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Survey of Herbicide Resistance and Seed Fate of Italian Ryegrass (*Lolium perenne ssp. multiflorum*) in Kentucky

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SURVEY OF HERBICIDE RESISTANCE AND SEED FATE OF ITALIAN RYEGRASS (*LOLIUM PERENNE SSP. MULTIFLORUM*) IN KENTUCKY

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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By

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Lexington, Kentucky

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ABSTRACT OF THESIS

SURVEY OF HERBICIDE RESISTANCE AND SEED FATE OF ITALIAN RYEGRASS (*LOLIUM PERENNE SSP. MULTIFLORUM*) IN KENTUCKY

Herbicide resistance is not a new problem for farmers in Kentucky, although the identification of herbicide resistant weed species continues to increase. Italian ryegrass (*Lolium perenne ssp. multiflorum*) is a problematic weed in Kentucky's soft red winter wheat that has historically been documented as herbicide resistant in isolated locations. A greenhouse herbicide screen was conducted to further understand the level of glyphosate, pinoxaden, and pinoxaden plus fenoxaprop resistance in Kentucky Italian ryegrass populations. The preliminary screen and dose responses indicate there is one Italian ryegrass population resistant to glyphosate and two populations resistant to pinoxaden along with pinoxaden plus fenoxaprop. Harvest weed seed control is a potential option being evaluated in Kentucky to control herbicide resistant Italian ryegrass in soft red winter wheat. One specific method of harvest weed seed control is the use of cage mills to destroy Italian ryegrass seed found within the fine chaff exiting the combine at harvest. This can only be an efficient and viable option of control if Italian ryegrass seed is retained on the seed head up to the time of harvest and is able to enter the combines chaff flow. Seed retention and dispersal of Italian ryegrass was evaluated prior to and at wheat harvest in 2020 and 2021 in Kentucky. Within one week of harvest, both the Italian ryegrass seed heads and the top layer of soil debris were collected from within a m^{-2} area for approximately each 0.2 ha of Italian ryegrass infestation in eight Kentucky wheat fields. For the preharvest and harvest samples, 11,000 of the Italian ryegrass seeds remained on the seed head up to wheat harvest and 85% of the seeds retained did enter the combine which makes mechanical harvest weed seed control a potential viable option of for Italian ryegrass control in Kentucky wheat.

KEYWORDS: Italian ryegrass, herbicide resistance, harvest weed seed control

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Introduction

Italian ryegrass *(Lolium perenne* ssp. *multiflorum)* is used widely as a pasture and cover crop species but is also considered a problematic weed along roadsides, in small grain crops, and within vegetable crops (Bond et al. 2014). This weed is becoming an increasing problem for soft red winter wheat (*Triticum aestivum*) growers in the mid-South areas of the United States because it is a winter annual that grows alongside the wheat. Italian ryegrass's ability to rapidly grow and hybridize allows it to quickly escape from roadsides, pastures, and grass waterways into row crop fields presenting problems for growers. As selection for herbicide resistance has increased in Italian ryegrass chemical control is becoming more difficult and alternative control methods need to be considered (Bond et al. 2014).

Since the late 1980's, Italian ryegrass has been inconsistently controlled by herbicides. The first herbicide resistance documentation for Italian ryegrass occurred in Oregon in 1987 to diclofop-methyl (Heap 2020). ACCase inhibitors, photosystem II inhibitors, ALS inhibitors, EPSP synthase inhibitors, glutamine synthase inhibitors, photosystem I electron diverter, and long chain fatty acid inhibitors are all the current herbicide sites of action that Italian ryegrass has had documented resistance worldwide (Heap 2020). New herbicide active ingredients are unlikely to be developed in the upcoming years so weed management tactics must be diversified to prolong the utility of current herbicides. New management techniques are also needed.

Italian Ryegrass (*Lolium perenne* ssp. *multiflorum*)

Italian ryegrass is a species in the *Lolium* genus of the Poaceae family. Italian ryegrass is known by other names such as annual ryegrass and common ryegrass (Lacefield et. al 2003). Within the genus *Lolium*, there are eight species of ryegrass: Italian ryegrass (*Lolium perenne ssp. multiflorum*), rigid ryegrass (*Lolium rigidum*), perennial ryegrass (*Lolium perenne*), poison ryegrass (*Lolium temulentum*), Dalmatian ryegrass (*Lolium subulatum*), Canary Islands ryegrass (*Lolium canariense*), hardy ryegrass (*Lolium remotum*), and Persian Darnel (*Lolium persicum*) (Barapour et al. 2017). Rigid ryegrass is known around the world as it was the first species to have herbicide resistance to multiple modes of action (Barapour et al. 2017). Italian ryegrass is commonly planted as a cover crop in the midwestern United States as it can quickly establish itself and accumulate substantial biomass both above and below ground, but in the western and southern parts of the United States, Italian ryegrass is considered to be weedy grass species (Lacefield et al. 2003). As a weed that can accumulate a large amount of biomass in the field, the increasing selection for herbicide resistant Italian ryegrass is a growing concern for wheat growers.

