Reduction of Ryegrass (Lolium multiflorum Lam.) Natural Re-Sowing with Herbicides and Plant Growth Regulators

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Received: 13 November 2020; Accepted: 10 December 2020; Published: 13 December 2020

Abstract: Ryegrass (Lolium multiflorum Lam.) is the main winter weed of crops in Southern Brazil. High competitiveness, adaptability, widespread resistance to herbicides and seed dormancy make the plant a permanent problem. Herbicides, as well as plant growth regulators, can be used as a management option for ryegrass seed production, however there is no consensus among authors at which stage of the plant the application is most effective. Thus, this study aimed to evaluate the production and physiological quality of ryegrass seeds in response to the application of herbicides and plant growth regulators in three stages of plant development (inflorescence emergence, flowering and fruit development). Each treatment consisted of applying two different doses of each of the active ingredients: ammonium glufosinate, clethodim, glyphosate, iodosulfuron-methyl, paraquat and 2,4-D (herbicides); ethephon and trinexapac-ethyl (plant growth regulators), still an untreated control, totaling 17 treatments for each stage of development. The experimental design used was randomized blocks, with three replications. The variables evaluated were: seed production (kg ha⁻¹), thousand seed weight (g), viability (%), germination (%), first germination count (%), dormant seeds (%) and dead seeds (%). The ryegrass seed production reduced 100% with clethodim, glyphosate, ammonium glufosinate or paraquat applied in the inflorescence emergence or flowering stages. In the fruit development stage, all treatments (herbicides and plant growth regulators) caused deleterious effects on seed production, the greatest effect occurred with paraquat (95%). Paraquat, ammonium glufosinate and clethodim affected the physiological quality of the seeds when applied in fruit development stage. This research demonstrated that the application of herbicides in the ryegrass reproductive stage decreases its seedbank replenishment (natural re-sowing), with the potential to harm its progeny.

Keywords: Italian ryegrass; seed quality; soil seedbank; weed management; weed seeds; late-season weed control; grassy weed
1. Introduction

Ryegrass (*Lolium multiflorum* Lam.), also known as Italian ryegrass, is characterized as an important and problematic weed from regions of the world’s temperate and subtropical climate. The ryegrass competes intensively for resources of the environment in wheat, oat or barley crops, mainly due to its morphophysiological similarities in development, such as plant height and canopy architecture, since it belongs to the same botanical family [1].

Competition reduces seed production and quality in the infested crop. The presence of ryegrass in wheat and barley crops has been reported with losses in many countries in the world such as Denmark [2], United States [3], Egypt [4], Argentina [5] and Japan [6]. In evaluations in a ryegrass-infested wheat area, reductions of 20% to 30% in yield were observed [5] and may reach a rate of up to 92% [7]. In Brazil, the presence of this weed can reduce wheat yield up to 62% [8].

The chemical management of weeds under no-tillage has become an inevitable discussion in agriculture, especially in relation to the potential for selection of herbicide-resistant weed biotypes [9,10]. Poaceae weeds are genetically diverse, with reproduction systems that facilitate rapid adaptation and that can lead to the selection herbicide-resistant biotypes [11–13]. Among the actions to reduce the risk of resistance, is the suppression of new additions of weed seeds to the soil seedbank, with a focus on preventing their production [10].

The “soil seedbank” promotes the persistence of weeds, serving as “seed reservoirs” [14]. Alternative methods of depleting weed seed banks can be adopted at various stages of the plant life cycle, such as preventing flowering and/or the formation of essential seed structures, reducing production and increasing mortality and thus preventing the adding of new propagules in the soil [15,16].

Some weeds are capable of producing a large number of seeds in a single life cycle, resulting in infestations in subsequent years. Ryegrass can produce 45,000 seeds/m² and more than 36,000 seeds/plant in infested wheat crops [17]. The success of annual weeds in cropping systems is related to the ability to germinate at high rates and the seed longevity [18]. The germination of ryegrass seeds starts and intensifies in the winter period, with the generation of several emergency plant flows [15,19,20].

A total prevention of seed return to the soil seedbank is a big challenge that will often require the use of multiple tools (e.g., herbicides, mechanical control, cultural strategies, physical weeding, and plant residue burning, among others) [10]. The use of herbicides has the potential to reduce the production and quality of ryegrass seeds and is a practice that can contribute to reducing the frequency of resistant ryegrass biotypes and, consequently, also of the soil seedbank [16,21]. The stage of plant development and the product to be applied can compromise productive parameters and the physiological potential of weed seeds [22–24].

Late-season herbicide applications for weed desiccation also offer potential opportunities to minimize viable seed production. Several chemicals applied in reproductive development stages can decrease or prevent the production of viable weed seeds [23,25]. The block that prevents seed maturation occurs mainly due to the interruption in photosynthesis and the photoassimilates transport to seeds, not allowing them to complete the process [23,26]. This results in a great incidence of the number of small, immature and malformed seeds.

*Lolium rigidum* is Australia’s leading winter crop weed [27], recognized for the rapid evolution of herbicide resistance [28] and for developing multiple resistance (seven mechanisms of action) [29]. Steadman et al. [25] evaluated the effect of applications of non-selective herbicides on the maturation of *L. rigidum* and observed reductions in the production and physiological quality of seeds, with differentiated efficacy regarding herbicides at the application stages. The species present in Brazil is *L. multiflorum*, which belongs to the same family and genus as *L. rigidum*.

