

Original Article: Interaction between Accase Inhibitors and Broadleaf Herbicides to the Control of Italian Ryegrass



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ABSTRACT

Management of Italian ryegrass is frequently done with tank-mix or sequential applications of herbicides. This study aimed to evaluate the interaction of haloxyfop-p-methyl or clethodim with 2,4-D, chlorimuron-ethyl, and cloransulam-methyl applied in a tank mix or sequential in the control of Italian ryegrass. The three experiments were performed in a factorial arrangement, and a randomized complete block was designed with four replications. Factor A consisted of the haloxyfop (62.35 g a.i. ha⁻¹), clethodim (108 g a.i. ha⁻¹) and chlorimuron (20 g a.i. ha⁻¹) (Experiment I); haloxyfop, clethodim and cloransulam (39.95g a.i. ha⁻¹) (Experiment II); haloxyfop, clethodim and 2,4-D (1005 g a.e. ha⁻¹) (Experiment III). Factor B consisted of tank-mix or sequential (same day) application. Treatments also included an untreated check and the isolated application of herbicides. Haloxyfop and clethodim alone, clethodim+2,4-D, haloxyfop+cloransulam, haloxyfop+chlorimuron, clethodim+cloransulam, clethodim+chlorimuron controlled Italian ryegrass. However, an antagonistic response of haloxyfop+2,4-D was observed. Based on this study, haloxyfop should not be associated with 2,4-D in the control of Italian ryegrass; however, the mixtures of ACCase+chlorimuron, ACCase+cloransulam, and clethodim+2,4-D were efficient in the control of the Italian ryegrass.

Introduction

The Italian ryegrass (*Lolium perenne* ssp. *multiflorum*), a weed found worldwide, is a significant problem in producing cereals and other crops (Preston *et al.* 2009). There are eight species in the *Lolium* genus, including *L. perenne* ssp. *multiflorum* (Terrell, 1968). The Italian ryegrass is an annual or biennial grass that can tolerate wide ranges of temperature and light regimes and adapt well to winter and springtime (Tehranchian *et al.* 2018). These characteristics offer high competition with other plants for

natural resources, such as nutrients, water, and sunlight (Bond *et al.* 2014).

In Brazil, Italian ryegrass is located southern region, and this species may cause an 18% to 56% decrease in production (Fleck *et al.* 1980; Trezzi *et al.* 2007). Besides, there are resistance reports to ALS (Acetolactate Synthase), EPSPs (5-enolpyruvylshikimate-3-phosphate), and ACCase (Acetyl CoA Carboxylase) inhibitors (Heap, 2020). This scenario increases the costs to control this species besides reducing control alternatives. The cost to control glyphosate resistance populations is around 48% higher than susceptible populations (Adegas *et al.* 2017).

The control options are limited in Italian ryegrass glyphosate-resistance populations. In this scenario, the ACCase inhibitors are the main herbicides for Italian ryegrass control (Grey et al. 2003). ACCase enzyme catalyzes acetyl-CoA's carboxylation to form malonyl-CoA, which fatty acids precursor (Kukorelli et al. 2013). When the ACCase enzyme is inhibited, the growing tissues present initial chlorosis, purplish color, with subsequent membrane damage (Délye, 2005; Kukorelli et al. 2013).

The problem worsens when Italian ryegrass and dicot weed species are present in the same cropping area, which is challenging to manage. In this case, herbicides association either in a tank mix or sequential application needs to be applied (Zhang et al. 1995; Damalas, 2004).

Once two or more herbicides are mixed in a tank mix, different interactions can occur between the herbicides (Colby, 1967). Sequential or tank-mix herbicidal applications can often present interaction issues that affect weed control efficacy by antagonistic effects (Colby et al. 1967; Leal et al. 2020, Leal et al. 2021). It can be attributed to changes in the amount of herbicide that reaches the site of action within plants through changes in its absorption/translocation or metabolism due to the presence of another herbicide (Damalas, 2004).

