


Article

Performance of Pinoxaden on the Control of Diclofop-Resistant Italian Ryegrass (*Lolium perenne* L. ssp. *multiflorum*) in Winter Wheat

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Received: 1 June 2018; Accepted: 6 July 2018; Published: 10 July 2018



Abstract: Resistance to herbicides in Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot) is an economically important herbicide-resistance issue in world agriculture. Diclofop-resistant ryegrass is the number one weed problem in Arkansas wheat. Field studies were conducted from 2006 through 2008 at Bredlow corner and Fayetteville, Arkansas, USA to determine the effect of herbicide programs consisting of pinoxaden (Axial) alone or in mixtures with preemergence and postemergence herbicides, along with tillage systems, for managing diclofop-resistant Italian ryegrass. Italian ryegrass control, wheat injury, and grain yield were evaluated. A single treatment with pinoxaden alone resulted in 58% ryegrass control and 1488 kg ha⁻¹ grain yield. The best treatment was metribuzin followed by mesosulfuron, which resulted in 2793 kg grain yield ha⁻¹, a 47% increase compared to pinoxaden alone. In Fayetteville (2006–2007 and 2007–2008), the best treatment was two applications of metribuzin, which yielded about 3700 kg grain ha⁻¹. A single application of pinoxaden resulted in 40% loss of grain yield compared to a single application of flufenacet + metribuzin and almost 50% yield loss compared to sequential application of metribuzin. Yield loss was attributed due to insufficient control of Italian ryegrass. The effect of tillage on Italian ryegrass was inconsistent between years and treatments. In the first year, for example, tillage × herbicide treatments did not differ. In the second and third year, control of Italian ryegrass was better in no-till than in conventional till in both locations. In conclusion, pinoxaden alone could not control diclofop-resistant Italian ryegrass sufficiently. Other herbicide modes of action need to be added to the management program of diclofop-resistant Italian ryegrass, especially one with residual activity such as metribuzin.

Keywords: ACCase inhibitor herbicide; cultural practices; graminicide herbicides; tillage; yield reduction

1. Introduction

Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot), a winter annual, is a problematic weed in small grains crops [1–3]. Reductions up to 61% in wheat grain yield were reported in the presence of Italian ryegrass (93 plants m⁻²) which caused reductions in wheat tillering capacity and promoted lodging [4]. In a six-year study, Bararpour and Oliver [5] reported a natural population of diclofop-resistant Italian ryegrass that caused 72% wheat yields reduction. Conventional

and organic wheat production systems are affected by infestations of Italian ryegrass and with the increase in resistance to herbicide modes of action and increased market for organic grain, alternative methods of control should be evaluated [3]. Bararpour et al. [6] reported Italian ryegrass in Arkansas capable of producing 45,000 seed plant⁻¹.

Historically, diclofop, an Acetyl CoA carboxylase (ACCase)-inhibiting herbicide, has been used for the control of Italian ryegrass [7,8]. Diclofop applied POST at 500 to 1500 g ai/ha controlled Italian ryegrass 81% to 100%, and increased wheat grain yield 20% to 60% [9–11]. Italian ryegrass is very competitive and has become progressively resistant to diclofop [12–14]. To mitigate this problem, producers have used acetate synthase (ALS) inhibitors (mesosulfuron, pyroxsulam) POST, a photosynthesis inhibitor (metribuzin) PRE, glyphosate preplant, and other herbicides. As evolution by selection dictates, Italian ryegrass has also evolved resistance to alternative herbicides. Perez and Kogan [15] reported a two- to four-fold resistance to glyphosate [15]. In 2003, a five-fold level of resistance to glyphosate was documented in Italian ryegrass in a filbert (*Corylus avellana* L.) orchard in Oregon [16,17]. In the United States, Nandula et al. [18] documented the first glyphosate-resistant (GR) Italian ryegrass in row crop production [17]. Thirty-two counties in Mississippi, and at least one county or parish in Arkansas, California, Louisiana, North Carolina, and Tennessee have GR Italian ryegrass populations [17,19,20].

Italian ryegrass resistance to ALS inhibitors is becoming widespread in the southern USA. In Mississippi, two sulfometuron-resistant populations of Italian ryegrass were documented by Taylor and Coats [21]. Resistance to mesosulfuron was detected just after one year of commercial use in Arkansas, presumably due to preselection with other ALS herbicides [22]. Italian ryegrass populations have been confirmed resistant to acetate synthase-inhibiting herbicides in Arkansas, California, Delaware, Georgia, Idaho, Kentucky, Missouri, North Carolina, and South Carolina [20].

