Suppression of *Lolium multiflorum* Lam. with *Vicia villosa* Roth combined with residual herbicides

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ABSTRACT

Lolium multiflorum Lam. (annual ryegrass) is a problematic weed species especially in no-tillage systems of the southern region of Buenos Aires province (Argentina). Increasing herbicide resistance cases have exacerbated the problem, requiring alternative control options based on an Integrated Weed Management approach (IWM). Field experiments were conducted in the south area of Buenos Aires province during 2017-2019 in order to evaluate both the suppressive effect of *Vicia villosa* (hairy vetch) over annual ryegrass when combined with residual herbicides, and the tolerance of the former to such herbicides. Annual ryegrass stand density and biomass at crop termination were reduced from 2 - 98% in response to combined hairy vetch plus herbicide treatments. Among the best chemical options, pyroxasulfone, acetochlor and S-metolachlor should be mentioned, reaching weed biomass control values of 97, 81 and 76%, respectively. Hairy vetch was partially affected by herbicides with a plant stand reduction ≤ 48%. No significant effect on biomass at crop termination was observed, except for diflufenican and trifluralin. The inclusion of hairy vetch as cover crop combined with residual herbicides could be a practical IWM practice for suppression of annual ryegrass populations resistant to ACCase, ALS and EPSPS inhibitors.

Keywords: cover crops, weed management, legumes, chemical control.

RESUMEN

Lolium multiflorum Lam. (raigrás anual) es una especie de maleza problemática, especialmente en los sistemas de labranza cero en el sur de Buenos Aires (Argentina). El aumento de los casos de resistencia a los herbicidas ha exacerbado el problema, requiriendo opciones de control alternativas basadas en un enfoque de manejo integrado de malezas (MIM). Los experimentos a campo se llevaron a cabo en el área sur de la provincia de Buenos Aires durante 2017-2019 con el fin de evaluar tanto el efecto supresor de Vicia villosa (vicia villosa) sobre raigrás cuando se combinan con herbicidas residuales y la tolerancia de la vicia villosa a tales herbicidas. La densidad de plantas y la biomasa al final del raigrás anual se redujeron del 2 al 98% en respuesta al cultivo de vicia villosa combinado con los tratamientos con herbicidas. Entre las mejores opciones químicas, se debe mencionar a pyroxasulfone, acetochlor y S-metolachlor, alcanzando valores de control de biomasa de raigrás del 97, 81 y 76%, respectivamente. La vicia villosa se vio parcialmente afectada por los herbicidas con una reducción del stand de implantación de ≤ 48%. No se observó ningún efecto significativo sobre la biomasa en el momento de terminación del cultivo, excepto con diflufenican y trifluralina. La inclusión de vicia villosa como cultivo de cobertura combinado con herbicidas residuales podría ser una práctica de MIM para la supresión de poblaciones de raigrás anual resistentes a los inhibidores de ACCase, ALS y EPSPS.

***Palabras clave:** cultivos de cobertura, manejo de malezas, leguminosas, control químico.*

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INTRODUCTION

Lolium multiflorum Lam. (annual ryegrass) is one of the most conspicuous and invasive weed species in winter cereal crops worldwide. In Argentina, this weed affects more than 1.7 million ha of wheat fields producing yield losses of 20-30% at a density of 100 to 250 plants m^{-2} (Scursoni *et al*., 2012, 2014). In Argentinian wheat crops, annual ryegrass management is strongly dependent on pre and post-emergent herbicides, such as diclofop-methyl, clodinafop-propargyl, pinoxaden, iodosulfuron-mesosulfuron plus metsulfuron-methyl, pyroxsulam plus cloquintocet-mexyl, flucarbazone sodium and flumioxazin (Gigón *et al*., 2017). In addition, chemical-based management has become increasingly complex and expensive due to the occurrence of multiple resistance biotypes, mainly to EPSPS, acetyl-CoA carboxylase (ACCase) and/or acetolactate-synthase (ALS) inhibitors (Heap, 2019). In addition, herbicide 'escapes' or delayed emergence of annual ryegrass can easily replenish the soil seedbank due to its high fecundity (González-Andujar and Fernández-Quintanilla, 2004). Chiefly, as a result of the increment of resistance cases, Argentinian farmers are urgently seeking for alternative management options to reduce both economic losses and environmental externalities (Gigón *et al*., 2017; Scursoni *et al*., 2019).

