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Selectivity of the Premixtures Flufenacet, Diflufenican and Flufenacet, Diflufenican, Metribuzin on Bread Wheat (*Triticum aestivum* L.) and Barley (*Hordeum vulgare* L.) and Efficacy on ALS/ACCase-Resistant Populations of *Lolium rigidum* Gaudin

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Abstract: The premixtures flufenacet plus diflufenican and flufenacet plus diflufenican plus metribuzin are two herbicides recently registered in Greece for weed control in bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) with application early post-emergence to the crop (1st–3rd leaf growth stage). To evaluate the selectivity of these new herbicides, pot experiments were conducted by applying flufenacet plus diflufenican at 240 + 120 g ai ha^{-1} and flufenacet plus diflufenican plus metribuzin at 119.7 + 119.7 + 44.8 g ai ha^{-1} to bread wheat and barley, at 1st (BBCH-11), 2nd (BBCH-12) and 3rd (BBCH-13) leaf growth stage. The efficacy of the herbicides at the above-mentioned rates in pre-emergence application was also tested on three ALS/ACCase herbicide-resistant populations of *Lolium rigidum* Gaudin in comparison with the pre-emergence herbicides prosulfocarb and chlorotoluron plus diflufenican at 3200 g ai ha^{-1} and 1380 + 92 g ai ha^{-1}, respectively. The results revealed decreased selectivity of both premixtures when applied at BBCH-11 for both winter cereals, with flufenacet plus diflufenican being less selective compared to flufenacet plus diflufenican plus metribuzin. Both herbicides highly controlled the three herbicide-resistant *L. rigidum* populations. The results indicated that both premixtures are effective chemical options for the management of herbicide resistant *L. rigidum*. To ensure crop safety and optimize efficacy, application at BBCH-12 is recommended.

Keywords: rigid ryegrass; phytotoxicity; herbicide injury

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1. Introduction

Herbicide resistance is a threat to food production; in winter cereals, herbicide resistance is increasing [1]. Particularly for rigid ryegrass (*Lolium rigidum* Gaudin), herbicide resistance has made post-emergence (POST) control ineffective [2,3]. Globally, this weed has evolved resistance to 13 modes of action in 12 countries (Australia, Chile, France, Greece, Iran, Israel, Italy, Saudi Arabia, South Africa, Spain, Tunisia, USA) [1]. In Greece, the common practice for the control of *L. rigidum* in winter cereals is the post-emergence application of acetyl-CoA-carboxylase (ACCase) or acetolactate synthase (ALS) inhibitors. However, ACCase and ALS herbicides are associated with the rapid evolution of herbicide resistance. As a result, *L. rigidum* has evolved resistance to ALS and/or ACCase inhibitors in Greece [4], and many farmers have shifted to the pre-emergence (PRE) herbicides prosulfocarb and chlorotoluron plus diflufenican to manage *L. rigidum*. Taking into consideration the necessity of management of herbicide resistant weeds and the need for a shift to herbicide groups different to ACCase and ALS inhibitors, the soil applied herbicides prosulfocarb, chlorotoluron, flufenacet, metribuzin and diflufenican are alternative chemical options to manage *L. rigidum* with resistance to ALS/ACCase herbicides in winter cereals [5,6]. Sometimes weather conditions such as heavy rains before crop emergence may decrease the selectivity of pre-emergence herbicides, as it has previously happened with prosulfocarb plus s-metolachlor or prosulfocarb and chlorotoluron plus isoxaben in wheat [7,8]. Recently, in Greece, another two herbicides, namely the double premixture flufenacet plus diflufenican and the triple premixture flufenacet plus diflufenican plus metribuzin, have been registered for weed control in bread wheat and barley [9] and in bread wheat, durum wheat and barley [10], respectively, with pre- or early post-emergence application to weeds. The time of application regarding the crop is from 1st to 3rd leaf growth stage [9,10], which correspond to BBCH 11 and BBCH 13, according to the extended BBCH-scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) [11].

