

2,4-D and saflufenacil application time on the quality of Italian ryegrass (*Lolium multiflorum*) seeds

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ABSTRACT: Italian ryegrass (*Lolium multiflorum* Lam.) is considered one of the main winter cover crops in southern Brazil; however, it is a weed species with a record of being resistant to several herbicides. The settlement of this species in agricultural areas occurs through the seed bank in the soil, and measures that interfere with seed production can assist in its management. The present study evaluated the phytotoxicity, production, and quality of Italian ryegrass seeds through the application of herbicides 2,4-D and saflufenacil at different stages of development. Three trials were conducted, a completely randomized experimental design for the first two and randomized blocks for the third, all with four replications. The treatments were arranged in a 3x3 factorial scheme, in which factor A was composed of the herbicides saflufenacil (35 g a.i. ha⁻¹), 2,4-D (1005 g a.e. ha⁻¹), plus control without application; and factor B consisted of the booting, anthesis, and maturation Italian ryegrass development stages. The phytotoxicity of the herbicides to Italian ryegrass did not exceed 30%, with saflufenacil causing the greatest injury at anthesis. The exceed the percentage of full seeds. The number of empty Italian ryegrass seeds was higher when saflufenacil was applied at anthesis. The application of 2,4-D at maturity reduced Italian ryegrass seeds. Key words: Germination, phytotoxicity, weed, seed bank management.

Influência da época de aplicação de 2,4-D e saflufenacil na qualidade de sementes de azevém (Lolium multiflorum)

RESUMO: O azevém (*Lolium multiflorum* Lam.) é considerado uma das principais plantas de cobertura hibernais do sul do Brasil, porém é uma espécie daninha com registro de resistência a diversos herbicidas. O estabelecimento dessa espécie nas áreas agrícolas ocorre através do banco de sementes no solo, sendo que medidas que interfiram na produção de sementes podem auxiliar no seu manejo. O objetivo do presente trabalho foi avaliar a fitotoxicidade, produção e qualidade de sementes de azevém mediante aplicação dos herbicidas 2,4-D e saflufenacil em diferentes estádios de desenvolvimento. Foram realizados três estudos, em delineamento experimental inteiramente casualizado para os dois primeiros, e blocos casualizados para o terceiro, todos com quatro repetições. Os tratamentos foram arranjados em esquema fatorial 3x3, em que o fator A foi composto pelos herbicidas saflufenacil (35 g i.a. ha⁻¹), 2,4-D (1005 g e.a. ha⁻¹), mais testemunha sem aplicação; e o fator B constituiu-se dos estádios de desenvolvimento do azevém emborrachamento, antese e maturação. A fitotoxicidade dos herbicidas ao azevém não superou 30%, sendo que o saflufenacil promoveu maior injúria e no estádio de antese. Ocorreu redução na produtividade de sementes vazias de azevém foi maior quando foi aplicação em antese de saflufenacil reduz o percentual de sementes cheias. O número de sementes vazias de azevém foi maior quando foi aplicado saflufenacil no estádio de antese. A aplicação de 2,4-D e m maturação reduziu a germinação e comprimento da radícula de azevém. Nessa perspectiva, estes herbicidas podem auxiliar na redução da produção e qualidade de sementes de azevém.

Palavras-chave: germinação, fitotoxicidade, planta daninha, manejo do banco de sementes.

INTRODUCTION

Italian ryegrass (*Lolium multiflorum* Lam.) is an annual plant belonging to the Poaceae family. It is widely used as forage, in southern Brazil, and is important during the lack of forage periods, entering the system as conserved forage (FLUCK et al., 2018). It has high nutritional quality, characterized by high protein and low structural carbohydrate content, thus being relevant in ruminant feeding (FLUCK et al., 2018). However, in addition to its forage quality,

Italian ryegrass has a highly competitive capacity, making it a problem in grain production areas, and it is also reported as a species (AGOSTINETTO et al., 2016). It is noteworthy that its growth habit is similar to that of winter cereals (LIU et al., 2016), which can promote a 20 to 30% reduction in wheat yield (SCURSONI et al., 2012), which justifies adopting control measures.