From an Integrated Weed Management (IWM) perspective, cultural management practices can help to increase crop competitiveness (Cornelius and Bradley, 2017). In this context, the inclusion of cover crops (CC) is a viable alternative for weed suppression in agricultural rotations. Cover crops can potentially generate two distinct weed suppressive effects, by competition during their active growing period, and also due to allelopathic and/or mulching effects generated by vegetal residues that could inhibit or delay germination of many weed species in the field (Pittman *et al*., 2019).

Hairy vetch (HV; *Vicia villosa* Roth) is an annual legume, very well adapted to temperate areas of Argentina (Renzi *et al*., 2019). The inclusion of HV within cereal crops rotations could benefit soil fertility due to its nitrogen fixation capacity. In addition, HV has been proposed as a 'good CC candidate' for weed suppression in semiarid environments (Renzi and Cantamutto, 2013; Akbari *et al*., 2019). A negative aspect for the use of HV as CC is its low initial growth rate (Holderbaum *et al*., 1990) which might derive into an incomplete suppression of highly invasive weeds, such as annual ryegrass. Thus, the use of HV could be improved if combined with the application of residual herbicides during autumn before wheat sowing. So far, no studies have been conducted to evaluate neither the suppressive effect of HV nor its tolerance to commonly used residual herbicides for annual ryegrass control in the southern region of Buenos Aires province (SBA). Therefore, the objectives of this study were to evaluate: (i) annual ryegrass suppression by HV combined with autumn-applied residual herbicides, and (ii) the tolerance of HV to such herbicides.

MATERIALS AND METHODS

Study sites

Field experiments were conducted on cereal crop fields of the SBA from 2017 till 2019. The experiments were performed at Coronel Dorrego (-38.832°S, -61.267°W) during 2017, Coronel Suárez (-37.444°S, -61.811°W) in 2018 and at Coronel Suárez, Coronel Pringles (-38.074°S, -61.158°W) and Claromecó (-38.803°S, -60.164°W) during 2019.

The soils at the experimental area are Typic argiudolls. Soils samples (0-20 cm) were taken in each experimental field and subjected to typical soil routine analysis. For C. Dorrego, the soil was a sandy loam, slightly alkaline (pH = 7.5), low organic matter (2.0%) and high in phosphorus (P) content (17.2 ppm P Bray & Kurtz). For C., the soil was a silty clay loam, neutral (pH = 6.8), medium OM (4.0 %), and high in phosphorus (P) content (18.0 ppm). For C. Pringles, the soil was a silty clay loam, neutral (pH = 7.2), medium OM (3.0 %), and high in phosphorus (P) content (19.0 ppm). For Claromecó, the soil was a sandy clay loam, neutral (pH = 6.4), medium OM (3.8 %), and high in phosphorus (P) content (26.2 ppm).

Experimental design

Hairy vetch cultivar 'Patagonia INTA' was sown at 25 kg pure live seed ha^{-1} in rows 17.5-cm apart at a seeding depth of 20-30 mm under no tillage system. Such a sowing rate is the typical density used by farmers. HV sowing was performed during autumn at all experimental fields, on June 7th, 2017 (C. Dorrego), March 3rd, 2018 (C. Suárez) and March 25th, 27th and 29th 2019 in C. Suárez, C. Pringles and Claromecó, respectively. Seeds were inoculated with the appropriate (commercially available) rhizobium group in a peat-based inoculum (*Rhizobium leguminosarum* bv *viciae*). Herbicides were applied one week before crop sowing in all pre-sowing cases.

The specificity of each experiment is provided below:

Experiment 1. A factorial design including twelve residual herbicides applied on HV pre-sowing (May 30th, 2017) (table 1) under high and medium annual ryegrass infestations (867 and 373 plants m^{-2}) was evaluated in C. Dorrego.

Experiment 2. A factorial design including seven residual herbicides applied at HV pre-sowing or pre-emergence (February 26th and March 3rd, 2018 respectively) (table 1) under a low annual ryegrass infestation level (66 plants m^{-2}) were studied in C. Suárez.

