



# Distribution and Control of Herbicide-Resistant Italian Ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] in Winter Wheat (*Triticum aestivum* L.) in North Carolina

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Italian ryegrass is consistently ranked as one of the most problematic weeds of winter wheat in the Southeastern United States. To determine the distribution of resistant Italian ryegrass biotypes, seed was collected from locations throughout North Carolina and screened with diclofop, pinoxaden, mesosulfuron, and pyroxsulam. Results identified evidence of resistance to diclofop at all locations sampled throughout the state. Resistance to mesosulfuron, pyroxsulam, and pinoxaden were confirmed in 11, 19, and five percent of sampled locations, respectively. Additionally, Italian ryegrass biotypes resistant to multiple and all herbicides tested were identified, eliminating POST herbicide application as an option for control. Adjusting tillage practices may be an option for sustainable weed management to maintain effective control and maximize crop yield. Companion studies were established in the Coastal Plain and Piedmont regions of North Carolina in 2013 and 2014 to evaluate the effect of tillage on Italian ryegrass efficacy with herbicides. Herbicide treatments consisted of pyroxasulfone PRE only, mesosulfuron, or pinoxaden and POST only applications of mesosulfuron plus pyroxasulfone or pinoxaden plus pyroxasulfone. Tillage treatments included no-till and conservation tillage. Treatments containing pinoxaden provided the greatest Italian ryegrass control, regardless of tillage system. The use of pyroxasulfone PRE controlled a higher percentage of Italian ryegrass in the Piedmont when compared to the Coastal Plain, which is believed to be due to multiple flushes during the growing season in the Coastal Plain. Herbicide treatment was still a significant factor in Italian ryegrass control, but Italian ryegrass seed head density was consistently lower in the no-till system. Tillage may be stimulating germination, allowing greater control with PRE herbicides. An integrated system of herbicides and tillage may allow for greater yield and reduce selection pressure on POST herbicides.

**Keywords:** Italian ryegrass, tillage, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot, herbicide resistance, mechanical management

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is a staple crop grown worldwide (Gupta et al., 2008). Growers around the globe produce over 26 billion bushels of wheat per year (United States Department of Agriculture, 2014). The United States produces ~2 billion bushels per year, of which 44 million are produced in North Carolina (United States Department of Agriculture, 2014). Winter wheat accounts for 75% of wheat production in the United States (Agriculture Marketing Resource Center, 2015). Winter wheat is planted in mid- to late- November and harvested around June in North Carolina (Weisz, 2013). As in many agronomic cropping systems, an effective weed management program plays an important role in a successful wheat growing season. Weed interference can cause significant yield reductions in winter wheat (Liebl and Worsham, 1987; Wilson and Wright, 1990).

Weedy *Lolium* spp. are a ubiquitous problem in wheat production worldwide (Llewellynn and Powles, 2001; Barros et al., 2005; Trusler et al., 2007). Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot], a common problem weed in Southeastern United States winter wheat production (Liebl and Worsham, 1987; Grey and Bridges, 2003; Grey et al., 2012). Studies conducted in Oregon showed yield losses of up to 60% that were attributable to increased densities of Italian ryegrass in winter wheat over the course of 2 years. These studies also investigated the effect of Italian ryegrass competition on wheat yield reduction by variety, however decreased yields were observed as Italian ryegrass populations increased, regardless of wheat variety (Appleby et al., 1976). Liebl and Worsham (1987) quantified yield losses associated with interference from Italian ryegrass reporting for every 10 Italian ryegrass plants  $m^{-2}$ , wheat yield was decreased by an average of 4.2%. In subsequent studies, Italian ryegrass has been shown to reduce wheat yield by >30% with as little as nine ryegrass plants  $m^{-2}$  present (Hashem et al., 1998; Scursoni et al., 2012).

