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Glyphosate-Resistant Italian Ryegrass (*Lolium perenne* L. spp. *Multiflorum*) Control and Seed Suppression in Mississippi

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Abstract: Italian ryegrass is a major weed problem in wheat (*Triticum aestivum* L.) production worldwide. Two separate studies were conducted in Stoneville, Mississippi to evaluate: (1) the efficacy of herbicides available to Mississippi producers for controlling glyphosate-resistant (GR) Italian ryegrass (control study), and (2) fall burndown herbicide seed suppression study. Results of the control study showed that flufenacet/metribuzin EPOST followed by (fb) pinoxaden LPOST (standard treatment) provided 93% control of GR Italian ryegrass. Some other treatments provided comparable Italian ryegrass control (92% to 97%) as the standard treatment in 2017. Italian ryegrass control in the seed suppression study was 100%, 100%, 67.5%, 97%, and 99.5% from the application of the following treatments: (1) *S*-metolachlor + flumioxazin + paraquat in October–November fb glyphosate + clethodim in January–February fb gramoxone as needed (weed-free check); (2) *S*-metolachlor + flumioxazin + paraquat in October–November; (3) field cultivator (disk) in October–November; (4) glyphosate + clethodim in January–February; and (5) field cultivator in October–November fb glyphosate + clethodim in January–February, respectively. The remaining Italian ryegrass from the application of treatments 3, 4, and 5 produced 65,700; 1008; and 9 seeds m^{-2}, respectively. Seed suppression study highlights the importance of 100% control that is required to manage GR Italian grass.

Keywords: acetyl-CoA; herbicide resistance; *Lolium perenne*; weed suppression; wheat production

1. Introduction

Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot) is among the top ten most abundant and problematic weeds for wheat production in the mid-southern United States [1,2]. Ryegrass weed spp., has a high capacity for evolving complex herbicide resistance patterns, making them one of the most troublesome weeds to control [3]. Hybrids of cultivated Italian ryegrass species and Italian ryegrass have been reported to escape along roadsides which have become problematic to control. Carey [4] and Van Wychen [5] reported that Italian ryegrass is the most troublesome and second most common weed for wheat producers in Mississippi. Italian ryegrass competitive indices including leaf development rate and plant height were reported to be greater than wheat [6]. Stone, et al. [7] reported that failure to control Italian ryegrass in wheat resulted in greater ryegrass root density, hence, creating an environment for an increased competition of ryegrass for moisture and nutrients [8].

Historically, Italian ryegrass has been controlled in crop production systems and along roadsides with herbicides [9]. Since 1998, populations of Italian ryegrass have been documented to be resistant to glyphosate, one of the most commonly used herbicides for burndown applications. Powles, et al. [10] reported the first evidence of glyphosate-resistance (GR) in rigid ryegrass. In an orchard in Australia (Orange, New South Wales, Australia), a rigid ryegrass (*Lolium rigidum* Gaud.) population was exposed to two to three applications of glyphosate year^{-1} for 15 yr and exhibited a 7 to 11-fold greater resistance than susceptible populations [11]. In a fruit orchard in Chile, rigid ryegrass populations exposed to three glyphosate applications every year for 8 to 10 years exhibited 2 to 4-fold greater resistance than susceptible populations [12]. In 2003, a 5-fold level of resistance to glyphosate was documented in Oregon [13]. The first GR Italian ryegrass in row crop production in the United States was documented by Nandula, et al. [14] and found two separate populations to be resistant to glyphosate rates up to 0.84 and 1.68 kg ae ha^{-1} [14]. In the mid-southern United States populations of GR Italian ryegrass have been documented in Arkansas, Louisiana, North Carolina, and Tennessee [15].

In wheat production, Italian ryegrass control has heavily relied on diclofop, an acetyl-CoA-carboxylase (ACCase-) inhibiting herbicide that only controls grass weed species [9]. Control options have become limited due to diclofop-resistant Italian ryegrass [16]. Aside from glyphosate, populations of Italian ryegrass in Mississippi have been found resistant or multiple-resistant to glyphosate, acetolactate synthase-inhibitors (ALS), and most recently, clethodim, an ACCase-inhibiting herbicide [15,17]. With populations evolving resistance to one or multiple herbicide chemistries, the ability for Italian ryegrass to mature and return seed back to the soil seedbank is becoming more prominent. In Arkansas, Bararpour, Norsworthy, Burgos, Korres, and Gbur [3] reported populations of Italian ryegrass plants producing 20,000 to 45,000 seed plant^{-1}. Natural infestations of Italian ryegrass of Arkansas were ±323 plants m^{-2}; furthermore, with interference due to densities of this magnitude wheat yield reductions were reported to be as high as 72% over 6 years [18].