Experiment 3. A completely randomized design (n=3) was implemented to study the tolerance of HV to the three most promising pre-emergent herbicides selected from EXP 1 and 2 (table 1). This experiment was performed during 2019 at three localities (C. Suárez, C. Pringles and Claromecó).

Experiments 1 and 2 were designed following a randomized complete block design with a split-plot arrangement (EXP 1: herbicides x *L. multiflorum* densities; EXP 2: herbicides x application timing). Main plots were herbicide treatments. Subplots were annual ryegrass densities and application timing in EXP 1 and 2 respectively. EXP 1 and 2 were carried out in 4 × 20 m plots, and EXP 3 in 3 × 5 m. Eleven herbicides were evaluated in EXP 1, seven in EXP 2, and three in EXP 3 (table 1) plus the untreated control (without herbicide). In addition, in EXP 1 a treatment without HV nor herbicide was included under a high level of weed infestation.

Herbicides were applied using self-propelled sprayers equipped with XR 8002 flat-fan nozzle tips (140 L ha^{-1} at 117 KPa and 6 km h^{-1}). HV was terminated with a rolled crimper previous to corn sowing in mid-November (EXP 1) and with glyphosate+2.4-D amine previous to sunflower seeding in mid-September (EXP 2).

Data collection

In Experiments 1-2, the stand of both HV and annual ryegrass established plants were assessed 4-5 weeks after sow-

Herbicide	Trade name	Formulation	Rate (g ai ha^{-1})	EXP	Application Time
Acetochlor	Trophy	90%, EC	900	1,2,3	PRES/PREE
Diflufenican	Brodal 50 SC	50%, SC	75	2	PRES/PREE
Flumioxazin	Sumisoya Flo	48%, SC	48	1,2	PRES/PREE
Imazamox	Sweeper 70DG	70%, WG	56	1	PRES
Imazapyr + Imazethapyr	Lightning	52,5% + 17,5%, WG	52	1	PRES
Imazethapyr	Pivot	10%, SL	100	1	PRES
Linuron	Linuron 50	50%, SC	500	1	PRES
Metribuzin	Sencorex 48	48%, SC	240	1	PRES
Oxyfluorfen	Koltar EC	24%, EC	192	1	PRES
Pendimethalin	Armadox 33E	33%, EC	660	1	PRES
Pyroxasulfone	Yamato	85%, WG	85	2,3	PRES/PREE
Pyroxasulfone + Flumioxazin	Yamato + Sumisoya Flo		48 + 85	2	PRES/PREE
S-metolachlor	Dual gold	96%, EC	960	1,2,3	PRES/PREE
Sulfentrazone	Authority	50%, SC	200	1	PRES
Trifluralin	Premerge	60%, EC	1080	2	PRES/PREE

Table 1. List of pre-sowing and pre-emergent herbicides used in the different experiments PRES: pre-sowing and PREE: pre-emergent.

ing (WAS) using 0.5 m^2 quadrats randomly distributed in each plot (n=4). HV and annual ryegrass biomass at crop termination were assessed at random using 0.16 m^2 quadrats (n=3). In Experiment 3, HV stand and biomass were evaluated after five and thirteen weeks after herbicide application (WHA). Samples were oven-dried at 65°C for 72 h. Percent stand and biomass reduction values were calculated by dividing the differences between treated and untreated plots by the untreated plot values.

The *L. multiflorum* seedling densities and biomass were plotted as a function of *L. multiflorum* and HV biomass, respectively. A regression analysis was performed using GraphPad Prism Software version 6.0 (GraphPad, San Diego, California, USA).

Weather data from each experiment were recorded with a meteorological station located < 1 km. Monthly rainfall totals and average monthly temperature for each year are presented in table 2.

Statistical analysis

The analyses of variance (ANOVA) were performed using InfoStat software (Di Rienzo *et al*., 2013). For analyses, the percent stand and biomass reduction were arcsine-square root transformed, and the untransformed data are presented in the tables for clarity. Regression analysis was performed to determine the significance of the relationship between *L. multiflorum* density and biomass, and between annual ryegrass and HV biomass. Goodness of fit values was performed using the normalized root mean square error (NRMSE) (Araya *et al*., 2017).