Italian ryegrass is a versatile species that has the ability to grow in a variety of environments. This winter annual bunchgrass germinates in the fall and spring when adequate moisture is available and when there have been 6 to 10 days with daytime temperatures of 10 to 30 degrees Celsius (Bond et al. 2014). Italian ryegrass can grow in soils with a pH from 5.0 to 7.9 with varying soil drainage and texture (Bond et al. 2014). These characteristics allow the plant to germinate quickly and be present under many different environmental conditions.

In one gram, there are an average of 494 seeds Italian ryegrass seeds (Lacefield et. al. 2003). Each Italian ryegrass plant then can produce approximately 300 seeds per plant (Mohler et al. 2021). Most of the seeds produced by Italian ryegrass plants will not have any primary dormancy and can germinate after entering the soil if there is adequate moisture, daytime temperatures are between 10 to 30 degrees Celsius for 6 to 10 days, and the soil pH is between 5 and 7.9 (Mohler et al. 2021, Bond et al. 2014). The combination of prolific seed production and minimal germination requirements for Italian ryegrass makes management of this species difficult.

Italian Ryegrass Uses

Recently throughout the United States, Italian ryegrass became a popular choice for a cover crop given that it can quickly establish itself in the fall prior to freezing winter temperatures and produce large amounts of biomass in the spring (Lacefield et. al. 2003). This makes it a popular cover crop because a plant that can establish itself and add biomass quickly can help benefit the soil profile and add organic matter with the residue it produces. When Italian ryegrass is planted as a cover crop, a significant issue is termination or achieving complete control of this cover crop in the spring. Since Italian ryegrass is becoming resistant to numerous herbicides, killing it as a cover crop can become more difficult in no-till production systems. The timing of a herbicide application in the spring and size of the Italian ryegrass can also make terminating it difficult (Green and Legleiter 2022). Italian ryegrass may have many positive qualities that allow it to be used as a forage or cover crop, but those benefits may not always justify the risk of it escaping and becoming a problematic weed in cereal crops.

The vigorous growth and quick establishment of Italian ryegrass also makes it a favorable plant for livestock producers as it can quickly accumulate large amounts of biomass that can be fed to their livestock. Another use for Italian ryegrass is for soil stabilization along roadsides. This grass will quickly germinate and can help hold roadside soils in place (Bond et al 2014). In the southeastern United States, Italian ryegrass is utilized as a common soil stabilizer and forage species (Bond et al. 2014). It is estimated that over 405,000 hectares of pasture lands contain Italian ryegrass within the southeastern United States for winter grazing (Bond et al 2014).   A third use for Italian ryegrass as a non-weed species is the use as cover crop in orchards or vineyards (Tehranchian et. al. 2017). Italian ryegrass in Oklahoma, was not a weed that presented problems in winter annual crops until Italian ryegrass cultivar known as 'Marshall' was introduced as a forage species (Barnes et al. 2001). This cultivar was developed and released to be more cold hardy, disease resistant, and mature later than previous cultivars of Italian ryegrass for farmers (Barnes et al 2001).

Italian Ryegrass Interference and Competition

Italian ryegrass is a cool season winter annual weed enabling it to compete with over-wintering grain crops. In a winter annual grain crop such as wheat, a monocot weed is highly problematic. Emergence of Italian ryegrass in Kentucky typically occurs from September through December, which makes this weed problematic in winter wheat systems (Martin and Green 2009). For every Italian ryegrass plant per 0.09 m^{-2}, the winter wheat yield can be reduced by 4 percent, which makes this the most problematic weed in winter wheat in Kentucky (Martin and Green 2009). Italian ryegrass has vigorous growth in early spring making it very competitive for sunlight, nutrients and water, which can lead to the Italian ryegrass outcompeting winter wheat or other crops (Bond et al 2014). In dense populations of Italian ryegrass (up to 118 plants/m^2) in winter wheat, yields have been shown to be reduced by up to 50% (Hoskins et al. 2005). This yield reduction, along with herbicide resistance, makes Italian ryegrass one of the most problematic weeds for winter wheat growers.

Herbicide Resistance in Crops and Weeds

Herbicide resistance is not new, but more and more weed species have resistance to one or multiple modes of actions. Herbicides are the most common weed management tool since they were developed (Shaner 2014). Herbicides select for naturally occurring resistant weed biotypes that survive a herbicide application and can result in large populations of resistant weeds if used repeatedly (Shaner 2014). The combination of a heavy reliance on herbicides and continuous use of herbicides with the same sites of action make preventing resistance selection difficult. The first example of herbicide resistance in the United States was spreading dayflower, *Commelina diffusa,* in Hawaii 1957 (Heap 2020). When the modes and sites of action are rotated, it can help prevent resistance selection to herbicides. Herbicides should not be the only tools of weed management.