Effective chemical control of Italian ryegrass is limited as this species has evolved resistance to all herbicides labeled in winter wheat (Grey and Bridges, 2003; Hoskins et al., 2005; Grey et al., 2012; Heap, 2020). The preemergence (PRE) herbicides applied for Italian ryegrass control in winter wheat include acetolactate synthase (ALS) (herbicide group [HG] 2)-, microtubule synthesis (HG 3)-, photosystem II (PSII) (HG 5)-, and very-long-chain fatty acid (VLCFA) (HG 15)-inhibiting herbicides. The postemergence (POST) herbicides applied for Italian ryegrass in winter wheat include acetyl CoA carboxylase (ACCCase) (HG 1)- and ALS-inhibiting herbicides. North Carolina Italian ryegrass populations evolved resistance to ACCCase- and ALS- inhibiting herbicides in 1990 and 2007, respectively (Heap, 2020). Multiple herbicide-resistant (HG 1 and 2) North Carolina Italian ryegrass populations were confirmed in 2007 as well (Heap, 2020). Resistance to the VLCFA-inhibiting herbicides remain unevolved in North Carolina (Everman, personal communication). Thus, North Carolina farmers growing winter wheat cannot solely rely on herbicides to sustainably control Italian ryegrass.

Mechanical control (i.e., tillage) is efficacious on Italian ryegrass in winter wheat (Oveson and Appleby, 1971; Moyer

et al., 1994; Bond et al., 2014). Tillage provides weed control by burying the seed deep within the soil profile resulting in seedlings never reaching the soil surface or seeds remaining dormant (Pollard and Cussans, 1981; Ball, 1992). However, much of the tillage efficacy research has focused on deep tillage (i.e., moldboard plow) which is not as commonly used anymore with the trend of adopting reduced- or no till agriculture. Previous research has also demonstrated implementing tillage can influence Italian ryegrass germination (Bueno et al., 2007; Trusler et al., 2007; Ichihara et al., 2009). Thus, tillage may impart enough of a stimulus to induce Italian ryegrass germination which allows for better control when a preemergence herbicide is applied (Stougaard et al., 1984; Shaw, 1996; Rasmussen, 2003). The combination of implementing tillage in addition to applying herbicides could increase the control of Italian ryegrass in North Carolina wheat.

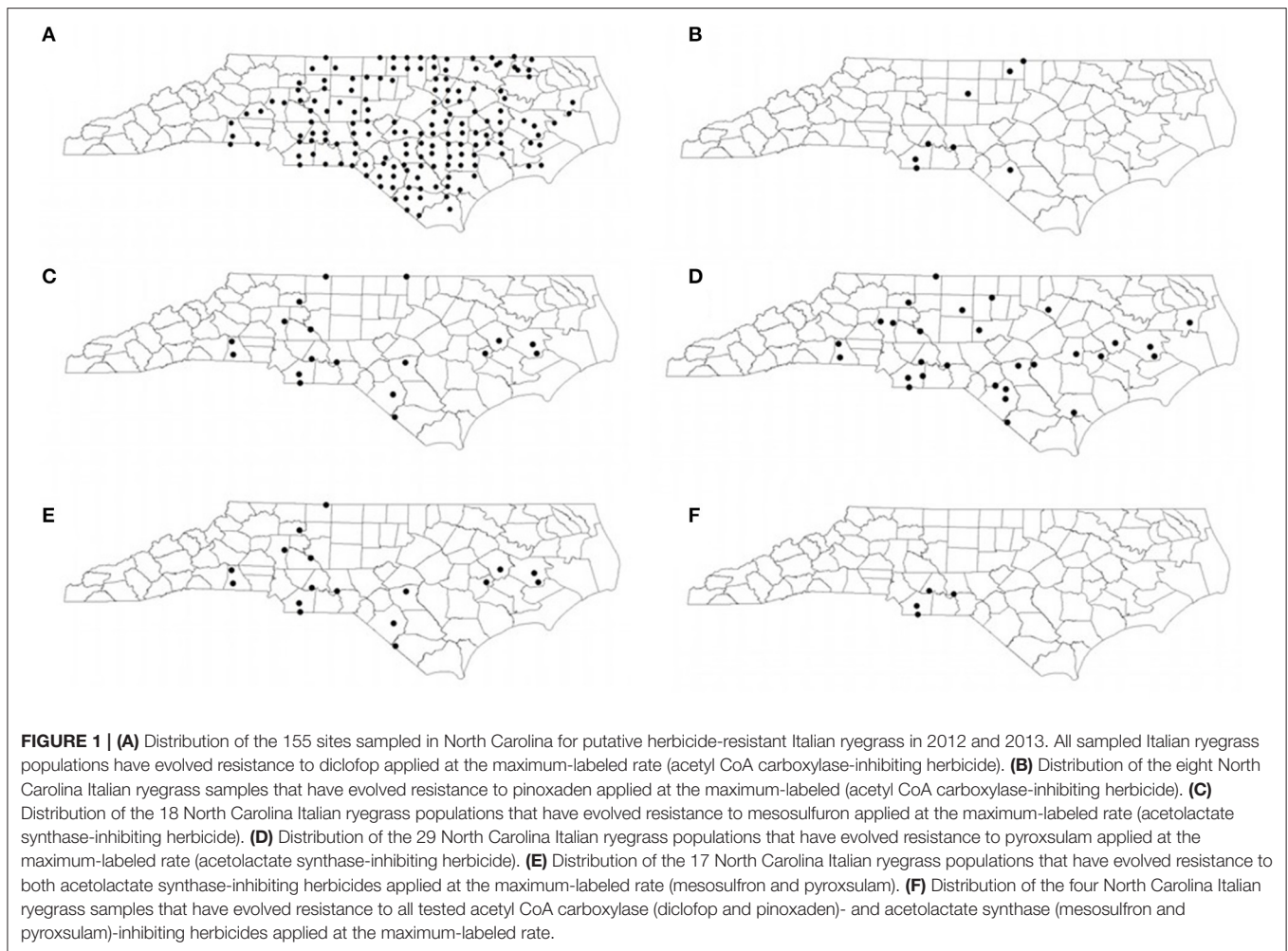
Currently, winter wheat is planted into no-till fields in the Piedmont regions, while planted into conservation tilled (>30% of residue left on soil surface) fields in the Coastal Plains regions of North Carolina and both regions are inhabited by herbicide-resistant Italian ryegrass populations. Thus, it is of interest to determine the efficacy of tillage and effective herbicides on North Carolina Italian ryegrass populations. The objectives of this research were to determine the distribution and the efficacy of conservation tillage practices of herbicide-resistant Italian ryegrass in North Carolina. The hypotheses of the research were that herbicide-resistant Italian ryegrass is pervasive across North Carolina and that tillage impacts Italian ryegrass control and density.

