

Research Article

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Critical period for Italian ryegrass (*Lolium perenne* L. spp. *multiflorum*) control in winter wheat in North Carolina

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Abstract

Field studies were conducted in North Carolina to determine the critical period of weed control (CPWC) for Italian ryegrass in winter wheat. Soft red winter wheat was planted in late fall in 2017 and 2018 in no-till fields near Salisbury, NC. Treatments consisted of allowing weeds to grow from crop emergence for different intervals until removal (“weedy”), maintaining “weed-free” conditions from crop emergence for the same intervals, and then letting the weeds emerge and compete with the crop for the duration of the season. In 2017, weed removal occurred in 2-wk intervals from crop emergence up to 18 wk after crop emergence (WAE) and 3-wk intervals up to 18 WAE in 2018. Additional biological measurements, including Italian ryegrass density and height, were collected at 6, 12, and 18 WAE to characterize the effect of crop-weed interactions on the CPWC and weed populations. Nonlinear regression analysis was conducted to relate the timing of weed removal and yield loss. The analysis was carried out using growing degree days (GDDs) accumulated at corresponding WAE. Italian ryegrass density ranged from 292 to 824 plants m⁻², which created intense competitive conditions with wheat. In the absence of weed control, yield loss surpassed 60%. Using 5% yield loss as an accepted threshold, the CPWC for Italian ryegrass in no-till planted wheat was estimated to be from 1,100 to 1,900 GDD. This relatively short period makes it possible to reduce weed control intensity if control actions are properly timed.

Introduction

Wheat is one of the most important crops in the world, occupying 17% of total crop acreage, feeding about 40% of the population, and providing 20% of total food calories in human nutrition (Gupta et al. 2008). In the southeastern United States, wheat production is often part of dual-cropping systems in which soybeans are planted following the harvest of winter wheat (Santos Hansel et al. 2019). Weed management in wheat production systems often represents a challenge for farmers, particularly in fields where grass weed species are present. Across the United States, weed interference can lead to winter wheat yield losses ranging from 3% to 34% (Flessner et al. 2021). Wheat is grown during cool seasons, favoring prolonged emergence periods from weed species such as Italian ryegrass, which are particularly difficult to control (Scursoni et al. 2012). Italian ryegrass is a vigorous, erect winter annual grass that is ranked as one of the most troublesome weeds in wheat production (Van Wychen 2023; Webster and MacDonald 2001). Failure to control Italian ryegrass results in reduced wheat yield, quality, or both (Hashem et al. 1998; Jones et al. 2021; Justice et al. 1994; Kinfe and Peeper 1991; Liebel and Worsham 1987; Stone et al. 1998). The adverse effects of Italian ryegrass on wheat productivity are present in many countries around the world (Gonzalez-Andujar and Saavedra 2003; Holm et al. 1977; Paynter and Hills 2009; Trusler et al. 2007). A survey conducted in North Carolina in 2015 displayed the widespread establishment of Italian ryegrass in the state, along with the presence of resistant biotypes (Jones et al. 2021).

Just a few selective grass herbicides are available to control Italian ryegrass in wheat fields in the United States, the most common being diclofop, mesosulfuron, pyroxsulam, and pinoxaden. Diclofop-methyl, an acetyl-CoA-carboxylase inhibitor (ACCase, categorized by the Weed Science Society of America [WSSA] as a Group 1 herbicide), has proved to be effective at controlling Italian ryegrass until the late 1980s when herbicide-resistant biotypes evolved (Stanger and Appleby 1989). Herbicide resistance to diclofop by Italian ryegrass has been reported in 24 instances across six different countries (Heap 2024). Pinoxaden is an ACCase-inhibiting herbicide labeled for the control of grass weed species in wheat crops in the United States. Contrasting with other selective grass herbicides such as diclofop and other ACCase inhibitors, pinoxaden is not antagonized by broadleaf herbicides and has a wider application

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window (Bararpour et al. 2018a). However, widespread use of ACCase inhibitors in wheat has resulted in herbicide resistance of *Lolium* spp. in 15 countries with 39 different biotypes of Italian ryegrass involved (Heap 2024). Additionally, Italian ryegrass resistance to acetolactate synthase (ALS, WSSA Group 2) inhibitors is becoming a common incidence, especially in the United States (Bararpour et al. 2018a). Herbicide-resistant Italian ryegrass populations have been confirmed in 16 states (Heap 2024). In North Carolina, herbicide resistance in Italian ryegrass has been confirmed for the following herbicide groups and herbicides: ACCase inhibitors (clethodim, diclofop, and pinoxaden [WSSA Group 1]); ALS inhibitors (imazamox, mesosulfuron, nicosulfuron, and pyroxulam [Group 2]); 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) inhibitors (glyphosate [Group 9]); and photosystem I electron diverters (paraquat [Group 22]) (Heap 2024).