Several studies have reported antagonistic effects when graminicides are applied either sequentially or as a tank-mix with a broadleaf herbicide. The mixture of graminicides with ALS or 2,4-D herbicides has been reported to cause antagonistic effects in grasses (Matzenbacher et al. 2015; Rustom et al. 2018; Leal et al. 2021). Antagonism was observed between quilazafop and 2,4-D; haloxyfop and 2,4-D or haloxyfop and cloransulam in *D. insularis* (Osipe et al. 2020; Leal et al. 2020; Leal et al. 2021), chlorsulfuron or 2,4-D and diclofop in *L. rigidum* (Han et al. 2013), metsulfuron or 2,4-D and clodinafop (Trezzi et al. 2007) as well as atrazine and glufosinate (Ulguim et al. 2019) in *L. multiflorum*. However, few studies on the interactions between chlorimuron, cloransulam,

or 2,4-D and ACCase herbicides to control Italian ryegrass.

Therefore, the objective of this study was to evaluate the interaction of both haloxyfop or clethodim (ACCase inhibitors herbicides) with 2,4-D, chlorimuron and cloransulam applied in tank-mix or sequential in the control of Italian ryegrass.

Materials and Methods

Plant materials and growth conditions

Experiments were conducted at a greenhouse using Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) plants. Seeds of Italian ryegrass were sown in a 1L polyethylene pot with Planosol soil (Santos et al. 2013) with the following chemical characteristics: pH 5.53; organic matter 0.49 dag kg⁻¹; P and K, 8.0 and 41.0 mg dm⁻³, respectively. After emergence, plants were thinned to keep only one plant per plot. The soil was weekly fertilized with nitrogen, phosphorus, and potassium (NPK, 5-20-20) and irrigated daily to maintain the soil at field capacity.

Three experiments were performed in a factorial arrangement, and a randomized complete block was designed with four replications (Table 1). Factor A consisted of the haloxyfop-p-methyl (62.35 g a.i. ha⁻¹), clethodim (108 g a.i. ha⁻¹) and 2,4-D (1005 g a.e. ha⁻¹) (Experiment I); haloxyfop-p-methyl (62.35 g a.i. ha⁻¹), clethodim (108 g a.i. ha⁻¹) and chlorimuron-ethyl (20 g a.i. ha⁻¹) (Experiment II); haloxyfop-p-methyl (62.35 g a.i. ha⁻¹), clethodim (108 g a.i. ha⁻¹) and cloransulam-methyl (39.95 g a.i. ha⁻¹) (Experiment III). Factor B consisted of tank-mix or sequential (same day) application (Table 1). Additional treatment with the application of isolated herbicides was also included and an untreated check.

Herbicides applications were initiated when plants were at 3 to 4 tillers. All treatments were sprayed using a CO₂ pressurized backpack sprayer equipped with four TeeJet XR110015 flat-fan nozzles (TeeJet Technologies, Springfield, IL, USA), which delivered 150 L ha⁻¹ of spray solution at 2.8 bar.

Table 1. Treatment herbicides and doses applied in Italian ryegrass.

Treatments	Association Type	Dose g ha ⁻¹ (a.e/ a.i)
Experiment I		
Haloxypop-p-methyl	-	62.35
Clethodim	-	108
2,4-D	-	1005
Haloxypop-p-methyl + 2,4-D	Sequential	62.35 + 1005
Clethodim + 2,4-D	Sequential	108.0 + 1005
Haloxypop-p-methyl + 2,4-D	Mixture	62.35 + 1005
Clethodim + 2,4-D	Mixture	108.0 + 1005
Untreated	-	-
Experiment II		
Haloxypop-p-methyl	-	62.35
Clethodim	-	108
Chlorimuron-ethyl	-	20
Haloxypop + Chlorimuron	Sequential	62.35 + 20
Clethodim + Chlorimuron	Sequential	108.0 + 20
Haloxypop + Chlorimuron	Mixture	62.35 + 20
Clethodim + Chlorimuron	Mixture	108.0 + 20
Untreated	-	-
Experiment III		
Haloxypop-p-methyl	-	62.35
Clethodim	-	108
Cloransulam-methyl	-	40
Haloxypop + Cloransulam	Sequential	62.35 + 40
Clethodim + Cloransulam	Sequential	108.0 + 40
Haloxypop + Cloransulam	Mixture	62.35 + 40
Clethodim + Cloransulam	Mixture	108.0 + 40
Untreated	-	-

To the haloxypop-p-methyl and clethodim was utilized a mineral oil 0,5% (v/v) and chlorimuron was utilized a vegetal oil 0,5% (v/v).