## MATERIALS AND METHODS

### Distribution of Herbicide-Resistant Italian Ryegrass in North Carolina

Italian ryegrass seeds were collected from wheat fields in North Carolina in the spring of 2012 and 2013. Sample locations were chosen on a longitudinal spacing of every 13 degree min, and a latitude spacing of every 10 degree min, resulting in a total of 239 locations selected for sampling. Sites were only sampled if an agricultural area was present within 3 km of the central grid point. When Italian ryegrass was found, seed heads from that location were collected and marked with the GPS coordinate of the location. Italian ryegrass seed was collected from 155 of the 239 locations (**Figure 1**). One hundred and thirty-six of these locations were sampled in 2012, with the other 20 being sampled in 2013. Collected seeds were sown into Fafard 2B potting mix in 9 by 13 cm flats in a greenhouse, with each flat containing one sampled Italian ryegrass population. After emergence, approximately three to four seedlings were transferred into 10 cm square pots filled with Fafard 2B potting mix. Overhead irrigation was supplied and light was supplemented by 1,000 watt metal halide bulbs for 12 h  $day^{-1}$ . Average day/night temperatures in the greenhouse were 25/15 C.

Once the plants reached three- to five-leaf stage, herbicide treatments were applied. Herbicide treatments consisted of: a non-treated check, two ACCCase- (diclofop-methyl [1,077 g



ai ha<sup>-1</sup>); pinoxaden [61 g ai ha<sup>-1</sup>]), and two ALS-inhibiting herbicides (pyroxsulam [18 g ai ha<sup>-1</sup> plus non-ionic surfactant at 0.5% v v<sup>-1</sup>]; mesosulfuron [15 g ai ha<sup>-1</sup> plus methylated seed oil at 1% v v<sup>-1</sup>]). Herbicide and adjuvant rates are based on the maximum-labeled rates of the respective treatment. One lethal rate of each herbicide was included as resistance to the selected herbicides has already evolved in North Carolina Italian ryegrass populations. Treatments were applied in a spray chamber calibrated to deliver 140 L ha<sup>-1</sup> of solution at 207 kPa 46 cm above the plant height with TeeJet TT8002 EVS nozzles (TeeJet® nozzles; Spraying Systems Co., Wheaton, IL). Treatments were arranged in a randomized complete block design with four replications and repeated once in time. Visual control ratings were taken at 14, 21, and 28 days after herbicide treatment. Control was estimated as a sum of total chlorosis, necrosis, and stunting on a rating scale ranging from 0 to 100%; where 0% equaled no control and 100% equaled complete control.