Previous research has evaluated the ryegrass seed production with glyphosate, glufosinate and paraquat application, but studies comparing them with other active ingredients are lacking. This work, in addition to testing the three mentioned, addresses the application of untested products such as clethodim, iodosulfuron-methyl and plant growth regulators in three reproductive development stages
of ryegrass. Furthermore, the impact of applications of these products on spikes, flowers and fruits (caryopsis) on the production and physiological quality of ryegrass seeds has been little studied. It is also important to highlight that there is little information related to the rate of application, in our study two doses were tested (recommended dose and reduced dose).

In these premises, the study of the effects of herbicide application or plant growth regulators at different stages of ryegrass development can be decisive in the adoption of strategies for a more efficient control of natural re-sowing of this weed. Thus, this study aimed to evaluate the production and physiological quality of ryegrass seeds, in response to the application of herbicides and plant growth regulators, in three reproductive stages of the plant.

2. Materials and Methods

2.1. Site Description

The study was conducted in the field, located in “Area 2” of Brazilian Agricultural Research Corporation (Embrapa Wheat), Coxilha/RS, Brazil. The area is located at latitude 28°10′58″ S and longitude 52°19′39″ W, with an average altitude of 721 m, average annual rainfall of 1803 mm and average annual temperature of 17.7 °C. The climate of the region according to the climatic classification of Köppen [30] is of the Cfa type (humid in all seasons, hot summer), with rains well distributed throughout the twelve months of the year [31,32]. The region is predominantly made up of Oxisols, which have very deep, homogeneous and highly weathered characteristics. The soil profile of the area used is classified as Dystrophic Red Humic Latosol [33].

2.2. Experimental Details and Treatment Descriptions

The treatments tested included six herbicides and two plant growth regulators, each in two doses of active ingredient (recommended dose and reduced recommended dose for those with recommendation and equivalent doses for those without recommendation) (Table 1) applied on ryegrass, in three stages of development according to the phenological scale for weed species (Biologische Bundesanstalt, Bundessortenamt and Chemical industry scale, BBCH), described by Hess et al. [34]: (1) inflorescence emergence (middle of heading, BBCH 55), (2) flowering (30%–50% of flowers open, BBCH 63 to 65) and (3) fruit development (caryopsis watery ripe, BBCH 71) (Figure 1).

Table 1. Treatments with herbicides or plant growth regulators, each in two doses of active ingredient, applied to ryegrass, in three stages of plant development (inflorescence emergence, flowering and fruit development). Passo Fundo/RS, Brazil, 2019.

| Treatment                     | Site of Action | Dose 1 | Reduced Dose 2 |
|-------------------------------|----------------|--------|----------------|
| Untreated control             | -              | -      | -              |
| Ammonium glufosinate          | GS inhibitor   | 400    | 200            |
| Clethodim                     | ACCase inhibitor | 108 | 54             |
| Glyphosate                    | EPSP inhibitor | 720    | 360            |
| Iodosulfuron-methyl           | ALS inhibitor  | 5      | 2.5            |
| Paraquat                      | PSI electron diverter | 400 | 200            |
| 2,4-D                         | Synthetic Auxin | 1340 | 670            |
| Ethephon                      | PGR 3          | 720    | 360            |
| Trinexapac-ethyl              | PGR 3          | 400    | 200            |

1 Recommended dose, g/L or g/kg of active ingredient (ai) or acid equivalent (ae). 2 Reduced recommended dose, g/L or g/kg of active ingredient (ai) or acid equivalent (ae). 3 PGR: plant growth regulator.

Each stage was considered as an experiment, being analyzed separately. An untreated control was added for each application moment. The one-year field experiments, started in September 2018, were conducted in plots (experimental unit-EU) measuring 5 m wide and 5 m long (25 m²), with a natural
infestation of ryegrass plants, originating from the soil seedbank. The origin of ryegrass occurrence was spontaneous, with a previous ryegrass-infested crop. The plants covered the soil completely, with no gaps for other plants. The average rainfall during the study was 175 mm. The experimental was arranged in a randomized complete block with three replications.

The germination test was performed to assess the physiological quality of ryegrass seeds, for which 50 seeds were distributed on two layers of moistened filter paper, contained in Petri dishes, with 2.5 times their weight with water. The plates were transferred to a seed germinator (Mangelsdorf, DeLeo - Porto Alegre/RS, Brazil) in a completely randomized design, with three replications, under a 12-h light

![Figure 1. Ryegrass stages of development at the time of treatments application: (a) BBCH 55 (inflorescence emergence: middle of heading), (b) BBCH 63 to 65 (flowering: 30%–50% of flowers open) and (c) BBCH 71 (fruit development: caryopsis watery ripe). The extended BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) scale for weed species was used [34].](image)

2.3. Procedure

The germination of ryegrass seeds from the soil seedbank is staggered due to the dormancy characteristic of the species. In order to ensure the correct application at the development stage and to standardize the size of plants in a growth stage, a mechanical mowing was performed, sectioning the plants and allowing a remnant of approximately 30 cm. The treatments were applied when 75% of plants from each EU reached the three stages of development previously described.

For the application of treatments, a backpack sprayer, pressurized with CO₂, was used, equipped with a spraying bar with six flat spray type XR 11002 VS, with a pressure of 2 Bar, which generated a spray volume of 150 L ha⁻¹. The height of the bar was 50 cm above the target. During the application the environmental conditions were 25 °C temperature, 60% relative humidity and wind speed below 7 km h⁻¹.