Visual control analysis

Visual assessments of weed control were performed using a scale from 0 to 100%, which 0% as no symptoms and 100% plant death (Frans et al. 1986), at 14, 21, and 28 days after application (DAA).

Analysis of chlorophyll a fluorescence

Chlorophyll, a fluorescence transient, was measured at 7 and 14 DAA in leaves using a Handy-PEA fluorimeter (Tsimilli-Michael and Strasser *et al.* 2008). Intact young leaves with a fully expanded first leaf still attached to the plant were kept in the dark for at least 20 min in specially provided clips to conduct parameters measurements (Table 2). The plotted fluorescence values were the average of eight measures of each treatment.

Table 2. Photosynthetic parameters deduced by the JIP-test analysis of the fluorescence transients (Strasser *et al.* 2004; Yusuf *et al.* 2010)

JIP Test Parameters	
ϕE_0	Quantum yield for electron transport (ET)
ϕR_0	Quantum yield for reduction of end electron acceptors at the PSI acceptor side
ABS/RC	Absorption flux (of antenna chlorophyll) per reaction center (RC)
DI_0/RC	Dissipation of an active RC
PI_{ABS}	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
PI_{TOTAL}	Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors

Dry mass analysis

At 28 DAA, the shoot was separated and placed in a paper bag. For drying, they were placed in an oven with forced air circulation at $60 \pm 5^\circ\text{C}$ until they reached a constant mass (~ 72 h); they were weighed to determine dry mass.

Statistical analysis

The data were checked for normality, and then ANOVA was performed. Since no significant effect ($p > 0.05$) between the tank mix and sequential was verified, data from each experiment were combined. When ANOVA indicated significant treatment effects, the means were separated at $p \leq 0.05$ and adjusted using Tukey. Statistical analyses were performed using the SAS 9.0 Statistical Software Program (SAS Institute Inc., Cary, NC).

Results and Discussion

Interaction between tank mix and sequential treatment (Association Type) was not significant for visual weed control and dry mass; therefore, data were pooled. The highest percentages of visual control were observed for the plants that received the haloxyfop or clethodim alone, which provided 80% of Italian ryegrass control at 14 DAA and about 99% control at 21 and 28 DAA (Figure 1). However, in Italian ryegrass, plants that receive haloxyfop or clethodim associated with 2,4-D reduced injury level.

At 14, 21 and 28 DAA, haloxyfop+2,4-D resulted in 9%, 18% and 14% of Italian ryegrass control, respectively, whereas clethodim+2,4-D provided 61%, 75% and 88% control, respectively (Figure 1). The application of clethodim+2,4-D did not affect Italian ryegrass dry mass compared to clethodim alone, indicating no antagonism for this mixture. In contrast, haloxyfop+2,4-D increased in 125% of dry mass compared to haloxyfop alone, thus indicating antagonism (Figure 1 D).