Due to the increasing evolution of herbicide-resistant Italian ryegrass, producers have adopted GR crop technologies to reduce input costs, equipment use, soil erosion, and number of herbicide applications and replaced conventional tillage practices with zero tillage or conservation tillage [17,21,23,24]. Conservation tillage promotes accumulation of plant residue at the soil surface that contributes to the inhibition of weed seed germination due to shading or cooler temperatures at the soil surface. It also increases the availability of soil nutrients (from enhanced microbial activities) and water by making the soil less vulnerable to erosion or evaporation processes [25]. In zero-till and low-input systems, Vencill and Banks [26] documented a rapid increase in soil seedbank due to inconsistent weed control [24]. Reduced tillage system favors Italian ryegrass proliferation, causing increased herbicide usage [27] and leading to selection of resistant biotypes. Like Italian ryegrass, the seedbank of common ragweed (*Ambrosia artemisiifolia* L.), common lambsquarters (*Chenopodium album* L.), horseweed (*Conyza Canadensis* (L.) Cronq), and sicklepod (*Senna obtusifolia* (L.) Irwin and Barneby) also increased in reduced tillage systems [26,28]. Increasing tillage and subsequent cultivation disrupts the soil and can stimulate or reduce the germination of certain weed seeds [26]. Also, tillage timing can be a factor in reduction of weed seed viability, moving seeds to different depths in the soil profile and creating an unfavorable environment during critical stages of reproduction [28]. Integrating herbicide programs within a tillage system improves overall long-term weed control and increases crop production.

Pinoxaden [29] (Axial[®] XL herbicide, Syngenta Crop Protection, LLC, Greensboro, NC, USA) is an ACCase-inhibiting herbicide labeled for POST control of grass weed species in wheat and barley [28]. It is a welcome addition to the limited options for grass control in wheat. Unlike diclofop and other ACCase-inhibitor herbicides, pinoxaden is not antagonized by broadleaf herbicides and has a wider window of application in wheat. With the rapid adoption of pinoxaden, and subsequent monitoring of ryegrass populations, we learned that 25% of Italian ryegrass populations are cross-resistant to diclofop and pinoxaden [29].

Preserving technologies and herbicide regimes are important for the future of agriculture. Developing programs that integrate tillage and herbicide technology, with correct identification of

weed species and understanding the biology of weed species are critical in increasing crop production to obtain maximum yield. Tillage and no-tillage systems can promote beneficial soil properties, but the type of system used for weed control can vary depending upon weed species. The objective of this research is to evaluate the integration of tillage into herbicide programs containing pinoxaden and other wheat herbicides for diclofop-resistant Italian ryegrass control and its effect on ryegrass soil seedbank.

2. Materials and Methods

2.1. Site Description and Crop Establishment

Two field experiments at two locations in Arkansas were conducted to investigate the efficacy of various herbicide combinations on diclofop-resistant Italian ryegrass. A tillage or no-tillage cropping system was included as an additional treatment in the second experiment. The first experiment was set up at the Agricultural Research Center, Fayetteville, Arkansas (36.09507° N, 94.17547° W) on Albaquults silt-loam soil (21% sand, 70% silt, 9% clay) with a pH of 5.8 and 0.5 organic matter content during 2005–2006 and 2007–2008. The second experiment was set up at Bredlow Corner (34.5343° N, 92.1013° W), Arkansas on a Yorktown silty clay (0% sand, 44% silt, 56% clay) soil with a pH of 5.8, and 1.8% organic matter content during 2006–2009. In the fall vegetation in all plots were mowed and two weeks later, the conventional plots were tilled to prepare the seedbed for planting wheat.

Glyphosate was broadcasted to the entire experimental area immediately after planting to burndown undesirable vegetation. Winter wheat 'Beretta 989' (©2011 AgriPro, Syngenta, Jasper, MO, USA) was planted at 112 kg ha⁻¹ seed in a 10 × 10 m experimental plot with row spacing at 18 cm in both locations. Wheat emerged approximately 10–14 days after planting (DAP); the desired crop stand was attained. Details of planting dates and crop emergence are shown in Table 1.

Table 1. Winter wheat planting, emergence and harvesting dates for both Fayetteville and Bredlow Corner field experiments.

Location	Year	Planting Date	Crop Emergence	Harvest
Fayetteville	2006–2007	10 October 2006	26 October 2006	22 May 2007
	2007–2008	15 October 2007	27 October 2007	19 May 2008
Bredlow corner	2006–2007	6 October 2006	21 October 2006	13 June 2007
	2007–2008	6 November 2007	16 November 2007	3 July 2008
	2008–2009	10 October 2008	26 October 2008	16 June 2009

2.2. Ryegrass Population and Herbicide Treatments

The experimental site had a uniform distribution of naturally occurring diclofop-resistant Italian ryegrass in both locations. The density of Italian ryegrass was estimated using a 0.5 m × 0.5 m quadrat at two randomly selected sampling points within each plot and was found to be 323 ± 12 and 600 ± 10 plants m⁻² at Fayetteville and Bredlow Corner, respectively.