In areas where Italian ryegrass occurs, weed management is defined based on local needs and restrictions. In areas with established crops, such

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as wheat (Triticum aestivum L.), management with herbicides is difficult due to reduced crop selectivity and cases of resistance to herbicides that inhibit Acetolactate Synthase (ALS) (HENCKES et al., 2019). There are currently 74 records of resistance to Italian ryegrass herbicides in the world (HEAP, 2024), five of which are in Brazil, with a highlight to cases of multiple resistance to the enzyme inhibitors Acetyl CoA Carboxylase (ACCase) and 5-Enolpyruvyl Shikimate-3-Phosphate Synthase (EPSPs), ACCase and ALS inhibitors, and ALS and EPSPs inhibitors (HENCKES et al., 2019). From this perspective, the rotating action mechanism limitation is highlighted, and knowledge of integrated weed management must be used to obtain satisfactory results.

One possibility for managing Italian ryegrass with multiple resistance is seed bank management. Italian ryegrass seeds have low persistence in the soil, with those found in the surface layer, above 5 cm, showing the greatest deterioration (CECHIN et al., 2020). The seed bank in the soil is made up of the seeds that remain viable in it, where the main form of entry are plants that are in the field producing seeds, and the exits occur due to germination, decomposition, predation, and physical movement (BÜHLER et al., 1997). Thus, the application of herbicides can negatively impact the entry of seeds into the bank by reducing seed rain, even for resistant species.

The application of glyphosate to horseweed (Conyza bonarienses L.) in the vegetative stage reduces seed production by up to 68.4% but makes the seeds unviable when applied at the beginning of the reproductive stage, making it an alternative to reduce the seed bank in the soil (PIASECKI et al., 2019). Like the horseweed, Italian ryegrass is a prolific plant species whose desiccation management is of fundamental importance to reduce seed rain in the soil (BAGAVATHIANNAN & NORSWORTHY, 2012). The production of susceptible Italian ryegrass seeds by the application of glyphosate at the seed development stage (caryopsis watery ripe) was reduced by approximately 90% (SCHAEFFER et al., 2020). However, the occurrence of resistant biotypes minimizes this result, and alternative herbicides used in desiccation may have an impact on Italian ryegrass seed production at different stages. Application of glyphosate plus clethodim on glyphosate-resistant Italian ryegrass promoted 97% of control, nevertheless one survival plant m⁻² produced 1008 seeds m⁻² (BARARPOUR et al., 2020), increasing seed bank.

Among herbicides, 2,4-D is an herbicide that plays an important role in pre-sowing desiccation management and is also used in postemergence management of wheat crops, to control difficult-to-control eudicot species such as Conyza spp. and Amaranthus spp., which are resistant to more than one mechanism of action (SOARES et al., 2012; KRUGER et al., 2010). Like 2,4-D, the herbicide saflufenacil is also commonly used in chemical control recommendations for weed desiccation and post-emergence management of wheat crops. The application of 2,4-D reduced Ambrosia artemisiifolia L. seed production by up to 80% (BAE et al., 2017). In this context, little is known about the effect of these herbicides on the seeds of weeds from the Poaceae family when used in the pre-sowing management of summer crops and post-emergence of winter crops, such as wheat.

Both herbicides are known to be selective to Italian ryegrass; however, they can cause mild or moderate phytotoxicity in the crop (ROSO et al., 2017). For 2,4-D, no reduction in the shoot dry mass (MSPA) of Italian ryegrass was observed, even when phytotoxicity was observed, while the application of saflufenacil, in addition to the phytotoxicity observed, resulted in a reduction in the Italian ryegrass MSPA (ROSO et al., 2017). However, little is known about the effect of these herbicides on seed quality. For instance, the selective herbicide aminopyralid applied at different stages on Italian ryegrass in general reduced seed weight, viability and the speed of seed germination by 1 to 2 days (BOBADILLA et al., 2020). Therefore, the purpose of the present study was to evaluate Italian ryegrass seed phytotoxicity, production, and quality through the application of the herbicides 2,4-D and saflufenacil at different stages of development of the weed plant.