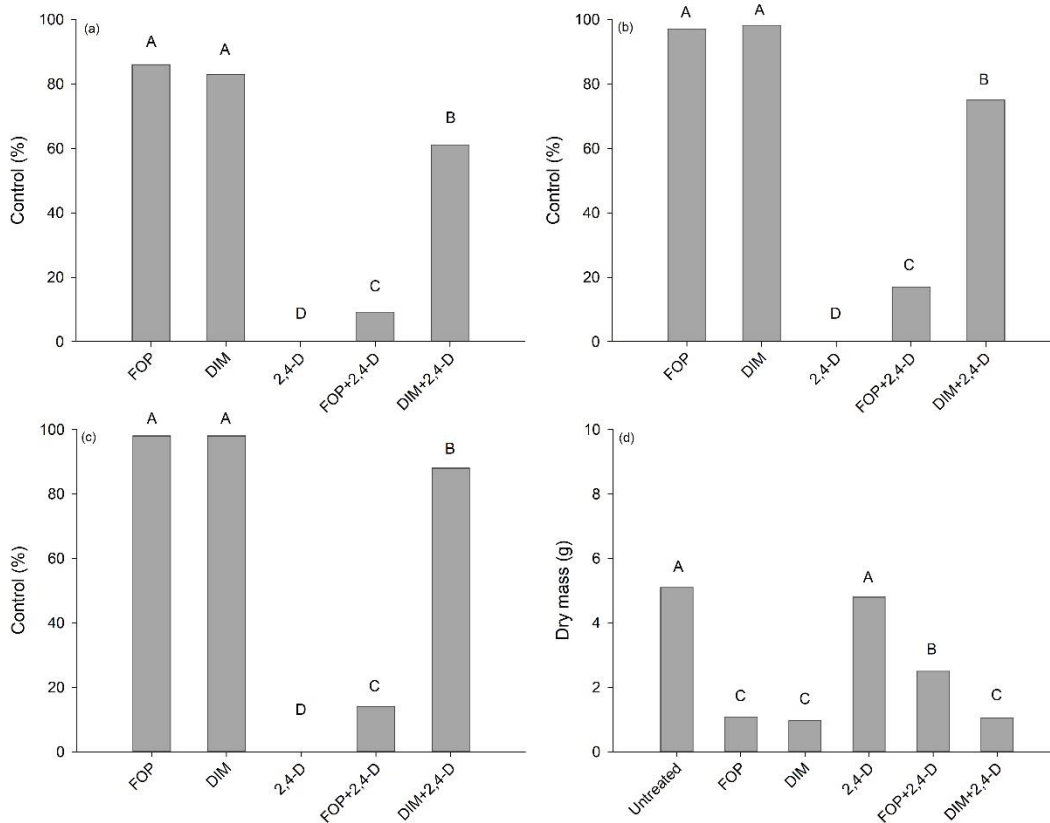


Figure 1. Plant control at 14(A), 21(B) and 28 (C) DAA and dry mass (D) for haloxyfop P-methyl (FOP), clethodim (DIM) and 2,4-D treatments applied to control *Lolium perenne* ssp. *Multiflorum*.

The 2,4-D-only treatment did not control Italian ryegrass because of selectivity of auxin herbicides as reduction translocation and/or rapid degradation of the exogenous auxin, alteration vascular anatomy of the dicot plants, or differential perception of auxin (Peterson et al. 2016).

Following previous studies, the pre-treatment using 2,4-D herbicide increased the metabolism of diclofop herbicide in *Lolium rigidum* (Han et al. 2013). Leal et al. (2020) indicated that the antagonism between 2,4-D and haloxyfop-P-methyl can be attributed to the increased metabolism observed in addition to the photosynthetic performance of sourgrass plants. Chl a fluorescence analysis presented an increase in photosynthetic performance (PI_{abs} and PI_{total})

in Italian ryegrass after 2,4-D application at seven and 14DAA (Figure 2). The fluorescence analysis is a technique that detects stresses caused by herbicides (Dayan and Zacaro, 2012; Sousa et al. 2014; Leal et al. 2020), as shown in this experiment.

In this study, haloxyfop+2,4-D, clethodim+2,4-D, haloxyfop, and clethodim treatments showed a reduction of the $\phi E0$ parameter (quantum yield of electron transport from QA- to the electron acceptor intersystem) and $\phi R0$ (Electron transport quantum yield of QA- for the final electron acceptor of the PSI) and, consequently, an increased heat dissipation (DI0/RC) and reduced in photosynthetic performance (PI_{abs} and PI_{total}) when compared to untreated, at 7 DAA (Figure 2 A).