A complete randomized block design with four replications was adopted for the first experiment at Fayetteville. The second experiment was established as a split-split plot design with three replications; whole-plot being the tillage regime (tilled vs. no-tilled) and sub-plot being herbicide treatment (Tables 2 and 3).

A CO₂-pressurized backpack sprayer with four 8002 flat-fan nozzles (TeeJet Technologies, Springfield, IL, USA) handheld boom calibrated to deliver 187 L ha⁻¹ at 276 kPa was used.

Table 2. Herbicide treatments for the control of diclofop-resistant Italian ryegrass in the first field experiment at Fayetteville, Arkansas.

Treatment ID	Herbicide Treatment	Application Rate	Growth Stage * at Application	Wheat	Ryegrass	Trade Name	Manufacturer
kg ai ha ⁻¹							
1	Pinoxaden †	0.060	21–22		×	Axial	Syngenta
2	Flufenacet fb	0.476	12–13	×		Axiom	Bayer CropScience
3	Metribuzin fb	0.280	12–13	×		Sencor	Bayer CropScience
	Metribuzin	0.280	22–23	×		Sencor	

* Refers to Zadoks et al. [30] growth scale for cereals and grasses; † adjuvant (Adigor) at 0.375% v/v; × denotes application of herbicides used; fb = followed by.

Table 3. Herbicide treatments for the control of diclofop-resistant Italian ryegrass in the second field experiment at Bredlow Corner, Arkansas.

Treatment ID	Herbicide Treatment	Application Rate	Growth Stage * at Application	Wheat	Ryegrass	Trade Name	Manufacturer
kg ai ha ⁻¹							
1	Pinoxaden †	0.060	21–22		×	Axial	Syngenta
2	Metribuzin fb	0.280	12–13	×		Sencor	Bayer CropScience
3	Pinoxaden ‡	0.060	21–22		×	Axial	Syngenta
	Mesosulfuron ‡ fb	0.069	21–22		×	Osprey	Bayer CropScience
4	Mesosulfuron ‡	0.069	14–22		×	Osprey	Bayer CropScience
	Mesosulfuron ‡	0.015	21–22		×	Osprey	Bayer CropScience
5	Metribuzin fb	0.280	12–13	×		Sencor	Bayer CropScience
	Mesosulfuron ‡	0.0134	21–22		×	Osprey	Bayer CropScience

* Refers to Zadoks et al. [30] growth stage scale for cereals and grasses; † adjuvant (Adigor) at 0.375% v/v; ‡ MSO (destiny) at 1% v/v; × denotes application of herbicides used; fb = followed by.

2.3. Data Collection and Statistical Analysis

Crop injury and weed control were scored on a scale of 0–100 (0 being no injury or weed control and 100 being complete crop death or weed control) on a weekly basis starting 8 weeks after crop emergence (WAE). All data were analyzed with JMP Pro 13.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, means were separated using Fisher's protected Least Significant Difference (LSD) ($\alpha = 0.05$). Because of environmental and climatic differences between years, data were analyzed for each individual year. The effect of tillage on Italian ryegrass control was inconsistent through years; therefore, data were analyzed separately by year. The effect of year or tillage on grain yield, adjusted at 13% moisture content, was not significant; hence, data were combined over tillage system and experimentation year.

3. Results

3.1. Bredlow Corner Trials

Significant differences ($\alpha = 0.05$) were observed for herbicide treatments on Italian ryegrass control throughout the growing period each year. Metribuzin fb pinoxaden provided better Italian ryegrass control (88.5% in 2006–2007; 87.7% in 2007–2008 and 83.5% in 2008–2009) compared to other herbicide

treatments at all evaluation times (Figure 1a–c). The control of Italian ryegrass declined for herbicide treatments consisting of a single active ingredient, except the mixtures containing metribuzin, toward the end the growing season (Figure 1a–c). Single applications of either pinoxaden or mesosulfuron controlled Italian ryegrass less than two applications of metribuzin, or other herbicide treatments, approximately 16 WAE (Figure 1a–c). The control of diclofop-resistant Italian ryegrass was significantly ($\alpha = 0.05$) less with a single pinoxaden application, which was 41% to 68% between n 2006 and 2009. Control of diclofop-resistant Italian ryegrass with pinoxaden alone declined with time from 78% to 55%, 80% to 68% and 65% to 41% for the years 2006–2007, 2007–2008 and 2008–2009, respectively (Figure 1a–c). Metribuzin followed by mesosulfuron was the only treatment that maintained control of diclofop-resistant Italian ryegrass at a satisfactory level (87–88%) at all evaluation times.