From each EU, 1 m² of ryegrass spikes were harvested, this performed when the seeds reached maturity (hard grain and/or dry straw). After harvest, the spikes were trailed in a plot combine harvester (Wintersteiger- Ried im Innkreis, Austria) and the seeds were stored in paper bags under ambient conditions until the evaluations, which were: seed production (kg ha⁻¹), thousand seed weight (TSW) (g), viability (%), germination (%), first germination count (FGC) (%), dormant seeds (%) and dead seeds (%), as described in the seed analysis rules (SAR) [35].

2.4. Seed Production and Quality

The evaluations of quality and production of ryegrass seeds were performed at seed analysis laboratory and weed science laboratory at Embrapa Trigo. The samples were weighed on a four-digit scale, thus obtaining the sample weight in g m⁻² and adjusted to kg ha⁻¹. The TSW was obtained following the guidelines of SAR [35].

The germination test was performed to assess the physiological quality of ryegrass seeds, for which 50 seeds were distributed on two layers of moistened filter paper, contained in Petri dishes, with 2.5 times their weight with water. The plates were transferred to a seed germinator (Mangelsdorf, DeLeo - Porto Alegre/RS, Brazil) in a completely randomized design, with three replications, under a 12-h light
photoperiod at 20 °C. The germination percentage (normal seedlings) was determined at 14 days after sowing on paper [35].

Together with the germination test, the FGC was performed five days after sowing to determine the seed vigor [36]. At the end of the germination test, the number of dead and dormant seeds was counted and the results were also expressed as a percentage.

Finally, in the germination test, the seeds considered dormant (“hard” in the soft compression with tweezer) were also evaluated by the tetrazolium test (TZ), to confirm their viability as described by Brazil [35]; thus, seeds viable by TZ were considered dormant and the percentage of non-viable seeds was added to the percentage of dead seeds.

The viability of ryegrass seeds was assessed by TZ. TZ is a biochemical test that determines the percentage of viable seeds based on the activity of dehydrogenase enzymes, regardless of the seed dormancy incidence [37]. First, the seeds were immersed in distilled water for 16 h at 20 °C. Then, the seeds were cut longitudinally from the embryo to 3 4 of the endosperm and transferred to a staining solution (0.5% of the salt 2, 3, 5 triphenyl tetrazolium chloride), in an oven at 30 °C for four hours. For the evaluation, the sectioned surfaces were observed in a stereomicroscope (Technical 35×). It was considered viable the seed that had the embryo with a reddish pink color [35].

2.5. Statistical Analysis

The experimental data obtained were subjected to analysis of variance (ANOVA) using the R software [38]. Data normality and homogeneity of the variances were checked by the Shapiro–Wilk and Levene’s tests, respectively. The abnormal data of seed production and TSW were square-root transformed. Abnormal percentage data were subjected to angular transformation (arcsine square-root) prior to analysis. For seed quality parameters, treatments that did not produce seeds were excluded from the analysis. When the ANOVA indicated significant treatment effects, the means were compared at p ≤ 0.05 using the Scott Knott test.

3. Results and Discussion

3.1. Inflorescence Emergence Stage

3.1.1. Seed Production

Significant differences between treatments were found only for seed production when herbicides and plant growth regulators were applied at this stage of development (middle of heading, BBCH 55).

Several treatments significantly reduced the production of ryegrass seeds (Table 2). Clethodim, glyphosate, ammonium glufosinate, iodosulfuron-methyl and paraquat, regardless of the dose evaluated, reduced seed production by 100%. A reduction close to 85% was observed with ethephon (720 g ha\(^{-1}\)). Trinexapac-ethyl, regardless of dose, reduced about 25% of seed production. 2,4-D and ethephon (360 g ha\(^{-1}\)), did not affect the seed production of ryegrass (Table 2).

The central principle of management of weed seed banks is to reduce the production of their seeds [39,40]. For this purpose, to control *Amaranthus palmeri*, Jha and Norsworthy [41] used ammonium glufosinate at the beginning of the inflorescence, with results similar to those observed in this work, where reductions of 78% to 95% in the production of seeds were observed. In another study, glyphosate applied at the same stage completely prevented the production of *Lolium rigidum* Gaud. seeds [25]; additional to that, in a study by Kleemann et al. [42], it was observed that clethodim, followed by cover plants, also provided efficient control and there was a decline in the weed seed bank. In *Sorghum halepense*, glyphosate (420 and 840 g ha\(^{-1}\)), clethodim (68 and 136 g ha\(^{-1}\)) and glufosinate (740 g ha\(^{-1}\)) at the boot stage, reduced seed production by 94% to 99% [26].

In the work of Christofoleti et al. [43] with ryegrass, clethodim (96 g ha\(^{-1}\)) and paraquat + diuron (300 + 150 g ha\(^{-1}\)), applied in pre-flowering, provided control above 90%. Herbicides such as paraquat can decrease seed yield by quickly inhibiting photosynthesis or compromising the transport
of photoassimilates to them [44]. In *Ambrosia trifida* and *A. artemisiifolia*, the application of ammonium glufosinate and glyphosate or glyphosate + dicamba, respectively, at the beginning of inflorescences, also resulted in a reduction in seed production of 78% to 99% [45–47].