Herbicide control options continue to be the most effective option for producers because of time and money. Bararpour, et al. [19] reported 93% control of GR Italian ryegrass with flufenacet + metribuzin at early POST (EPOST) fb pinoxaden late POST (LPOST) on one- to two-leaf Italian ryegrass. In a seed suppression study, Bararpour, et al. [20] reported 100% control and seed suppression with treatments containing *S*-metolachlor + flumioxazin + paraquat in October–November fb glyphosate + clethodim in January–February fb paraquat as needed; and *S*-metolachlor + flumioxazin + paraquat in October–November. These studies emphasize the importance of achieving 100% control of Italian ryegrass and ways to prevent seed returns to the soil seedbank. The high capacity of seed production will lead to increased seed deposition into the soil seedbank and may increase the evolution of herbicide-resistant populations of Italian ryegrass [3]. Combining these factors will ultimately lead to herbicide failure and cause technologies that are available to producers to collapse.

Heavy reliance on weed control programs by the use of herbicides may cause more herbicide chemistries to fail in providing acceptable control. Italian ryegrass continues to cause problems for producers in fall and spring burndown applications. The use of tillage, delayed planting, increased seeding rates, narrow rows, and crop rotation are some of the cultural practices for controlling herbicide-resistant Italian ryegrass [21]. Therefore, research should be conducted by incorporating cultural practices with herbicide programs to help mitigate Italian ryegrass seed production, thereby, decreasing the return of seed to the soil seedbank to delay the evolution of herbicide resistance, and to preserve herbicide technologies that are available. The objective of this study was to evaluate the efficacy of herbicides available to Mississippi producers for controlling GR Italian ryegrass. Additionally, we investigate the weed management strategy targeting GR Italian ryegrass seed production or seed deposition to the soil seedbank in Mississippi in order to facilitate the solution of current weed problems (preventing/reducing the occurrence of herbicide resistance, reducing weed fecundity and soil seedbank).

2. Materials and Methods

2.1. Glyphosate-Resistant Italian Ryegrass Control Study

A two-year field study was conducted (2016–2017 and 2017–2018) at the Mississippi State University Delta Research and Extension Center in Stoneville, MS on Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) with 1.7% organic matter and pH 7.1. The experimental design was a randomized complete block design with 12 treatments and four replications (Table 1). Research plots contained a uniform, natural infestation (±39 plants m^{-2}) of GR Italian ryegrass. The plot size was 3 × 10 m. The herbicide treatments, application rates, and application timings, trade names, and manufacturer information are listed in Table 1. A CO_2-pressurized backpack sprayer with four 110,015 TTI nozzles (TeeJet Technologies, Springfield, IL, USA) mounted on a handheld boom calibrated to deliver 140 L ha^{-1} at 276 kPa was used for applying the treatments. Italian ryegrass control at five different timings was recorded 3, 5, 7, 11, and 15 weeks after treatment application (WAT). Weed control was scored on a scale of 0 to 100 where 0 is no weed control and 100 being complete weed control or plant death.

Table 1. Herbicide treatments for the glyphosate-resistant Italian ryegrass control study in Stoneville, Mississippi from 2016 to 2017 [a].

Treatment [a] ID	Herbicide Treatment	Application Rate	Application Time [b]	Trade Name	Manufacturer
		kg ai ha^{-1}			
T1	mesosulfuron	0.015	EPOST	Osprey	Bayer CropScience, St. Louis, MO, USA
T2	flufenacet/metribuzin + pinoxaden	0.286 + 0.06	EPOST	Axiom +Axial XL	Bayer CropScience, St. Louis, MO, USA; Syngenta, Greensboro, NC, USA
T3	flufenacet/metribuzin fb mesosulfuron	0.286 fb 0.015	EPOST fb LPOST	Axiom fb Osprey	Bayer CropScience, St. Louis, MO, USA
T4	mesosulfuron fb mesosulfuron	0.015 fb 0.015	EPOST fb LPOST	Osprey fb Osprey	Bayer CropScience, St. Louis, MO, USA
T5	metribuzin fb mesosulfuron	0.105 fb 0.015	EPOST fb LPOST	TriCor fb Osprey	Bayer CropScience, St. Louis, MO, USA
T6	metribuzin fb metribuzin	0.105 fb 0.105	EPOST fb LPOST	TriCor fb TriCor	Bayer CropScience, St. Louis, MO, USA
T7	pyroxasulfone	0.09	EPOST	Zidua	BASF Crop Protection, Research Triangle Park, NC, USA
T8	mesosulfuron	0.015	LPOST	Osprey	Bayer CropScience, St. Louis, MO, USA
T9	pyroxasulfone fb metribuzin	0.09 fb 0.105	EPOST fb LPOST	Zidua fb TriCor	BASF Crop Protection, Research Triangle Park, NC, USA; Bayer CropScience, St. Louis, MO, USA
T10	mesosulfuron fb metribuzin	0.015 fb 0.105	EPOST fb LPOST	Osprey fb TriCor	Bayer CropScience, St. Louis, MO, USA
T11	flufenacet/metribuzin fb pinoxaden (standard)	0.286 fb 0.06	EPOST fb LPOST	Axiom fb AxialXL	Bayer CropScience, St. Louis, MO, USA; Syngenta, Greensboro, NC, USA
T12	Nontreated check	-	-	-	-

