height reduction
	cm	%
TAM 200	20.7	61.6
Mesa	18.8	58.6
Chisholm	22.2	57.3
Hawk	19.0	57.2
Vona	19.7	57.1
Pioneer 2180	17.0	56.8
Redland	19.5	56.6
TAM W-101	17.4	55.0
Brule	19.3	55.0
Sandy	20.1	54.9
Abilene	17.2	52.7
Pioneer 2157	18.8	51.8
Newton	16.6	51.7
Arkan	23.6	51.7
Siouxland	22.7	51.1
TAM 107	20.3	50.7
Larned	23.1	49.7
NK 812	19.2	46.6
Cimarron	19.9	46.5
Karl	21.9	44.0
LSD (0.05)	1.3	5.9

CHAPTER III

GROWTH RESPONSE OF WHEAT (*Triticum aestivum*) CALLUS TO IMAZAPYR AND *IN VITRO* SELECTION FOR RESISTANCE

Growth Response of Wheat (*Triticum aestivum*) Callus to Imazapyr and In Vitro Selection for Resistance.[1]

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Abstract. There were no differences between intact wheat plants and wheat callus response to varying concentrations of imazapyr. Fifty percent growth inhibition of wheat callus occurred with 0.05 and 0.10 uM imazapyr. Calli with growth rates exceeding a calculated upper prediction interval were obtained from selection experiments. As imazapyr concentration increased, the free pool content of isoleucine, leucine, and valine decreased from 123 to 53, 195 to 66, and 287 to 69 picomoles mg^{-1}, respectively. Comparison of a callus growing well to one growing poorly on 2.0 uM imazapyr showed an increase in total free pools of

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amino acids and an increase in the percent isoleucine, leucine, and valine. Plants have been regenerated and seed derived from these regenerates is currently being evaluated.

Nomenclature: Imazapyr, (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2yl]-3-pyridinecarboxylic acid; imazaquin, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2yl]-3-quinolinecarboxylic acid; wheat, *Triticum aestivum* L. cv. 'Bobwhite'.

Additional index words. selection, herbicide resistance, amino acids, imidazolinones.

INTRODUCTION

The use of in vitro selection with plant cell cultures to recover mutations that confer resistance to herbicides with low mammalian toxicity, a broad spectrum of activity, and environmental safety, is a relatively new approach to develop selective weed control strategies in crops (4). To implement this strategy the mode of action of the herbicide must be a fundamental biochemical process which is inherent to both cultured cells and whole plants. The long range goal of this research is to develop wheat lines tolerant to imazapyr to permit imazapyr use for control of established stands of field bindweed (Convolvulus arvensis) in wheat fields.

Imazapyr effectively controls field bindweed at 0.56 kg ha^{-1}, however wheat is not tolerant (2, 6). Imazapyr is a member of the imidazolinone herbicide family, which inhibit the synthesis of the branched chain amino acids isoleucine, leucine, and valine by inhibiting the activity of acetohydroxyacid synthase (1, 9, 12, 13, 14). Since the mode of action is a fundamental biochemical process, *in vitro* selection for mutations conferring imazapyr resistance has been successful in maize (*Zea maize*) (4, 12).

Maize cell cultures and intact plants respond in a similar way to imidazolinones (1, 12). *In vitro* selection for imazaquin resistant maize was successfully implemented by placing maize callus tissue on medium with low levels of imazaquin and increasing the imazaquin concentration as the growth rate of the callus increased (12).

The first objective of this research was to determine if the responses of wheat callus tissue and intact wheat plants to imazapyr were similar. The second objective was to characterize the growth and amino acid metabolism of callus subjected to *in vitro* selection.

MATERIALS AND METHODS

Callus versus whole plant responses. Bobwhite wheat callus was grown on Murashige and Skoog (10) basal medium, as modified by Sears and Deckard (11), with two percent sucrose and 2.3 uM 2,4-D. Imazapyr was filter sterilized and added after autoclaving to obtain 0, 0.01, 0.10, 1, 10, and 100 uM concentrations. Four pieces of callus, each weighing approximately 110 mg, were placed on each petri plate and maintained in the dark at 25°C. The tissue was sampled after 14 days for free pool amino acid analysis.