Table 2. Production and quality of ryegrass seeds in response to the application of herbicide or plant growth regulators doses in the inflorescence emergence stage (middle of heading, BBCH 55). Passo Fundo/RS, Brazil, 2019.

| Treatment                  | Dose \(^1\) | Seed Production | TSW  | Viability \(^3\) | Germination Test (%) \(^2\) |
|----------------------------|-------------|-----------------|------|-------------------|-----------------------------|
|                            | g ha\(^{-1}\) | kg ha\(^{-1}\) | g    | %                 | V  | G  | D  | M  |
| Untreated control          | -           | -               | 580.5 a | 1.94 ns            | 77 ns               | 68 ns | 69 ns | 11 ns | 20 ns |
| 2,4-D                      | 1340        | 589.7 a         | 1.97  | 82                 | 71 | 74 | 17 | 9 |
| 2,4-D                      | 670         | 566.1 a         | 2.04  | 81                 | 64 | 68 | 13 | 19 |
| Ethephon \(^4\)           | 360         | 544.2 a         | 2.02  | 83                 | 67 | 70 | 13 | 17 |
| Trinexapac-ethyl \(^4\)   | 200         | 438.2 b         | 1.97  | 75                 | 71 | 73 | 14 | 13 |
| Trinexapac-ethyl \(^4\)   | 400         | 434.5 b         | 1.97  | 74                 | 55 | 61 | 23 | 16 |
| Ethephon \(^4\)           | 720         | 77.2 c          | 1.69  | 79                 | 63 | 73 | 10 | 17 |
| Clethodim                  | 54          | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| Clethodim                  | 108         | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| Glyphosate                 | 360         | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| Glyphosate                 | 720         | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| Ammonium glufosinate       | 200         | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| Ammonium glufosinate       | 400         | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| Iodosulfuron-methyl        | 2.5         | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| Iodosulfuron-methyl        | 5           | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| Paraquat                   | 200         | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| Paraquat                   | 400         | 0.0 d           | -     | -                  | -  | -  | -  | -  |
| CV (%)                     | -           | 16.4           | 7.2   | 6.7               | 8.7 | 6.6 | 17.1 | 24.2 |

Note: Means followed by the same letter in column are not different using Scott Knott test (\(p \leq 0.05\)). \(^{ns}\): not significant. CV: coefficient of variation. TSW: thousand seed weight. \(^1\) g/L or g/kg of active ingredient (ai) or acid equivalent (ae). \(^2\) Vigor (V), germinated seeds (G), dormant seeds (D) and dead seeds (M). \(^3\) Evaluation by tetrazolium test (TZ). \(^4\) Plant growth regulators.

3.1.2. Seed Quality

The TSW, viability, vigor, germination, dormant seeds and dead seeds of ryegrass were not affected in the applications of ethephon, 2,4-D and trinexapac-ethyl in the inflorescence emergence stage. The other treatments did not produce seeds for seed quality analysis.

3.2. Flowering Stage

3.2.1. Seed Production

The seed production in the plots that received the treatments ethephon (360 and 720 g ha\(^{-1}\)) and 2,4-D (670 g ha\(^{-1}\)) was higher than that obtained in the control (untreated). Results of seed production similar to the control were obtained with the application of 2,4-D (1340 g ha\(^{-1}\)) and trinexapac-ethyl (200 and 400 g ha\(^{-1}\)). The treatments with clethodim, ammonium glufosinate, paraquat and glyphosate provided a total reduction in the production of ryegrass seeds and iodosulfuron-methyl caused reductions of more than 90% (Table 3). In the flowering of ryegrass, the products directly affected the reproductive system because the anthers are extruded from the spikelet.

In another similar study, the seed production of *Avena fatua* was completely prevented with glyphosate (880 g ha\(^{-1}\)) applied in anthesis [48]. The maximum reductions in seed yield (100%) that occurred in this study were also with glyphosate (360 and 720 g ha\(^{-1}\)), but in addition other herbicides also completely prevented seed production at the flowering stage, paraquat (200 and 400 g ha\(^{-1}\)), ammonium glufosinate (200 and 400 g ha\(^{-1}\)) and clethodim (54 and 108 g ha\(^{-1}\)). Kumar and Jha [49]
observed that the application of glyphosate, glufosinate or paraquat at the beginning of flowering reduced the seed production of *Kochia scoparia* (L.) Schrad by 99%.

Several other studies have also reported that a single application of glyphosate, 2,4-D, dicamba, glufosinate or paraquat in flowering and in the early stages of seed development of *Abutilon theophrast*, *Datura Stramonium*, *Chenopodium album*, *Amaranthus retroflexus*, *Amaranthus palmeri*, *Senna obtusifolia*, *Ipomoea lacunosa*, *Sida spinosa* and *Echinochloa crus-galli* resulted in an 80% to 99% reduction in seed production [50–53].

The results of this work demonstrate that an application of some herbicides (clethodim, ammonium glufosinate, paraquat, glyphosate or iodosulfuron-methyl) during the ryegrass flowering period can be useful to reduce the replenishment of seeds to the soil seedbank and, thus, decreasing the evolution of resistance to herbicides. In addition, the application of 2,4-D (670 g ha$^{-1}$) and ethephon at the same stage should be avoided, since their application stimulates the production of viable ryegrass seeds.

