EVALUATION OF ITALIAN RYEGRASS HERBICIDE RESISTANCE AND
POTENTIAL UTILITY OF HARVEST WEED SEED CONTROL

Travis R. Legleiter
Amber Herman
University of Kentucky Research and Education Center, Princeton

OBJECTIVES

Italian ryegrass (annual ryegrass) continues to be problematic in Kentucky wheat acres and has shown rapid increases in infestations over the past several growing seasons. This weed species has proved to be the most problematic weed for Kentucky wheat growers with our previous research identifying at least one population of glyphosate-resistant and one population of pinoxaden (Axial XL) resistant annual ryegrass in Kentucky wheat fields.

Since the identification of the single population of pinoxaden resistant ryegrass from Simpson County in 2017, we have observed numerous wheat fields through the state with late season ryegrass escapes. In addition to those escapes we have also received multiple complaints of failed glyphosate burndowns of this weed. The number of complaints of failed burndowns increased exponentially in 2021 and 2022.

Herbicide resistance in ryegrass is inevitable and Kentucky wheat acres are on the brink of widespread herbicide resistance. The lack of potential postemergence herbicides and limits of currently effective preemergence herbicides call for additional control tactics such as harvest weed seed control. Rigid ryegrass seed destruction at harvest has been implemented by Australian farmers for over a decade with much success. The investigation of the potential of this technology in Kentucky wheat acres is warranted at this time as Kentucky wheat farmer continue to struggle with annual ryegrass and herbicide resistance.

Objectives:

1. Conduct dose response studies on ryegrass populations that showed lack of control in initial greenhouse screenings
2. Investigate ryegrass seed retention, seed rain, and combine dispersal to further understand the utility of harvest weed seed control

METHODS & MATERIALS

Objective 1: 22 populations of Italian ryegrass were screened against a susceptible population of ryegrass in 2020/21 using glyphosate, pinoxaden, and pinoxaden plus fenoxaprop. Three populations (Daviess 1, Pulaski 1, and Simpson 1) had significantly lower control using glyphosate and eight populations (Hickman 1, Simpson3, Simpson 4, Simpson 5, Simpson 6, Todd 1, Todd 2, and Todd3) had significantly lower control with pinoxaden as compared to the susceptible population. The suspected populations were further screened under greenhouse conditions using dose response techniques against a susceptible population to further quantify potential herbicide resistance within the populations. The three dose response studies (glyphosate, pinoxaden, and pinoxaden plus fenoxaprop ) included rates from a 1/16 fold to a 16 fold rate of the labeled field rate. A complete list of rates and products can be found in Table 1. All trials were evaluated at 28 days after application and results subjected to a dose response analysis using the drm package in R.

Objective 2: Commercial grower wheat fields with ryegrass escapes were evaluated for annual ryegrass seed rain June of 2020 and 2021 prior to wheat harvest. A 1m² area was evaluated for every 0.5 acre of infestation within each field evaluated. Within the 1 m² area all ryegrass seed heads were collected and all debris on the soil surface immediately within the 1m² collected using a vacuum. Ryegrass seed was then separated and cleaned of all other debris within the samples. Ryegrass seed samples were weighed and counted to achieve a distribution of seed retained on the seed head and seed that had “rained” to the soil surface just prior to wheat harvest.
A field located at the University of Kentucky Research and Education Center was further evaluated for distribution of ryegrass during wheat harvest in 2020 and 2021. Samples were collected from below the combine header, from the chaff behind the combine, and from the combine grain tank. Four samples per 1 acre of infestation were collected during harvest in each year. Ryegrass seed was separated from all other debris, grain, and chaff within the collected samples. Ryegrass seed samples were weighed and counted to achieve a distribution of ryegrass seed that shattered at the combine header, seed contained within the chaff, and seed contained within the grain tank.

RESULTS AND DISCUSSION

Objective 1. The dose response curves created based on visual control 28 days after glyphosate application revealed that two of the suspected populations had visually different curves than the susceptible populations (Figure 1). The Pulaski 1 and Simpson 1 populations required a significantly higher dose of glyphosate to reach 50% control as compared to the susceptible populations, while the Daviess 1 population was similar to the susceptible. These results confirm that at least two additional (in addition to populations confirmed in 2017) populations of Italian ryegrass are expressing glyphosate-resistance in Kentucky.