Whole plant responses to imazapyr were evaluated by using hydroponics. Wheat seedlings were grown in water for four days, to deplete the nutrient reserves in the endosperm, and then transferred to one-half strength Hoaglands solution (8). The same imazapyr concentrations used for the callus treatments were used for the hydroponic treatments. Seedlings were grown in a controlled environment chamber with a 25°C day/20°C night and a 12 hour photoperiod. After three days, 8 to 10 mm long root tips were removed for free pool amino acid analysis.

The experimental design was a randomized complete block with three replications. Free pool amino acids were extracted using the methods of Bieleski and Turner (3). Internal standards, of 50,000 pmol alpha-aminobutyric acid and 25,000 pmol norleucine were added to correct for experimental error during extraction and quantification. Further purification was obtained using cation exchange column (1) and 10,000 mw ultrafiltration. Amino acid analyses were performed by using pre-column derivatization with phenylisothiocyanate (5). Reverse phase high performance liquid chromatography was used to separate and quantify free pool amino acid derivatives in each sample (7). Data were subjected to analysis of variance and means separated using protected LSD's.

Establishment of growth inhibition curve. Bobwhite wheat callus was grown as previously described. Imazapyr concentrations of 0, 0.01, 0.05, 0.1, 1, 10, and 100 uM were used to determine which concentration inhibited callus growth by 50 percent. Growth rates were determined by recording calli fresh weights at the beginning of each transfer period. Calli were transferred to fresh medium every 14 days for five transfer periods. All tissue cultures were grown in the dark at 25°C. Data were subjected to analysis of variance.

In vitro selection for imazapyr resistance. Bobwhite wheat callus was grown as previously described. The 2,4-D concentration was reduced from 3.4 uM to 2.3 uM after the fourth transfer to allow differentiation of the callus tissue and eventual regeneration of plants. As the growth rate of the treated callus increased to near the growth rate of the control, the imazapyr concentration was increased. Tissue was sampled for amino acid analysis upon completion of the selection strategy. Free pool amino acids were analyzed as described previously. The experimental design was a randomized complete block paired across treatments with 10 replications and four subsamples per replication. Data were subjected to analysis of variance procedure and evaluation of individual growth rates was performed using prediction intervals (15).

RESULTS AND DISCUSSION

Callus versus whole plant responses. There were no imazapyr treatment by tissue type interactions, thus data were pooled across tissue type for each concentration of imazapyr. Because the response of wheat callus and intact plants was similar, *in vitro* selection of imazapyr resistance in wheat should be possible.

The free pool valine content decreased from 1060 in the untreated to 580 picomoles mg^{-1} with 10 uM imazapyr (Table 1). There was no decrease in the level of leucine or isoleucine. With imazapyr at 10 uM, there was an increase in phenylalanine. This data agrees somewhat with Anderson and Hibberd (1) who reported a decrease in valine and leucine but not isoleucine. Also free pool quantities of many amino acids were elevated. The differences between Anderson and Hibberd's results and ours may be attributable to their use of suspension cultures whereas we used callus cultures. Because suspension cultures are less organized and submerged in the treatment, response to imazapyr may be quicker.

Establishment of growth inhibition curve. As expected, growth rates of wheat callus cultures decreased with increasing imazapyr concentration after 14 days (Figure 1). Similar results were observed after five transfers (70 days), with little or no growth occurring in cultures growing on imazapyr at 1, 10, and 100 uM. Growth was inhibited about 50 percent with imazapyr at 0.05 and 0.1 uM imazapyr compared to the untreated. Based on these results *in vitro* selection experiments were initiated at 0.1 uM imazapyr.

<u>In</u> <u>vitro</u> selection for imazapyr resistance. At transfer four there was no significant difference in growth rate between the treated calli (35.0 mg day^{-1}) and the control (35.6 mg day^{-1}), therefore the imazapyr concentration was increased from 0.1 to 1 uM (Table 2). Growth rates between the treated and control calli were similar at transfer seven, so the imazapyr concentration was increased. The treated calli were split and a portion placed on medium containing 2, 5, and 10 uM imazapyr. At transfer 9 the differences in growth rates between the control calli and calli growing on imazapyr at 2, 5, and 10 uM had decreased (Table 2). Thus, at transfer 10 the 2,4-D concentration was reduced to 1.1 uM to prevent loss of embryogenic callus and enhance plant regeneration.