### Tillage Impact on Italian Ryegrass Control, Density, and Wheat Yield

The study locations during the 2013–2014 growing season were at a private farm near Hertford, North Carolina (36.18N,

–76.38 W) and at the Piedmont Research Station in Salisbury, North Carolina (35.70 N, –80.62 W). These two locations represent the Coastal Plains (Hertford) and the Piedmont (Salisbury) regions of North Carolina. The Hertford location is tilled prior to wheat planting, while the Salisbury had been in continuous no till for ~30 years before research was initiated. The soil of the field location near Hertford, North Carolina is a Roanoke silt loam (fine, mixed, semiactive, thermic Typic Endoaquult). The soil of the field at the Piedmont Research Station is a Lloyd clay loam (fine, kaolinitic, thermic Rhodic Kanhapludult). Planting at all locations was done in the fall of the respective year with a 3 m wide grain drill. Winter wheat was seeded at a rate of 72 seeds meter<sup>-1</sup>, with a 19 cm row spacing. Locations were planted twice with one study being conservatively-tilled and the other study being no-tilled prior to sowing. No tillage was further implemented on the tillage studies. Tillage was conducted with a chisel plow in Hertford, while tillage was conducted with a coulter-blade plow with a rolling-spike harrow attachment in Salisbury. Each study was tilled and planted on the same day. The experimental design was a randomized complete block with four replications. Plots were 3 m wide by 10 m long for all studies. Five herbicide programs

**TABLE 1** | Herbicide treatment, application timing, and rates applied in no-till and conventional tillage systems for Italian ryegrass control.

Herbicide program	Timing	Rate (g ai ha <sup>-1</sup> ) <sup>a</sup>
Non-treated		
Pyroxasulfone	PRE	74.4
Pyroxasulfone fb mesosulfuron <sup>a</sup>	PRE fb POST	74.4 fb 15
Pyroxasulfone fb pinoxaden	PRE fb POST	74.4 fb 60.5
Mesosulfuron <sup>a</sup> + pyroxasulfone	POST	15 + 74.4
Pinoxaden + pyroxasulfone	POST	60.5 + 74.4

<sup>a</sup>Included MSO at a rate of 0.25% v v<sup>-1</sup>.

MSO, methylated seed oil; PRE, pre-emergence; fb, followed by; POST, post-emergence.

were included in the experiment (**Table 1**). Treatments were applied with a CO<sub>2</sub>-pressurized backpack sprayer calibrated to deliver 140 L ha<sup>-1</sup> of solution at 207 kPa 46 cm above the plant height with TeeJet XR11002 nozzles (TeeJet<sup>®</sup> nozzles; Spraying Systems Co., Wheaton, IL). Italian ryegrass control was visually assessed at the postemergence herbicide timing and prior to wheat harvest. Italian ryegrass control was visually assessed on a rating scale ranging from 0 to 100%, where 0% equaled no control and 100% equaled complete control. Italian ryegrass densities were estimated prior to winter wheat harvest by averaging the frequency of seed heads recorded in three one-meter<sup>2</sup> areas within a plot.

## Statistical Analysis

All statistical analyses were performed using Statistical Analysis Software, SAS 9.3 (SAS Institute, Inc., NC, USA).

## Distribution of Herbicide-Resistant Italian Ryegrass in North Carolina

A herbicide-susceptible Italian ryegrass population was identified based on control ratings and was defined as a population from a location sample where there was complete control observed across all replications and in both runs. The pinoxaden+mesosulfuron- and pyroxasulam-susceptible Italian ryegrass populations were collected from Stokes and Carteret County, North Carolina, respectively. No diclofop-susceptible Italian ryegrass populations were collected from the 155 sampled. Once a herbicide-susceptible Italian ryegrass population was determined, Italian ryegrass control data were subjected to ANOVA using PROC GLIMMIX and treatment means were separated using Dunnett's Procedure ( $P < 0.05$ ) to separate Italian ryegrass populations that exhibited lower control than the selected susceptible populations. Moreover, Italian ryegrass populations were concluded to be herbicide-resistant if control was <50% as the discriminating rates of the applied herbicides should result in complete control. Outliers were not removed as they represent the variability of resistance within sampled locations most likely due to segregation (Poirier et al., 2014). Variability between runs was not significant, therefore runs were combined for analysis.