Dose response curves based on visual control 28 days after pinoxaden and pinoxaden plus fenoxaprop showed that at least three populations had different curves than the susceptible population (Figure 2 and 3, respectively). Todd 1, Todd 2, and Todd 3 all required significantly greater doses of pinoxaden and pinoxaden plus fenoxaprop to reach 50% control as compared to the susceptible population. While the Simpson 3, Simpson 4, Simpson 5, and Simpson 6 curve all visually look different than the susceptible, the doses of both pinoxaden and pinoxaden plus fenoxaprop to achieve 50% control were similar to the susceptible. The similarity in doses to achieve 50% control while appearing visually different is likely due to high variability in the response of the Simpson populations to each dose of pinoxaden and pinoxaden plus fenoxaprop. This indicates that while the four populations cannot be officially considered resistant, the populations are very likely in the beginning stages of resistance selection with a mixture of both susceptible and resistant plants existing across those field populations. While the three Todd populations can be confirmed resistant to pinoxaden and pinoxaden plus fenoxaprop with a high level of resistance occurring within each population.

Objective 2. Seed retention and rain collections were analyzed from eight locations collected in 2020 and 2021 from across 4 wheat growing counties in Kentucky. At all locations seed retention and rain collections were conducted within three days prior to the wheat field harvest operation. Across locations, the majority of seed was retained within the ryegrass seed head prior to harvest. The samples showed that a mean of 1069 Italian ryegrass seeds/ft\(^2\) remained on the seed heads prior to wheat harvest as compared to 138 Italian ryegrass seeds/ft\(^2\) found in the soil surface (Figure 4). This study conducted across eight site years indicates that Italian ryegrass will retain 89% of its seeds up to the time of soft red winter wheat harvest in Kentucky.

Italian ryegrass seed dispersal at harvest was evaluated in 2020 and 2021 at the UKREC location. Difference in Italian ryegrass seed distribution during the 2020 wheat harvest were not found with 660 seeds/ft\(^2\) occurring within the chaff exiting the combine, 464 seeds/ft\(^2\) within the grain tank, and 414 seeds/ft\(^2\) shattered at the combine header (Figure 5). In 2021, differences between the three collections did occur with 72 seeds/ft\(^2\) passing through the combine with the chaff, 71 seeds/ft\(^2\) occurring in the grain tank, and 6 seeds seeds/ft\(^2\) shattering at the combine header (Figure 5). In 2021 the amount of Italian ryegrass seed that shattered at the combine header was significantly lower as compared to number of seed found in the chaff collection and the grain tank.

As the objective of this research was to observe the potential efficacy of harvest weed seed control, the Italian ryegrass seed entering into the combine versus seed shattering at the head was the primary focus. Therefore, the Italian ryegrass seed in the grain tank samples and the chaff collection were combined as both proportions had successfully entered the combine at harvest. Using this comparison 414 Italian ryegrass seeds/ft\(^2\) shattered at the head-
er and was significantly less than the 1123 Italian ryegrass seeds/ft² that entered the combine in 2020 (Figure 6). Similarly in 2021, 6 Italian ryegrass seeds/ft² shattered at the header and was significantly less as compared to the 142 Italian ryegrass seeds/ft² that entered the combine (Figure 6). When comparing the number of ryegrass seeds entering the combine versus seed shattering at the head or being deposited back into the soil seed bank, the results support the concept that harvest weed seed control may be a viable option for Italian ryegrass in Kentucky wheat.

**CONCLUSION**

Dose response studies confirmed at least 2 additional glyphosate resistant and 3 pinoxaden (Axial XL) and pinoxaden plus fenoxaprop (Axial Bold) resistant populations of Italian ryegrass have been found in Kentucky. Additionally, several populations showed indications of early selection of pinoxaden and pinoxaden plus fenoxaprop resistance events are occurring within the populations. The resistant populations or populations showing early stages of resistance selection occurred in either Simpson or Todd Counties where a large proportion of wheat is grown in Kentucky and have historically dealt with ryegrass as a problematic weed species in this crop. Many growers within this region have relied heavily on pinoxaden based herbicides for postemergence control of ryegrass, and thus it is not surprising to find a high proportion of pinoxaden resistance occurring in this region. Looking toward the future, it should be assumed that pinoxaden resistance in ryegrass will continue to occur and spread in wheat growing regions of Kentucky.