The release of herbicide resistant crops brought about the idea that the threat of new cases of herbicide resistant weeds would diminish. This was the thought because weed scientists believed the unique mode of action of glyphosate would make it unlikely that weeds would develop resistance (Shaw 2016). Rather, more cases of herbicide resistance are being found. The selection pressure of millions of hectares all using the same modes of action in herbicide resistant crops resulted in more herbicide resistant weed biotypes (Shaw 2016). If herbicides continue to be the main source of weed management, herbicide resistance in weeds will also continue to increase.

Weeds can become resistant to herbicides mechanisms that are known along with mechanisms that are unknown. Herbicides are primarily known to have resistance in two different mechanisms: target site resistance and nontarget site resistance. Target site resistance is when the binding site for an herbicide has structurally changed or the binding site for the herbicide is overexpressed, likely through gene amplification (Baucom 2016). Nontarget site resistance generally is the prevention of the herbicide from reaching its site of action. Translocation of the herbicide, absorption, and enhanced metabolisms are all examples of ways plants can have nontarget site resistance. Nontarget site resistance can come with the increased threat of multiple resistance.

Resistance problems in weeds are hard to control when different nations or regions have varying regulations on herbicide use. A herbicide can have a labeled maximum rate in one country that is different from another country's maximum labeled rate. For example, the herbicide diclofop has a registered use rate of 375 grams of active ingredient per hectare in Australia, 640 grams of active ingredient per hectare in the United States, and 900 grams of active ingredient per hectare in France (Manalil 2011). This is a very large difference in labeled rates for one herbicide. Many times higher rates are not used in applications as many growers cut herbicide rates to reduce input cost. These varied labeled rates and rate cutting can result in a more rapid selection for resistance. Studies have shown that repeated low-rate applications select for resistance faster (Manalil 2011). This means that lower rates do not help prevent resistance but instead quicken the pace at which the weeds select for resistance. Following labeled use rates for herbicides is important in order for herbicides to remain effective in killing the targeted weeds and to slow the selection of resistance in the targeted weeds.

Herbicide Resistance in Italian Ryegrass

In the United States, Italian ryegrass has been reported to have herbicide resistance to multiple sites of action across numerous states. To date, 17 states have reported resistance in Italian ryegrass from seven different sites of action in the United States; additionally, 11 other countries have reported herbicide resistant Italian ryegrass (Heap 2020). Resistance is the ability of a plant to withstand an herbicide application rate that would normally kill the wild type plant (Ellis et al. 2010). As early as 1987, herbicide resistance has been reported in Italian ryegrass in 17 different states with resistance to six different sites of action (Heap 2020). Within Kentucky, two reports from the International Survey of Herbicide Weeds show herbicide resistance to ACCase inhibitors and ALS inhibitors (Heap 2020). Beyond having populations of Italian ryegrass with only one type of resistance, it is possible for some of these populations to have resistance to more than one herbicide with the same site of action. When Italian ryegrass is proven to have resistance to multiple herbicides within the same site of action, it is considered to have cross-resistance.

The repetitive use of the same herbicides to control Italian ryegrass is the biggest factor in selecting for resistance. In some places, Italian ryegrass has been a nuisance weed in orchards and has been subject to intensive repetitive applications of glyphosate and populations have been confirmed to display glyphosate resistance (Avila-Garcia and Mallory-Smith 2011). When controlling weeds in such an intensely managed system, herbicides must be varied because repeated applications of only one herbicide of the same site of action can quickly select for resistance. Orchard managers in California repeatedly applied the same herbicide until that no longer worked due to the repetitive selection of herbicide resistant plants (Tehranchian et. al. 2017). Italian ryegrass along roadsides or in orchards as a cover crop are subjected to some of the most repetitive herbicide applications for management, which is contributing to the selection for herbicide resistance.

Italian ryegrass has resistance to seven different herbicide modes of action, and the resistance events have occurred through multiple mechanisms. Since the adoption of glyphosate resistant crops, the use of glyphosate has drastically increased. The increasing reliance on glyphosate has likely resulted in Italian ryegrass now having increased documentation of resistance to glyphosate. Some resistant biotypes documented to have glyphosate resistance have an altered target site and have reduced penetration into the leaf surface (Baucom 2016). Cross resistance is not possible for populations resistant to glyphosate because it is the only EPSPS herbicide, but for populations resistant to ACCase inhibitors there are cases of cross resistance occurring. For Italian ryegrass biotypes that have documented resistance to ACCase inhibitors, studies have shown that the target site of the herbicide has mutated resulting in an amino acid substitution (Tehranchian 2017). An additional Italian ryegrass resistance events to ACCase inhibitors can occur through altered metabolism, absorption, and translocation which enables the plant to detoxify the herbicide rapidly prior to reaching its target site (Ellis 2010). Many mechanisms of Italian ryegrass resistance to herbicides are being discovered and will continue to be discovered as Italian ryegrass continues to be managed in ways that allow for selection of resistant biotypes.