## Tillage Efficacy on Italian Ryegrass

Italian ryegrass control and seed head density data were subjected to ANOVA using PROC GLM and treatment means were separated using Fisher's Protected LSD ( $P < 0.05$ ). PROC CORR was also conducted to determine if Italian ryegrass seed density, control prior to winter wheat harvest, and winter wheat yield data were correlated.

## RESULTS AND DISCUSSION

### Distribution of Herbicide-Resistant Italian Ryegrass in North Carolina

It is important to note that this survey method is predisposed to select for herbicide resistance. When a field was visited, samples were collected only when Italian ryegrass was visible above the crop canopy, meaning it is likely that these plants were escapes from previous herbicide applications. The four herbicides screened can be broken down into their mode of action group, either ACCase or ALS-inhibiting herbicides.

### Acetyl CoA Carboxylase-Inhibiting Herbicides

No single Italian ryegrass population could be identified as susceptible to diclofop (**Figure 1**). This result was not unexpected as diclofop has been used extensively and recurrently in North Carolina winter wheat production (Heap, 2020; Everman, personal communication; Kuk and Burgos, 2007). Resistance to pinoxaden was not confirmed in North Carolina until 2007 (Heap, 2020). While pinoxaden has the same mode of action as diclofop, pinoxaden can control diclofop-resistant Italian ryegrass populations, and resistance has not been reported to be as common in similar surveys (Kuk and Burgos, 2007; Salas et al., 2013; Bararpour et al., 2018). Out of the 155 sampled populations in North Carolina, eight Italian ryegrass populations exhibited controls levels below those of the herbicide-susceptible population when treated with pinoxaden, elucidating the evolution of resistance in the select populations (**Figure 1**). The distribution of pinoxaden-resistant Italian ryegrass populations are isolated in Southwestern North Carolina. Since all population showed signs of resistance to diclofop, these four populations are cross-resistant to the tested ACCase-inhibiting herbicides.

### Acetolactate Synthase-Inhibiting Herbicides

Eighteen Italian ryegrass populations exhibited controls levels below those of the herbicide-susceptible population when treated with mesosulfuron, elucidating the evolution of resistance in the select populations (**Figure 1**). Resistance to pyroxasulam was more common than resistance to mesosulfuron in the sampled Italian ryegrass populations. Twenty-nine Italian ryegrass populations exhibited controls levels below those of the herbicide-susceptible population when treated with pyroxasulam, elucidating the evolution of resistance and that resistance is widespread throughout the state (**Figure 1**). Out of the 155 sampled populations in North Carolina, 17 Italian ryegrass populations were cross resistant to both ALS-inhibiting herbicides (**Figure 1**).

Four Italian ryegrass populations were found to have evolved resistance to all four of the tested herbicides (**Figure 1**). The



four multiple-herbicide-resistant Italian ryegrass populations are isolated in Southwest North Carolina. These four locations were identified in Richmond, Stanly, and Union counties. Chemical control of Italian Ryegrass in winter wheat would likely be limited or impossible exclusively with POST herbicides. Findings from this survey can be a valuable resource to growers when making decisions concerning Italian ryegrass control in winter wheat. While resistance may not be present on every farm near one of the sample locations, the possibility should be taken into account when designing a herbicide program.

While the herbicide resistance survey was conducted 6–7 years ago, the distribution of the herbicide-resistant Italian ryegrass populations would likely be similar today. Wheat production in North Carolina has declined (2015: 600,000 ha; 2020: ~100,000 ha) since this work was conducted and minimal (if any) herbicides are applied to the wheat planted, depending on grain price (United States Department of Agriculture, 2014, 2019). While pyroxasulfone (not included in the original survey) has been applied to the wheat grown within North Carolina since the research was conducted, no control failures have been reported with this herbicide. While that does not suggest that pyroxasulfone-resistant Italian ryegrass populations have not evolved within North Carolina, the lack of control failure complaints suggest that pyroxasulfone remains efficacious on North Carolina Italian ryegrass populations.