RESULTS

As observed in table 2, registered rainfall in 2017 was higher than the average historical value (+11%). The high amount of precipitation registered during late summer and autumn did not allow for HV early sowing, thus the growing period was shortened to winter-spring seasons in EXP 1. During 2018-2019, rainfall values were lower than historic records; however, no detrimental effects on HV establishment were observed (EXP 2-3).

Evaluation of annual ryegrass and HV stand densities

For untreated controls, annual ryegrass densities were more than 5-fold greater in EXP 1 (867 and 373 plants m^{-2} for high and medium infestation respectively) compared to EXP 2 (66 plants m^{-2}) ($P<0.05$) (figure 1). HV densities of untreated controls were 61, 83 and 52 plants m^{-2} in EXP 1, EXP 2 and EXP 3, respectively.

As observed in table 3, during 2017 (EXP 1) no significant interactions were observed between weed infestation level and herbicides treatments for both annual ryegrass ($P = 0.92$) and HV stand densities ($P = 0.85$). Among the evaluated herbicides, oxyfluorfen and acetochlor reduced ryegrass stand compared to the untreated control (table 3). The former also reduced the HV stand ($P<0.01$) not affecting its biomass ($P>0.05$). Neither acetochlor, imazamox, imazethapyr, imazapyr+imazethapyr, S-metolachlor, metribuzin nor pendimethalin affected HV stand (table 3).

The interaction between herbicide treatment and application timing was highly significant for HV stand (EXP 2, table 4). Annual ryegrass density was reduced by > 95% compared to the untreated control except for the case of diflufenican. Stand reductions of HV were < 25% (compared to the untreated control) using acetochlor, diflufenican, pyroxasulfone+flumioxazin or trifluralin (in pre-sowing) or pyroxasulfone, acetochlor and S-metolachlor (in pre-emergence) (table 4 and figure 2). However, both acetochlor and trifluralin applied in pre-emergence increased the damage over HV compared to pre-sowing applications (table 4).

Evaluation of annual ryegrass and HV biomass at crop termination

Annual ryegrass biomass increased with plant density. A polynomial model was adjusted to describe weed biomass related

Monthly	Rainfall (mm)					Temperature (°C)				
	EXP 1	EXP 2	EXP 3			EXP 1	EXP 2	EXP 3		
	C. Dorrego	C. Suarez	C. Suarez	C. Pringles	Claromeco	C. Dorrego	C. Suarez	C. Suarez	C. Pringles	Claromeco
January	24	52	88	4	139	26.4	22.3	21.4	23.1	21.4
February	120	28	69	0	82	20.6	22.1	20.3	21.8	20.5
March	140	42	90	74	58	19.3	17.9	17.0	17.9	17.6
April	129	73	16	25	26	14.7	16.2	15.8	15.9	16.0
May	62	56	48	37	47	11	9.3	10.5	10.8	11.9
June	52	0	44	32	13	7.9	6.7	8.0	8.7	10.7
July	15	33	0	1	57	7.5	6.1	6.5	6.4	7.7
August	47	16	1	3	44	8.6	7.6	7.9	8.6	9.2
September	101	112	8	21	38	10.4	11.6	11.2	11.5	10.7
October	30	47	40	53	86	15.5	13.0	13.7	14.6	12.7
HV Growing season	720	459	404	250	451	14.2	13.3	13.2	13.9	13.8
30 yr avg	527	652	652	585	623	13.2	11.8	11.8	12.3	13.6

Table 2. Accumulated monthly rainfall (mm), average monthly temperatures (°C) and sum/average of precipitation/temperature during the hairy vetch (HV) growing season (Jan-to-Oct) compared to the historic average values.