Flufenacet is an oxyacetamide residual herbicide applied pre- or early post-emergence. It controls grasses and certain broadleaf weeds acting on cell division by inhibiting the elongase enzyme that is essential for building very long chain fatty acids (VLCFA) in plants [12,13]. When soil-applied, it is absorbed by roots and shoots and translocates to the upper part of the plant [14]. Flufenacet has been used for the control of annual grasses in various crops such as maize, wheat, rice, cotton, sunflower, soybeans, tomato and potato, either alone or in mixtures with other herbicides [12,15]. It is considered moderately soluble in water and moderately mobile [16]. Flufenacet has been registered as a premix with metribuzin in maize and soybean [5,17]. Diflufenican is a pyridinecarboxamide herbicide that inhibits phytoene desaturase (PDS) in the carotenoid synthesis causing degradation of chlorophyll. When soil-applied, it makes a soil layer on the soil surface from where weeds can absorb it; once it is absorbed, it inhibits the phytoene desaturase production hindering the biosynthesis of carotenoid, which leads to the destruction of chlorophyll and cell membrane rupture with the consequent death of broadleaf weeds [18]. As a result, the herbicide causes bleaching and necrosis of the tissues, leading to plant death. It is applied pre- or early post-emergence in wheat and barley for the selective control of grasses and broadleaf weeds [12]. Diflufenican is absorbed principally by the shoots of germinating grasses and broadleaf weed seedlings that become bleached before tissue necrosis. It has a low aqueous solubility and is slightly mobile in soil [16]. Metribuzin is a triazinone chemical family of PS-II inhibitors. It is a pre- and post-emergence herbicide for the selective control of grasses and broadleaf weeds in cereals and a range of other crops [19–21]. It is predominantly absorbed by the roots and the leaves with translocation acropetally in the xylem [12]. It can be applied post-emergence in winter wheat to control grass and broadleaf weeds. Metribuzin has high solubility in water and is regarded mobile in soil with high leachability [16].

Herbicides should control weeds being selective and safe to crops without causing herbicide injury that may affect crop growth and yield production. Selectivity depends on the different response of the plants to the same herbicide [22]. Among selectivity factors, the capacity of the plants to metabolize/detoxify the herbicide is important [22,23]. The growth stage of both weeds and crops can affect herbicide performance in terms of its efficacy and selectivity [24,25]. The timing of herbicide post-emergence application is therefore crucial to increase crop tolerance and avoid any crop injury [26,27].

To our knowledge, there has been limited experimental data obtained on the selectivity of flufenacet plus diflufenican and flufenacet plus diflufenican plus metribuzin regarding the timing of their application to winter cereals. Moreover, there has been limited information published on the efficacy of these herbicide on the herbicide-resistant populations of *L. rigidum* of Greece. Therefore, the present study was conducted to evaluate the bread wheat and barley response to flufenacet plus diflufenican and to flufenacet plus diflufenican plus metribuzin applied early post-emergence (EPOST) at BBCH-11, BBCH-12 and BBCH-13 and, moreover, to evaluate their efficacy on three Greek populations of *L. rigidum* resistant to ALS/ACCase inhibitors in comparison with the pre-emergence herbicides prosulfocarb and chlorotoluron plus diflufenican. The information aims to providing knowledge and recommendation to the farmers on crop safety and control of *L. rigidum*.

2. Materials and Methods

2.1. Herbicide Treatments

Flufenacet plus diflufenican and flufenacet plus diflufenican plus metribuzin applied EPOST at BBCH-11, -12 and -13 were tested for their selectivity on bread wheat and barley (Table 1). Moreover, the above-mentioned herbicides along with prosulfocarb and chlorotoluron plus diflufenican applied PRE were evaluated for their efficacy against three *L. rigidum* populations resistant to ALS/ACCase inhibitors.

Table 1. Herbicide active ingredients (ai), grouped by HRAC (Herbicide Resistance Action Committee), mode of action (MoA), product information (trade name, type of formulation, % ai and manufacturer) and herbicide rates applied in this study *.