## Tillage Impact on Italian Ryegrass Control, Density, and Wheat Yield

Tillage was evaluated in separate companion trials placed adjacent to each other in each location and year, so results cannot be directly compared; however, trends can be observed between tillage types. No differences in control were observed at the POST application for the three treatments containing pyroxasulfone PRE in either tillage system with control ranging from 76 to 80% in the no-till and 81–88% in the tilled system (Table 2).

Late season Italian ryegrass control, prior to harvest, varied greatly across herbicide programs and locations, but was not impacted by year. The greatest Italian ryegrass control was observed when pinoxaden was applied POST (Table 3). Reduced efficacy of mesosulfuron in both tillage systems when compared to pinoxaden indicates an established or emerging issue with resistance to the ALS-inhibiting herbicides (Kuk and Burgos, 2007). Although ALS-resistant Italian ryegrass plants may be present, treatments of mesosulfuron plus pyroxasulfone applied total POST resulted in greater control than a single PRE application of pyroxasulfone when averaged over locations and years. Two-pass herbicide programs provided higher levels of control for all treatments, however only one two-pass program was significantly greater than its total POST counterpart (Table 4). A significant interaction of herbicide program and location averaged over years was also observed for late season Italian ryegrass control. Trends were similar to the herbicide program analysis, but differences in Italian ryegrass response in Hertford and Salisbury due to ALS-resistant biotypes are more apparent (Table 4). The greatest Italian ryegrass control was observed where pyroxasulfone was followed by an application of

**TABLE 2 |** Italian ryegrass control at the postemergence timing in winter wheat averaged across locations (Hertford and Salisbury, North Carolina) and years (2013 and 2014); experiments were separated by tillage environment.

Herbicide program	Timing	No-Till		Till	
		%			
Non-treated		0	B	0	b
Pyroxasulfone	PRE	79	A	81	a
Pyroxasulfone fb mesosulfuron	PRE fb POST	80	A	86	a
Pyroxasulfone fb pinoxaden	PRE fb POST	76	A	88	a
Mesosulfuron + pyroxasulfone	POST	0	B	0	b
Pinoxaden + pyroxasulfone	POST	0	B	0	b

Columns that share the same letters are not statistically different based on Fisher's LSD ( $P < 0.05$ ).

PRE, pre-emergence; fb, followed by; POST, post-emergence.

**TABLE 3 |** Italian ryegrass control prior to winter wheat harvest across both experiment locations (Hertford and Salisbury, North Carolina) and years (2013 and 2014); experiments were separated by tillage environment.

Herbicide program	Timing	No-Till		Till	
		%			
Non-treated		0	D	0	d
Pyroxasulfone	PRE	52	C	69	c
Pyroxasulfone fb mesosulfuron	PRE fb POST	54	C	77	bc
Pyroxasulfone fb pinoxaden	PRE fb POST	95	A	97	a
Mesosulfuron + pyroxasulfone	POST	70	B	79	b
Pinoxaden + pyroxasulfone	POST	87	A	93	a

Columns that share the same letters are not statistically different based on Fisher's LSD ( $P < 0.05$ ).

PRE, pre-emergence; fb, followed by; POST, post-emergence.

pinoxaden regardless of location or tillage system. In the tilled system, the total POST pinoxaden program provided similar levels of control, however control in the no-till system was lower at both locations. Pyroxasulfone applied PRE provided 35 and 69% control at Hertford and Salisbury, respectively, in the no-till system, and 57 and 84% control at Hertford and Salisbury, respectively, in the tilled system. Applying mesosulfuron POST or mesosulfuron plus pyroxasulfone POST did not significantly improve Italian ryegrass control at either location for no-till or tilled systems (Table 4).