[a] Treatments 1, 3, 4, 5, 8, and 10 were supplemented with Activator 90 at 0.25% v/v + urea-ammonium nitrate at 3.3% v/v; EPOST application was 14 December 2016 and 10 November 2017 on one- to two-leaf Italian ryegrass; LPOST (spring application) was 21 March 2017 and 16 March 2018 on three- to four-tiller Italian ryegrass. [b] fb = followed by; EPOST = early POST; LPOST = late POST.

2.2. Glyphosate-Resistant Italian Ryegrass Seed Suppression Study

Seed suppression study was conducted based on the reasoning that the evolution of herbicide-resistant weeds from seed deposition (input) of the un-controlled weeds into soil seedbank is greater than the output (seed loss from soil seedbank through germination and weed control programs, predation, decay, etc.). Italian ryegrass seed suppression study was conducted in 2016–2017 at the Delta Research and Extension Center in Stoneville, MS on Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) with 1.7% organic matter and pH 7.1. The experimental design was a randomized complete block design with six treatments and four replications (Table 2). The plot size was 3 × 10 m. The herbicide treatments, application rates, application timings, trade names, and manufacturer information are listed in Table 2. A CO_2-pressurized backpack sprayer with four 110,015 TTI nozzles (TeeJet Technologies, Springfield, IL, USA) mounted on a handheld boom calibrated to deliver 140 L ha^{-1} at 276 kPa was used for applying the treatments. Weed control was scored on a scale of 0 to 100 where 0 is no weed control and 100 being complete weed control or plant death. Additionally, the number of plant m^{-2}, number of spikes m^{-2}, and the total number of seeds m^{-2} were also recorded from each replication. The total number of seeds m^{-2} were calculated by [(number of spikes m^{-2}) × (number of spikelets per spike) × (number of seeds per spikelet)].

Table 2. Herbicide treatment programs for the glyphosate-resistant Italian ryegrass control and seed suppression in the field experiment at Stoneville, Mississippi in 2017.

Treatment ID	Treatment [a]	Application Rate	Application Timing	Trade Name	Manufacturer
		kg ai ha^{-1}			
T1	*S*-metolachlor	1.43	October–November	Dual Magnum	Syngenta, Greensboro, NC, USA
	flumioxazin	0.072	October–November	Valor SX	Valent, Walnut Creek, CA, USA
	paraquat	1.12	October–November	Gramoxone SL	Syngenta, Greensboro, NC, USA
	glyphosate	1.27	January–February	Roundup PowerMax	Bayer CropScience, St. Louis, MO, USA
	clethodim	0.14	January–February	Select Max	Albaugh, LLC, Ankeny, IA, USA
	paraquat	1.12	As needed	Gramoxone SL	Syngenta, Greensboro, NC, USA
T2	*S*-metolachlor	1.27	October–November	Dual Magnum	Syngenta, Greensboro, NC, USA
	flumioxazin	0.072	October–November	Valor SX	Valent, Walnut Creek, CA, USA
	paraquat	1.12	October–November	Gramoxone SL	Syngenta, Greensboro, NC, USA
T3	Field cultivator (disk)		October–November		
T4	glyphosate	1.13	January–February	Roundup PowerMax	Bayer CropScience, St. Louis, MO, USA
	clethodim	0.127	January–February	Select Max	Albaugh, LLC, Ankeny, IA, USA
T5	Field cultivator (disk)		October–November		
	glyphosate	1.27	January–February	Roundup PowerMax	Bayer CropScience, St. Louis, MO, USA
	clethodim	0.14	January–February	Select Max	Albaugh, LLC, Ankeny, IA, USA
T6	Untreated control		-	-	-

[a] Treatments 1, 2, 4, and 5 were supplemented with Activator 90 at 0.5% *v/v*.

2.3. Statistical Analysis

Statistical analysis was performed in SAS (SAS Institute Inc., Cary, NC, USA) using GLM procedure. In the Italian ryegrass control study, weed control data collected at five different timing were treated as dependent variables and herbicide treatments were treated as independent variables. Additionally, weed control data from 2016–2017 and 2017–2018 for Italian ryegrass control study were analyzed separately, because of significant differences in the year. For Italian ryegrass seed suppression study percent weed control, plants m^{-2}, number of spikes m^{-2}, and the total number of seeds m^{-2} were dependent variables and number of herbicide treatments were independent variables. For the data that met the assumptions of ANOVA, means were separated using Fisher's protected Least Significant Difference (LSD) ($\alpha = 0.05$).