	Herbicide Ai	HRAC Group	Mode of Action (MoA)	Product Information	Herbicide Rate (g ai ha^{-1}) #
1.	flufenacet + diflufenican	15 + 12	Inhibition of very long chain fatty acid synthesis + Inhibition of Phytoene Desaturase	Fosburi 600 SC (40% + 20% ai) Bayer AG (Leverkusen, Germany)	240 + 120
2.	flufenacet + diflufenican + metribuzin	15 + 12 + 5	Inhibition of very long chain fatty acid synthesis + Inhibition of Phytoene Desaturase + Inhibition of photosynthesis at PS-II Serine 264 Binders	Herold Trio SC (17.1% + 17.1% + 6.4% ai) Bayer AG (Leverkusen, Germany)	119.7 + 119.7 + 44.8
3.	prosulfocarb	15	Inhibition of very long chainfatty acid synthesis	Boxer 80 EC (80% ai) Syngenta Crop Protection AG) (Basel, Switzerland)	3200
4.	chlorotoluron + diflufenican	5 + 12	Inhibition of photosynthesis at PS-II Serine 264 Binders + Inhibition of Phytoene Desaturase	Carmina Max SC (60% + 4% ai) Nufarm GmbH & Co KG (Linz, Austria)	1380 + 92

* Prosulfocarb and chlorotoluron plus diflufenican were tested only in the efficacy trial; # grams active ingredient per hectare.

2.2. Plant Material

Seeds of the Greek cultivars *Elisavet* and *Ippolytos* of bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), respectively, provided by the Institute of Plant Breeding and Genetic Resources were used to evaluate the selectivity of flufecanet plus diflufenican and flufenacet plus diflufenican plus metribizun applied at three different growth stages. Three populations of *L. rigidum* gathered from three locations from north of Greece with proven resistance to ALS and ACCase inhibitors [4] and a susceptible population of *L. rigidum* with susceptibility to ALS and ACCase inhibitors (Table 2) were used to study the efficacy of the two herbicides.

Table 2. Location of collection, population code and HRAC Group of herbicide resistance evaluated in the present study.

Location of Collection	Population Code	Resistance to HRAC Group
Volvi	S	no resistant (susceptible)
Arethousa	GR9	1 and 2
Drama	GR20	1 and 2
Kilkis	GR24	1 and 2

2.3. Selectivity Trial

A pot experiment was conducted in October 2022 and repeated in January 2023 under glasshouse conditions to study the selectivity of the premixtures flufenacet plus diflufenican and flufenacet plus diflufenican plus metribuzin on bread wheat and barley. Seeds of the cultivars *Elisavet* (bread wheat) and *Ippolytos* (barley) were seeded on different dates (with a week time interval) in pots (diameter 12 cm, height 20 cm) filled with sandy loam soil (52% sand, 14% clay, 34% silt, pH 7.9, Electric Conductivity (EC) 0.451 mS cm^{-1}, 1.2% organic matter content) to obtain plants at the 1st (BBCH-11), 2nd (BBCH-12) and 3rd (BBCH-13) leaf growth stage at herbicide application. After emergence, plants were thinned to five plants per pot at similar growth stage within each pot. When bread wheat and barley reached the above-mentioned growth stages, flufenacet plus diflufenican at 240 + 120 g ai ha^{-1} and flufenacet plus diflufenican plus metribuzin at 119.7 + 119.7 + 44.8 g ai ha^{-1} were applied the same day (Table 1). A boom AZO sprayer (AZO-sprayers, P.O. Box 350-6710 BJ EDE, The Netherlands) equipped with six nozzles (TeeJet Turbo TwinJet TTJ60-11002, TeeJet Technologies, Glendale Heights, IL, USA) calibrated to deliver 300 L ha^{-1} at 250 kPa was used for herbicide applications. At spraying, plant height was on average 5 cm, 11 cm and 19 cm for bread wheat and 7 cm, 12 cm and 22 cm for barley, at BBCH-11, -12 and -13, respectively. Pots were arranged on the glasshouse bench in a randomized complete block design separately for each cereal species and for each BBCH growth stage. There were untreated controls for each cereal species and for each BBCH growth stage. Each treatment was replicated six times. Plants were left to reach tillering stage (based on the untreated controls), which was almost 55 days after sowing (DAS) on average for both trials and for each growth stage at spraying; at that point, plant height was assessed for each treatment. Thereafter, plants were cut at ground level and air-dried, and then plant dry weight was recorded. Any injury symptom that appeared within the period from herbicide application to the end of each experiment was recorded. Pots were irrigated with the same amount of water as needed during the experiments. No artificial lighting or heating was used during the experiment.