MATERIALS AND METHODS

Three experiments were carried out, from May 2018 to June 2019, in a greenhouse and laboratory belonging to the Universidade Federal de Santa Maria (UFSM). The experimental design was completely randomized, with 4 replications. In all experiments, treatments were arranged in a 3x3 factorial scheme, in which factor A was composed of the herbicides saflufenacil at 35 g a.i. ha⁻¹ (Heat[®], WG, 700g i.a. kg⁻¹, BASF S.A.) and 2,4-D at 1005 g a.e. ha⁻¹ (U 46[®], SL, 670g e.a. L⁻¹, Sumitomo Chemical Brasil Indústria Química S.A.), plus control with no application of herbicide. The factor B consisted of the booting, anthesis, and

maturation stages of Italian ryegrass development (ZADOKS et al., 1974).

For the first experiment, carried out in a greenhouse, the experimental units were composed of pots with a 5-L capacity, filled with sieved soil, whose fertility was corrected through previous soil analysis. Each experimental unit received ten Italian ryegrass seeds, and after emergence, thinning was carried out to a population of three plants per pot. The treatments were applied using a knapsack sprayer, pressurized to CO_2 , equipped with AIXR 110015 spray nozzles, calibrated to apply a 150 L ha⁻¹ spray volume.

The variables analyzed were phytotoxicity, total number of seeds, empty seeds (%), and full seeds (%). The phytotoxicity variable was evaluated at 7, 14, 21 and 28 days after application (DAA), at different stages of Italian ryegrass development, on a percentage scale where zero indicates absence of injuries and 100 indicates plant death (SBCPD, 1995). The seeds were collected from their dehiscence, the number of seeds per plant was counted manually. After harvesting and counting, the seeds were stored in a cold chamber belonging to the UFSM Seed Analysis Laboratory, until the other experiments were carried out.

The second experiment was conducted in a BOD-type growth chamber belonging to the UFSM Seed Research Didactic Laboratory, calibrated for 20 and 30 °C temperatures during the night and day, respectively, and 16 hours of light for 14 days. The experimental design used was completely randomized, with 4 replications each, with 50 Italian ryegrass seeds from the first experiment harvest, considering the same treatments. The experimental units (EU) were made up of gerbox boxes, in which Italian ryegrass was sown on honeycombed germitest paper and hydrated with distilled water, equivalent to 3 times the weight of the paper. To standardize germination and overcome dormancy, the seeds underwent the cold exposure process for 7 days at 5 °C and 0.2% KNO₃ was added to distilled water.

The third experiment consisted of the emergence test in sand, with a randomized block design and four replications. Each replication consisted of 25 seeds from the harvest of the first experiment, considering the same treatments, and the EUs consisted of polyvinyl chloride (PVC) cylinders approximately 3 centimeters (cm) high, arranged in four trays with the bottom covered with a thin layer of sand, for better seed allocation. After the seeds were placed inside the cylinders, they were filled with sand to the edge and dormancy was overcome with the addition of 0.2% KNO₃ to the distilled water used to hydrate the seeds, carried out in an uncontrolled

environment. The sand used underwent the washing, sieving, and drying process.

The tests were carried out according to the Rules for Seed Analysis (Regras para Análise de Sementes, RAS) (BRASIL, 2009), the variables analyzed in both experiments were the standard germination test (TPG) at 7 and 14 days after sowing (DAS), epicotyl and radicle length at 7 DAS in paper substrate, and epicotyl in sand. All data was subjected to analysis of variance (P \leq 0.05), of normality, using the Shapiro-Wilk test (P \leq 0.05), and homogeneity, using the O'Neill-Matthews test (P \leq 0.05). For emergence data in sand at 7 and 14 DAS, it was necessary to transform the data using the equation logX + 0.5. Subsequently, means were compared using the Scott-Knott test (P \leq 0.05).

RESULTS AND DISCUSSION

An interaction was observed between the factors for the variables phytotoxicity and percentage of empty and full seeds, while for the number of seeds per plant there was no interaction, only the effect of the herbicide factor. For the experiments carried out with Italian ryegrass seeds, an interaction was observed for germination on germitest paper at 7 and 14 DAS, but emergence tests in sand did not demonstrate a significant difference between treatments. For the variables epicotyl and radicle length in germitest paper, there was an interaction between the factors; however, for the variable epicotyl length in sand, there was no significant difference between treatments.