3. Results and Discussion

3.1. Glyphosate-Resistant Italian Ryegrass Control Study

In 2016–2017, rating 1 was taken three-weeks-after treatment application (WAT) and all herbicide treatments provided >92% GR Italian ryegrass control except T11 (flufenacet/metribuzin EPOST fb pinoxaden LPOST) which accounted for 66% control (Figure 1A). The highest control of GR Italian ryegrass (98%) was provided by T5 (metribuzin EPOST fb mesosulfuron LPOST) at 3WAT. Rating 2 was made 5WAT and T11 (flufenacet/metribuzin EPOST fb pinoxaden LPOST) provided 95% of GR Italian ryegrass control. Mesosulfuron EPOST fb metribuzin LPOST (T10), when rated at 5WAT, had 91% GR Italian ryegrass control and accounted for the least control compared to the all other treatments except T1 (mesosulfuron EPOST). Treatment 1 (mesosulfuron EPOST), T10 (mesosulfuron EPOST fb metribuzin LPOST), and T11 (flufenacet/metribuzin EPOST fb pinoxaden LPOST) showed lowest (89–90%) GR Italian ryegrass control at 7WAT. Treatments 2, 3, and 9 showed 97% of GR Italian ryegrass control at rating 3 that was taken 7WAT. Late POST herbicide application was applied on 21 March 2017 when Italian ryegrass had 3–4 tillers with an average height of tillers ranging between

5–8 cm. Rating 4 was made two weeks after LPOST during which T8 (mesosulfuron LPOST) provided the lowest 66% GR Italian ryegrass control. Treatment 1, 4, and 10 provided 83% to 89% control of GR Italian ryegrass when rated at 11WAT (Figure 1D). Glyphosate-resistant Italian ryegrass control by T2, T3, T5, T6, T7, and T9 was between 95% to 98% for rating 4 (11WAT) and were greater than T1, T4, and T10. The final herbicide performance evaluation (rating 5) was made 15WAT EPOST and T2, T3, T5, T6, T7, T9, and T11 showed GR Italian ryegrass control of 92% to 97% which were greater (8–28%) than T1, T4, T8, and T10. The T1 and T4 showed 4% to 16% greater GR Italian ryegrass control compare to T8 and T10. A single application of mesosulfuron LPOST (T8) in 2017 was the weakest treatment (69%) for GR Italian ryegrass control (Figure 1E).