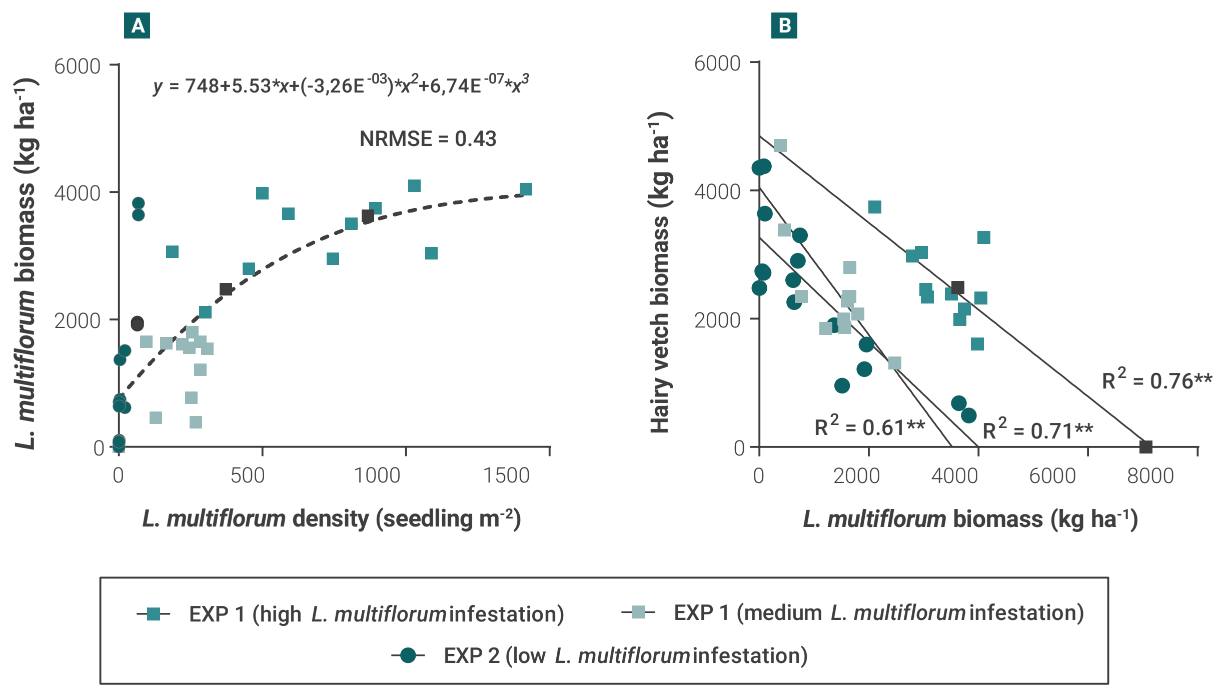

Figure 1. *Lolium multiflorum* biomass (kg ha^{-1}) as a function of seedling density evaluated at hairy vetch (HV) termination (a), and HV biomass related to *L. multiflorum* biomass (b). Black indicates the untreated controls.

to seedling density (figure 1a). Annual ryegrass biomass was significantly influenced by herbicides and it was negatively correlated to HV biomass (figure 1b). Under a high ryegrass infestation, HV reduced the weed biomass by half (7060 vs 3627 kg ha^{-1}; P<0.01) without the application of herbicides. HV biomass at medium and high ryegrass infestation levels were not affected by herbicides' treatments (P = 0.62, 2503 kg ha^{-1}).

Among the evaluated herbicides, acetochlor significantly reduced ryegrass biomass compared to the control (table 3, 4). For the rest of the cases, the reduction was on average < 50% in 2017 (EXP 1) but higher than 65% in 2018 (EXP 2). Acetochlor, S-metolachlor, pyroxasulfone and flumioxazin plus pyroxasulfone reduced annual ryegrass biomass > 85%, in both application timing, compared to the untreated control (table 4). The herbicides were more effective at a low-medium ryegrass infestation compared to the highest infestation level (table 3, 4). No significant differences in HV biomass among herbicide treatments, except for pre-sowing and pre-emergent applications of diflufenican or trifluralin in pre-emergence (table 3, 4; figure 2).