The effectiveness of herbicide treatments was reflected on wheat grain yield as plots that received only a single pinoxaden application produced the lowest wheat yield, averaged across the years and tillage systems, equal to 1505 kg ha^{-1} (Figure 2) compared to the other treatments. The plot treated with metribuzin fb mesosulfuron yielded highest (2825 kg/ha). A single application of mesosulfuron resulted in 30% more grain yield (2134 kg/ha) compared to a single application of pinoxaden.

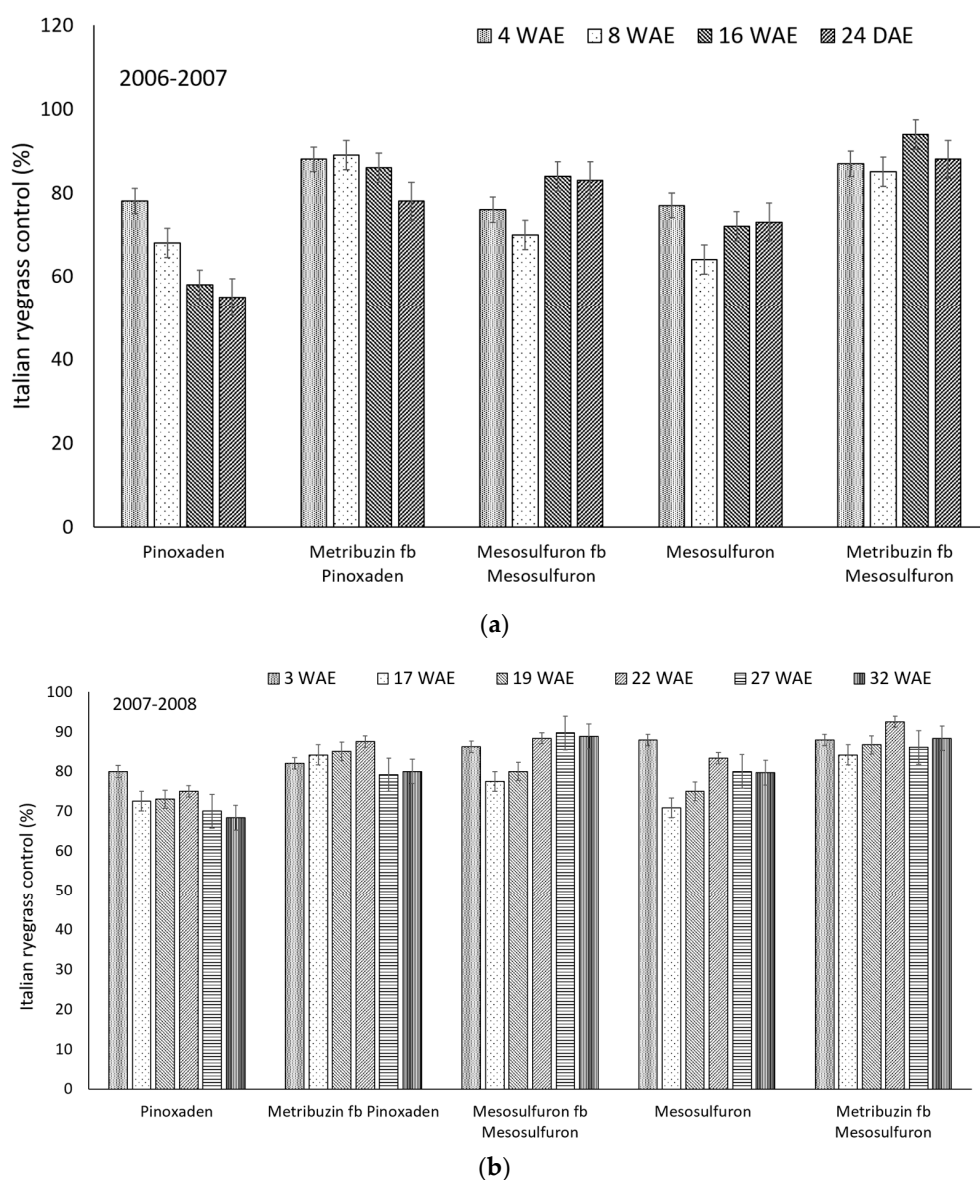


Figure 1. Cont.