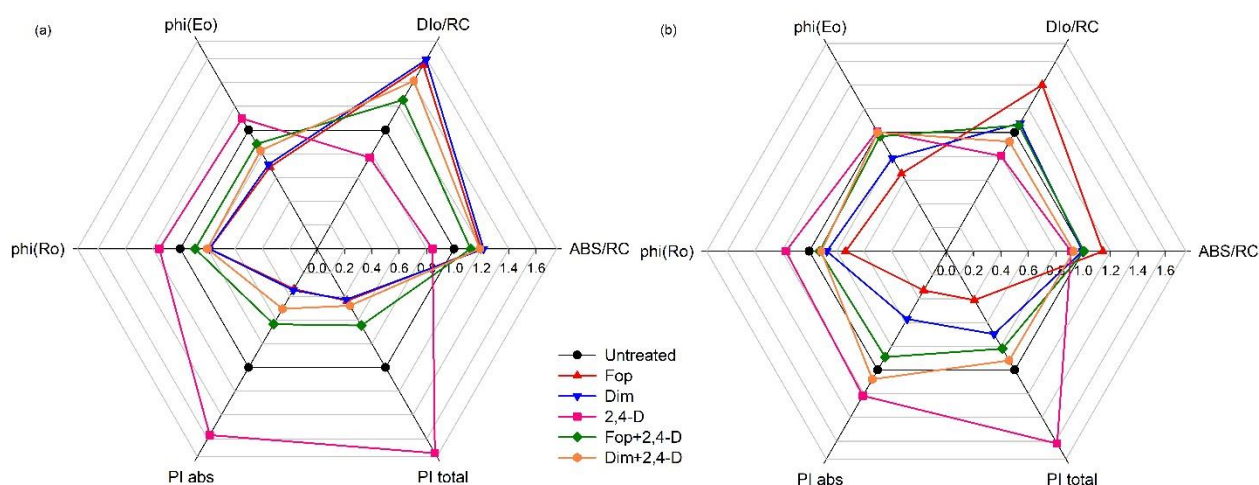


Figure 2. Chlorophyll a fluorescence transient of dark-adapted leaves for haloxyfop-P-methyl (FOP), clethodim (DIM), and 2,4-D treatments applied to control *Lolium perenne* ssp. Multiflorum at 7 (A) and 14DAA (B). Data correspond to the photosynthetic parameters deduced by the JIP-test analysis of the normalized fluorescence transients using the control reference. (n=8).

However, 14 DAA, clethodim, and haloxyfop alone provided 40% and 70% reduction in the photosynthetic performance, respectively, while the haloxyfop+2,4-D and clethodim+2,4-D exhibited the same behavior as the untreated plants. This shows that physiologically the graminicides in a tank mix with 2,4-D do not have the same effect on plant metabolism compared to the herbicide alone. These results confirm the decrease in the Italian ryegrass control after haloxyfop+2,4-D and clethodim+2,4-D application at 14DAA.

ACCase inhibitors herbicides are often antagonized when applied with other herbicides (Blouin et al. 2010; Han et al. 2013; Peterson et al. 2016, Leal et al. 2020; Leal et al. 2021). Our findings agree with previous studies that reveal the following antagonistic effects of tank-mix of clodinafop and 2,4-D in the control of *Lolium multiflorum* (Trezzi et al. 2007) and haloxyfop and 2,4-D in the control of *Digitaria insularis* (Leal et al. 2020).

The interactions between pesticides can occur before, during, or after herbicide application. These can interact physically or chemically in the spray solution or biologically in the plant (Zhang et al. 1995).

However, the antagonism effect may be maximized or minimized according to the interval between herbicide applications adopted in the management program (Trezzi et al. 2007, Leal et al. 2020). In this study, there was no interaction between the tank mix and sequential applications of the herbicides, suggesting that the reduced effect observed when applying 2,4-D + haloxyfop was not related to the incompatibility of the herbicide in the tank mix. Nevertheless, when incompatibility between herbicides occurs in spray solutions (tank mix), the sequential herbicide application might be an alternative. Our study showed that the sequential application on the same day was not enough to interrupt the antagonism between the herbicides, suggesting the need for a longer interval between the applications of herbicides to eliminate the antagonistic effects.

The mixture of chlorimuron+haloxyfop provided 70% and 85%, and haloxyfop alone provided 86% and 98% Italian ryegrass control at 14 and 21DAA, respectively, but there was no difference in the control and dry mass at 28DAA. The mixture of chlorimuron+clethodim showed similar control to clethodim alone at 14, 21, and 28DAA (Figure 3). In Italian ryegrass control, these results suggested no antagonism between chlorimuron and ACCase herbicides.

This same result was found by fluorescence analysis at seven and 14DAA, when all treatments showed a reduction of the parameters $\phi E0$ and $\phi R0$ and, consequently, a decline in photosynthetic performance compared to the untreated, except for chlorimuron application alone (Figure 4). Chlorimuron alone did not affect Italian ryegrass, which may explain the non-effects of this herbicide on the chlorophyll a

fluorescence in any of the evaluations (Figure 4). It suggests that fluorescence is a potential stress indicator that can indicate physiological disturbances after herbicide application.