Herbicide	annual ryegrass		hairy vetch	
	Stand	Biomass	Stand	Biomass
	% Reduction†			
Acetochlor	65**	62**	5ns	0ns
Flumioxazin	44*	21ns	47**	8ns
Imazamox	20ns	45*	11ns	6ns
Imazethapyr	26ns	28*	14ns	7ns
Imazapyr + Imazethapyr	30ns	16ns	10ns	5ns
Linuron	33*	29*	22*	6ns
S-metolachlor	32*	39*	3ns	3ns
Metribuzin	33*	21ns	6ns	1ns
Oxyfluorfen	76**	26*	44**	7ns
Pendimethalin	44*	30*	4ns	6ns
Sulfentrazone	42*	26*	21*	16ns
Herbicide x ryegrass infestation	ns	ns	ns	ns
High ryegrass infestation	30	14	10	6
Medium ryegrass infestation	43	48	21	6
LSD (0.05)	*	**	**	ns

Table 3. Percent reduction of hairy vetch (HV) and annual ryegrass stand densities (seedling m^{-2}) and biomass at crop termination as a result of residual herbicide treatments applied at pre-sowing of HV (EXP 1, 2017). Epigraph: '**' indicates significance at p<0.01, '*' at p<0.05 and ´ns´ not significant difference (p > 0.05). † Relative to untreated control.

Herbicide	Application timing	annual ryegrass		hairy vetch	
		Stand	Biomass	Stand	Biomass
		% Reduction†			
Acetochlor	pre-sowing	96**	95**	23*	0ns
Diflufenican		8ns	3ns	16ns	68**
Flumioxazin		94**	65**	32*	0ns
Pyroxasulfone		98**	97**	33*	0ns
Pyroxasulfone + Flumioxazin		98**	98**	20*	0ns
S-metolachlor		98**	96**	35**	0ns
Trifluralin		68**	68**	16ns	0ns
Acetochlor	pre-emergence	97**	85**	35*	0ns
Diflufenican		3ns	2ns	40**	61**
Flumioxazin		96**	53**	48**	10ns
Pyroxasulfone		98**	97**	12ns	0ns
Pyroxasulfone + Flumioxazin		96**	96**	39*	0ns
S-metolachlor		97**	93**	33*	0ns
Trifluralin		82**	32*	40**	47**
Herbicide x Application timing		ns	*	**	*
pre-sowing		80	75	28	10
pre-emergence		81	65	35	17
LSD (0.05)		ns	**	ns	ns

Table 4. Percent reduction of hairy vetch (HV) and annual ryegrass stand densities (seedling m^{-2}) and biomass at crop termination as a result of residual herbicide treatments applied at pre-sowing or pre-emergence of HV (EXP 2, 2018). Epigraph: '**' indicates significance at p<0.01, '*' at p<0.05 and ´ns´ not significant difference (p>0.05). † Relative to untreated control.

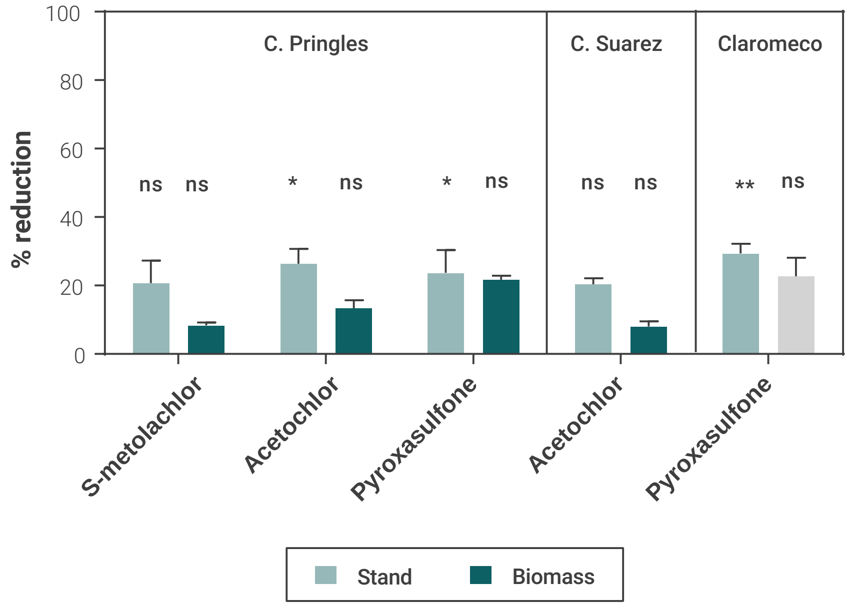

Figure 2. Influence of residual herbicide on hairy vetch (HV) stands densities (seedling m-2) 3 to 4 weeks after sowing and biomass reduction at 90 days after application (mean±SE) in EXP 3. '**' indicates significance at p<0.01, at p<0.05, and ´ns´ not significant difference (p>0.05) compared to the untreated control.