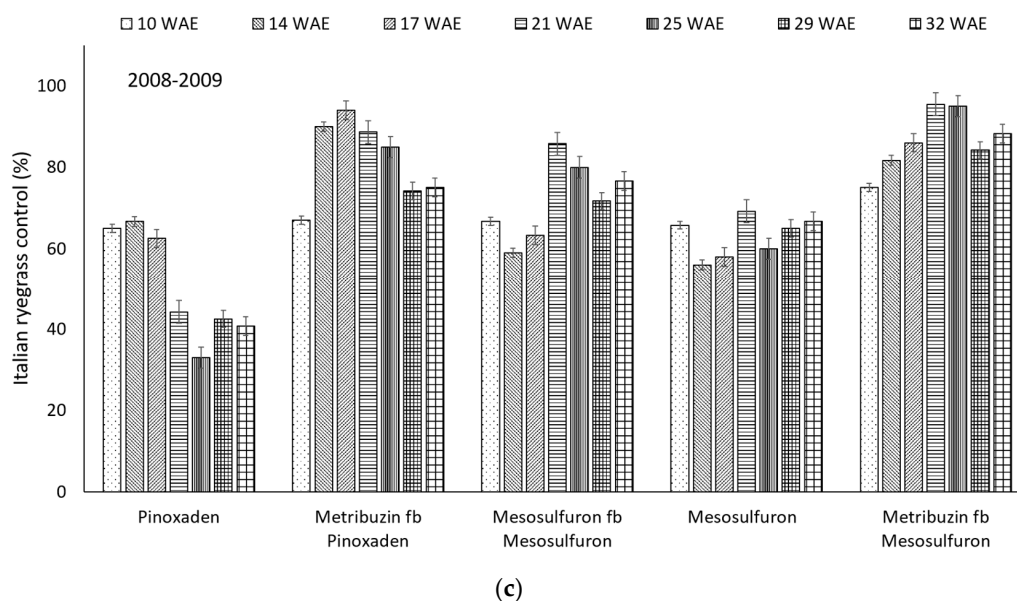


Figure 1. (a) Effects of herbicide program on percentage Italian ryegrass control in 2006–2007 experimental period. Vertical bars represent LSD at 5% significance level (LSD for 4, 8, 16 and 24 WAE = 5, 9, 9, 15 respectively); (b) Effects of herbicide program on percentage Italian ryegrass control in 2007–2008 experimental period. Vertical bars represent Least Significant Difference (LSD) at 5% significance level (LSD for 3, 17, 19, 22, 27 and 32 WAE = 3, 5, 6, 3, 9, 8 respectively); (c) Effects of herbicide program on percentage Italian ryegrass control in 2008–2009 experimental period. Vertical bars represent LSD at 5% significance level (LSD for 10, 14, 17, 21, 25, 29 and 32 WAE = 2, 3, 5, 5, 6, 6 respectively). LSD = Least Significant Difference, WAE = Weeks after emergence, DAE = Days after emergence.

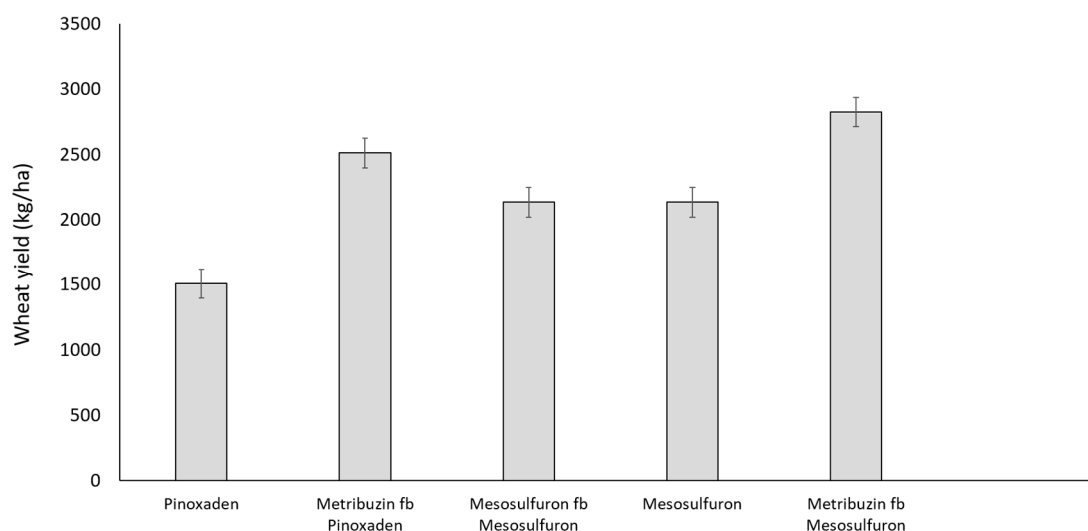


Figure 2. Effects of herbicide program on grain yield at Bredlow corner averaged across experimentation years and tillage systems. Vertical bars represent Least Significant Difference (LSD) at 5% significance level (LSD = 225.7).