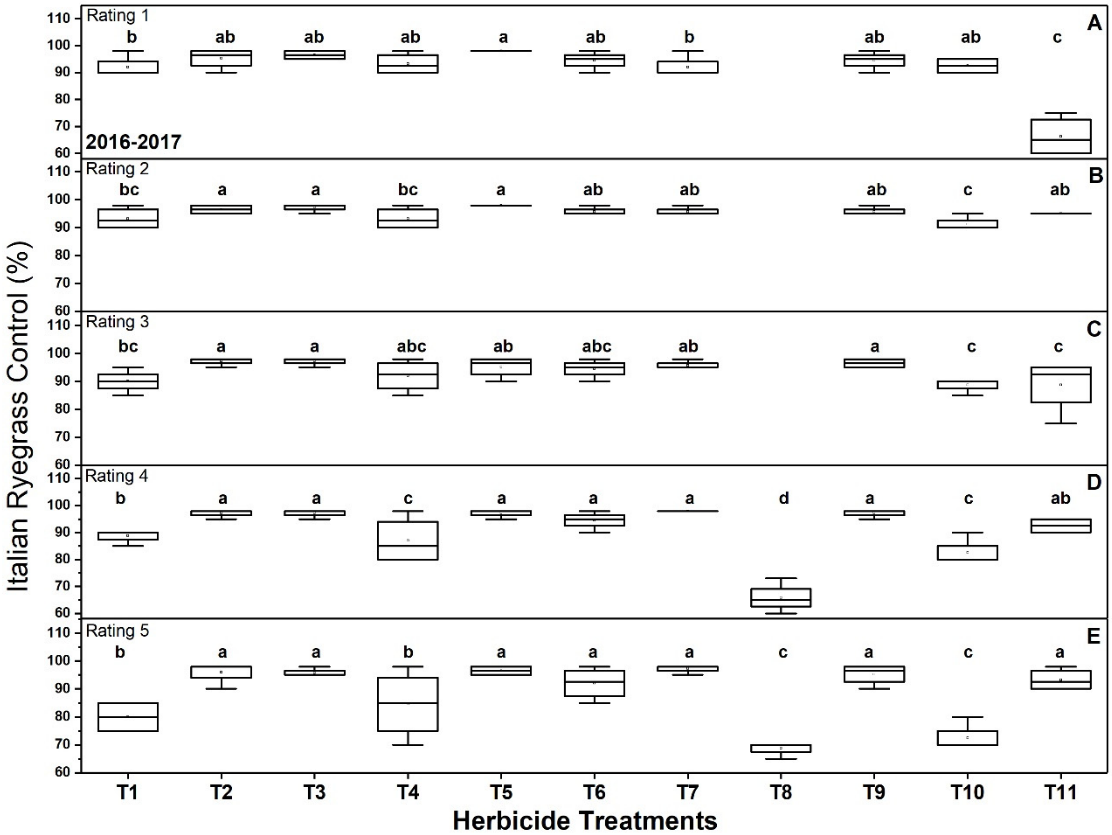

Figure 1. Glyphosate-Resistant Italian ryegrass Control Study in 2017 showing percent weed control. Herbicide treatments were T1 = mesosulfuron, T2 = flufenacet/metribuzin + pinoxaden, T3 = flufenacet/metribuzin followed by (fb) mesosulfuron, T4 = mesosulfuron fb mesosulfuron, T5 = metribuzin fb mesosulfuron, T6 = metribuzin fb metribuzin, T7 = pyroxasulfone, T8 = mesosulfuron, T9 = pyroxasulfone fb metribuzin, T10 = mesosulfuron fb metribuzin, T11 = flufenacet/metribuzin fb pinoxaden (standard). rating 1 in (**A**) was 3 weeks after treatment application (3WAT), rating 2 in (**B**) was 5WAT, rating 3 in (**C**) was 7WAT, rating 4 in (**D**) was 11WAT and rating 5 in (**E**) was 15WAT. Letters show the least squared difference between the treatments at $\alpha = 0.05$.

In 2017–2018, 3WAT, T1 (mesosulfuron EPOST), T2 (flufenacet/metribuzin + pinoxaden EPOST), T4 (mesosulfuron EPOST fb mesosulfuron LPOST), T10 (mesosulfuron EPOST fb metribuzin LPOST), and T11 (flufenacet/metribuzin EPOST fb pinoxaden LPOST) showed 69% to 80% of GR Italian ryegrass control (Figure 2A). However, all other treatments (T3, T5, T6, T7, and T9) were rated below 46% for GR Italian ryegrass control. Flufenacet/metribuzin + pinoxaden EPOST (T2) showed 90% of GR Italian ryegrass control when rated 5WAT and provided 15% to 23% greater control than T3, T5, T6, and T9. Glyphosate-resistant Italian ryegrass control among treatments T1, T2, T4, T7, T10, and T11 was comparable and provided 81% to 90% control (Figure 2B). Seven weeks after EPOST in 2018 GR Italian ryegrass control for all treatments were rated to be less than 81% with lowest GR Italian ryegrass control observed in T6 (56%) (metribuzin EPOST fb metribuzin LPOST) followed by T5 (63%) < T3 and T9 (67%). Late POST treatment application was made on 16 March 2018 and rating 4 was conducted two weeks after the treatment application or 11WAT of EPOST (Figure 2D). Mesosulfuron LPOST (T8) was applied to GR Italian ryegrass when it was three- to four-tillers growth stage and rating 4 made two weeks after LPOST showed only 66% control. Similarly, in T5 and T6 LPOST application of mesosulfuron and metribuzin provided 67% and 69% of GR Italian ryegrass control, respectively. Lowest GR Italian ryegrass control was for T5 and T8 when compared among all treatments and were lower than T2, T9, and T11 (Figure 2D). Among all treatment flufenacet/metribuzin + pinoxaden EPOST (T2) provided the highest 90% control and was rated as the best treatment (Figure 2D). Final herbicide performance evaluation rating 5 showed that T2 (flufenacet/metribuzin + pinoxaden EPOST) maintained 91% of GR Italian ryegrass control which was followed by T11 (flufenacet/metribuzin EPOST fb pinoxaden LPOST) at 87% control. All other treatments showed >76% of GR Italian ryegrass control and poorest performance of 60% control was for T6 (metribuzin fb metribuzin).