A weed with the ability to cross-pollinate is one that presents more concerns around herbicide resistance. Italian ryegrass is a grass species that has the ability to cross-pollinate, especially with perennial ryegrass (*Lolium perenne)* and rigid ryegrass (*Lolium rigidum)* (Barapour et al. 2017, Terrell 1968). Hybridizing with perennial and rigid ryegrass and creating the potential for a future hybrid ryegrass with herbicide resistance is troublesome. Italian ryegrass may also hybridize with members of the *Festuca* genus like red fescue (*Festuca rubra*) tall fescue (*Festuca pratensis*) and meadow fescue (*Festuca ovina)* (Terrell 1968). Italian ryegrass's ability to cross-pollinate to other members of its genus as well as members of the *Festuca* genus present potential risks of spreading herbicide resistance to other species beyond Italian ryegrass.

Economics of Herbicide Resistance

Weed control can be one of the larger expenses growers can have when managing their crops. Herbicide resistance complicates weed management plans and can make it more costly. Some growers may try to minimize their expenses by only applying herbicides in severe infestations scenarios or every other year (Trsuler 2007). Although most Kentucky grain crops are produced with no-till systems, tillage is an alternative management technique for control of some weeds. However, there are additional costs associated with owning and operating tillage implements. Rate cutting is another way whereby growers try to reduce weed control costs, though this comes at risk of poor control (Manalil 2011).

Herbicide resistance is increasing the cost of weed control. Less options, specifically herbicides, are available for control as more herbicide resistance is documented. Italian ryegrass has such a large effect on the yield of winter wheat, up to a

60% reduction in some scenarios, that controlling weeds like it are necessary to maintain profitable yields (Barapour et al. 2017). Rigid ryegrass is a closely related weed that is resistant to numerous herbicides making it the most economically important weed in the agriculture world (Barapour et al. 2017). Beyond the expense of herbicides, the loss in yield from weeds like Italian ryegrass can impact growers profits. Italian ryegrass has reduced yield as much as 6050 kilograms per hectare (Martin and Green 2009). If herbicides will not control weeds that can compete and reduce yields, other options need be explored.

Alternative Methods of Italian Ryegrass Control

A monocot crop that competes with a monocot weed like Italian ryegrass leaves a smaller selection of usable herbicides Italian ryegrass seeds can be easily spread by the combine at harvest, overwinter, and then compete with winter wheat or corn; this is just one example of how no-till in Kentucky can lead to infestations the following growing season (Martin and Green 2009). With the occurrence of Italian ryegrass herbicide resistance and due to Kentucky's no-till production systems there are opportunities for Italian ryegrass infestations with limited options for control. Tillage is often recommended and is very important in the world today with herbicide resistance becoming prevalent (Renton and Flower 2015). Other cultural practices besides tillage can help reduce the amount of Italian ryegrass in a field but may not be as effective as herbicides. Delayed planting, increased seedling rates, narrow row widths, and rotating crops all have been practiced to reduce the Italian ryegrass populations in fields that have shown herbicide resistance (Bond et al. 2014). Implementing cultural practices can be helpful in managing the Italian ryegrass populations in a field, especially as more resistance has been documented, as the selection of herbicides that can still control Italian ryegrass diminish.

As weed resistance to herbicides continues to expand, the methods of weed control must diversify. New or different weed control practices come at varying costs. Australia is leading the development of new management practices for control of rigid ryegrass with the implementation of harvest weed seed control. Harvest weed seed control targets control of weed seeds at the time of harvest to reduce the number of weed seeds that go back into the soil's seed bank (Walsh et. al. 2013). Harvest weed seed control methods are only effective if the targeted weed retains its seed up to the time of harvest (Walsh and Powles, 2014). Narrow windrow burning, chaff carts, bale direct systems, or Harrington Seed Destructors are all harvest weed seed control options for control that are being developed to prevent weed seeds from reaching the seed bank (Tidemann 2017). All options are worth exploring but are dependent on the type of cropping system.