3.2. Fayetteville Trials

The percentage control of diclofop-resistant Italian ryegrass by the single application of pinoxaden was the lowest amongst the other herbicide programs evaluated. More specifically, the control of Italian

ryegrass 4 WAE was 75% in 2006–2007 (Figure 3a) and the lowest was 39% in 2007–2008 (Figure 3b). This activity declined with time in both years.

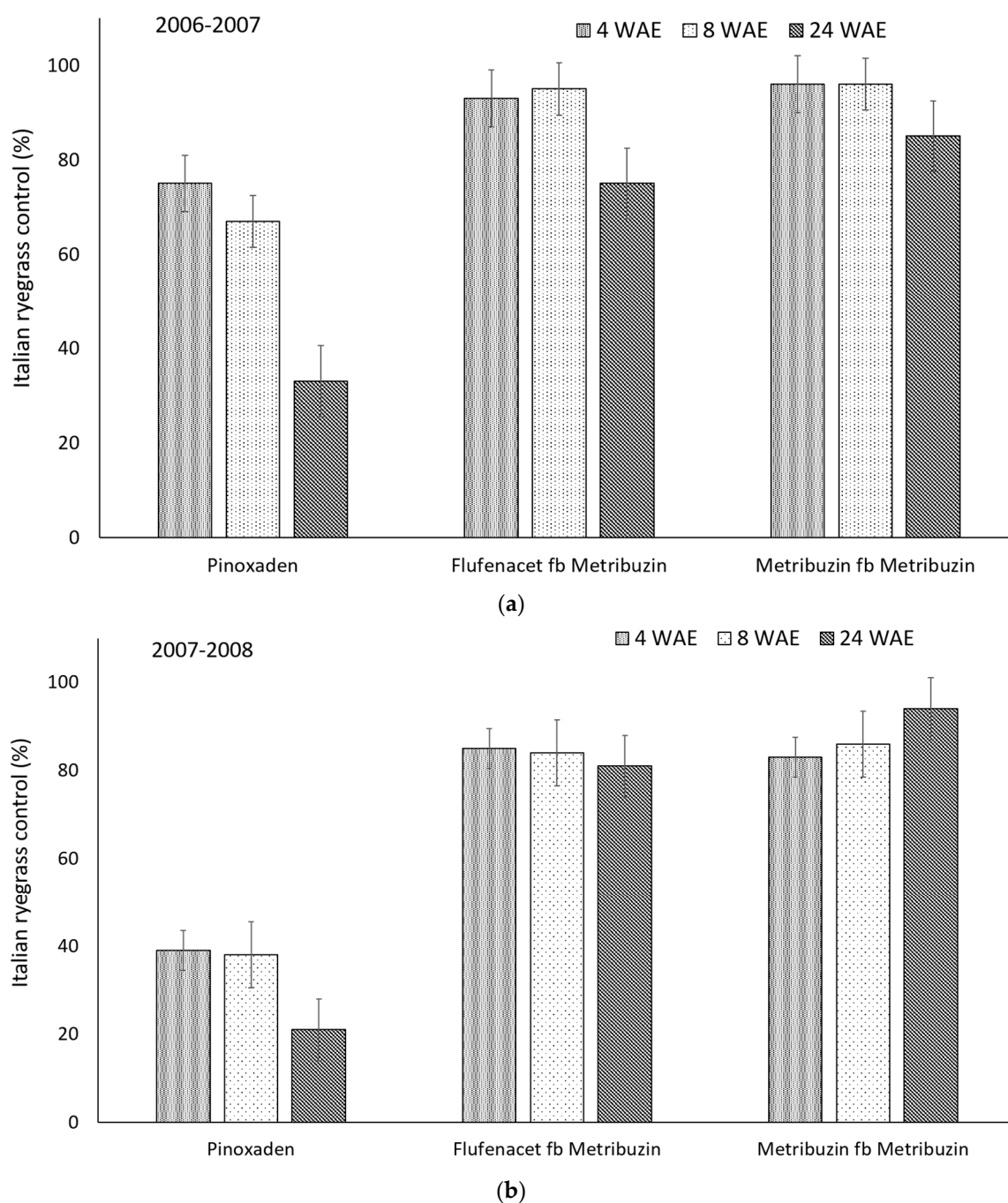


Figure 3. (a) Effects of herbicide programs on percentage Italian ryegrass control at Fayetteville 2006–2007 trials. Vertical bars represent LSD at 5% significance level (LSD for 4, 8, and 24 WAE = 11, 10, 18 respectively). (b) Effects of herbicide programs on percentage Italian ryegrass control at Fayetteville 2007–2008 trials. Vertical bars represent Least Significant Difference (LSD) at 5% significance level (LSD for 4, 8 and 24 WAE = 10, 17 and 16 respectively).

Flufenacet fb metribuzin controlled Italian ryegrass 93% four [4] WAE in 2006–2007, declining to 75% at 24 WAE (Figure 3a). On the contrary, the efficacy of this treatment remained at 81–85% throughout the entire 2007–2008 growing season.