**Table 3.** Production and quality of ryegrass seeds in response to the application of herbicide or plant growth regulators doses in the flowering stage (30%–50% of flowers open, BBCH 63 to 65). Passo Fundo/RS, Brazil, 2019.

| Treatment           | Dose 1 | Seed Production | TSW   | Viability³ | Germination Test (%)² |
|---------------------|--------|-----------------|-------|------------|-----------------------|
|                     | g ha$^{-1}$ | kg ha$^{-1}$ | g     | %          | V G D M                |
| Ethephon 4          | 360    | 587.4 a         | 2.13 a | 90 a       | 67 a 77 a 13 b 10 b    |
| 2,4-D               | 670    | 561.6 a         | 2.03 a | 97 a       | 70 a 76 a 9 b 15 b     |
| Ethephon 4          | 720    | 506.5 a         | 2.40 a | 77 b       | 76 a 80 a 9 b 11 b     |
| Trinexapac-ethyl 4  | 400    | 445.0 b         | 1.81 b | 84 a       | 60 b 66 b 21 a 13 b    |
| Untreated control   | -      | 414.4 b         | 2.27 a | 91 a       | 70 a 74 a 8 b 18 b     |
| Trinexapac-ethyl 4  | 200    | 380.4 b         | 2.03 a | 75 b       | 61 b 70 a 20 a 10 b    |
| 2,4-D               | 1340   | 358.7 b         | 2.19 a | 85 a       | 72 a 79 a 13 b 8 b     |
| Iodosulfuron-methyl | 2.5    | 27.1 c          | 1.75 b | 81 b       | 54 b 63 b 13 b 24 a    |
| Iodosulfuron-methyl | 5      | 20.7 c          | 1.66 b | 67 b       | 43 b 49 c 19 a 32 a    |
| Clethodim           | 54     | 0.0 d           | -     | -          | - - - -                |
| Clethodim           | 108    | 0.0 d           | -     | -          | - - - -                |
| Glyphosate          | 360    | 0.0 d           | -     | -          | - - - -                |
| Glyphosate          | 720    | 0.0 d           | -     | -          | - - - -                |
| Ammonium glufosinate| 200    | 0.0 d           | -     | -          | - - - -                |
| Ammonium glufosinate| 400    | 0.0 d           | -     | -          | - - - -                |
| Paraquat            | 200    | 0.0 d           | -     | -          | - - - -                |
| Paraquat            | 400    | 0.0 d           | -     | -          | - - - -                |
| CV (%)              | -      | 18.3            | 4.6   | 6.7        | 10.9 8.6 19.4 41.7     |

Note: Means followed by the same letter in column are not different using Scott Knott test ($p \leq 0.05$). CV: coefficient of variation. TSW: thousand seed weight. ¹ g/L or g/kg of active ingredient (ai) or acid equivalent (ae). ² Vigor (V), germinated seeds (G), dormant seeds (D) and dead seeds (M). ³ Evaluation by tetrazolium test (TZ). ⁴ Plant growth regulators.

3.2.2. Seed Quality

The TSW was significantly reduced with the herbicide iodosulfuron-methyl and the trinexapac-ethyl regulator, with 23% reductions in the dose of 2.5 g ha$^{-1}$ and 27% in the dose 5 g ha$^{-1}$ of iodosulfuron-methyl and 20% reduction for trinexapac-ethyl at dose of 400 g ha$^{-1}$, when applied during flowering. The other treatments did not affect the seed weight (Table 3). Some herbicides such as triasulfuron, flumetsulam or MCPA, applied at the early flowering stage of *Raphanus raphanistrum* also reduced weight of the seeds [54]. In another study, tribenuron and MCPA reduced the seed weight of *Fallopia convolvulus*, *Galium spurium* and *Thlaspi arvense* after treatment with sublethal doses in early flowering stage [55].

The viability of the seeds produced was performed by TZ, which indicated that the herbicide iodosulfuron-methyl (2.5 and 5 g ha$^{-1}$), trinexapac-ethyl (200 g ha$^{-1}$) and ethephon (720 g ha$^{-1}$) reduced the percentage of viable seeds. The application of herbicide during flowering or at the beginning of
seed formation has the potential to decrease the production of viable weed seeds, eventually allowing the depletion of the soil seedbank [23,41,52,53].

In the germination test, iodosulfuron-methyl significantly reduced the germination and vigor of ryegrass seeds, regardless of the dose for vigor and there was a greater reduction in germination at the highest dose (5 g ha⁻¹). Such as tribenuron-methyl (5.63 g ha⁻¹), applied during the early flowering stage of *Amaranthus retroflexus* L. the herbicide also significantly reduced the percentage of seed germination to about 45% [56]. Trinexapac-ethyl reduced the vigor and trinexapac-ethyl (400 g ha⁻¹) reduced germination. The percentage of dormant seeds increased when applied trinexapac-ethyl (200 and 400 g ha⁻¹) and iodosulfuron-methyl (5 g ha⁻¹). Seed mortality increased only with the application of iodosulfuron-methyl (2.5 and 5 g ha⁻¹) in flowering stage (Table 3).