In the early 2000s, herbicide-based strategies were the only practical means for controlling Italian ryegrass, but it was recognized that integrating herbicide programs within a tillage system could result in improved overall long-term weed control and increased crop production (Bailey and Wilson 2003; Bararpour et al. 2018a). The adoption of no-till or conservation tillage in integrated weed management programs has become regular practice among wheat producers (Bond et al. 2014; Carpenter and Gianessi 1999; Cerdeira and Duke 2006; Taylor and Coats 1996). Conservation tillage and no-till can inhibit weed seed germination due to shading or cooler temperatures at the soil surface, which may result in Italian ryegrass population reductions (Bararpour et al. 2018a). Furthermore, the integration of tillage alongside the application of a preemergence herbicide could enhance Italian ryegrass control because tillage-induced seed germination may expose seedlings to the herbicide (Jones et al. 2021). However, previous studies have indicated that reduced tillage systems may favor Italian ryegrass proliferation (Kegode et al. 1999), which could result in a rapid increase in the soil seedbank and the need for herbicide applications. Consequently, subsequent herbicide use could lead toward selection of resistant biotypes (Cerdeira and Duke 2006).

Improvement of weed management strategies requires a better understanding of weed-crop interactions (Scursoni et al. 2012). Understanding the emergence dynamics of weed populations and competition is essential for successful weed control and high-yielding crops (Scursoni et al. 2012). One beneficial strategy for weed management programs is the use of the critical period for weed control (CPWC), which is defined as the period in a crop's growth cycle during which weeds must be controlled to prevent yield losses (Knezevic et al. 2002). The CPWC is made up of two different components: the critical timing of weed removal (CTWR) and the critical weed-free period (CWFP). Theoretically, weed competition outside of the CPWC does not contribute to crop yield loss greater than the set threshold. Studies regarding the CPWC for wheat have been previously conducted in Brazil, where general weed control (Silva et al. 2016) and control of Italian ryegrass specifically (Galon et al. 2019), were assessed. Another study conducted in England examined the CWFP for organically grown wheat (Welsh et al. 1999). However, studies of the CPWC in wheat have not been previously conducted in the United States, and thus it is valuable to determine the CPWC for Italian ryegrass, which has become one of the most common and troublesome weed species in wheat production (Van Wyken 2023). Thus, the objective of this study is to determine whether a defined CPWC for

Italian ryegrass exists in wheat production and if one does exist, determine its duration in the crop's growth cycle.

Materials and Methods

Field studies were established to evaluate the CPWC for Italian ryegrass in wheat during the winter growing seasons of 2017–2018 and 2018–2019 at the North Carolina Department of Agriculture & Consumer Services Piedmont Research Station in Salisbury, NC (35.691633°N, 80.637698°W). The soil at the experiment location is a Lloyd clay loam (USDA-NRCS 2020). The wheat variety used was 'DynaGro Shirley', which is a well-adapted and high-yielding soft red winter wheat in the North Carolina Piedmont region (Weisz 2013). Wheat was drill-planted on a no-tilled field at a rate of 1.31 million seeds ha⁻¹ on November 15, 2017, and October 30, 2018. Plots were 9 m long and 3 m wide with planted rows spaced at 19 cm. Standard management practices for fertilization were in accordance with the North Carolina Small Grain Production Guide (Post 2021).