Each method of harvest weed seed control aims to remove the weed seed from the field or destroy the weed seed before it can enter the seed bank. Chaff carts are a cart attached to combine and collecting all the chaff exiting the combine. Narrow windrow burning is conducted using a chute mounted to the combine to deposit the chaff exiting the combine in narrow rows that are burned when conditions are ideal (Walsh and Powles, 2007). Bale direct harvest systems bale the chaff that exits the combine (Walsh and Powles, 2007). Lastly a seed destructor, like the Harrington Seed Destructor, aim to destroy all the weed seed in the fine chaff that exits the combine (Walsh et al, 2012).

The Integrated Harrington Seed Destructor, a tool that required no towing like the previous Harrington Seed Destructor, has proved helpful in reducing the number of viable rigid ryegrass seeds that reach the soil seed bank (Tidemann 2017). The cost of the Integrated Harrington Seed Destructor at $160,000 USD and the risk of managing windrow burning, are large hurdles for weed control. The use of a seed destructor controlled 95 ± 0.8% of rigid ryegrass seed in an Australia (Walsh et al., 2012). New weed control options must be economically and risk feasible for growers if they are to be implemented on larger scales.

The Harrington Seed Destructor is one of the methods of harvest weed seed control from Australia that is now being studied in the United States. It has been tested in the Southern United States to determine its efficacy at controlling common weeds in soybean and rice fields (Schwartz-Lazaro et. al. 2017). In Australia, it is used on rigid ryegrass and they have found that approximately 70 to 80 percent of rigid ryegrass seed will enter the combine at harvest to be deposited back onto the field with the chaff (Walsh and Powles 2007). When the Seed Destructor was tested in Australia, 95 percent of the rigid ryegrass seeds in the chaff were destroyed. In Australia, 58 percent of the Lolium ssp. ryegrass seed was retained on its seed head (Shergill et al., 2020). The Seed Destructor therefore is a very efficient harvest weed seed control tactic. With the close relation between rigid ryegrass and Italian ryegrass and similarity in seeds, it may be an option for Italian ryegrass control here in the United States.

Summary and Objectives

With the continuing use of herbicides on Italian ryegrass, the selection for resistance has grown since the first case in 1987. The limited number of herbicides available for use in a grass crop such as wheat with a grass weed makes weed management difficult. The fear of Italian ryegrass becoming resistant to most herbicides like its relative, rigid ryegrass, has brought this issue forward. Italian ryegrass can quickly establish itself and it has the ability to hybridize with multiple species within its genus of *Lolium* and the genus *Festuca*. Producing offspring with resistance or hybrids with resistance is a large threat with this species already having resistance to several modes of action. The objectives of this research are to evaluate seed retention and rain of Italian ryegrass prior to winter wheat harvest, evaluate seed shatter and dispersal of Italian ryegrass at wheat harvest, and to evaluate the presence of herbicide resistance in Italian ryegrass throughout Kentucky.

CHAPTER 2.

Introduction

Weed management in Kentucky row crops is primarily conducted with the application of herbicides. The repeated applications of the same herbicide sites of action have selected for herbicide resistant species over time. Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) is a winter annual that is problematic weedy grass in Kentucky winter wheat (*Triticum aestivum*) fields. Since 1987 there have been 35 documented cases of herbicide resistance in the United States with Italian ryegrass (Heap 2022). Both postemergence, herbicide applications after the crop has emerged, and burndown applications, herbicide applications to prepare the seedbed for planting, are failing to control Italian ryegrass leaving farmers with very few options. Weed management with heavy reliance on herbicides in winter wheat might not be enough to control Italian ryegrass in the coming years.

Soft red winter wheat is a winter annual crop grown in Kentucky primarily within a corn-soybean-wheat or wheat-soybean rotation and is Kentucky's fourth most valuable cash crop (Lee et al., 2009). Weeds are the most important pest in wheat worldwide (Oerke, 2006). Italian ryegrass could be the most detrimental weed species in Kentucky winter wheat. Currently, Italian ryegrass has been documented worldwide as resistant to ACCase inhibitors, photosystem II inhibitors, ALS inhibitors, EPSP synthase inhibitors, glutamine synthase inhibitors, photosystem I electron diverters, and long chain fatty acid inhibitors (Heap 2022). Kentucky currently only documented cases of ACCase inhibitors and ALS inhibitors resistance (Heap 2022).

There are many different species of ryegrass but only a few are problematic weed species. Italian ryegrass, sometimes known as annual ryegrass, is just one of eight species within the genus *Lolium* (Bararpour et al., 2017). Rigid ryegrass (*Lolium rigidum*) is another weedy ryegrass of the *Lolium* genius that is resistant to 12 different herbicide sites of action worldwide (Heap 2022). Italian ryegrass can spread into wheat fields because it is used as roadside vegetative cover, a cover crop, forage species, and ground cover in orchards (Bond et al., 2014, Tehranchian et al., 2018). These areas of beneficial use are often in close proximity to row crop fields such as growing in grass waterways within and adjacent to crop fields. One reason Italian ryegrass causes such problems in wheat fields is the fact that it is more competitive in root and shoot growth than wheat (Stone et al., 1998). One Italian ryegrass plant per 0.09 m^{-2} can decrease wheat yield by four percent (Martin et al., 2009). Italian ryegrass's competitiveness along with increasing instances of herbicide resistance, makes it a troublesome weed.