There were no differences in isoleucine, leucine, and valine content between the control callus and 2 uM imazapyr at the end of the selection (Table 3). However, there was a reduction of all three amino acids at 5 and 10 uM imazapyr when compared to the control and 2 uM.

The growth rate of two, five, and two calli at 0, 2, and 5 uM imazapyr, respectively, exceeded the upper prediction interval (Figure 2). At 10 uM, there were no calli with growth rates exceeding the prediction interval. A comparison of free pool amino acid content shows an increase in total free pool amino acid content of control callus compared to the resistant and the resistant to the susceptible calli (Table 4). The increase in total free pool indicates good growth. The mole percentages of isoleucine, leucine, and valine were similar in the control and the resistant calli, but were reduced 240, 130, and 230 percent, respectively, in susceptible tissue when compared to resistant tissue. This indicates increased production of these amino acids in the resistant tissue in the presence of what were initially lethal concentrations of imazapyr.

Plants were regenerated from calli growing on 1, 2, 5, and 10 uM imazapyr. Selfed seed were recovered from plants regenerated from 1 uM. These seed have been planted for seed increase and bioassays are being conducted to determine whole plant tolerance to imazapyr.

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Table 1. Free pool amino acid content of wheat callus and roots at five imazapyr concentrations[1].

Amino acid	Imazapyr concentration (uM) 0.0	0.01	0.1	1.0	10.0	LSD (0.05)
	picomoles mg^{-1}					
ASX[2]	2620	2430	3190	3680	1560	NSD
GLX[3]	2270	2460	3010	3190	2150	NSD
Serine	1230	1090	1250	1110	1120	NSD
Glycine	1890	1930	1540	1510	1300	NSD
Histidine	730	700	650	770	570	NSD
Arginine	5990	5140	4780	5970	3950	NSD
Threonine	430	390	420	440	370	NSD
Alanine	7740	9670	11850	8750	11410	NSD
Proline	2330	2640	3180	3160	1460	1130[4]
Tyrosine	280	320	330	360	270	NSD
Valine	1060	890	980	800	580	200
Methionine	230	240	240	300	260	NSD
Cystine	80	70	100	100	90	NSD
Isoleucine	410	380	410	430	400	NSD
Leucine	620	570	650	560	480	NSD
Phenylalanine	290	280	290	290	420	110
Lysine	1810	1760	2220	2240	2050	NSD

[1]Tryptophan not quantified; There was no callus by root interaction; therefore data are pooled over tissue type.

[2]ASX = pooled value for Asparagine and Aspartic acid.

[3]GLX = pooled value for Glutamine and Glutamic acid.

[4]LSD P=0.10; not significant at P=0.05

Table 2. Growth rate of wheat callus through 9 transfers to fresh media with various 2,4-D and imazapyr concentrations

Media situation				
	Concentration		Growth	
Transfer	2,4-D	Imazapyr	Control	Treated
	— uM —		— mg day^{-1} —	
1	3.4	0.1	initial weight	
2	3.4	0.1	20.7	21.9
3	3.4	0.1	23.4	22.6
4	3.4	1.0	35.6	35.0
5	2.3	1.0	36.9	35.5
6	2.3	1.0	71.4	60.4*
7	2.3	2.0	23.5	17.5*
8	2.3	2.0	29.1	14.1*
	2.3	5.0		12.1*
	2.3	10.0		13.8*
9	2.3	2.0	33.8	26.8*
	2.3	5.0		17.1*
	2.3	10.0		19.6*

*Significant difference ($P<0.05$) between treated and control tissue.

Table 3. Effect of imazapyr concentration in culture medium on isoleucine, leucine, and valine content of wheat callus after 10 transfers (130 days) to fresh media.

Imazapyr concentration	Isoleucine	Leucine	Valine
uM		picomoles mg^{-1}	
0	110	200	230
2	120	200	290
5	60	80	110
10	50	70	70
LSD (0.05)	50	90	110

Table 4. Comparison of free pool amino acid[1] content between control, resistant, and susceptible calli.