There were statistical differences between the times of application for the treatments with the herbicide saflufenacil, where in the evaluations at 7 and 14 DAA they showed greater phytotoxicity when applied to the booting (Table 1). In the evaluation at 28 DAA, greater phytotoxicity was observed when the herbicide saflufenacil was applied at anthesis (Table 1). Although, saflufenacil is considered selective to plants from the Poaceae family, injuries are frequent, such as the reduction in yield observed in corn (*Zea mays* L.) after the application of saflufenacil in post-emergence, especially when associated with the herbicide glyphosate (GALON et al., 2020).

The herbicide saflufenacil, despite presenting higher initial phytotoxicity values when applied at booting, demonstrated greater damage to Italian ryegrass plants when applied at anthesis (Table 1). It is noteworthy that the phytotoxicity values were less than 30% at the end of the evaluations, confirming the herbicide selectivity. Previous research evaluating Italian ryegrass control through the application of 2,4-D and saflufenacil

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Table 1 - Phytotoxicity (%) of saflufenacil and 2,4-D in *Lolium multiflorum* at 7, 14, 21, and 28 days after application (DAA) at different stages of development. Santa Maria/RS, 2019.

Treatments		7 DAA			14 DAA-			21 DAA			28 DAA	
	\mathbf{B}^1	A^2	M^3	В	А	М	В	А	М	В	А	М
Saflufenacil	46 Aa^4	6 Ab	-	23 Aa	15 Ab	-	12 Aa	26 Aa	-	5 Ab	28 Aa	-
2,4-D	6 Ba	0 Aa	-	8 Ba	10 Aa	-	3 Ba	1 Ba	-	2 Ba	2 Ba	-
Control	0 Ba	0 Aa	-	0 Ca	0 Ba	-	0 Ba	0 Ba	-	0 Ba	0 Ba	-
CV(%)		44.75						39,.2				

¹booting ²anthesis ³maturation ⁴Means followed by the same capital letters in the column, comparing herbicides for each stage, and lower-case letters in the line, comparing stages for each herbicide, do not differ according to the Scott-Knott test ($P \le 0.05$).

herbicides showed that there was no efficacy by using these herbicides, reaffirming the selectivity for Italian ryegrass (GALON et al., 2020; MARCHIORETTO et al., 2018). The application of saflufenacil to Italian ryegrass plants showed higher phytotoxicity than the 2,4-D herbicide and the control (Table 1). The 2,4-D herbicide presented low values (<10%) for phytotoxicity, not statistically different from the control treatment in most evaluations (Table 1). Phytotoxicity at the maturation stage was not evaluated, as the plants were in senescence.

A statistical difference can be observed for the total numbers of seed production, where the treatments that received the herbicides present lower values when compared to the control, not differing from each other (Table 2). It is noteworthy that there was a reduction in seed production, 30.12 and 33.67% for the herbicides saflufenacil and 2,4-D, respectively. Similar results were observed with the application of glyphosate in horseweed, with the herbicide application in the vegetative stage, reducing seed production by 68.4% in relation to the control without application and when applied in the reproductive stage, there was no seed production (PIASECKI et al., 2019).

No differences were observed between the times of application for the treatments with the application of 2,4-D in the evaluation of empty and full seed percentage, and, in general, this treatment did not differ from the control for both variables (Table 2). However, different results were observed when 2,4-D was applied to wheat, and at the time before tillering and at booting they caused yield losses, with a greater number of sterile grains per spike and consequently a lower number of full grains per spike (SOLIGO et al., 2022). In treatments that received the herbicide saflufenacil application, a reduction in the percentage of empty seeds was observed when applied in the booting phase, and an increase in the percentage of empty seeds when applied at the anthesis of Italian ryegrass plants (Table 2). Similarly, aminopyralid applied at booting and anthesis stages of Italian ryegrass reduced seed viability (BOBADILLA et al., 2020).

Plants that received the application of herbicides in the maturation phase showed no differences in relation to the treatment without herbicides (Table 1). It is also noteworthy that, in general, the comparison between saflufenacil and 2,4-D was similar, except when the herbicides were

Table 2 - Total number of seeds, number of empty seeds (%) and number of full seeds (%) per *Lolium multiflorum* plant after 2,4-D and saflufenacil application. Santa Maria/RS, 2019.