As herbicide resistant crops were released, like glyphosate resistant soybeans, glyphosate became the most widely used herbicide. With this increased use glyphosate resistant weeds have developed (Livingston et al., 2015). Italian ryegrass is just one example of a weed to have recently documented populations of herbicide resistance as a result of the overuse of glyphosate.

Herbicides are the most common pesticide used in wheat and postemergence herbicides are the most common weed management practice in wheat (Flessner et al., 2021). Furthermore, Kentucky primarily grows no-till wheat and instead of tillage, glyphosate is a popular burndown option for farmers. From personal experiences, pinoxaden is a popular postemergence herbicide for farmers. Italian ryegrass for many reasons is a weed farmers must scout for and learn to manage in Kentucky winter wheat fields. It has increasing cases of herbicide resistance, which lessens the number of options for chemical weed management for farmers.

An objective of this study was to screen populations to confirm suspected glyphosate, pinoxaden, and pinoxaden plus fenoxaprop herbicide resistance in Italian ryegrass populations from across Kentucky. All 3 herbicides are common herbicides used to control Italian ryegrass in or before wheat in Kentucky. This objective was completed by performing an initial herbicide screen and to determine which populations had significantly less control than the susceptible population. The populations with significantly less control where then further tested in dose response studies.

Materials and Methods

Population Collection

Seed from 23 populations of Italian ryegrass were collected from Kentucky corn, soybean and wheat fields in May and June of 2020 (Table 2.1). Of the 23 populations, 20 were collected from fields where a postemergence herbicide had failed in winter wheat, 2 were collected from fields where a burndown application had failed prior to soybean planting, and 1 came from a field where a burndown failed prior to corn planting. The populations from wheat fields were collected as mature seed heads prior to wheat harvest. The populations from the corn and soybean fields were collected as entire plants and transplanted into greenhouse pots to finish growing and allowed to produce seed. The seed heads from all populations were hand threshed and put through an air column seed blower to clean any excess debris from the seed. The susceptible population, which was confirmed in the initial herbicide screen, used in all experiments was a cover crop annual ryegrass variety known as “King”.

Greenhouse Procedures and Herbicide Applications

For all experiments, 0.25 grams of seed from each Italian ryegrass population collected was broadcast into a plastic greenhouse flat measuring 25.4 centimeters by 25.4 centimeters, containing Berger BM 1 potting mix. After the seeds were broadcast in the flats, an additional fine layer of potting mix was sprinkled over the seeds. The flats were watered daily, fertilized weekly with Miracle Gro All Purpose Plant Food and maintained at 15.6° to 26.7° Celsius. ValuTek High Pressure 150-Watt sodium lamps in the greenhouse were used to simulate daylight. Once emerged, the seedlings were transplanted into 14 centimeters long cones, with one Italian ryegrass seedling per 53 cm^3 cone. The plants were allowed to grow and reach between one and four tillers prior to herbicide application. There was a large spread in plant size as some populations grew quicker than other populations. Prior to all applications, 4 plants at random were selected and plant heights recorded. All applications, both in the initial screen and dose response were made using a spray chamber (Devries Generation 3 Spray Booth). The glyphosate application was made at a spray volume of 140 L/ha, while the pinoxaden and pinoxaden plus fenoxaprop were applied at a spray volume of 94 L/ha. The 140 L/ha applications were made with a TeeJet XR 11002 nozzle and for the 94 L/ha applications a TeeJet 8002EVS nozzle was used.

Initial Herbicide Screen

All 23 populations were evaluated in the initial screen in addition to the known susceptible population. This initial screen was designed as a randomized complete block with 5 replications. There were 2 separate runs were performed in the greenhouse. Each Italian ryegrass population was subjected to applications of glyphosate (Group 9), pinoxaden (Group 1), and pinoxaden plus fenoxaprop (Group 1). The screen was conducted by applying a 1- and 2-fold labeled rate of each herbicide (Table 2.1). A rate of 10 g/L Amsol AMS was added in the glyphosate mix. Visual evaluations based on a scale of every 5 percent from 0 to 100 percent were conducted 28 days after application. No control was represented by 0 percent and 100 percent represented complete weed death or full control. All plant material was harvested 28 days after application plants and fresh weights recorded. All samples were placed in a dryer (Grieve Corporation SC-400) at 55° Celsius for 2 to 3 days before dry weights were recorded.