Amino acid	Control pmol mg^{-1}	Control Mol%	Resistant pmol mg^{-1}	Resistant Mol%	Susceptible pmol mg^{-1}	Susceptible Mol%	LSD (0.05)
ASX[2]	570	4.2	550	6.7	280	4.1	150
GLX[3]	1050	7.8	1080	13.1	340	5.0	500
Serine	240	1.8	280	3.4	120	1.7	30
Glycine	330	2.4	300	3.6	170	2.4	60
Histidine	210	1.6	130	1.6	60	0.8	80
Arginine	4190	31.1	1350	16.4	1900	27.7	630
Threonine	120	0.9	180	2.2	20	0.2	20
Alanine	3870	28.7	3440	41.8	3140	45.8	930
Proline	1670	12.4	300	3.7	400	5.9	390
Tyrosine	90	0.7	10	0.2	40	0.5	10
Valine	310	2.3	210	2.5	70	1.1	40
Methionine	40	0.3	10	0.1	20	0.3	10
Cysteine	20	0.2	10	0.1	10	0.2	NSD
Isoleucine	160	1.2	100	1.2	30	0.5	10
Leucine	260	1.9	100	1.2	60	0.9	20
Phenylalanine	100	0.7	50	0.6	20	0.3	10
Lysine	250	1.9	140	1.7	170	2.5	80
Total	13490		8210		6860		1030[4]

[1]Tryptophan not quantified.

[2]ASX = pooled value for Aspartic acid and Asparagine.

[3]GLX = pooled value for Glutamic acid and Glutamine.

[4]LSD for total P=0.10

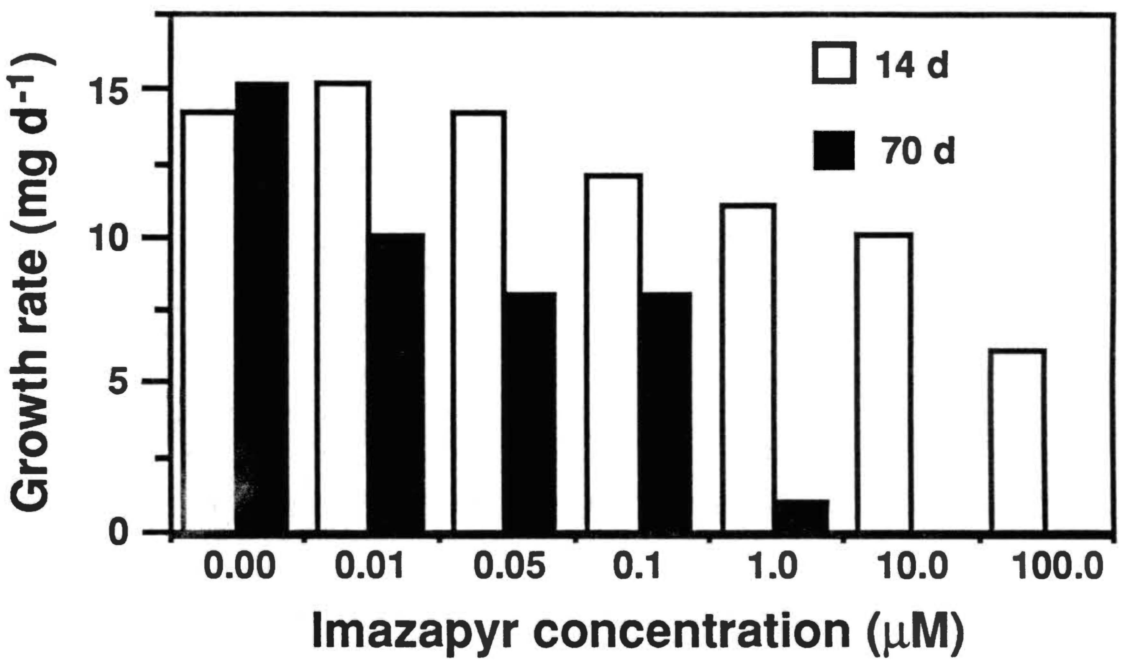

Figure 1. Growth rate of wheat callus 14 and 70 days after initiation on varying concentrations of imazapyr.