Cloransulam+haloxyfop and cloransulam+clethodim treatment presented a reduction control in Italian ryegrass of 31-23% and 12-13% compared to the herbicides alone at 14 and 21DAA, respectively (Figure 5). However, the cloransulam+haloxyfop produced a neutral response at 28DAA while cloransulam+clethodim showed a 14% reduction control compared to clethodim alone (Figure 5). Dry mass analysis of both mixtures exhibited the same results visualized for herbicides alone (Figure 5). In addition, the results showed that the fluorescence parameters, PI_{abs} and PI_{total} , decreased in mixed herbicides, clethodim, and haloxyfop alone at 7DAA and 14DAA (Figure 6). In Italian ryegrass control, these results suggested no antagonism between cloransulam and ACCase herbicides.

Although mixing two or more herbicides can provide multiple benefits, such as a broad spectrum of weed control, costs reduction (Blouin et al. 2010), and improved resistance management, it is essential to understand the interaction between herbicides to offer effective control.

In this study, cloransulam+ACCase, chlorimuron+ACCase, and clethodim+2,4-D mixtures or sequential applications were effective in controlling Italian ryegrass (Figure 3 and 5).

Even though the association of ACCase inhibitors herbicides and cloransulam or chlorimuron do not have an antagonistic effect in the control of Italian ryegrass, control is slower compared to the ACCase inhibitors herbicides alone until 21 days after application. This information is relevant when there are plants in advanced stages of development or unfavorable environmental conditions, which may provide an advantage for the regrowth of these plants.

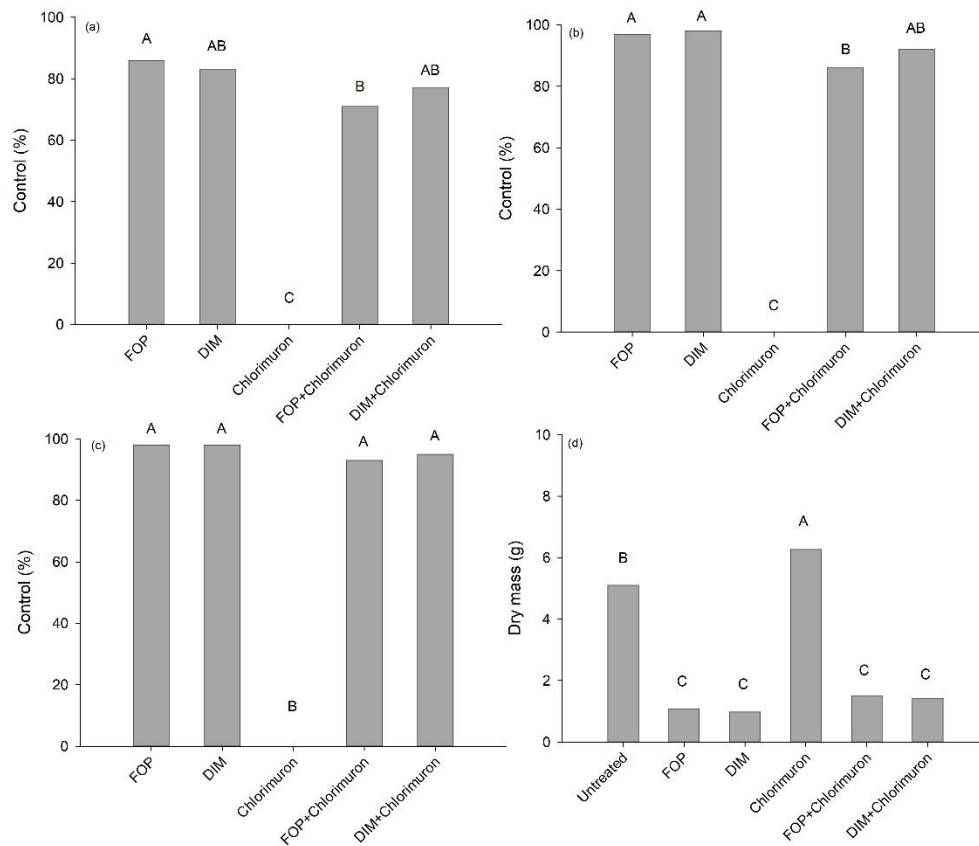


Figure 3. Plant control at 14(A), 21(B) and 28 (C) DAA and dry mass (D) for haloxyfop-P-methyl (FOP), clethodim (DIM) and chlorimuron treatments applied to control *Lolium perenne* ssp. *Multiflorum*