Italian ryegrass seed head density was affected by a significant year, location, and herbicide program interaction. The highest Italian ryegrass seed head density was observed at Hertford in 2013 with 336 and 476 seed heads  $m^{-2}$  in the no-till and tilled systems, respectively (Table 5). Within each year, seed head density was greatest in Hertford compared to Salisbury within each tillage system (Table 5). This high density of Italian ryegrass in the non-treated at Hertford is likely due to a heterogeneous distribution of ALS-resistant Italian ryegrass plants, and the impacts of such high densities are apparent in all efficacy evaluations taken in this study. The large range of densities within a tillage system (4–336 and 0–476 seed heads  $m^{-2}$  in no-till

**TABLE 4** | Italian ryegrass control in conservation and no tillage systems prior to winter wheat harvest as influenced by herbicide program and location averaged over years.

Herbicide program	Timing	No-Till				Till			
		Hertford		Salisbury		Hertford		Salisbury	
%									
non-treated		0	C	0	C	0	c	0	c
pyroxasulfone	PRE	35	C	69	B	57	b	84	ab
pyroxasulfone fb mesosulfuron	PRE fb POST	69	BC	73	B	78	ab	80	b
pyroxasulfone fb pinoxaden	PRE fb POST	93	A	99	A	96	a	99	a
mesosulfuron + pyroxasulfone	POST	40	C	67	B	68	b	86	ab
pinoxaden + pyroxasulfone	POST	87	A	87	B	88	a	97	a

Columns that share the same letters are not statistically different based on Fisher's LSD ( $P < 0.05$ ).

PRE, preemergence; fb, followed by; POST, postemergence.

**TABLE 5** | Italian ryegrass seed head density counts prior to winter wheat harvest across separated by experiment locations (Hertford and Salisbury, North Carolina) and years (2013 and 2014); experiments were separated by tillage environment.

Herbicide program	Timing	No-Till				Till											
		2013		2014		2013		2014									
		Hertford	Salisbury	Hertford	Salisbury	Hertford	Salisbury	Hertford	Salisbury								
Plants m <sup>-2</sup>																	
Non-treated		299	AB	94	DE	167	CD	78	EFG	476	a	118	bc	128	bc	59	cd
Pyroxasulfone	PRE	250	BC	43	EFG	71	EFG	25	EFG	152	b	23	d	61	cd	20	d
Pyroxasulfone fb mesosulfuron	PRE fb POST	169	CD	22	EFG	20	EFG	38	EFG	54	cd	13	d	45	cd	11	d
Pyroxasulfone fb pinoxaden	PRE fb POST	4	EFG	9	EFG	15	EFG	7	G	0	d	0	d	3	d	2	d
Mesosulfuron + pyroxasulfone	POST	336	A	59	EFG	93	DEF	48	EFG	413	a	17	d	72	bcd	24	d
Pinoxaden + pyroxasulfone	POST	28	EFG	43	EFG	18	EFG	15	EFG	9	d	1	d	3	d	2	d

Columns that share the same letters are not statistically different based on Fisher's LSD ( $P < 0.05$ ).

PRE, pre-emergence; fb, followed by; POST, post-emergence.

**TABLE 6** | Winter wheat yield averaged across experiment locations (Hertford and Salisbury, North Carolina) and years (2013 and 2014); experiments were separated by tillage environment.

Herbicide program	Timing	No-Till		Till	
		Timing	tons ha <sup>-1</sup>	Timing	tons ha <sup>-1</sup>
Non-treated		3.3	C	3.2	c
Pyroxasulfone	PRE	3.8	BC	4.3	a
Pyroxasulfone fb mesosulfuron	PRE fb POST	4.0	B	4.3	a
Pyroxasulfone fb pinoxaden	PRE fb POST	4.6	A	4.9	a
Mesosulfuron + pyroxasulfone	POST	3.2	C	3.6	b
Pinoxaden + pyroxasulfone	POST	4.3	AB	4.9	a

Columns that share the same letters are not statistically different based on Fisher's LSD ( $P < 0.05$ ).