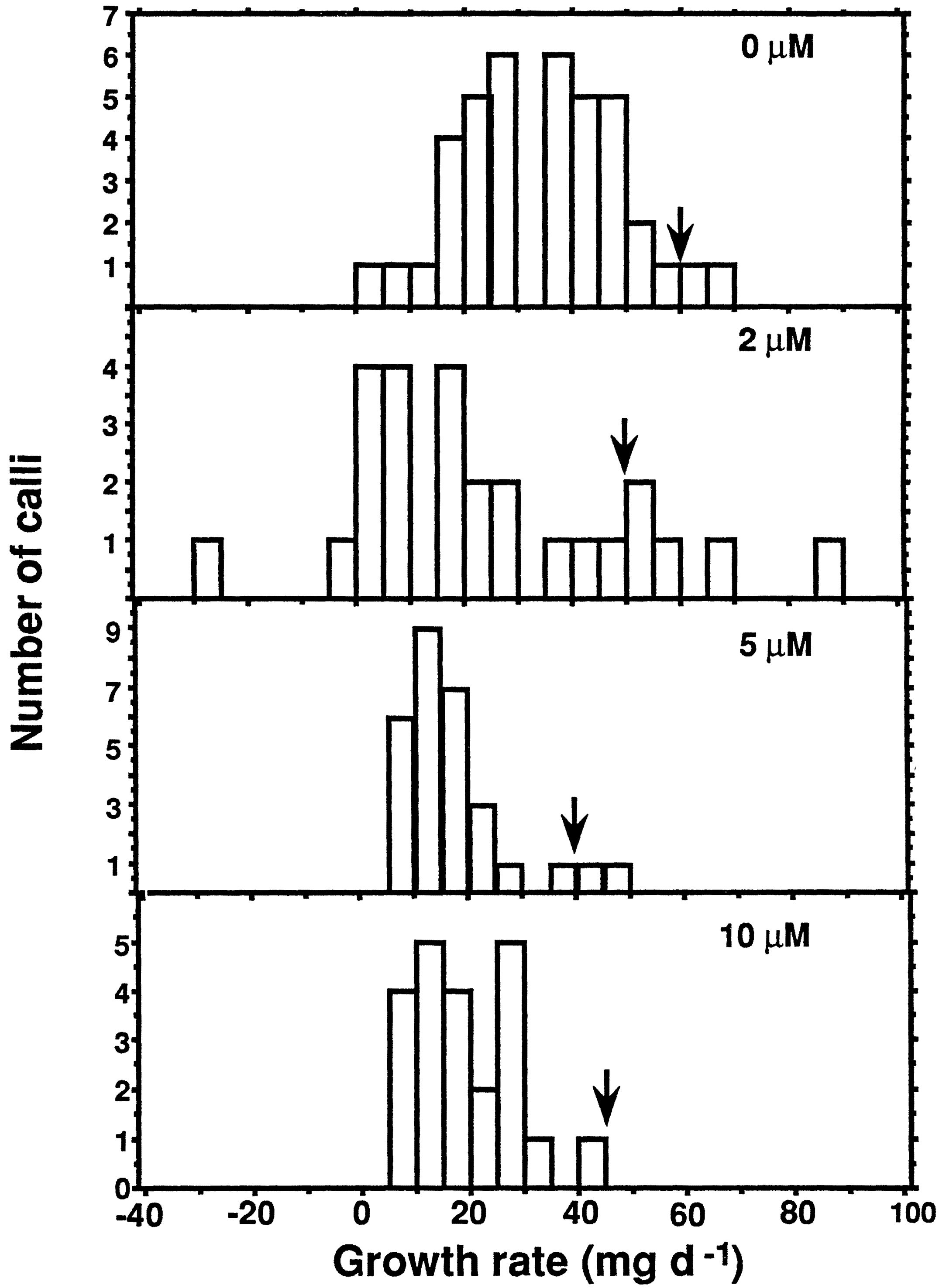

Figure 2. Growth rate distribution of calli surviving nine transfers to fresh media containing 0 to 10 uM imazapyr, bars to right of arrow indicate calli with growth rates exceeding the upper prediction interval.

VITA

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Candidate for the Degree of

Doctor of Philosophy

Thesis: INVESTIGATIONS ON THE POTENTIAL USE OF IMIDAZOLINONES FOR FIELD BINDWEED CONTROL IN WINTER WHEAT AND IN VITRO SELECTION FOR IMAZAPYR RESISTANCE IN WHEAT

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