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PYRAFLUFEN-ETHYL AND FLORASULAM EFFICACY AGAINST GLYPHOSATE RESISTANT HORSEWEED (*Conyza canadensis*) BIOTYPES

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Abstract

The confirmation of glyphosate resistance (GR) biotypes of Conyza canadensis (horseweed) in orchards and vineyards in Greece highlighted the need of alternative methods and tools for the management of this important species. Herbicides with a different mode of action ought to be studied in terms of their efficacy against the herbicide resistant biotypes. The aim of this study was the efficacy evaluation of the herbicides pyraflufen ethyl, florasulam, MCPA and glyphosate, against several horseweed biotypes. Seeds from several biotypes of C. canadensis were sampled and sown in pots. Assessments of fresh and dry weight of horseweed seedlings were carried out 7, 14, 21 and 28 days after treatment (DAT). Pyraflufen ethyl alone or in combination with florasulam, resulted in an exceptional efficacy (>95%) against horseweed, even at 7 DAT. It was also noticeable that the mean accumulation of fresh weight in the treatments with pyraflufen ethyl was significantly reduced compared to the untreated plants (44 to 59% lower at 3 DAT). The findings of the present study reveal the high potential of pyraflufen-ethyl alone or with florasulam for the effective management and control of GR Conyza spp. biotypes. Further research on pot and field experiments is expected to highlight the dynamics of PPO inhibitors against GR horseweed.

Key words: *glyphosate, herbicides, horseweed, pyraflufen ethyl.*

INTRODUCTION

Conyza canadensis (L.) Cronq. (henceforth horseweed) is a summer annual weed species belonging to the genus *Conyza* (Travlos & Chachalis, 2013). The prolific fecundity of this species, with up to 200,000 seeds per individual plant (Palma-Bautista et al., 2020), the wide seed dispersal due to wind and the capacity of germination in a wide range of environmental conditions (Nandula et al., 2006) makes the management of this weed a serious challenge for farming systems globally. Nowadays, horseweed poses a major threat for the sustainability of orchards, vineyards and field crops (Weaver, 2001), especially in no-tillage or minimum tillage farming systems (Travlos et al., 2009).
In the globe and specifically in countries of the Mediterranean basin, farmers have reported several herbicide applications failures to control *Conyza* spp. (Travlos & Chachalis, 2013). This was mainly the result of over-reliance on glyphosate and subsequently the consecutive use of this herbicide as the only reliable solution for mitigation of horseweed infestations (Amaro-Blanco et al., 2018).
Glyphosate [N-(phosphonomethyl) glycine] is a non-selective, post-emergent, water-soluble and broad-spectrum herbicide (VanGessel, 2001), which blocks the shikimic acid pathway by inhibiting the 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS; EC 2.5.1.19) (González-Torralva et al., 2012).
This active ingredient has been characterized as the most important herbicide globally (Travlos et al., 2017b), while the last decades has been consecutively used in many agricultural and non-agricultural situations (Gonzalez-Torralva et al., 2010).
Nevertheless, the repeated applications of glyphosate, even 2-3 times in the same cultivation period, have contributed to the persistent evolution of glyphosate resistance cases in many weed species (Sansom et al., 2013). Currently, there are 48 glyphosate resistant weed species (GR) globally (Heap, 2020).

Therefore, alternative to glyphosate techniques and methods are required, in order to reduce the glyphosate reliance and mitigate GR evolution (Duke & Powles, 2008). Recently, extensive research focuses on long-term management of GR through evaluation of herbicide rotation regimes (Amaro-Blanco et al., 2018) or improvement of glyphosate applications through optimized use of adjuvants (Palma-Bautista et al., 2020), along with thorough research about the sustainability of farming systems through integration of novel integrated weed management techniques such as false seedbed (Kanatas et al., 2020a) and proper decision-making for weed management (Kanatas et al., 2020b).

Under this context, the scope of this study was the evaluation of the efficacy of alternative to glyphosate chemical herbicides against GR horseweed biotypes in Greece. This research included previously reported important active ingredients against dicot weeds, including pyraflufen ethyl, florasulam and MCPA. Pyraflufen ethyl is an effective, post-emergent, contact herbicide, inhibitor of protoporphyrinogen oxidase (PPO), which causes rapid necrosis in plant tissues and has been used against broad-leaf weeds in cereals and as desiccant in potato crop (Ivany, 2005; Miura et al., 2003). Florasulam is a post-emergent herbicide belonging to the triazolopyrimidine chemical family, inhibiting the acetolactate synthase enzyme (ALS) (Travlos et al., 2014). MCPA is a systemic, post-emergent herbicide used for the control of several annual and perennial weeds.