Data were subjected to ANOVA for both plant height and dry weight separately for bread wheat and barley and separately for each growth stage at herbicide application. Data were analyzed as a two-way factorial experiment (time × herbicides). Since there was not significant interaction between time and herbicides, data were pooled over time. Means of data were separated using Fisher's protected LSD test at $p = 0.05$ level of significance. Means and standard errors are presented in the tables. GenStat Release 10.1 (10th Edition) Data Analysis Software was used for the data statistical analysis.

2.4. Efficacy Trial

A pot experiment was conducted in October 2022 and repeated after a week under glasshouse conditions to study the efficacy of flufenacet plus diflufenican, flufenacet plus diflufenican plus metribuzin, prosulfocab, and chlorotoluron plus diflufenican (Table 1) on three *L. rigidum* populations (GR20, GR9, GR24) originating from north of Greece from cereal fields with proven resistance to ALS and ACCase (Table 2). A susceptible (S) population originated from a field with olive groves from Volvi area north of Greece was also included in this study (Table 2). An untreated control of each population was used. Twenty seeds of each population were sown in pots (diameter 12 cm, height 20 cm) filled with the same soil used in selectivity experiment. All herbicides were applied 1 DAS with the boom AZO sprayer used in selectivity experiment at 300 L ha^{-1} at 250 kPa. Efficacy of herbicides was visually evaluated 3 weeks after sowing, taking into account the weed survival and growth and using a 0 to 100% scale, where 0% = no symptom, and 100% = dead plant, in comparison to the untreated controls. Pots were arranged in a completely randomized design with four replicates. No artificial lighting or heating was used during the experiment.

Data were analyzed as a two-way factorial experiment (time × herbicides) separately for each *L. rigidum* population. Since there was not significant interaction between time and herbicides, data were pooled over time. Data were subjected to ANOVA for each population, and means of data were separated using Fisher's protected LSD test at $p = 0.05$ level of significance. Means and standard errors are presented in the tables. The GenStat Data Analysis Software package was used for the data statistical analysis.

3. Results

3.1. Selectivity Trial

3.1.1. Selectivity in Bread Wheat

- Plant height.

Bread wheat treated at BBCH-13 revealed 31.7 cm and 30.4 cm height after the application of the triple and the double mixture, respectively, with no difference compared to the untreated control (32.1 cm). Plants treated at BBCH-12 and at BBCH-11 revealed significant height reductions; more specifically, at the end of the trials, plants treated with the triple and the double premixture at BBCH-12 reached 34.4 cm and 29.4 cm height, respectively, compared to 37.1 cm of the untreated control. At this point the effect of the double premixture was comparable to that of the triple premixture. Plants treated at BBCH-11 exhibited greater height reductions, with plant height of 23.4 cm and 16.9 cm for the triple and the double mixture, respectively, compared to 30.9 cm for the untreated control. The results revealed greater effect of flufenacet plus diflufenican on plant height compared to the triple mixture; that was also observed in plants treated at BBCH-12 (Table 3).

Table 3. Bread wheat height (cm) as affected by herbicides applied at BBCH-11, -12 and -13.

Treatment	Bread Wheat Growth Stage at Herbicide Application		
	BBCH-11	**BBCH-12**	**BBCH-13**
untreated control	30.9 a * (0.88) #	37.1 a (1.32)	32.1 (0.59)
flufenacet + diflufenican + metribuzin	23.4 b (1.90)	34.4 b (1.84)	31.7 (0.79)
flufenacet + diflufenican	16.9 c (1.58)	29.4 c (2.24)	30.4 (0.77)
LSD ## (5%)	3.26	1.97	ns **

* Means followed by the same letter within each column are not statistically different at 5% level of significance; # standard error of mean (in parenthesis); ## least significant difference; ** ns = not significant.

- Plant dry biomass.

Assessments in plant dry weight did not reveal differences between the untreated control and the herbicide-treated plants when herbicides were applied at BBCH-13. At this point, plant dry weight ranged between 0.122 and 0.124 g plant^{-1}. In contrast, significant dry weight reductions occurred when bread wheat was treated at both BBCH-12 and BBCH-11. Herbicide applied at BBCH-12 resulted in 0.161 g and 0.159 g plant^{-1} for the triple and the double mixture comparable to 0.202 g plant^{-1} of the untreated control, with no significant difference between the two herbicides. When bread wheat was treated at the younger growth stage (BBCH-11), significant reductions in plant dry weight were recorded particularly in plants treated with the double mixture. Plant dry weight was 0.139 g, 0.100 g and 0.079 g plant^{-1} for the untreated control, the triple and the double mixture, respectively (Table 4).