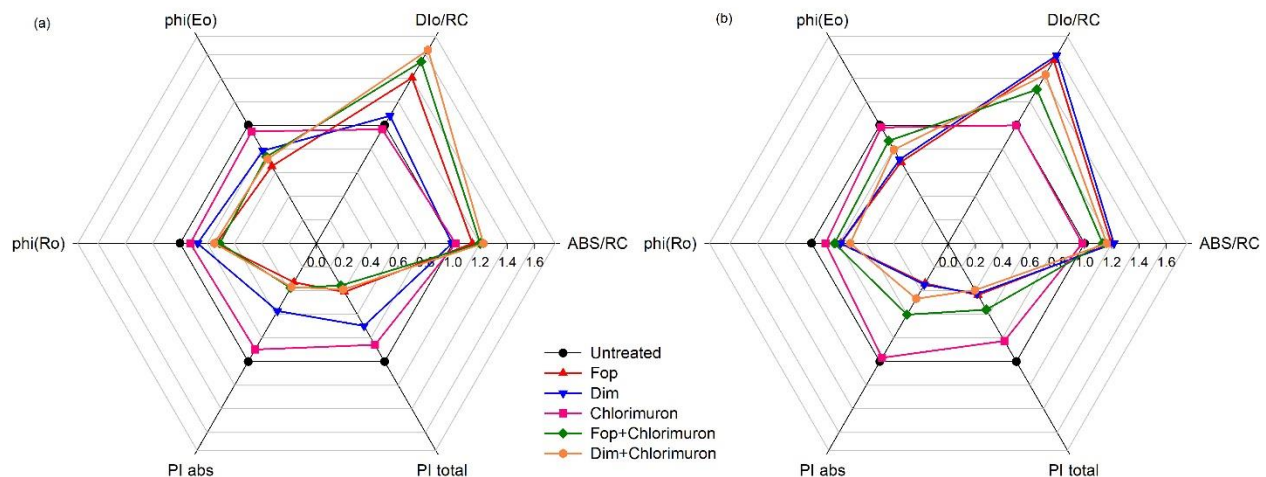


Figure 4. Chlorophyll a fluorescence transient of dark-adapted leaves for haloxyfop-P-methyl (FOP), clethodim (DIM), and chlorimuron treatments applied to control *Lolium perenne* ssp. *Multiflorum* at 7 (A) and 14DAA (B). Data correspond to the photosynthetic parameters deduced by the JIP-test analysis of the normalized fluorescence transients using the control reference. (n=8).