This is among the first researches to our knowledge that highlight several strands of alternative herbicide control in confirmed glyphosate-resistant *C. canadensis* in Greece. The objectives of this study were as follows: (1) to determine the susceptibility of GR horseweed biotypes at the rosette stage to chemical herbicides in comparison to untreated plants and plants treated with glyphosate; and (2) to evaluate the efficacy of pyraflufen-ethyl alone or in tank mix with florasulam, and MCPA plus florasulam against the GR horseweed biotypes.

MATERIALS AND METHODS

A pot experiment was conducted in a glass-greenhouse in the Agricultural University of Athens during September 2019. The prior activities to the herbicide treatments included the collection of seed samples of *C. canadensis* from perennial crops in Askri and Larisa region and then seed sowing. Overall, three horseweed biotypes, potentially glyphosate-resistant, were sampled from vineyards (A1, A2, A3) and two more from apple orchards (L1, L2).

For each weed population, 18 plastic pots were used. Each 11-cm-deep and 9.5-cm-wide pot was first filled with a mix of herbicide-free soil from the field of the Agricultural University of Athens and common peat substrate (1:1, v/v) and then 20 seeds were placed on their substrate surface. Following the sowing, pots were properly irrigated, organized into 5 groups (one for each population) of 6 pots each and maintained in the greenhouse. When the seedlings reached the rosette stage (7 to 12 cm diameter, 10 to 15 leaves), each pot of the aforementioned groups was treated with the herbicides reported in Table 1.

The plants were sprayed using a custom-built, compressed-air, low-pressure flat-fan nozzle experimental sprayer, calibrated to deliver 300 l ha^{-1} at 250 kPa.

Table 1. Herbicide treatments tested *in situ* for efficacy against glyphosate-resistant *Conyza canadensis* biotypes

Active ingredient	Treatment	HRAC group	Dose (g a.e. or a.i. ha^{-1})
Control	Untreated	-	-
Glyphosate	T1	G	1800*
Pyraflufen ethyl	T2	E	22
Pyraflufen ethyl + florasulam	T3	E + B	22 + 5
Pyraflufen ethyl (x/2) + florasulam	T4	E + B	11 + 5
MCPA + florasulam	T5	O + B	1200 + 5

g a.i. ha^{-1}, grams of active ingredient per hectare.
*g a.e. ha^{-1}, grams of acid equivalent per hectare.
HRAC, Herbicide-Resistance Action Committee; G, EPSPS inhibitors; B, ALS inhibitors; O, synthetic auxins; E, PPO inhibitors

Three pots from each group was left as untreated control. Fresh and dry weight assessments were conducted at 3, 7, 14, 21 and 28 days after treatment (DAT) with cutting of the above ground biomass of 5 individual plants per treatment. Samples were then dried at 60°C for 48 hours and their dry weight was recorded in order to determine the biomass and thus evaluate the efficacy of each herbicide.
The experimental design was a completely randomized design with a split-plot arrangement, and biotypes were the main plots and herbicide treatments were the subplots. The experimental data were analyzed using the STATGRAPHICS Centurion XVII Version statistical software (Statpoint Technologies Inc., The Plains, VA, USA). All data were subjected to multiple ANOVA. Treatment means were separated using Fisher's protected LSD test at $P<0.05$.

RESULTS AND DISCUSSIONS

The fresh and dry weight measurements revealed that there were significant differences among the treatments. According to the results, all surveyed horseweed biotypes were quite resistant to glyphosate, while the L1 biotype demonstrated the higher levels of resistance.

Table 2. Data records of dry weight of untreated and glyphosate treated *C. canadiensis* plants for the six populations (A1, A2, A3, L1 and L2) over time. Data are presented as the average weight of 5 samples for each treatment and population over time

Average weight (g)		Control	Glyphosate
		Dry weight	Dry weight
14 DAT	A1	0.51	0.24
	A2	0.52	0.48
	A3	0.77	0.67
	L1	0.52	0.47
	L2	1.10	0.98
21 DAT	A1	0.71	0.49
	A2	0.58	0.53
	A3	1.07	0.98
	L1	0.89	0.77
	L2	0.83	0.78
28 DAT	A1	0.39	0.26
	A2	0.83	0.77
	A3	0.83	0.75
	L1	1.25	0.94
	L2	0.91	0.81

Several horseweed plants survived glyphosate application at 14 and 28 DAT, indicating the glyphosate resistance of these biotypes (Figure 1). Although the glyphosate-treated plants managed to survive better than the pyraflufen-ethyl treated ones, the former displayed a reduction in their biomass over time when compared to the control, as estimated by using the dry-weight data presented in Table 2. In particular, four biotypes (A2, A3, L1 and L2) displayed resistance to glyphosate, while A1 was rather susceptible population.