The sequential metribuzin application was the best treatment for diclofop-resistant Italian ryegrass control in the Fayetteville test. This treatment controlled Italian ryegrass 96% and 85% at 4 WAE and 24 WAE, respectively in 2006–2007. This treatment was also the most effective in 2007–2008 season with 94% control 24 WAE.

The lowest yield, among all herbicide treatments, was obtained when pinoxaden was applied alone (Table 4). This treatment produced similar yield as the weedy check, which produced plots 1506 kg/ha) in 2007–2008 period (Table 4). The plots treated with flufenacet fb metribuzin produced approximately 38% more grain yield compared to plots treated with pinoxaden alone (Table 4). The application of metribuzin fb metribuzin produced the highest grain yield in 2006–2007 and 2007–2008 cropping seasons with up to 5084 kg/ha yield (Table 4).

Table 4. Effects of herbicide program on grain yield (kg ha⁻¹) at Fayetteville in 2006–2007 and 2007–2008 experimental periods. LSD = Least Significant Difference.

Year	Control	Pinoxaden	Flufenacet fb Metribuzin	Metribuzin fb Metribuzin	LSD ($\alpha = 0.05$)
2006–2007	1067.1	2761.9	4707.7	5084.4	752.4
2007–2008	1506.5	1945.9	3138.5	3766.2	564.3

3.3. Effects of Tillage on Italian Ryegrass Control

Significant effect of tillage treatments was observed in 2007–2008 ($\alpha = 0.05$) and 2008–2009 ($\alpha = 0.057$) at Breadlow Corner. Italian ryegrass control was lower under tilled conditions than no-till regardless of herbicide program (Figure 4a,b).



Figure 4. Cont.

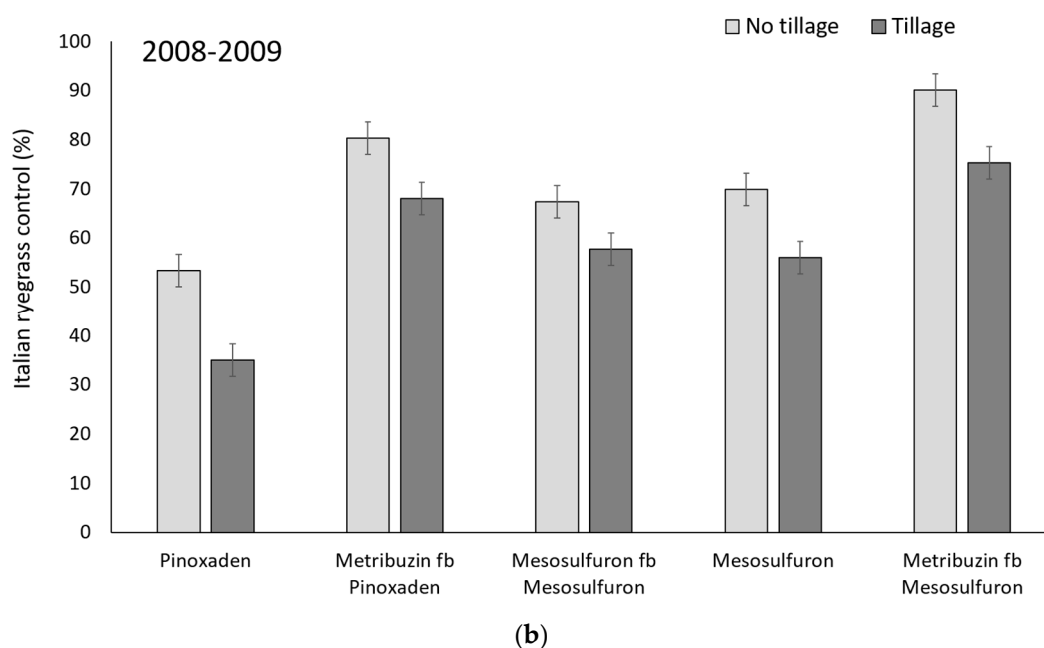


Figure 4. (a) Effects of herbicide program \times tillage on percentage Italian ryegrass control in 2007–2008. Vertical bars represent Least Significant Difference (LSD) at 5% significance level (LSD for tillage and no tillage treatments = 3); (b) Effects of herbicide program \times tillage on percentage Italian ryegrass control in 2008–2009. Vertical bars represent LSD at 5% significance level (LSD for tillage and no tillage treatments = 9).