The reality of inevitable widespread resistance to pinoxaden, calls for alternative practices to control ryegrass in wheat. One potential non-chemical control method is the use of harvest weed seed control at harvest. The successful use of harvest weed seed control depends on seed being retained on ryegrass seed heads prior to wheat harvest and being taken into harvest equipment and contained within the chaff of the crop that is distributed behind the combine. Results of this research show that at least 89% of ryegrass seed is retained on the seed head prior to harvest. Additionally, across two years 73 to 96% of ryegrass seed successfully entered the combine to be either deposited in the grain tank or exit with chaff for possible control with a harvest weed seed control tactic. The number of seed entering the combine was significantly greater both years as compared to the number of seed shattering at the combine head. These results indicate that Italian ryegrass in Kentucky wheat is a good candidate for harvest weed seed control and that it could be an additional tool for wheat growers dealing with herbicide resistant ryegrass. Additional research is ongoing to further evaluate the utility of both a seed control unit and chaff lining for control of Italian ryegrass in Kentucky.

**ACKNOWLEDGEMENTS**

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Table 1. Rates of glyphosate (Roundup PowerMax 3), Pinoxaden (Axial XL), and Pinoxaden + Fenoxaprop (Axial Bold) evaluated in the dose response study.

<table>
<thead>
<tr>
<th>Rate</th>
<th>Glyphosate (g ae/ha)</th>
<th>Roundup PowerMax3 (fl oz/A)</th>
<th>Pinoxaden (g ai/ha)</th>
<th>Axial XL (fl oz/A)</th>
<th>Pinoxaden + Fenoxaprop (g ai/ha)</th>
<th>Axial Bold (fl oz/A)</th>
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<tbody>
<tr>
<td>0x Rate</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>1/16th Rate</td>
<td>58</td>
<td>1.25</td>
<td>4</td>
<td>1.025</td>
<td>4 + 2</td>
<td>0.9375</td>
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<td>1/8th Rate</td>
<td>105</td>
<td>2.5</td>
<td>8</td>
<td>2.05</td>
<td>7 + 4</td>
<td>1.875</td>
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<tr>
<td>1/4th Rate</td>
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<td>5</td>
<td>15</td>
<td>4.1</td>
<td>15 + 7</td>
<td>3.75</td>
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<tr>
<td>1/2x Rate</td>
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<td>10</td>
<td>30</td>
<td>8.2</td>
<td>30 + 15</td>
<td>7.5</td>
</tr>
<tr>
<td>1x Rate</td>
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<td>20</td>
<td>61</td>
<td>16.4</td>
<td>60 + 30</td>
<td>15</td>
</tr>
<tr>
<td>2x Rate</td>
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<td>40</td>
<td>121</td>
<td>32.8</td>
<td>120 + 59</td>
<td>30</td>
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<tr>
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<td>80</td>
<td>241</td>
<td>65.6</td>
<td>240 + 119</td>
<td>60</td>
</tr>
<tr>
<td>8x Rate</td>
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<td>160</td>
<td>482</td>
<td>131.2</td>
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</tr>
<tr>
<td>16x Rate</td>
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<td>320</td>
<td>964</td>
<td>262.4</td>
<td>960 + 479</td>
<td>240</td>
</tr>
</tbody>
</table>

Figure 1. Dose response curves of 3 suspected glyphosate-resistant and one susceptible (SUS) Italian ryegrass population based on visual evaluations 28 days after glyphosate application.
Figure 2. Dose response curves of 8 suspected pinoxaden-resistant and one susceptible (SUS) Italian ryegrass populations based on visual evaluations 28 days after pinoxaden (Axial XL) application.

Figure 3. Dose response curves of 8 suspected pinoxaden and fenoxaprop-resistant and one susceptible (SUS) Italian ryegrass population based on visual evaluations 28 days after pinoxaden plus fenoxaprop (Axial Bold) application.
Figure 4. Location of Italian ryegrass seed prior to Kentucky’s soft red winter wheat harvest combined over eight sites in 2020 and 2021.

* Means followed a different letter are significantly different. Tukey HSD α=0.05

Figure 5. Distribution of Italian ryegrass seed during harvest of soft red winter wheat in Kentucky in 2020 and 2021. Distribution points include ryegrass seed shattered at combine header, seed deposited in combine grain tank with wheat, and seed discharged from the combine with crop chaff.

* Means within a year followed a different letter are significantly different. Tukey HSD α=0.05
Figure 6. Distribution of Italian ryegrass seed during harvest of soft red winter wheat in Kentucky in 2020 and 2021. Distribution points include ryegrass seed shattered at combine header and seed that successfully entered the combine.

* Means within a year followed a different letter are significantly different. Tukey HSD $\alpha=0.05$