Figure 1. Visual evaluation of glyphosate efficacy at 14 DAT

Regarding T5 (MCPA + florasulam), this treatment showed similar results to T1, indicating that this tank mix of post-emergent herbicides was not very effective for the control of GR horseweed. The mean accumulation of fresh weight at 21 DAT in T5 was only 19% lower than the glyphosate-treated plants (data not shown), while progressively the control was higher. This finding is in accordance with the results of Travlos et al. (2014) who recommended that florasulam in mix with penoxsulam provided long-term control of *C. canadensis* even four months after the application. Promising and relevant are also the results about florasulam when combined with aminopyralid against broad-leaf weeds in maize (Travlos & Apostolidis, 2017a).
Seedlings treated either with pyraflufen-ethyl alone or in tank mix with pyraflufen-ethyl and florasulam failed to survive 7 DAT, while the plant injury was visible even at 3 DAT (Figure 2).
Our data suggest that survival of *C. canadensis* was significantly affected by the contact herbicide pyraflufen-ethyl, which provided high control of the weed. This result is opposite to the conclusion of Shrestha et al. (2008) who stated that pyraflufen-ethyl provides partial control of *C. canadensis* and *C. bonariensis* in California.

Figure 2. Comparison of the several treatments at 3 DAT on A1 biotype

The factors of treatment and biotype had a statistically significant effect (P=0.0001, P=0.0472, respectively) on fresh weight of plants at 3 DAT (Figure 3).

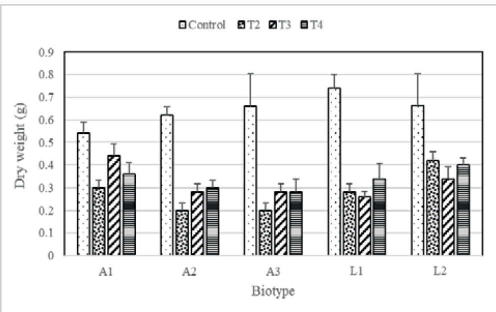

Figure 3. Comparison of the efficacy of pyraflufen-ethyl alone (T2) or in mixture with florasulam (T3, T4) to untreated plants at 3 DAT. The bars indicate standard errors of the means

There was a statistically difference between the untreated control and the treatments with pyraflufen-ethyl (T2, T3, T4) the same day. In particular, the mean accumulation of fresh weight in plants treated only with pyraflufen-ethyl (T2) was significantly reduced by 59% compared to the untreated plants. The application of pyraflufen-ethyl at recommended rate plus florasulam (T3) was strongly depended on biotype characteristics and led to moderate reduction of fresh weight accumulation between 26 and 71% compared to control, while the half rate of pyraflufen-ethyl plus florasulam (T4) provided only 44% mean biomass reduction 3 DAT (Figure 3). Nevertheless, the results of this study show that a dose of 11 g a.i. ha^{-1} of pyraflufen-ethyl, when this herbicide is combined with florasulam, provides adequate efficacy against GR horseweed, indicating that increasing the application doses give little advantage. Ivany (2005) recommended that an application rate of pyraflufen-ethyl between 10 and 15 g a.i. ha^{-1} can provide acceptable desiccation results in *Solanum tuberosum* cultivars. Our results are partially in agrrement to those of Amaro-Blanco et al. (2018) who concluded that pyraflufen ethyl at a rate of 22 g a.i. ha^{-1} can effectively reduce weed soil cover, while it doesn't provide satisfactory reduction of horseweed seed production.

Unfortunately, we can't rely on the discovery of new herbicide modes of action soon, so the integration of innovative herbicide mixtures and the adoption of herbicide rotation regimes are imperative (Heap & Duke, 2018). The evaluation and utilization of effective alternatives to glyphosate for the control of GR *C. canadensis* has been proposed by Sansom et al. (2013) and Mora et al. (2019). Under this context, our results suggest that the active ingredients pyraflufen-ethyl and florasulam may be important substitutes or additives to glyphosate. Another PPO inhibitor, oxyfluorfen, has been also reported to be effective against *C. canadensis* (Norsworthy et al., 2009). The application of various chemical herbicides is critical for horseweed, since this species' germination period is extended throughout spring and summer (Bajwa et al., 2016). The timing of treatment is important as well, because horseweed plants are more susceptible in younger rather than in more advanced growth stages (Shrestha et al., 2008). Finally, we propose the utilization of different herbicide modes of action and their appropriate rotation, along with adoption of more integrated weed management technologies, for the management of the noxious weed *C. canadensis* (Travlos et al., 2014; Palma-Bautista et al., 2020).

CONCLUSIONS

Of the herbicides evaluated, pyraflufen-ethyl provided 100% control of glyphosate-resistant *C. canadensis* 7 DAT when either applied alone or in combination with the ALS inhibitor, florasulam.

This study suggests that glyphosate resistant weeds can be managed through an integrated weed management scheme that includes novel tank mixtures with both contact and systemic herbicides.

The susceptibility of horseweed biotypes from annual and perennial crops to pyraflufen-ethyl poses a significant tool for mitigation of this species infestations.

Glyphosate was the least effective herbicide even when applied at high rate of 1.8 kg ha^{-1}, while the mix of MCPA with florasulam provides acceptable efficacy only 21 DAT.

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