Treatments	Total number of seeds	Empty seeds (%)			Full seeds (%)		
		\mathbf{B}^1	A^2	M^3	В	А	М
Saflufenacil	$230.07~\mathrm{B^4}$	14.39 Bc	39.21 Aa	25.62 Ab	85.61 Aa	60.79 Bc	74.38 Ab
2,4-D	218.37 B	35.78 Aa	37.79 Aa	36.07 Aa	64.22 Ba	62.21 Ba	63.93 Aa
Control	329.22 A	29.73 Aa	29.73 Ba	29.73 Aa	70.27 Ba	70.27 Aa	70.27 Aa
CV (%)	26.43		16.13			7.36	

¹booting ²anthesis ³maturation ⁴Means followed by the same capital letters in the column, comparing herbicides for each stage, and lower-case letters in the line, comparing stages for each herbicide, do not differ according to the Scott-Knott test ($P \le 0.05$).

applied at booting. For application in the Italian ryegrass development phase, at anthesis, an increase in the proportion of empty seeds was observed for both herbicides compared to the control treatment. During this period, the application of 2,4-D can inhibit floral initiation, reducing the number of seeds per ear and consequently, yield (DERSCHEID, 1952). The results observed at this research can provide tools for Italian ryegrass seed bank management, with reduces on seed deposits on soil. Additionally it is important understanding how application of 2,4-D and saflufenacil can interfere on seed quality and thus, reducing Italian ryegrass germination and emergence.

For the germination test results, no statistical difference was observed in the treatments applied with saflufenacil between the application times. The 2,4-D, when applied to Italian ryegrass at the anthesis stage, did not show changes in the percentage of germination when compared to the control. For the booting and maturation stages, a reduction in the germination percentage was noted with the application of 2,4-D (Table 3). The 2,4-D applied at boot stage on Italian ryegrass reduced speed germination on field trial for around one day, comparing to control (BOBADILLA et al., 2020).

The lowest seed germination values came from plants that received application of 2,4-D during maturation, considering that there was a difference for the control and for the other stages of application. The saflufenacil treatment in booting and saflufenacil and 2,4-D in anthesis obtained the highest germination means, with a difference for the control without application (Table 3). For this result, we understood that saflufenacil at booting does not cause damage to seed quality, as it is still protected by the leaf sheath. As for saflufenacil and 2,4-D at anthesis, we believed that pollen sterility occurs and thus less seed fertilization, and the plant invests its resources in fertilized ovules, as observed for rice (*Oryza sativa* L.), where morphological and anatomical changes occurred in the pollen grain after exposure to herbicides (MORAES et al., 2013).

The treatment with 2,4-D at maturation was the only one to obtain a mean germination value less than 50% at 7 DAS (Table 3). Bearing in mind that the same treatment reduces the quantity of seeds per plant (Table 2). We can see that at this stage the Italian ryegrass plants that received application of 2,4-D suffered irreversible injuries, thus not only reducing the quantity but also the vigor of the produced seeds. A similar result was observed when applying herbicides in the pre-harvest of wheat, where the application of paraquat and glyphosate at the beginning of maturation reduced the germination and emergence of harvested seeds (FIPKE et al., 2018). In germination at 14 DAS, the 2,4-D treatment in maturation differed from all other treatments, with 76% germination and was 15.5% lower in relation to the control (Table 3). The application of glyphosate, paraquat + diuron and glufosinate to Italian ryegrass resulted in a reduction in the germination test, and for the first count test there was a decrease in germination for paraquat + diuron and glufosinate (CAMPOS et al., 2012).

For the epicotyl length variable, there was a difference between treatments, where the greatest lengths were in treatments with saflufenacil at booting and 2,4-D at anthesis, differing from the control (Table 4). These characteristics can favor plants in competition, due to accelerated growth related to greater seed vigor. For applications in maturity, the two herbicides differed from the control, with a reduction in the growth of seedlings from plants that received herbicide application. From this perspective, direct damage caused by herbicides to seeds may occur.

The application of 2,4-D during Italian ryegrass maturation reduced the radicle length by 51.13% in relation to the control (Table 3),

 Table 3 - Lolium multiflorum germination on germitest paper from that received treatments with herbicides, in days after sowing (DAS).

 Santa Maria/RS, 2019.