The experimental design was a randomized complete block replicated four times. The first-year treatments consisted of weed interference periods up to the 18th week after crop emergence (WAE), resulting in nine "weedy" and "weed-free" treatments (2, 4, 6, 8, 10, 12, 14, 16, 18 WAE). The second-year weed interference periods were increased from 2 to 3 wk because the first-year field observations noted slow plant growth in 2-wk intervals. In the second year, treatments consisted of six "weedy" and "weed-free" treatments up to the 18th WAE (3, 6, 9, 12, 15, 18 WAE). In weedy treatments, Italian ryegrass was allowed to grow in the plots up to the corresponding WAE, after which Italian ryegrass was removed using pinoxaden, and maintained weed-free for the remainder of the season by hand-weeding. In weed-free treatments, plots were maintained free of weeds, including Italian ryegrass, up to the corresponding WAE treatment using a mix of chemical control and hand-weeding, after which Italian ryegrass was left to grow for the remainder of the season. Herbicides, as opposed to or additional to hand removal, have been used to remove emerged weeds in critical period studies (Contreras et al. 2022; Everman et al. 2008). Two control treatments were included for comparison, consisting of weedy or weed-free all season, where Italian ryegrass was either present or removed throughout the entire growing season. Italian ryegrass was controlled with pinoxaden (Axial XL, 50 g ai L⁻¹; Syngenta Crop Protection, Greensboro, NC) at a rate of 60 g ai ha⁻¹ using a CO₂ pressurized backpack sprayer calibrated for an output of 140 L ha⁻¹ at 207 kPa with XR11002 flat-fan nozzles (TeeJet Technologies, Glendale Heights, IL). Broadleaf weeds were controlled early in the season with a premix of thifensulfuron methyl at 21 g ai ha⁻¹ + tribenuron methyl at 10.5 g ai ha⁻¹ (Harmony Extra SG, 103 g ai kg⁻¹; FMC Corporation, Philadelphia, PA) on the entire study area using the previously described methods.

Wheat and Italian ryegrass height were recorded at the time of each weed removal. Heights were measured from the soil surface to the newest fully expanded leaf of the plant. Italian ryegrass plant density was recorded using a 0.5-m² square in which each plant was counted by isolating the crown. Due to differences in weed removal timing between the two years, biological data were compared on similar removal periods, which were 6, 12, and 18 WAE, and a final Italian ryegrass density count was collected at 24 WAE. Growing degree days (GDDs) were employed to establish a standardized measure of time across different years. GDDs were calculated using Equation 1:

Table 1. Accumulated growing degree days at the corresponding weeks after wheat emergence averaged between the 2017–2018 and 2018–2019 winter wheat growing seasons in Salisbury, North Carolina.^a

WAE	GDD
0	0
1	300
2	368
3	429
4	553
5	582
6	583
7	667
8	721
9	818
10	868
11	980
12	1,116
13	1,322
14	1,428
15	1,496
16	1,594
17	1,675
18	1,826
19	1,895
20	2,177
21	2,328
22	2,279
23	2,765
24	3,048

^aAbbreviations: GDD, growing degree days; WAE, weeks after wheat emergence.

$$GDD = \left[\frac{(T_{MAX} + T_{MIN})}{2} \right] - T_{BASE} \quad [1]$$

where T_{MAX} represents the daily maximum air temperature, T_{MIN} is the daily minimum temperature, and T_{BASE} is the threshold temperature below which growth ceases (McMaster and Wilhem 1997). The T_{BASE} for wheat is 0 C (McMaster and Wilhem 1997), and total GDDs were calculated by adding the accumulating daily GDD values from the time of crop emergence until 24 WAE. GDD accumulation with weeks after emergence is shown in Table 1. Biological measurements were similar between the two years at the corresponding GDD; therefore, data were pooled across GDD. Wheat was harvested using a small-plot combine at the end of the season and adjusted to a 12.5% moisture content to obtain final yield values. These yields were then standardized based on percentage, with the all-season, weed-free treatment set as 100% yield.

Data were subjected to ANOVA using the GLM procedure with SAS (v. 9.4; SAS Institute, Cary, NC) to test the statistical significance of the fixed effects (i.e., year, weedy and weed-free treatments and their interactions). Replications and interactions involving replications were considered random effects in the model. A residual analysis was conducted to check for homogeneity of variance and normal distributions using the UNIVARIATE procedure with SAS. Means were separated by using Tukey's honestly significant difference test ($P < 0.05$). The statistical analysis of the CPWC was performed with R software (R Core Team 2019) using the DRC (dose response curve) statistical package (Ritz and Streibig 2005) following a procedure described by Knezevic and Datta (2015). Weed interference

Table 2. Estimates for the four-parameter logistic equation describing the critical periods for Italian ryegrass control in no-till winter wheat in North Carolina based on pooled data from the 2017–2018 and 2018–2019 growing seasons.^{a,b}

Critical timing of weed removal				Critical weed-free period			
C	D	X	E	C	D	X	E
44.13	−5.38	1,376.42	56.02	42.22	8.02	1,300.19	61.13

^aPooled data are represented by combining data from both growing seasons.