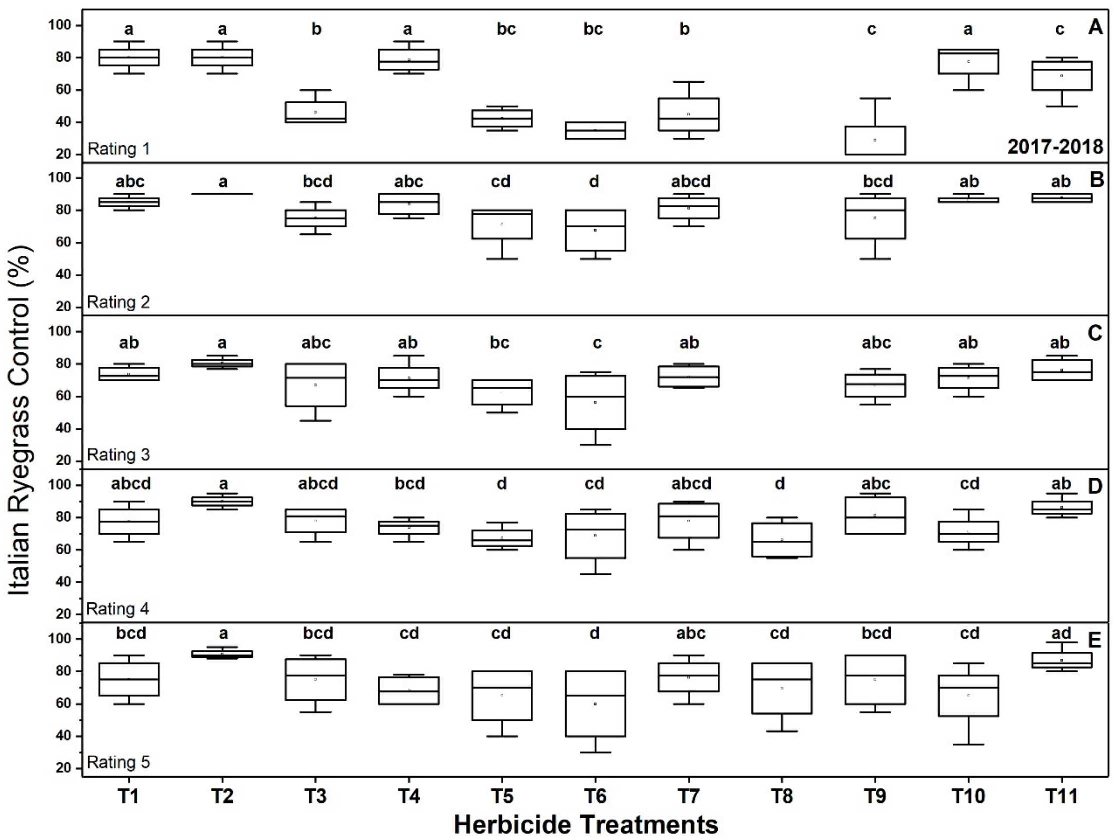

Figure 2. Glyphosate-resistant Italian ryegrass Control Study in 2018 showing percent weed control. Herbicide treatments were T1 = mesosulfuron, T2 = flufenacet/metribuzin + pinoxaden, T3 = flufenacet/metribuzin followed by (fb) mesosulfuron, T4 = mesosulfuron fb mesosulfuron, T5 = metribuzin fb mesosulfuron, T6 = metribuzin fb metribuzin, T7 = pyroxasulfone, T8 = mesosulfuron, T9 = pyroxasulfone fb metribuzin, T10 = mesosulfuron fb metribuzin, T11 = flufenacet/metribuzin fb pinoxaden (standard). rating 1 in (**A**) was 3 weeks after treatment application (3WAT), rating 2 in (**B**) was 5WAT, rating 3 in (**C**) was 7WAT, rating 4 in (**D**) was 11WAT, and rating 5 in (**E**) was 15WAT. Letters show the least squared difference between the treatments at $\alpha = 0.05$.

Single herbicide application program included mesosulfuron EPOST (T1), T2 (flufenacet/metribuzin + pinoxaden EPOST), T7 (pyroxasulfone EPOST), and T8 (mesosulfuron LPOST). Only T2 provided >90% GR Italian ryegrass control during two years of study. The application timing of T2 was EPOST targeted at one- to two-leaf stage of Italian ryegrass. Flufenacet herbicide in flufenacet/metribuzin + pinoxaden is reported to have herbicide residual activity and inhibit the synthesis of fatty acids for wax and phospholipid formation in plant leaves [22]. Metribuzin herbicide in T2 is a photosynthesis inhibitor; whereas, pinoxaden inhibits the enzyme Acetyl-CoA-Carboxylase (ACCase), resulting in interruption of synthesis of fatty acids and impacting the formation of biomembranes [23,24]. The multi-mode of action and herbicide residual activity provided by T2 resulted in the highest GR Italian ryegrass control. Several studies have reported 84 to 96% Italian ryegrass control with flufenacet/metribuzin herbicides [16,19,20,25–28]. Glyphosate-resistant Italian ryegrass control in 2017–2018 using mesosulfuron and pyroxasulfone ranged between 45% to 85% (Figure 2A–E). Mesosulfuron is a member of sulfonylurea group of herbicides and its mode of action is inhibition of acetolactate synthase (ALS); whereas, pyroxasulfone herbicide blocks lipid biosynthesis through inhibition of several very-long-chain fatty acids [29,30]. Kuk and Bugos [29] reported that the resistance mechanism of Italian ryegrass to mesosulfuron is partly due to an alteration in the target enzyme, ALS. Mesosulfuron LPOST (T8) was applied to GR Italian ryegrass when it had three- to four-tillers and it is possible that ALS inhibition was not 100%, therefore control ranged from 66% to 70%. Hulting, Dauer, Hinds-Cook, Curtis, Koepke–Hill and Mallory–Smith [28] studied Italian ryegrass control with pyroxasulfone, flufenacet, and flufenacet + metribuzin and reported that pyroxasulfone applications controlled Italian ryegrass between 65% to 100% and flufenacet and flufenacet + metribuzin treatments provided similar control as that of pyroxasulfone.