4. Discussion

4.1. Effects of Herbicide Treatment on Diclofop-Resistant Italian Ryegrass and Grain Yield

Pinoxaden is an ACCase-inhibitor herbicide for selective grass weed control in wheat and barley (*Hordeum vulgare* L.) (Porter et al. [31]). Its phenylpyrazolin structure is different from the cyclohexanedione and aryloxyphenypropanoate (AOPP) chemical structures, which allows it to bind at a different locus in the acetyl coenzyme-A carboxylase (ACCase) protein. Diclofop belongs to the AOPP chemical family of ACCase inhibitors; therefore, it was hypothesized that diclofop-resistant ryegrass is not cross-resistant to pinoxaden. A survey of Italian ryegrass in Arkansas by Kuk et al. [22] has shown 25% cross-resistance between diclofop and pinoxaden. The diclofop-resistant ryegrass population at the field sites fell into the 25% proportion, being cross-resistant to pinoxaden. The recommended rate of pinoxaden did not control 1- to 2-tiller Italian ryegrass and resulted in the lowest yields, not different from the weedy check. It has been recommended that appropriate management strategies should be developed for the control of diclofop (and other)-resistant Italian ryegrass which usually involve the use of alternative herbicide treatments as an immediate course of action [22]. Our research demonstrated that planting no-till wheat is an effective tool for reducing ryegrass population size, which improves the efficacy of herbicides.

The amount of yield loss incurred with insufficient ryegrass control demonstrates the importance of managing this weed. Cross-resistant populations, when treated with pinoxaden, can incur close to 50% yield loss. This highlights the importance also of resistance testing. Characterization of the resistance profile of field populations will allow the growers to plan an appropriate herbicide treatment. Pinoxaden is still effective on 75% of the diclofop-resistant populations. The grower just needs to know the nature of his ryegrass population.

The high competitive ability of ryegrass is due to its high tillering that chokes the wheat. Under heavy ryegrass infestation yield loss can be up to 92% (Hashem et al. [32]). According to these authors one Italian ryegrass plant among 100 winter wheat plants/m² caused 33% yield loss.

Italian ryegrass populations in the southern United States have evolved cross- and multiple resistance to various herbicide classes including ACCase and ALS inhibitors, and even to glyphosate. There is evidence that enhanced herbicide metabolism is partially responsible for resistance to diclofop, pinoxaden, mesosulfuron, and pyroxsulam in some Italian ryegrass populations [33]. If this is true, it is possible that cross-resistance and multiple-resistance can ensue because of cross-reactivity of some detoxifying enzymes.

Due to the obligatory outcrossing reproductive nature of Italian ryegrass, plants exhibiting multiple resistance mechanisms could hybridize and produce offspring carrying new combination of resistance genes that may enable new resistant phenotypes [34]. Because of increasing resistance to ALS inhibitors, on top of resistance to ACCase inhibitors, the remaining herbicide options for Italian ryegrass control include pendimethalin and flufenacet plus metribuzin [33] and, recently, pyroxasulfone.

4.2. Effects of Tillage \times Herbicide Treatment on Italian Ryegrass Control

In the past few years, there has been a growing trend towards reducing tillage in cropping systems to allow stubble retention, earlier planting and improved soil structure. However, the adoption of conservation tillage systems will change weed control practices [35]. No-tillage significantly reduced the ryegrass population size across years. In this case, the efficacy of herbicides would be improved because of better coverage per plant with respect to foliar herbicides and higher dosage per plant with respect to soil-applied herbicides. Further, tillage systems exert a significant influence on the vertical distribution of weed seeds in the soil seed bank. As stated by Korres et al. [36] the persistence of the soil seedbank depends on a wide range of interacting biotic and abiotic factors including burial depth. Under tilled conditions, a large proportion of the seedbank is buried beyond the emergence zone and would lay dormant. New deposits of seeds each year increases the seedbank size. Therefore, tillage practices could serve as a conservation medium for the seeds of Italian ryegrass. Under no-tillage, the deeply buried seeds are not excavated and will eventually lose viability with time. Those in the emergence zone will be controlled by herbicides. New seeds deposited on the soil surface either germinate and are controlled, or are subjected to predation or other avenues of losses. Thus, no-tillage help reduce ryegrass population.

5. Conclusions

In conclusion, the diclofop-resistant Italian ryegrass at the study locations was cross-resistant to pinoxaden. A single pinoxaden application did not control this population. The best herbicide treatments for diclofop-resistant ryegrass are split application of metribuzin, a premix of saflufenacet and metribuzin, or saflufenacet followed by metribuzin. No-tillage reduces the ryegrass population size of ryegrass more than conventional tillage and enhances the efficacy of wheat herbicides.

Author Contributions: Investigation and Methodology, T.B.; Formal analysis, N.E.K., T.B.; Writing original draft: N.E.K., T.B., R.R.H.; Writing review and Editing: N.R.B., N.E.K., T.-M.P.T.

Funding: This research received no external funding

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

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