AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2019 – 2021

TITLE: Using Nuclear Magnetic Resonance to Investigate the Mechanisms of Glufosinate Resistance in Italian Ryegrass from Willamette Valley

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EXECUTIVE SUMMARY:
Herbicide-resistant weeds are an increasing problem in Oregon's agriculture and beyond. Glufosinate-resistant Italian ryegrass is one of the multiple documented cases of resistance but requires further attention because glufosinate is an effective option to manage resistance to other herbicides. A glufosinate-resistant Italian ryegrass biotype from Oregon known to metabolized glufosinate was used in this study and compare to a susceptible biotype. We hypothesized that metabolism could be enhanced by pre-treatment with 2,4-D, a known cytochrome P450 inducer, and thus glufosinate-resistant plants exposed to 2,4-D would become more tolerant to glufosinate. The overall goal of this project is to investigate the mechanism of glufosinate resistance. Results from this work could provide important insights into the management and novel management approaches to herbicide-resistant weeds.

OBJECTIVES:
1) To study the response of glufosinate metabolism to stress inducers 2,4-D.
2) To develop a novel method to identify glufosinate metabolites in glufosinate-resistant Italian ryegrass from Oregon.

PROCEDURES:
Objective 1: Objective 2: 2,4-d pre-treatment

Two greenhouse experiments were performed in 2020. The glufosinate susceptible and resistant biotypes of Italian ryegrass were germinated in potting mix and individually transplanted into 36 cell plots. Plants were grown under natural light conditions. Uniform 3 leaf-stage seedlings were treated with herbicides using commercial formulations. Plants were treated with glufosinate (Rely 280) plus ammonium sulfate at two rates, 0.35 and 0.86 kg active ingredient ha^{-1}. Treatments with 2,4-D (Saber) at 2.13 kg acid equivalent ha^{-1} were applied at three-time points relative to the glufosinate application: 3 days prior, 1 day prior, and in tank-mix. A glufosinate only treatment and nontreated were included for comparison. Chlorophyll content and chlorophyll fluorescence (dark-adapted) were measured at 3 days after treatment. A visual estimate of plant control and above-ground biomass was recorded two weeks after treatment. The experiment was organized as a 2 by 9 factorial organized as a randomized complete block design. The first factor was the plant biotypes, and the second factor the treatments. The experiment included four replicates, and each replicates consisted of six plants of each biotype. The experiment was repeated. Data were submitted to ANOVA and means separated by Turkey's test.

Objective 2: This objective was not further pursued based on the results of the first objective.

SIGNIFICANT ACCOMPLISHMENTS:

Treatments tested significantly affected Italian ryegrass biotypes, but not interaction among treatment and biotypes was observed. Results are present were analyzed by biotype. The glufosinate rate was the most notable effect in both biotypes. Glufosinate reduced chlorophyll content compared to nontreated plant on both biotypes in a rate-dependent manner (Table 1). In general, higher chlorophyll content was observed in the resistant biotype. Glufosinate at 0.86 kg ai rate resulted in chlorophyll levels below 97 $mg.cm^2$ for either biotype, while chlorophyll content was above 135 $mg.cm^2$ when plants were treated with glufosinate 0.35 kg ai ha^{-1}. Treatments reduced chlorophyll fluorescence on both biotypes in a rate-dependent manner.

Glufosinate (0.35 kg ai ha-1) controlled the resistant biotype by 41 to 65%, and the susceptible biotype by 66 to 75% (Table 1). In either biotype, there was no effect of 2,4-D treatment. The control increased to 84 to 95% when plants were treated with glufosinate at 0.86 kg ai ha^{-1}, but no effect on 2,4-D was noted. The same trend was noted with biomass, indicating that 2,4-D did not influence the effect of glufosinate in both biotypes and rates tested.

Table 1. Italian ryegrass glufosinate-resistant (R) and susceptible (S) response to pre-treatment with 2,4-D.

		Chlorophyll		Fluorescence		Control		Biomass	
		$mg.cm^2$		(Fv/Fm)		%		g. $plant^{-1}$	
Treatment	2,4-D	R	S	R	S	R	S	R	S
nontreated	-	434 a	433 a	0.77 a	0.76 a	0 d	0 c	2.1 a	2.2 a
	-	258 b	114 bc	0.61 ab	0.45 b	41 c	75 b	0.89 b	0.53 b
glufosinate	3 d	168 bcd	157 b	0.52 bc	0.18 c	53 c	75 b	0.44 b	0.30 b
0.35 kg	1 d	213 bc	135 b	0.44 bc	0.38 b	65 b	74 b	0.57 b	0.23 b
	0 d	211 bc	142 b	0.60 b	0.41 b	42 c	66 b	0.65 b	0.46 b
	-	97 cd	50 c	0.40 bcd	0.19 c	84 a	95 a	0.56 b	0.29 b
glufosinate	3 d	21 d	11 c	0.17 d	0.17 c	92 a	92 a	0.25 b	0.14 b
0.86 kg	1 d	49 d	16 c	0.25 d	0.15 c	90 a	94 a	0.40 b	0.28 b
	0 d	77 d	43 c	0.36 cd	0.19 c	83 a	92 a	0.42 b	0.32 b

Means followed by the same letter are not statistically different according to Tukey's test ($P<0.05$).

BENEFITS & IMPACTS:

The most significant benefit from this work is that 2,4-D does not impact the performance of glufosinate on Italian ryegrass. Both herbicides are frequently used, and in certain tree crops like hazelnuts, used in tank-mixture, this finding reassures that growers can continue that practice. As for the resistance mechanism, these data strongly suggest that cytochrome P450 is not involved in the metabolism of glufosinate in the tested biotype. A previous study with certain Italian ryegrass populations from Oregon indicated that metabolism was involved in the resistance mechanisms (Brunharo et al. 2019), and glufosinate metabolism is also observed in many weed species (Everman et al. 2009).

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

None at this time

FUTURE FUNDING POSSIBILITIES:

REFERENCES

Brunharo CA, Takano HK, Mallory-Smith CA, Dayan FE, Hanson BD (2019) Role of glutamine synthetase isogenes and herbicide metabolism in the mechanism of resistance to glufosinate in *Lolium perenne* L. spp. *multiflorum* biotypes from Oregon. J Agric Food Chem 67:8431-8440

Everman WJ, Mayhew CR, Burton JD, York AC, Wilcut JW (2009) Absorption, translocation, and metabolism of 14C-glufosinate in glufosinate-resistant corn, goosegrass (Eleusine indica), large crabgrass (Digitaria sanguinalis), and sicklepod (Senna obtusifolia). Weed Sci 57:1-5