Table 4. Bread wheat dry weight (g plant^{-1}) as affected by herbicides applied at BBCH-11,-12 and -13.

Treatment	Bread Wheat Growth Stage at Herbicide Application		
	BBCH-11	**BBCH-12**	**BBCH-13**
untreated control	0.139 a * (0.0064) #	0.202 a (0.0254)	0.122 (0.0073)
flufenacet + diflufenican + metribuzin	0.100 b (0.0075)	0.161 b (0.0199)	0.124 (0.0050)
flufenacet + diflufenican	0.079 c (0.0059)	0.159 b (0.0254)	0.122 (0.0075)
LSD ## (5%)	0.0146	0.0205	ns **

* Means followed by the same letter within each column are not statistically different at 5% level of significance; # standard error of mean (in parenthesis); ## least significant difference; ** ns = not significant.

- Injury symptoms

Apart from plant stunting, injury symptoms consisted of bleaching symptoms that appeared 2–3 days after herbicide application. Symptoms were observed in all treated plants regardless of the time of herbicide application; however, they were slight on plants treated at BBCH-13 and more pronounced at BBCH-11; furthermore, symptoms were more evident in plants treated with flufenacet plus diflufenican compared to the triple mixture.

3.1.2. Selectivity in Barley

- Plant height.

Herbicide-treated barley at BBCH-13 revealed height of 33.1 cm and 32.9 cm for the triple and the double mixture, respectively, no different to the untreated control (33.1 cm). When herbicides were applied at BBCH-12, small but significant height reductions were observed between the untreated control (31.4 cm) and the triple and double herbicide-treated barley (30.0 cm and 29.3 cm). At this point, there was no significant different effect on plant height between the two herbicides. Height reductions were more pronounced when herbicides were applied at BBCH-11. At this point, the untreated control exhibited 32.7 cm height, and plants treated with the triple mixture showed 27.8 cm height, whereas those treated with the double mixture exhibited 22.6 cm. The results revealed significantly greater height reduction in flufenacet plus diflufenican compared to flufenacet plus diflufenican plus metribuzin when barley was treated at the younger growth stage (Table 5).

Table 5. Barley height (cm) as affected by herbicides applied at BBCH-11, -12 and -13.

Treatment	Barley Growth Stage at Herbicide Application		
	BBCH-11	**BBCH-12**	**BBCH-13**
untreated control	32.7 a * (0.52) #	31.4 a (0.56)	33.1 (1.24)
flufenacet + diflufenican + metribuzin	27.8 b (0.77)	30.0 b (0.35)	33.1 (0.92)
flufenacet + diflufenican	22.6 c (1.10)	29.3 b (0.53)	32.9 (0.85)
LSD ## (5%)	2.05	1.09	ns **

* Means followed by the same letter within each column are not statistically different at 5% level of significance; # standard error of mean (in parenthesis); ## least significant difference; ** ns = not significant.

- Plant dry biomass.

Assessments in plant dry weight did not reveal differences between the untreated control and the herbicide-treated plants when herbicides were applied at BBCH-13 and at BBCH-12. Plant dry weight ranged between 0.130 g and 0.143 g plant^{-1} and between 0.126 g and 0.135 g plant^{-1} when barley was sprayed at the 3rd and at the 2nd leaf growth stage, respectively. A reduction in selectivity occurred when herbicides were applied at BBCH-11, reducing plant dry weight to 0.110 g and 0.097 g plant^{-1} for the triple and double premixture, respectively, compared to 0.126 g plant^{-1} for the untreated control. The same effect was observed between the double and the triple mixture (Table 6).

Table 6. Barley dry weight (g plant^{-1}) as affected by herbicides applied at BBCH-11, -12 and -13.