Multiple herbicide application programs included T3, T4, T5, T6, T9, T10, and T11. Flufenacet/metribuzin EPOST fb pinoxaden LPOST (T11) was the only herbicide application program that provided 93% and 87% control of GR Italian ryegrass in 2016–2017 and 2017–2018, respectively. Flufenacet + metribuzin was reported to control the Italian ryegrass population ranging from 66% to 97% [8,16]. Pinoxaden is an ACCase–inhibiting herbicides and has been reported previously to control diclofop-resistant Italian ryegrass [19]. A single application of pinoxaden resulted in 58% Italian ryegrass control; whereas, metribuzin fb pinoxaden showed Italian ryegrass control ranging between 84% to 89% [19]. Mesosulfuron EPOST fb mesosulfuron LPOST (T4) and metribuzin EPOST fb mesosulfuron LPOST (T5) showed GR Italian ryegrass control ranging between 65% to 97% which is similar to that reported by Bararpour, Korres, Burgos, Hale, and Tseng [19]. Ryegrass control by application of metribuzin EPOST fb metribuzin LPOST ranged between 79% to 94% [25]. Among all single and dual herbicide application programs T2 and T11 showed >87% control at 15WAT over two years of the study period. It is important to note that resistance to ACCase and ALS inhibitors in Italian ryegrass present a serious threat to wheat growers. Herbicide treatment program including flufenacet/metribuzin and pinoxaden either applied EPOST or in a combination of EPOST and LPOST provided GR Italian ryegrass control; however, this control was highly variable and inconsistent. Limited herbicide options for Italian ryegrass control in wheat, resistance to most currently available herbicides, and diminishing prospects for novel modes of action being commercialized emphasize the need for 100% seed suppression of GR Italian ryegrass [9].

3.2. Glyphosate-Resistant Italian Ryegrass Seed Suppression Study

In the seed suppression study, T1 had *S*-metolachlor + flumioxazin + paraquat applied in October–November fb an application of glyphosate and clethodim in Jan-Feb and paraquat applied as need showed 100% GR Italian ryegrass control (Figure 3A). Similarly, T2 had *S*-metolachlor + flumioxazin + paraquat applied in October–November also provided 100% GR Italian ryegrass control. Treatment 3 was a field cultivator treatment and GR Italian ryegrass control was lowest (68%) (Figure 3A). Treatment 4 and T5, glyphosate + clethodim without and with field cultivator showed 97% and 99.5% control, respectively. Plots that received T3 (field cultivation) had 18 GR Italian ryegrass plants m^{-2}, which produced 292 spikes m^{-2} (each spike had 25 spikelets and each spikelet had 9 seeds) further producing 65,700 seeds m^{-2} (Figure 3B–D). Similarly, T4 with 97% control had 1 GR Italian ryegrass plant m^{-2} which accounted for 18 spikes m^{-2} (each spike had 14 spikelets and each spikelet had four seeds) resulting in producing 1008 seeds m^{-2}. Plots that received T5 (field cultivator in October–November fb glyphosate + clethodim in January–February with 99.5% GR Italian ryegrass control) average 0.009 plants m^{-2}. The remaining GR Italian ryegrass plants were able to produce 0.75 spike m^{-2}. Each spike had six spikelet's and each spikelet had two seeds. Therefore, the remaining GR Italian ryegrass plants produced 9 seeds m^{-2}. The untreated control had 39 GR Italian ryegrass plants m^{-2} which produced 454 spike m^{-2} (each spike had 27 spikelets and each spikelet had 8 seeds); therefore, GR Italian ryegrass plants in the untreated check plot produced 98,064 seeds m^{-2} (Figure 3B–D).