^bThe four-parameter logistic equation is $Y = [C + (D - C)] / [1 + \exp(B(\log X - \log E))]$, where Y indicates relative yield as a percentage, C is the lower limit, D is the upper limit, X is the time expressed as growing degree days, and E is the number of growing degree days giving a 50% response between upper and lower limits, also known as the inflection point, or ED_{50} .

treatment comparisons were based on a nonlinear regression analysis in which timing of weed removal and weed-free periods were associated with relative yield. The curves associated with weed interference periods were fit to a four-parameter log-logistic model (Equation 2):

$$Y = [C + (D - C)] / [1 + \exp(B(\log X - \log E))] \quad [2]$$

where Y indicates relative yield as a percentage, C is the lower limit, D is the upper limit, X is the time expressed as GDD, and E is the GDD giving a 50% response between upper and lower limits, also known as the inflection point, or ED_{50} (Knezevic and Datta 2015). The CTWR and CWFP curves were graphically represented using a four-parameter logistic regression model with Sigmaplot software (v. 14.0; Systat Software, San Jose, CA), appropriate for graphical representation of weedy and weed-free curves (Knezevic et al. 2002).

Results and Discussion

Although weed interference treatments varied between growing seasons, there were no differences ($P = 0.58$) in relative yield values between growing seasons. Therefore, data were pooled and presented for both years combined. The parameter estimates of the set of curves are presented in Table 2. The acceptable yield loss threshold defining the CPWC was 5%.

Wheat yield decreased as the duration of Italian ryegrass interference increased (Figure 1). With combined growing seasons, the CTWR or the beginning of the CPWC for Italian ryegrass in wheat is estimated at 1,100 GDD (Figure 1). The CWFP or the end of the CPWC for Italian ryegrass in wheat is estimated at 1,900 GDD (Figure 1). Therefore, the CPWC for Italian ryegrass in wheat is between 1,100 and 1,900 GDD.

Italian ryegrass heights were not different across years in the corresponding WAE treatments; therefore, height data were pooled together. Italian ryegrass postemergence control timing is recommended at the 2- to 3-tiller stage of the weed, when plant height is around 8 to 10 cm (Grey and Bridges 2003). This coincides with the CTWR being at 1,100 GDD, because the height of Italian ryegrass during this period was 6.4 cm on average (Table 3), suggesting a similar plant growth stage (2- to 3-tiller) to that when Italian ryegrass is commonly controlled. Postemergence applications of various herbicides at the 2- to 3-tiller stage provided at least 88% control of Italian ryegrass in Georgia (Grey and Bridges 2003). Similarly, Italian ryegrass subjected to various postemergence herbicides between the 1- and 3-tiller stage was controlled by 83% to 99% in Illinois (Hoskins et al. 2005). Italian

Table 3. Italian ryegrass density and height, and wheat height with (weedy) and without (weed-free) Italian ryegrass competition at various weeks after wheat emergence and corresponding growing degree days.^{a,b}

WAE	GDD	Italian ryegrass				Wheat			
		Density ^c		Height		Weedy		Weed-Free	
						Height		Height	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
		Plants m ⁻²		cm		Plants m ⁻²		cm	
6	582	292 a	38	4.3 a	0.2	8.3 a	0.3	8.6 a	0.3
12	1,116	304 a	57	6.4 a	0.5	8.6 a	0.3	9.2 a	0.4
18	1,826	599 b	37	16.9 b	0.6	28.6 b	1.7	29.8 b	1.5
24	3,048	824 c	37	–	–	–	–	–	–

^aAbbreviations: GDD, growing degree days; SE, standard error; WAE, weeks after wheat emergence.

^bDifferent letters within the same column indicate a significant difference across GDD.

^cPlant density was measured in a 0.5-m² quadrant.