Treatment	Barley Growth Stage at Herbicide Application		
	BBCH-11	**BBCH-12**	**BBCH-13**
untreated control	0.126 a * (0.0118) #	0.133 (0.0061)	0.142 (0.0065)
flufenacet + diflufenican+ metribuzin	0.110 ab (0.0105)	0.126 (0.0049)	0.130 (0.0033)
flufenacet + diflufenican	0.097 b (0.0140)	0.135 (0.0038)	0.143 (0.0054)
LSD ## (5%)	0.0181	ns	ns **

* Means followed by the same letter within each column are not statistically different at 5% level of significance; # standard error of mean (in parenthesis); ## least significant difference; ** ns = not significant.

- Injury symptoms

The injury symptoms were similar to those observed in bread wheat;, along with stunting, bleaching spots observed in all the herbicide treatments regardless of the time of herbicide application. Symptoms were more pronounced when herbicides were applied at BBCH-11 and on plants treated with flufenacet plus diflufenican.

3.2. Efficacy Trial

The GR9 and GR24 populations were totally controlled (98–100%), whereas the GR20 population was highly controlled (>90%) by all pre-emergence herbicides. Some plants that survived in GR20 population were severely injured and were unlikely to recover. The results revealed the high efficacy of both flufenacet plus diflufenican and flufenacet plus diflufenican plus metribuzin for the control of *L. rigidum* (Table 7).

Table 7. Efficacy (% control *) of four pre-emergence herbicides on a susceptible populations of *L. rigidum* and on three ALS/ACCase-resistant populations.

	Treatment	g ai ha^{-1}	*L. rigidum* Population Code			
			GR20	**GR9**	**GR24**	**S**
1	flufenacet + diflufenican + metribuzin	119.7 + 119.7 + 44.8	93 ^ (1.62) #	98 (0.50)	100 (0.25)	100 (0.20)
2	flufenacet + diflufenican	240 + 120	95 (1.34)	100 (0.18)	100 (0.26)	100 (0.30)
3	prosulfocarb	3200	91 (2.58)	100 (0.30)	100 (0.25)	98 (0.46)
4	chlorotoluron + diflufenican	1380 + 92	90 (2.67)	98 (0.50)	100 (0.26)	98 (0.50)
	LSD ## (5%)		ns **	ns	ns	ns

* Based on a 0 to 100% scale of efficacy (0% = no control, 100% = dead plants); ^ decimal numbers of means were rounded to the nearest whole number; # standard error of mean (in parenthesis); ## least significant difference; ** ns = not significant.

4. Discussion

Both herbicides exhibited decreased selectivity when applied at BBCH-11 in both bread wheat and barley. Presumably, plants treated at 1st leaf growth stage had a lower ability to metabolize the herbicides. Regarding the effect of the timing of herbicide application, increased phytotoxicity in wheat has been reported with EPOST treatments of metribuzin (0.43 to 0.69 kg ha^{-1}) compared to applications at full tillering stage as reviewed by [28]. Difference in herbicide selectivity has been also observed between PRE and POST applications of metribuzin in wheat; crop injury by metribuzin at 0.43 kg ai ha^{-1} was 90% in PRE application, compared to 30% and 20% injury when metribuzin treatment was delayed to two-leaf and three-tiller growth stage, respectively [29]. Moreover, metribuzin at 420 g ai ha^{-1} caused less injury to wheat when the herbicide was applied at tillering than at the two- or three-leaf stages [5]. In contrast, in another study, application of metribuzin at 105 g and 210 g ai ha^{-1} at PRE, two-leaf, early and late spring growth stage revealed the least wheat injury at early spring application [21]. Although the EPOST (12–15 DAS) application of flufenacet at 180–480 g ai ha^{-1} controlled *Phalaris minor* Retz., it caused phytotoxicity to wheat [28]. In general, these results indicated an increased injury when metribuzin and flufenacet were applied at younger growth stage of wheat.

Bleaching spots observed on plants treated with the double or the triple mixture were due to diflufenican herbicide, since bleaching is a typical injury symptom of a carotenoid inhibitor, whereas the height and dry weight reductions were possibly due to flufenacet and/or to metribuzin or to interaction between herbicides. Diflufenican, however, is rapidly metabolized by cereals, it can cause small transient phytotoxicity symptoms on leaves in the form of white/yellow spots streaks or bands, as those recorded in the present study [12,30]. Injury symptoms such as leaf burning, stunting and stand reduction were reported in wheat after metribuzin was applied at 420 g ha^{-1} early post-emergence at three to five tillers, however, with no effect on crop yield [20]. Another study reported combination of stunting and chlorosis injury symptoms on wheat after the pre-emergence application of flufenacet plus metribuzin [31]. As stated on the product label of flufenacet plus diflufenican plus metribuzin, transient injury symptoms in the form of chlorosis and retardation of growth may occur on plants, however, with no effect on crop yield [16].