Dose Response Studies

Populations that had significantly less control when compared to the susceptible population in the initial screen were further evaluated in a dose response experiment. Dose response trials were conducted for glyphosate, pinoxaden, and pinoxaden plus fenoxaprop. There were 3 populations plus the susceptible in the glyphosate dose response and 8 populations plus the susceptible in the pinoxaden and pinoxaden plus fenoxaprop dose responses. The glyphosate dose response experiment had 5 replicates and was run twice. Amsol AMS was added to the glyphosate mix at a rate of 20 g/L. This was larger rate of Amsol AMS than used in the initial herbicide screen because larger rates of glyphosate were used in the dose response study. The population Daviess 1 in the second run only had 3 replicates due to a shortage of seed. The pinoxaden and pinoxaden plus fenoxaprop dose response had 5 replicates and was run once. The dose response studies were designed as a randomized complete block experiment.

For all dose responses, the Italian ryegrass populations were subjected to 10 rates of the herbicide in question ranging from 0 to 16-fold of the recommended rates (Table 2.2). Visual evaluations based on a scale every 5 percent from 0 to 100 percent were conducted at 28 days after application. No control was represented by 0 percent and 100 percent represented complete weed death or full control. All plant material was harvested 28 days after application plants and fresh weights recorded. They were then dried in a dryer (Grieve Corporation SC-400) for 2 to 3 days at 55° Celsius before dry weights were recorded.

Data Analysis

Before any analyses were conducted, all data were checked for normality and equal variances by examining the Conditional Pearson Residuals. The fixed effects in both the initial herbicide screen and the dose responses were the herbicide rates. Each site was ran separately with the herbicide rates as fixed effects and replicated as random effects. The susceptible population's percent visual control 28 days after application was compared to each population's percent visual control to determine if a population had significantly lower or greater control in the initial herbicide screen. Data was evaluated using SAS 9.4 (SAS Institute Inc) PROC GLIMMIX for analysis of variance. The means were separated using Tukey's HSD with alpha at 0.05.

Dose response curves were developed for glyphosate, pinoxaden and pinoxaden plus fenoxaprop using both dry weight and 28 DAT visual evaluations from the dose response experiment. Dose response curves were developed in R using version 4.1.2 (R Core Team 2021) using the drm function from the drc package (v 4.1.2; Ritz et al. 2015). All dose response curves were generated in R using a 3-parameter model which estimates the bottom (minimum response), top (maximum response), and EC50. LD50 values, the dose required for 50 percent control, were also calculated in R utilizing the drc package and LD50 means were separated with alpha at 0.05.

Results and Discussion

Initial Herbicide Screen

There were no interactions between treatments in run 1 and run 2 of the initial herbicide screen, thus runs 1 and 2 were pooled for further analysis. Multiple populations had significantly lower control as compared to the susceptible population for each chemical, at both the 1- and 2-fold application rates (Table 2.1). These results from the initial herbicide screen indicated that there may be new populations of Italian ryegrass within Kentucky that are resistant to one or more of the herbicides included in the initial screen.

There were three populations that had 70 percent or less control with glyphosate and were significantly less than control of the susceptible population at 88 or 97 percent control with 1x and 2x, respectively (Table 2.1). Two populations, Pulaski 1 and Simpson 1, were controlled significantly less with glyphosate at both rates while Daviess 1 was controlled significantly less only at the 2x rate of glyphosate (Table 2.1). Eight of the 24 populations had 47 percent or less control with pinoxaden at both the 1x and 2x rates and were significantly less than the susceptible at 85 percent control at the 1x rate and 96 percent control at the 2x rate (Table 2.1). Six populations had 18 percent or less control with pinoxaden plus fenoxaprop at the 1x rate and were significantly less than the susceptible at 75 percent control (Table 2.1). Seven populations had 44 percent or less control with pinoxaden plus fenoxaprop at the 2x rate and were significantly less than the susceptible at 80 percent control (Table 2.1). The six populations that had significantly less control for pinoxaden plus fenoxaprop were the same populations that had reduced control with pinoxaden.

Glyphosate Dose Response

Three populations (Daviess 1, Pulaski 1, and Simpson 1) were included in the glyphosate dose response studies. Each had reduced control with glyphosate as compared to the susceptible population in the initial herbicide screen. Pulaski 1 and Simpson 1 had significantly greater LD50 based on dry biomass compared to the susceptible (Table 2.3 and Figure 2.1). This indicates that a greater rate of glyphosate was needed to achieve 50 percent control when measured by dry weight, 28 days after glyphosate application. Pulaski 1 also had a significantly greater LD50 based on visual control rating as compared to the susceptible population (Table 2.3). Conversely, the Simpson 1 visual control LD50 was not significantly different than the susceptible population, despite the LD50 value being considerably greater numerically (Table 2.3). A combination of a large standard error for the Simpson 1 population and extrapolation of the LD50 due to a lack of effective control even at the greatest dose in the experiment (Figure 2.2) likely contributed to the contradicting outputs. Despite the inability to separate the visual control LD50 of Simpson1 from the susceptible population, the combination of a visual differentiation of curves in Figure 2.2 and a significant difference in dry weight biomass between Simpson 1 and the susceptible population's LD50 indicates Simpson 1 is likely resistant. Therefore, two populations from this experiment were considered resistant to glyphosate based on dry weights and visual control ratings taken 28 days after glyphosate application.