DISCUSSION

For the environmental conditions of the SBA, annual ryegrass densities at high, medium or even low infestation levels can reduce wheat yield in more than 60, 30 and 10% respectively (Gigón *et al*., 2017).

Pre-sowing and pre-emergent herbicides applied under adequate soil conditions and with low level of residues (≈ 30% cover) were capable of reducing annual ryegrass stand by 65-76% (acetochlor and oxifluorfen) in 2017, and more than 95% (acetochlor, S-metolachlor, flumioxazin, pyroxasulfone and pyroxasulfone plus flumioxazin) compared to the control in 2018 (table 4).

Our findings agree with Boutsalis *et al*. (2014), Cornelius and Bradley (2017), and Khalil *et al*. (2019) who reported that pyroxasulfone provided substantial control on annual ryegrass. Tharp and Kell (2000) observed reductions of 94% on annual ryegrass stands with S-metolachlor applications. Observed differences between suppression levels with acetochlor and S-metolachlor in 2017 and 2018 were possibly due to contrasting infestation levels. As indicated by Boutsali *et al*. (2014), a lower annual ryegrass density resulted in better overall control with pyroxasulfone applied at pre-sowing and pre-emergence in south-eastern Australia wheat fields.

No significant differences in the HV biomass between medium and high *L. multiflorum* infestation levels (P = 0.62, 2503±1020 kg ha^{-1}) could be explained by the principle of ´facilitation´ (Vandermeer, 1989). The presence of tendrils allows HV to climb over ryegrass plants thus improving competition for light (Aarssen *et al*., 1986). Highly competitive species and/or cultivars generally have better ability to access resources such as light, soil moisture and nutrients, thus suppressing the growth of weed species (Latif *et al*., 2019; Akbari *et al*., 2019).

HV was less sensitive to the evaluated herbicides than annual ryegrass. Cornelius and Bradley (2018) showed that HV proved to be one of the CC species least affected by herbicide carryover applied in soybean (e.i. S-metolachlor, acetochlor, pyroxasulfone, flumioxazin, sulfentrazone, and imazethapyr). As observed in our work, only diflufenican (at pre-sowing and pre-emergence) and trifluralin (at pre-emergence) reduced HV biomass. However, no phytotoxic effects on HV were mentioned with diflufenican in post-emergence (Renzi and Cantamutto, 2013) and trifluralin in pre-sowing (Graham, 2006). Despite some herbicides reducing the HV stand, no biomass reduction was observed at crop termination compared to the untreated control (table 3, 4; figure 2). This response may have occurred due to the compensatory growth of HV and also to some extent by the sowing density typically used by farmers in the SBA. This sowing rate almost doubles the minimal seedling densities recommended by previous study conducted by Renzi *et al*. (2017). Therefore, further studies should be performed to evaluate the sowing density effect on HV compensatory behaviour.

HV as cover crop plus residual herbicides provided acceptable suppression of annual ryegrass. Thus, it could be a valid alternative for cereals and summer crops rotations of the SBA. Our results point out an opportunity for growers and farmers to select HV as cover crop based on additional ecosystem benefits, mainly nitrogen fixation (Renzi *et al*., 2019). A weed-suppressive cover crop can limit weed seed rain, reducing population growth and ultimately weed pressure in future cash crop (Baraibar *et al*., 2018. Annual ryegrass seedbank viability is no more than two years (Narwal *et al*., 2008), so CC+residual herbicides could be a highly effective management strategy to reduce the presence of dense ryegrass infestations in the short-term.

CONCLUSIONS

Among the evaluated alternatives, acetochlor, S-metolachlor and pyroxasulfone showed the best performance without significant HV biomass reductions. Despite the remaining chemical options provided partial controls (30-40%) of annual ryegrass, as well as, affecting the HV stand they should not be discarded as they may allow the selection of wide spectrum of herbicides with different modes of actions (MOAs) mainly when a typical high sowing density is adopted by farmers. Alternative MOAs combinations with HV could also be used for managing 'difficult to control weeds' that are actually emerging in the southern region of Buenos Aires province.

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CONFLICTS OF INTEREST

None.

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