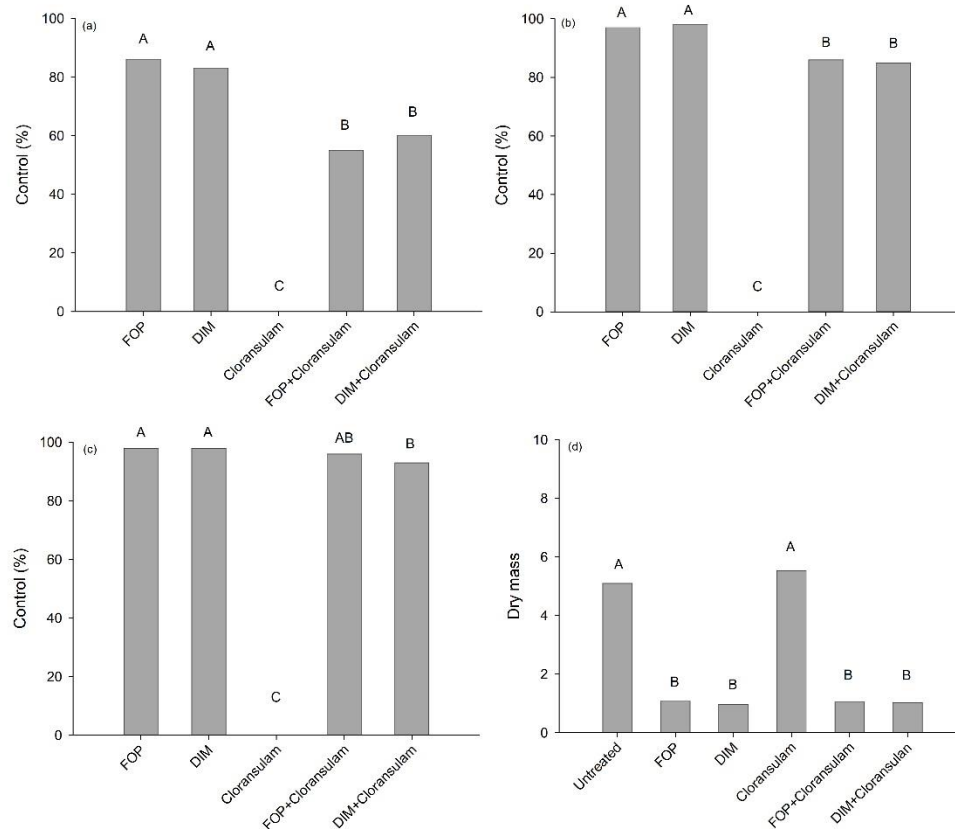


Figure 5. Plant control at 14(A), 21(B) and 28 (C) DAA and dry mass (D) for haloxyfop-P-methyl (FOP), clethodim (DIM) and cloransulam treatments applied to control *Lolium perenne* ssp. *Multiflorum*.

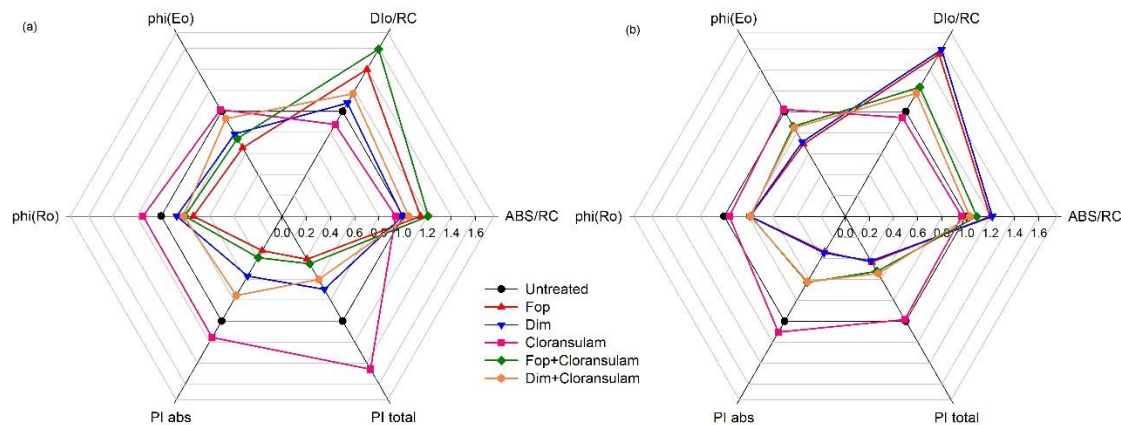


Figure 6. Chlorophyll a fluorescence transient of dark-adapted leaves for haloxyfop-P-methyl (FOP), clethodim (DIM), and cloransulam treatments applied to control *Lolium perenne* ssp. *Multiflorum* at 7 (A) and 14DAA (B). Data correspond to the photosynthetic parameters deduced by the JIP-test analysis of the normalized fluorescence transients using the control reference. (n=8).

Conclusions

Based on the results of this study, haloxyfop should not be applied associated with 2,4-D, due to a reduction in the control of Italian ryegrass; however, the mixtures of ACCase+ chlorimuron, ACCase+cloransulam, and clethodim+2,4-D are an excellent alternative to control this species, and broadleaf species when present in the same area.

Some future work could be suggested to obtain the best interval between the 2,4-D and haloxyfop herbicide applications to eliminate the antagonistic effects in Italian ryegrass.

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Conflict of interest

No conflicts of interest have been declared.

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