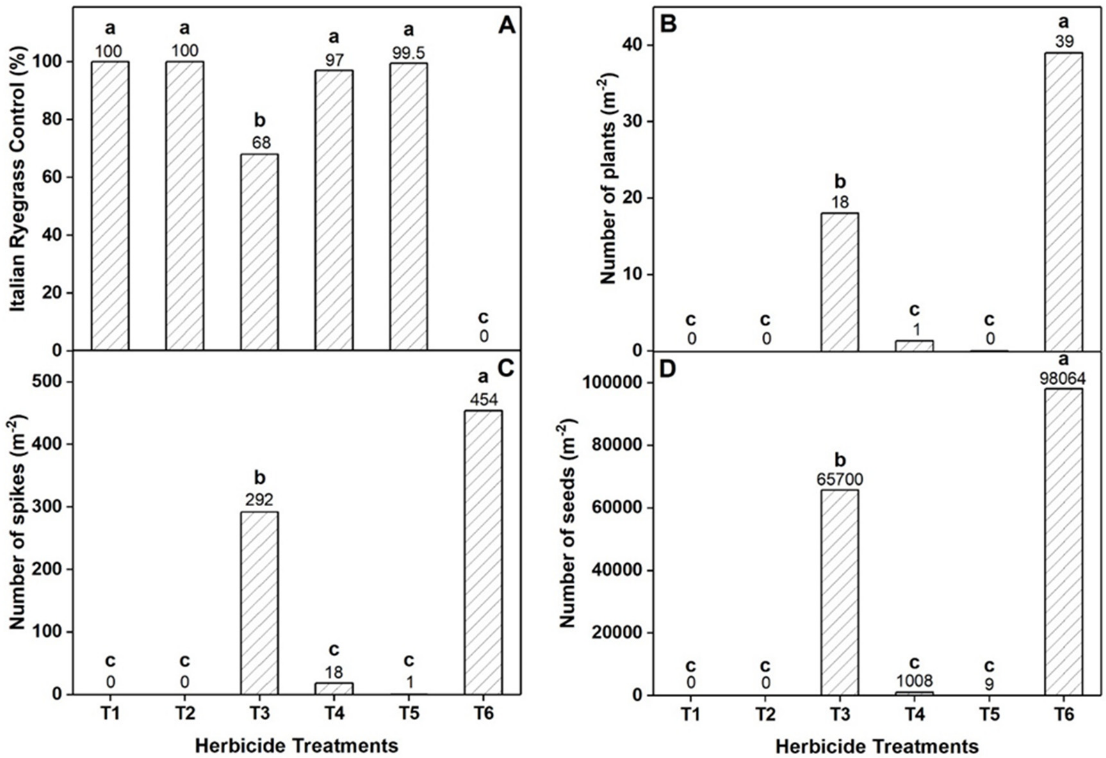

Figure 3. Glyphosate-resistant Italian ryegrass seed suppression study in 2017 showing percent weed control (**A**), number of plants m^{-2} (**B**), number of spikes m^{-2} (**C**), and number of seeds m^{-2} (**D**). Herbicide treatment details are provided in Table 2. Letters show the least squared difference between the treatments at $\alpha = 0.05$.

One of the most critical issues for weed scientists today is the management of herbicide-resistant weeds in a long-term spatiotemporal scale. An efficient weed management program should focus on eliminating crop-weed interference but also maintain this result for as long as possible by preventing/delaying the occurrence of herbicide-resistant weeds. One of the ways of doing this is to stop weed seed deposition. We believe that if the input (seed deposition to the soil seedbank from the remaining or un-control weeds) is greater (>) than the output (seed loss from soil seedbank through germination and weed control programs, predation, decay, etc.), the resistant-weeds will be evolved and the technology or herbicide control program will be lost.

Therefore, one of the best ways to preserve the technology or to maintain the technology for a longer period is to stop weed seed deposition to the soil seedbank (no seeds means no weeds or no herbicide resistance weeds). In this study, it seems that weed control programs that provided around 90% control were a good program. However, the seed suppression study showed that the plots which received a treatment that controlled 97% of GR Italian ryegrass, deposited >1008 seed to the soil seedbank. This means that resistance will be developed in a matter of time since the input (seed deposition to the soil seedbank) is greater than the output (seed withdrawal from soil seedbank). Weed pressure may be decreased or eradicated if weed management programs can lead to decreasing the input vs. output (INPUT < OUTPUT). Therefore, for a long-term weed management strategy, weed seed production must be targeted as well. This study highlights the importance of complete eradication of GR Italian ryegrass so that the deposition to seedbank is reduced to zero. Complete eradication of weeds, although difficult to achieve can promote the stability of herbicide control technology for a longer period of time.

4. Conclusions

Flufenacet/metribuzin EPOST followed by (fb) pinoxaden LPOST (standard treatment) provided 93% control of GR Italian ryegrass. Some other treatments provided comparable Italian ryegrass control (92% to 97%) as the standard treatment in 2017. Seed suppression study highlights the importance of 100% GR Italian grass so that the evolution of herbicide-resistant can be stopped. One of the best ways, to preserve the technology for as long as possible is to stop weed seed deposition to the soil seedbank (no seeds means no weeds or no herbicide resistance weeds).

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