3.3. **Fruit Development Stage**

3.3.1. Seed Production

All herbicides and plant growth regulators sprayed at the fruit development stage decreased seed production, to a greater or lesser extent (Table 4). The greatest reductions in seed production were with the application of paraquat, glyphosate, ammonium glufosinate and clethodim, regardless of the dose, and iodosulfuron-methyl (5 g ha⁻¹). The other treatments also negatively affected the production of ryegrass seeds, but to a lesser extent, close to 50%, compared to the control (Table 4).

| Treatment                 | Dose ¹ | Seed Production | TSW | Viability ³ | Germination Test (%) ² |
|---------------------------|--------|-----------------|-----|-------------|------------------------|
|                           | g ha⁻¹ | kg ha⁻¹          | g   | %           | V | G | D | M |
| Untreated control         | -      | 823.4 a         | 2.09 b | 78 b | 51 a | 53 b | 19 b | 28 b |
| Trinexapac-ethyl ²        | 200    | 557.3 b         | 2.00 b | 88 a | 63 a | 66 a | 25 b | 9 b  |
| 2,4-D                     | 1340   | 520.2 b         | 1.80 c | 89 a | 58 a | 59 b | 20 b | 21 b |
| 2,4-D                     | 670    | 514.6 b         | 1.97 b | 85 a | 55 a | 57 b | 18 b | 25 b |
| Trinexapac-ethyl ²        | 400    | 349.8 c         | 2.01 b | 86 a | 61 a | 67 a | 21 b | 12 b |
| Ethephon ²                | 360    | 348.8 c         | 2.32 a | 89 a | 73 a | 73 a | 16 b | 11 b |
| Ethephon ²                | 720    | 321.8 c         | 2.22 a | 88 a | 69 a | 70 a | 16 b | 14 b |
| Iodosulfuron-methyl       | 2.5    | 309.5 c         | 1.81 c | 71 b | 47 a | 49 b | 19 b | 32 a |
|                          | 5      | 193.4 d         | 1.71 c | 63 c | 31 b | 38 c | 25 b | 37 a |
| Clethodim                 | 108    | 152.8 d         | 1.53 d | 43 d | 19 b | 22 c | 29 b | 49 a |
|                          | 54     | 137.3 d         | 1.80 c | 48 d | 31 b | 38 c | 22 b | 40 a |
| Ammonium glufosinate      | 200    | 126.4 d         | 1.52 d | 51 d | 34 b | 35 c | 23 b | 42 a |
| Glyphosate                | 720    | 96.2 d          | 1.37 d | 79 b | 34 b | 36 c | 19 b | 45 a |
| Glyphosate                | 360    | 90.9 d          | 1.73 c | 79 b | 34 b | 36 c | 31 a | 33 a |
| Ammonium glufosinate      | 400    | 74.0 d          | 1.21 d | 37 d | 10 c | 13 d | 34 a | 53 a |
| Paraquat                  | 200    | 45.3 d          | 1.40 d | 77 b | 7 c | 9 d | 43 a | 48 a |
|                          | 400    | 21.9 d          | 1.34 d | 73 b | 9 c | 10 d | 51 a | 39 a |

Note: Means followed by the same letter in column are not different using Scott Knott test (𝜈 ≤ 0.05). CV: coefficient of variation. TSW: thousand seed weight. ¹ g/L or g/kg of active ingredient (ai) or acid equivalent (ae). ² Vigor (V), germinated seeds (G), dormant seeds (D) and dead seeds (M). ³ Evaluation by tetrazolium test (TZ). ⁴ Plant growth regulators.
decreased seed production when applied after anthesis, in milky grain and pasty grain [25], similar to the results obtained in this investigation.

3.3.2. Seed Quality

Seed weight can be an indicator of seedling vigor [25]. In the evaluation of TSW, another physical attribute of seed quality, the results indicated greater reductions in response to the application of the herbicides ammonium glufosinate, paraquat, glyphosate and clethodim. Ammonium glufosinate (400 g ha\(^{-1}\)) reduced the weight of seeds by 42%, not significantly differing from glyphosate (720 g ha\(^{-1}\)), with a 34% reduction and paraquat (200 and 400 g ha\(^{-1}\)), 32% and 35%, respectively, and clethodim (108 g ha\(^{-1}\)) and ammonium glufosinate (200 g ha\(^{-1}\)) in 27%. Increase in TSW was observed in the application of ethephon regardless of the applied dose. Trinexapac-ethyl and 2,4-D (670 g ha\(^{-1}\)) did not affect TSW.

Smaller but significant reductions in TSW were observed in the spraying of 2,4-D (1340 g ha\(^{-1}\)), iodosulfuron-methyl (2.5 and 5 g ha\(^{-1}\)), glyphosate (360 g ha\(^{-1}\)) and clethodim (54 g ha\(^{-1}\)) at this same stage of development. A similar result was obtained in a study with wheat, in which ammonium glufosinate and clethodim decreased the weight of the seeds when applied in a pasty grain stage [22]. Ammonium glufosinate and clethodim may have caused greater stress on plants, interfering with the transport of photoassimilated compounds to the seed and consequently affecting TSW [22].

In another study on wheat, doses of glyphosate at the milky grain stage were evaluated. It was found that there was a reduction in the weight of the grain when the herbicide was applied in higher doses (840 g ha\(^{-1}\)) [58]. The size of seeds suggests that the seeds could still be immature when the development is interrupted by the herbicides applied [23]. The application of glyphosate in the initial seed development was also seen in Senna obtusifolia with a 73% reduction in seed weight and at the same stage in Sesbania exaltata the seed weight was also reduced by 46% [24].