Crops are enriched with detoxification mechanisms (enzymes of cytochrome P450-dependent hydroxylases and glutathione S-transferases or sugar conjugates with sugar) to metabolize and tolerate herbicides [23]. Crops detoxify flufenacet by glutathione (GSH) conjugation and by the formation of flufenacet oxalate [32,33] and metribuzin either via deamination and dethiomethylation or by glucoside and homoglutathione conjugations [34–36]. Moreover, crops such as wheat can tolerate metribuzin compared to some weed species such as downy brome (*Bromus sterilis* L.) due to their ability for more rapid metabolism of the herbicides and to less herbicide absorbance [34]. Regarding diflufenican, differential uptake between cereals and weeds along with the increased ability of the crops to metabolize the herbicide contribute to herbicide selectivity [12]. Diflufenican is rapidly metabolized via the nicotinamide and nicotinic acid to CO_2 [12].

Environmental conditions such as low temperature, rainfall after herbicide treatment and soil texture are other interactive factors that can affect the absorption, translocation, accumulation, metabolism and sequestration of herbicides leading to crop injuries and lower yield [28]. The effect of low temperatures on herbicide absorption and translocation, on plant growth and retardation of metabolism due to deviation from the optimal temperature range required by the enzymes to express their activity may decrease herbicide selectivity [37,38]. Previous studies have reported enhanced phytotoxicity of metribuzin in wheat under cold weather following herbicide application [19,20]. Reduced grain yield has been reported when wheat was treated with metribuzin at 280 g ha^{-1} at the four-tiller growth stage and after four days following application low temperatures of −20 °C existed for 2 consecutive days [39]. Rainfall or irrigation are likely to influence herbicide movement into the soil with effect on the crop related to the physicochemical properties of the soil. Flufenacet is moderately soluble in water and moderately mobile; diflufenican has low aqueous solubility and low leachability in soil, whereas metribuzin has high solubility and high leachability [16]. Metribuzin applied 0.42 to 0.56 kg ai ha^{-1} PRE on a sandy soil with 2% organic matter resulted in stunted and chlorotic plants along with a reduction in wheat stand by 20% (at 0.56 kg ai ha^{-1}) [40]. The effect of soil texture and rainfall on herbicide selectivity was highlighted in another study where higher wheat injury was observed after rainfall on a sandy loam than on a silty clay when metribuzin was applied PRE; lighter soil texture and rainfall resulted in higher wheat injury due to movement of metribuzin into the soil and to greater herbicide absorption by the emerging seedlings [29]. Herbicide injury in the form of minor stunting was observed in a fine sandy loam soil from flufenacet plus metribuzin at 0.480 + 0.12 kg ha^{-1} in wheat applied PRE, however, in this study wheat plants outgrew the stunting and recovered without grain yield loss [41]. Crop stunting (4 to 13%) has been reported in maize in soils with greater than 75% sand after the application of flufenacet plus metribuzin [42]. Low temperature may also affect herbicide efficacy; decreased absorption of metribuzin in downy brome (*Bromus tectorum* L.) and in jointed goatgrass (*Aegilops cylindrica* Host.) has been reported at cooler temperatures [43]. Herbicide selectivity and efficacy issues due to various soil types and adverse environmental conditions can make farmers cautious with the use of pre-emergence herbicides to control weeds. The soil used in the present study was sandy loam with low organic matter content; that might have contributed to some extent to the phytotoxicity observed on bread wheat and barley.

Comparing the two premixtures, the double premixture of flufenacet plus diflufenican was more aggressive to bread wheat and barley compared to the triple, although the latter contained the two herbicides plus metribuzin. The lower selectivity of the double premixture was possibly attributed to the higher recommended field rate of flufenacet (240 g ai ha^{-1}) compared to that of the triple (119.7 g ai ha^{-1}). Moreover, the lower selectivity of both herbicides observed in bread wheat compared to barley particularly when both herbicides were sprayed at BBCH-11 may be attributed to the small difference in growth between the two species at herbicide spraying. Although both species were at the same growth stage (BBCH-11 or BBCH-12) when herbicide-treated, barley plants were slightly taller (7 cm compared to 5 cm for bread wheat) presenting a slight step ahead in growth compared to bread wheat and that possibly affected herbicide tolerance.