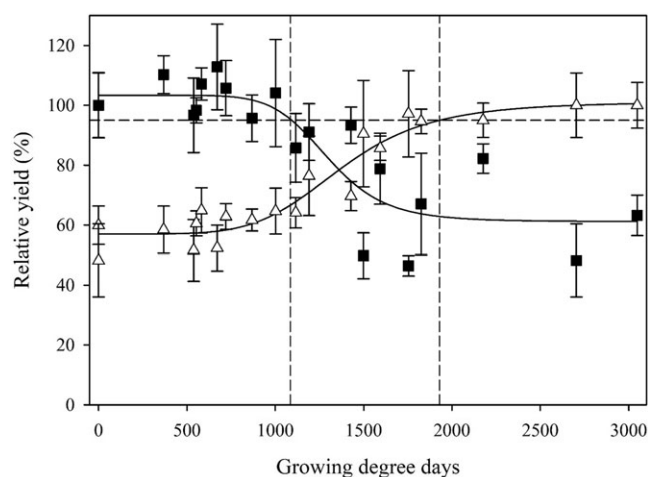


Figure 1. Critical period for Italian ryegrass control in no-till winter wheat in North Carolina pooled by growing season (2017–2018 and 2018–2019). Yield is expressed as a percentage of weed-free wheat yield using a four-parameter logistic model (Knezevic et al. 2002) to determine the critical timing for weed removal (Δ) and the critical weed-free period ($\blacksquare$) in growing degree days (GDDs). The horizontal line (—) denotes the 5% acceptable yield loss. The vertical lines (---) denote the start (1,100 GDD) and end (1,900 GDD) of the critical period for weed control.

ryegrass treated with flufenacet during the 1- to 2-leaf stage and that treated with mesosulfuron during the 3- to 4-leaf stage exhibited control rates of 87% and 92%, respectively (Bararpour et al. 2018b). These studies exemplify the need for well-timed and effective weed control, which concurs with the estimated CTWR.

Similar to Italian ryegrass height, density was not different between years at the same WAE treatments, suggesting a similar emergence pattern (Table 3). Furthermore, plant density was not different between 6 and 12 WAE (582 and 1,116 GDD, correspondingly) collection dates; however, Italian ryegrass density did increase by 18 WAE (1,816 GDD). This coincides with recorded evidence that Italian ryegrass has prolonged emergence periods during the wheat growing season (Scursoni et al. 2012). Traditional plant emergence models were not used because they were not the focal point of the study; however, these results give way to possible emergence pattern assumptions in which germination of Italian ryegrass continues throughout the growing season. The initial flush of weed emergence was observed

during the first 1,116 GDD, after which Italian ryegrass emergence continues between 1,116 and 1,816 GDD (Table 3). This period parallels wheat's reproductive growth stages related to accumulated GDD (Haun 1973; NDAWN 2017), beginning with the emergence of the flag leaf up to a completed flowering. An elevated weed density during this period could adversely affect the reproductive growth of wheat, leading to a reduction in overall yield production.

Previous studies of Italian ryegrass density in wheat have shown that 50 plants per square meter can reduce wheat yields by as much as 50% (Stone et al. 1999). Earlier research in North Carolina reported wheat yield was reduced by 4.2% for every 10 Italian ryegrass plants per square meter (Liebel and Worsham 1987). The same levels of yield reductions were not observed in this study because the previously mentioned weed densities were maintained throughout the growing season. On a further note, weed control this late in the season becomes a problem because selective grass herbicides must be used at specific wheat growth stages, most of them before flag leaf development (Scursoni et al. 2012).

There were no differences in height of Italian ryegrass between the two crop years, but overall means were different in between weeks (Table 3), with heights of 4.3 cm at 582 GDD, 6.4 cm at 1,116 GDD, and 16.9 cm at 1,826 GDD. Italian ryegrass increased in height by nearly 3-fold, between 1,116 and 1,826 GDD, which took place during the CPWC. This period of rapid growth of Italian ryegrass may intensify its consumption of resources, and crop-weed competition becomes critical during this timeframe, when limited resources could reduce wheat yields.

Wheat height was recorded in weedy and weed-free plots at similar GDD to understand the effect of Italian ryegrass competition on wheat height. Heights were similar over the years, so they were pooled across GDD. There was no difference in wheat height between 582 and 1,126 GDD, but it increased at 1,826 GDD (Table 3). Wheat growing in competition with Italian ryegrass and wheat growing in weed-free plots did not differ in plant height ($P > 0.05$). These results are different than reported in a previous study that Italian ryegrass reduces wheat height when they are in competition (Hashem et al. 1998).

Practical Implications

The result of this experiment provides valuable insights into the best timing for postemergence control of Italian ryegrass in winter

wheat crops in North Carolina, helping to minimize potential yield losses. With increasing reports of herbicide resistance by Italian ryegrass, it is crucial to pinpoint the ideal window for effective control. The data clearly define a CPWC for Italian ryegrass in wheat crops in the region. Specifically, to limit yield losses to 5%, control measures should be applied between 1,100 and 1,900 GDDs. Controlling Italian ryegrass at the right time boosts wheat productivity and reduces unnecessary herbicide use. Future studies should investigate how tillage practices affect the CPWC for Italian ryegrass in wheat, and how different tillage methods influence the weed's emergence and growth patterns.

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