Treatments		Germination (%)					
		7 DAS7			14 DAS		
	\mathbf{B}^1	A^2	M ³	В	А	М	
Saflufenacil	94.0 Aa ⁴	97.0 Aa	84.5 Aa	97.5 Aa	99.0 Aa	92.0 Aa	
2,4-D	69.5 Bb	95.0 Aa	47.0 Bc	86.5 Bb	98.0 Aa	76.0 Bc	
Control	80.5 Ba	80.5 Ba	80.5 Aa	91.5 Ba	91.5 Aa	91.5 Aa	
CV (%)		11.48		6.08			

¹booting ²anthesis ³maturation ⁴Means followed by the same capital letters in the column, comparing herbicides for each stage, and lower-case letters in the line, comparing stages for each herbicide, do not differ according to the Scott-Knott test ($P \le 0.05$).

Treatments	Epicotyl length on paper (cm)					
	B^1	A^2	M ³			
Saflufenacil	5.10 Aa ⁴	5.10 Aa	4.50 Ba			
2,4-D	4.30 Bb	5.20 Aa	4.30 Ba			
Control	4.40 Ba	4.40 Ba	4.40 Aa			
CV (%)		6.71				
	Radicle length on paper (cm)					
	В	А	М			
Saflufenacil	1.52 Aa	1.36 Aa	1.28 Aa			
2,4-D	1.52 Aa	1.45 Aa	0.65 Bb			
Control	1.33 Aa	1.33 Aa	1.33 Aa			
CV (%)		12.91				

Table 4 - Epicotyl and radicle length of Lolium multiflorum from plants that received herbicide treatments. Santa Maria/RS, 2019.

¹booting ²anthesis ³maturation ⁴Means followed by the same capital letters in the column, comparing herbicides for each stage, and lower-case letters in the line, comparing stages for each herbicide, do not differ according to the Scott-Knott test ($P \le 0.05$).

indicating that the injuries reduce the quality of plant seeds that received this treatment. Therefore, the seedlings resulting from this treatment will have their establishment hampered when in competition with other plants with greater vigor, as they tend to have greater difficulty obtaining the resources for their growth and development. A previous study observed that the application of herbicides before physiological maturity can interfere with the filling and quality of wheat seeds, resulting in lower vigor and germination (JASKULSKI & JASKULSKA, 2014).

When the aim is to cultivate Italian ryegrass as forage or for seed production, saflufenacil can become an ally, as, when well positioned, it does not cause seed unviability and phytotoxicity is recovered. However, when applied at anthesis, a lower percentage of full seeds was observed (Table 2). Although, there is a reduction in the quantity of seeds produced when the plants received treatments with herbicides (Table 1), it may be recommended to apply saflufenacil until booting in the case of high weed infestation, a stage where higher percentage of full seeds was obtained. Therefore, it can be an alternative to efficiently control eudicot weeds.

For cases in which the presence of Italian ryegrass is undesirable, the application of herbicides to control other weed species must be carried out when the weed is in the anthesis stage for 2,4-D and saflufenacil. This is justified because herbicide treatments reduced the number of seeds per plant when applied at this stage, resulting in a reduction in the percentage of full seeds (Table 2). Thus, it will be possible to obtain a significant suppression of Italian ryegrass seeds, reducing the supply of the soil seed bank and consequently, reducing the Italian ryegrass seeds in the soil seed bank.

Managing the Italian ryegrass seed bank is important in view of the various reports of herbicide resistance (HENCKES et al., 2019) which make control difficult. In the case of glyphosate-resistant Italian ryegrass, control with this herbicide mixed with clethodim promoted the production of 1008 seeds m⁻² in field conditions, even with control of over 95% (BARARPOUR et al., 2020). Therefore, attention to the possibilities of reducing seed quality is necessary in proactive integrated weed management. In this research, application of 2,4-D and saflufenacil had differences in production of full and empty seeds (Table 2), with implications for germination and seed quality (Tables 3 and 4). Thus, it can help with Italian ryegrass seed bank management.

CONCLUSION

There was a reduction in Italian ryegrass seed yield when herbicides 2,4-D and saflufenacil were applied. The application of 2,4-D and saflufenacil at anthesis reduces the percentage of full seeds. The application of 2,4-D at maturity reduces Italian ryegrass germination.

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The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors have contributed equally to the conception and writing of the manuscript.

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