Treatments at BBCH-13 for crop safety may come in contrast to the optimum timing of herbicide application for the effective control of *L. rigidum* and other weeds that emerge along with barley or wheat. Any delay to apply the herbicides (e.g., at BBCH-13) to avoid herbicide injury can decrease weed efficacy since weeds may manage to emerge and grow beyond the critical time of herbicide treatment. Flufenacet, diflufenican and metribuzin are specifically soil-acting herbicides acting more effectively pre-emergence. This was evident when flufenacet was applied in wheat 12–15 DAS and a failure to control broad-leaf weeds that germinated along with the crop was detected [28]. Another study revealed that the most consistent control of Italian ryegrass (*Lolium mulitflorum* Lam.) was achieved with pre-emergence treatments of the premixture flufenacet plus metribuzin compared to POST applications [5]. In general, delayed application of herbicides that are absorbed via roots and the hypocotyl shoots results in poor efficacy [44].

Regarding herbicide efficacy, the present study revealed the high control of ALS/ACCase herbicide-resistant *L. rigidum* when the premixtures were applied pre-emergence to weeds. The two premixtures exhibited similar efficacy to prosulfocarb and to chlorotoluron plus diflufenican. The latter two herbicides are very effective against *L. rigidum* or *Lolium perenne* L. and have been adopted by Greek farmers [8,45].

Furthermore, the herbicides tested have an additional advantage as mixtures of active ingredients; they can slow down the selection of herbicide-resistant biotypes [46,47] and broaden the range of weed species controlled [48,49]. The tank mixture of flufenacet plus metribuzin at 120 + 140 g ai ha^{-1} in wheat resulted in increased weed control, lower crop phytotoxicity and increased grain yield compared to flufenacet single application at 180 or 240 g ai ha^{-1} [28]. Another study showed that flufenacet plus triallate or plus pryroxaflutole provided consistently higher control level ($\geq$85%) of *Bromus diandrus* Roth. in wheat compared to single application of flufenacet [50]. Having in mind that *L. rigidum* can rapidly evolve cross-resistance to several wheat-selective herbicides and to several modes of action [6], the need for using herbicide mixtures instead of a single active ingredient is increasing. Intervention with new herbicides such the two premixtures of the present study can help delay or manage herbicide resistance. Furthermore, these new chemical options decrease the possibility of crop injury due to any unexpected heavy rains occurring before crop emergence, since they are applied EPOST to the crop.

The results of the present study lead to the recommendation of applying these herbicides at BBCH-12 to combine crop selectivity and optimize weed control. Although these two new herbicides were proved very effective against *L. rigidum*, integrated weed management should be applied, since cases of *Lolium* spp. with resistance to flufenacet, metribuzin, prosulfocarb and chlorotoluron have been already reported [6,51–54]. Finally, the results of the present study should be compared with field experiments that include data on crop yield. The present study was terminated 55 DAS and did not examine the effect of the herbicides on crop yield. Furthermore, field studies under different soil types and weather conditions should provide more information in terms of selectivity and the efficacy of the two herbicides studied, considering that both herbicides are marketed in a single application rate and any reduction in their field rate is not applicable [9,10].

5. Conclusions

The results of the present study indicate that flufenacet plus diflufenican and flufenacet plus diflufenican plus metribuzin can be used as alternative herbicides to manage the ALS/ACCase herbicide resistance of *L. rigidum*. Precautions, however, should be taken in terms of their selectivity to bread wheat and barley regarding the timing of their EPOST application; to ensure crop safety and optimize efficacy, application at BBCH-12 is recommended particularly for flufenacet plus diflufenican and when herbicides are applied to sandy soils. Reducing the risks of further evolution of herbicide resistance, farmers are encouraged to rotate these two herbicides with each other and/or with prosulfocarb or chlorotoluron plus diflufenican and to apply integrated weed management with crop rotations.

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