PRE, pre-emergence; fb, followed by; POST, post-emergence.

and tilled, respectively) makes statistical treatment separation difficult. However, clear trends can be observed. Similar to late season control ratings, treatments with pinoxaden POST resulted in the lowest Italian ryegrass seed head densities, regardless of tillage system. In 6 out of 8 instances, mesosulfuron plus

pyroxasulfone POST was not significantly different from the non-treated, however there was only one instance of treatments being significantly different where the same herbicides were applied sequentially (Table 5). Although the seed head densities were not significantly different due to the high degree of variability, the lower densities where a PRE was followed by a POST compared to the total POST combination emphasizes the value of a PRE herbicide to control Italian ryegrass. Correlation analysis results detected a relatively high negative correlation between Italian ryegrass control prior to harvest and Italian ryegrass seed head density in the no-till ( $R = -0.63$ ;  $P < 0.0001$ ) and tilled systems ( $R = -0.50$ ;  $P < 0.0001$ ).

Winter wheat yield was significantly affected by year and herbicide program for both tillage systems. There was a significant location effect for the no-till system, and a significant year by location interaction for the tilled system. When averaged over herbicide program in the no-till system, wheat yield was greater in 2014 (4.2 tons ha<sup>-1</sup>) compared to 2013 (3.6 tons ha<sup>-1</sup>) and at Salisbury (4.8 tons ha<sup>-1</sup>) compared to Hertford (3.0 tons ha<sup>-1</sup>). In the tilled system, the yield was also greater in Salisbury (5.4 tons ha<sup>-1</sup>) than Hertford (2.8 tons ha<sup>-1</sup>) when averaged over location and herbicide program. The year by location interaction

for wheat yield in the tilled system showed a similar trend, however greater yields in Hertford were observed in 2014 and in Salisbury in 2013 (data not shown). The effects of environment on crop yield are well-documented and come as no surprise (Bassett et al., 1989; Laidig et al., 2017). Although not part of the analysis, of interest was that higher yields were observed, in general, in the tilled system, regardless of year and location (data not shown) (Šíp et al., 2013).

Winter wheat yield was affected by herbicide program, therefore results are averaged over years and locations within each tillage system. Treatments containing pinoxaden gave the greatest level of Italian ryegrass control, and subsequently resulted in the greatest winter wheat yield in both no-till and tilled systems (Table 6). Following the trends in Italian ryegrass seed head density, the lowest yields were observed in the non-treated and where mesosulfuron was applied as part of a total POST program. Pyroxasulfone followed by mesosulfuron applied to winter wheat did not yield significantly different from the highest yielding in the tilled system. Correlation analysis identified a relatively high negative correlation between Italian ryegrass seed head density and winter wheat yield in the no-till and tilled systems ( $R = -0.69$ ;  $P < 0.0001$  and  $R = -0.61$ ;  $P < 0.0001$ , respectively).

The results of the tillage system study gives clear conclusions on the importance of herbicide program to control Italian ryegrass. Effective POST herbicides are critical to maximize control and reduce Italian ryegrass seed head. The negative correlation between seed head density and winter wheat yield reveals the importance of reducing populations, not just improving control. In addition, the use of an effective PRE herbicide preserved winter wheat yield potential even where herbicide-resistant biotypes occur (Bond et al., 2014; Liu et al., 2016). Looking closely at Italian ryegrass seed head density and control data in the different tillage systems, trends emerge which suggest greater germination in the tilled system. The use of a

PRE in the tilled system resulted in a lower percentage of Italian ryegrass when compared to the no-till system. The higher density may be due to stimulated germination due to tillage at planting (Forcella and Lindstrom, 1988; Chauhan et al., 2006; Bueno et al., 2007). This may have enabled the PRE treatment to control more Italian ryegrass, leaving less for the POST treatment to control, explaining the differences in late season Italian ryegrass control, seed head density, and yield of two-pass and one-pass herbicide programs containing mesosulfuron.

To further investigate the impact of tillage on Italian ryegrass, future studies should investigate the role of light and tillage on germination, as well as the occurrence of multiple germination flushes throughout the growing season. The distribution of herbicide-resistant Italian ryegrass populations in North Carolina should be sampled again in the future with the inclusion of screening pyroxasulfone. Since pyroxasulfone is one of the only effective herbicides to control Italian ryegrass, North Carolina wheat farmers have likely recurrently and extensively applied the herbicide. Over reliance of pinoxaden and pyroxasulfone since the herbicide screen was first conducted could have selected for resistant Italian ryegrass populations within the state (Kaundun, 2013; Busi et al., 2018; Heap, 2020).

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

EJ conducted analysis and writing. ZT conducted research and writing. WE supervised research, analysis, and writing. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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