The viability of ryegrass seeds was reduced in the application of clethodim, ammonium glufosinate and iodosulfuron-methyl (5 g ha\(^{-1}\)) at the fruit development stage (Table 4). Jha and Norsworthy [41] demonstrated that the application of glufosinate, 2,4-D or dicamba in the reproductive stage of Amaranthus palmeri also reduced seed viability.

For a weed to recover, it must disperse viable seeds in the area, therefore reducing its number of seeds is an important practice to avoid re-infestation. Damage to the inflorescence reduces the formation of seeds and interrupts the development of the growing embryo, decreasing viability [59]. The herbicide glyphosate did not affect the viability of ryegrass seeds in the fruit development stage (Table 4), Steadman et al. [25] achieved the same result in Lolium rigidum. The viability of ryegrass seeds at the fruit development stage was also increased in comparison to untreated control (Table 4). The applications of trinexapac-ethyl, 2,4-D and ethephon, regardless of the dose, increased the percentage of viable seeds. Plant growth regulators, such as ethephon and trinexapac-ethyl are products that generate a greater translocation of reserve for seeds, and thus can result in a greater number of individuals able to survive [60], such as increased viability.

The paraquat (200 and 400 g ha\(^{-1}\)) and ammonium glufosinate (400 g ha\(^{-1}\)), were the ones that most reduced seed germination and vigor, followed by glyphosate (360 and 720 g ha\(^{-1}\)), iodosulfuron-methyl (5 g ha\(^{-1}\)), clethodim (54 and 108 g ha\(^{-1}\)) and ammonium glufosinate (200 g ha\(^{-1}\)) with minor reductions in germination and vigor, but significant in relation to the control. Highlight for paraquat with reductions of 82% for germination and 85% for vigor, regardless of dose and ammonium glufosinate (400 g ha\(^{-1}\)) of 76% and 80% for germination and vigor.

Similar results of herbicidal effect on the physiological quality of ryegrass seeds were found by Campos et al. [61] who obtained total germination control with paraquat + diuron (500 + 250 g ha\(^{-1}\)) and ammonium glufosinate (600 g ha\(^{-1}\)). The application of ammonium glufosinate by Da Rosa Ulghim et al. [62] in ryegrass post-flowering also significantly reduced germination and seed viability of the weed. In sorghum, the herbicide glyphosate applied in pre-harvest had a detrimental effect on the germination of its seeds [63].
The herbicide clethodim drastically decreased the vigor (36%) (first germination count) of wheat seeds when applied in the grain filling stage [22]. In the same crop, Bellé et al. [64] found that desiccation in pre-harvest with paraquat and glyphosate in the stages of soft mass and hard seed mass led to reduced germination, with a greater effect when paraquat was used.

In crops such as rice [65] and wheat [66], paraquat has also reduced germination and seed vigor, affecting initial seedling development. The product used in post-flowering, to prevent the development of Lolium spp. seeds, can inhibit the germination of mature seeds by 80% to 100% [15,67,68], equivalent results to those found in this research.

Herbicides may have caused a decrease in seed reserves and, consequently, vigor. Thus, the seed may have undergone changes in the endosperm and embryo imposed by the application of paraquat, ammonium glufosinate and clethodim, for example, in this case it is already known that herbicides used in pre-harvest desiccation (before physiological maturation) may accumulate in the seed, reducing its germination and vigor [69].

Seed vigor was not affected by the application of trinexapac-ethyl, 2,4-D, ethephon and iodosulfuron-methyl (2.5 g ha\(^{-1}\)). In contrast, seed germination was increased by applications of ethephon and trinexapac-ethyl (regardless of dose) at the fruit development stage. 2,4-D and iodosulfuron-methyl (2.5 g ha\(^{-1}\)) did not affect seed germination.

Treatments with paraquat (200 and 400 g ha\(^{-1}\)), ammonium glufosinate (400 g ha\(^{-1}\)) and glyphosate (360 g ha\(^{-1}\)) showed the highest percentage of dormant seeds (Table 4). In sunflower, the application of paraquat increased the outer cell wall thickness of the endosperm cell layer of the seed coat and this fact was associated with increased seed dormancy [70].

Finally, the number of dead seeds was increased with the application of paraquat, ammonium glufosinate, clethodim, glyphosate and iodosulfuron-methyl. The highest mortality of ryegrass seeds (53%) occurred with the application of ammonium glufosinate (400 g ha\(^{-1}\)), not differing statistically from paraquat, ammonium glufosinate (200 g ha\(^{-1}\)), clethodim, glyphosate and iodosulfuron-methyl.

### 3.4. Practical Implications

In the subsequent paragraphs the practical aspects of the study are commented and discussed. In this study recommendations by Norsworthy et al. [10] were followed, to manage resistant weeds, whose guidelines are the prevention of seed production and its increase in the soil bank. Herbicide applications in weeds at the end of the season (“escapes”), due to the time-spreading emergence, can potentially reduce additions to the seed bank in the growing season. The results presented here suggest that the herbicides clethodim, glyphosate, ammonium glufosinate and paraquat applied in the stages of inflorescence emergence or flowering (anthesis) and iodosulfuron-methyl only in the inflorescence emergence stage, can be used effectively with the objective of avoiding the total replenishment of the seed bank and thus collaborate with the depletion of the species bank. It is important to highlight a secondary effect of the clethodim application, in addition to totally inhibiting the production of seeds, it prevented regrowth and, consequently, the appearance of new plants in the area, an indirect effect that also contributed to the reduction of the re-sowing potential of the species.

The control of weed seed production came to be considered a tool capable of reducing the spread of weed resistant to herbicides, preventing the establishment, spatial distribution and accumulation of seeds in the soil [71]. In addition, it also has the potential to delay the evolution of resistance by reducing the number of plants exposed, due to the selection pressure by the herbicide. This inhibition of weed seed production is important, particularly for species that have already developed resistance to glyphosate, such as ryegrass.

This study also demonstrated that the use of lower doses (reduced doses) of most tested herbicides is recommendable to manage ryegrass seeds in the reproductive stage. According to the information obtained, the use of a reduced dose of herbicide (paraquat, ammonium glufosinate, clethodim and glyphosate) is equally efficient (in relation to the recommended dose of the same herbicide), for the management of ryegrass seeds in the stages of inflorescence emergence and
flowering. For iodosulfuron-methyl, the effect of the dose is indifferent in relation to seed production, but to reduce the quality of seeds in the flowering stage the recommended dose is more efficient. However, at the fruit development stage, the recommended doses of the herbicides were more efficient in reducing the weight, viability and germination of the seeds, but there was no difference between the doses for the seed production and dead seeds. In the case of paraquat, the reduced dose should be used regardless of the ryegrass reproductive stage.

*Lolium* species, due to their genetic diversity and hybridization potential, are at high risk of developing resistance to other herbicide action sites currently used [17,72,73]. One of the best ways to preserve herbicide technology for as long as possible is to stop the deposition of weed seeds in the soil seedbank, since without the presence of seed it means that there are no herbicide-resistant weeds or weeds in the area [3].

With the information obtained in this study, it can also be stated that seeds added to the seed bank after a spray treatment would, therefore, be less competitive in subsequent crops as was observed in the applications of paraquat, clodethodim and ammonium glufosinate, whose ryegrass seeds had vigor and germination minors. The reduction in seed weight and viability, which occurred when spraying ammonium glufosinate and clodethodim, for example, can also affect the presence of the plant species in the following season. In addition to negatively impacting production, also affecting the quality of ryegrass seeds, it further emphasizes the importance of the practice for integrated and sustainable management.

Decreasing the potential for weed infestation in the soil seedbank can also be an effective means of reducing its impact on subsequent crops and assisting integrated control practices. The eradication of weed plants, although difficult to achieve, can promote the stability of herbicide control technologies for longer periods [3,74], therefore, once the weed species is observed at a reproductive stage, a ryegrass control operation should be implemented, in order to reduce its natural re-sowing totally or partially.

This research demonstrated that the annual contributions of ryegrass seeds to the soil seedbank can be significantly reduced by a single application of herbicides (glyphosate, ammonium glufosinate, paraquat, clodethodim or iodosulfuron-methyl) at the stages of inflorescence emergence, flowering or fruit development.

4. Conclusions

The application of clodethodim, glyphosate, ammonium glufosinate and paraquat in the stages of inflorescence emergence or flowering and iodosulfuron-methyl in the inflorescence emergence, suppressed the ryegrass natural re-sowing and consequently the replenishment of the soil seedbank. In the fruit development stage, all the products applied caused negative effects on seed production, especially paraquat. The greatest reduction in thousand seed weight at the fruit development stage occurred with the application of ammonium glufosinate (400 g ha⁻¹). The herbicides paraquat, ammonium glufosinate and clodethodim reduced seed germination and vigor, paraquat and ammonium glufosinate increased the percentage of dormant seeds and paraquat, ammonium glufosinate, clodethodim, glyphosate and iodosulfuron-methyl increased the percentage of dead seeds, when applied in the ryegrass fruit development stage. The reduced dose of the herbicides paraquat, ammonium glufosinate, clodethodim, iodosulfuron-methyl and glyphosate can be used for the management of ryegrass seeds at the stages of inflorescence emergence and flowering (except iodosulfuron-methyl at the flowering), fulfilling the same effect as the recommended dose of herbicide.

**Author Contributions:** Conceptualization, A.H.S., O.A.S., J.A.G.B., D.K.R. and N.C.L.; methodology, A.H.S., O.A.S., J.A.G.B., D.C.S., D.K.R. and N.C.L.; validation, F.M.d.S., N.C.L. and L.V.; formal analysis, A.H.S., O.A.S., D.C.S. and F.M.d.S.; investigation, A.H.S., O.A.S., D.C.S. and J.A.G.B.; resources, L.V.; data curation, A.H.S., D.K.R. and D.C.S.; writing—original draft preparation, A.H.S., O.A.S. and F.M.d.S.; writing—review and editing, A.H.S., N.C.L. and L.V.; visualization, D.K.R., F.M.d.S., N.C.L. and L.V.; supervision, N.C.L. and L.V.; project administration, A.H.S., N.C.L. and L.V.; funding acquisition, L.V. All authors have read and agreed to the published version of the manuscript.
Acknowledgments: To Programa de Suporte à Pós-Graduação de Instituições Comunitárias de Ensino Particulares (PROSUC) of the CAPES for the scholarship and financial support, to the Empresa Brasileira de Pesquisa Agropecuária–Embrapa Trigo, Embrapa/Bayer Project for the funding and to the University of Passo Fundo (UPF).

Conflicts of Interest: The authors declare no conflict of interest.

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