Pinoxaden and Pinoxaden Plus Fenoxaprop Dose Response

The eight populations included in the pinoxaden and pinoxaden plus fenoxaprop dose response were Hickman 1, Simpson 3, Simpson 4, Simpson 5, Simpson 6, Todd 1, Todd 2, and Todd 3, as each had reduced control with pinoxaden and pinoxaden plus fenoxaprop as compared to the susceptible population in the initial herbicide screen (Table 2.1).

Four of the eight populations, Simpson 4, Todd 1, Todd 2 and Todd 3, had visually different curves at 28 days as compared to the susceptible population when looking at the pinoxaden dry weight dose response curve (Figure 2.3). By analysis, only the Todd 3 population had a significantly greater LD50, or pinoxaden rate to achieve 50 percent control as indicated by harvested plant dry weight, when compared to the susceptible (Table 2.4). Two of the same populations, Todd 1 and Todd 3, were uniquely different in the pinoxaden visual control dose response curve (Figure 2.4). Todd 1 and Todd 3 both had significantly greater LD50's for visual control rating LD50's as compared to the susceptible population (Table 2.4). This indicates that these two populations would need a greater dose of pinoxaden to achieve 50 percent control as compared to the susceptible population. Todd 3 had a significantly greater LD50 value for both the dry weight and visual control evaluations indicating resistance to pinoxaden. Simpson 6 had a large standard error for its 28 days after application visual ratings, which is likely why it has such a large LD50 but is not significantly different than the susceptible population. This is likely because this population is early in its selection for resistance, though further investigation is needed. For Todd 1 the LD50 values for visual control, was significantly greater than the susceptible population, thus further investigation may be needed to confirm resistance since dry weight was not significant (Table 2.4).

Three populations, Todd 1, Todd 2, and Todd 3, had visually different response curves for pinoxaden plus fenoxaprop by harvested plant dry weights when compared to the susceptible population (Figure 2.5). The susceptible population's dry weight dose response curve was almost a horizontal line indicating a lack of dose response for the susceptible population at the pinoxaden plus fenoxaprop rates evaluated. The susceptible population is likely too susceptible to pinoxaden plus fenoxaprop and no LD50 comparisons could be made. Even the lowest herbicide rates controlled the susceptible population. Due to the lack of a dose response curve of the susceptible population, a viable pinoxaden plus fenoxaprop LD50 based on dry weights for the susceptible population could not be produced. Thus, comparisons of dry weight LD50 values between the suspected populations and susceptible populations could not be conducted.

However, the three Todd populations along with Simpson 5 were visually different when looking at the pinoxaden plus fenoxaprop visual control dose response curves (Figure 2.6). The Simpson 3 visual control dose response curve is essentially a horizontal line and does not fit a dose response curve. As a result it did not produce a viable LD50 value for visual control (Table 2.4). Simpson 5, Todd 1, Todd 2, and Todd 3 all had significantly greater visual control LD50 values when compared to the susceptible (Table 2.4). These four populations require a greater rate of pinoxaden plus fenoxaprop to achieve 50 percent visual control as compared to the susceptible population. These LD50 values based on visual control evaluations, indicated possible cross resistance events to the two group 1 herbicides. Although further investigation evaluating each herbicide separately would be needed to confirm cross resistance. Additionally, further investigation into the Simpson 3 populations is warranted due to all rates of pinoxaden plus fenoxaprop included in this dose response experiment failed to achieve control of the ryegrass plants.

Summary

The initial herbicide screen confirms the suspicions that there is increasing herbicide resistance cases in Italian ryegrass populations in Kentucky. Populations that had significantly less control went through further testing utilizing glyphosate, pinoxaden and pinoxaden plus fenoxaprop dose responses. Results of the dose response experiments confirmed that multiple new isolated populations of Italian ryegrass in Kentucky are resistant to either glyphosate or pinoxaden, and potentially some cross resistance events occurring in pinoxaden plus fenoxaprop tank mixes. These confirmations of glyphosate, pinoxaden and pinoxaden plus fenoxaprop resistance in Italian ryegrass are some of the first confirmation of these events in Kentucky. Resistance to group 1 herbicides, ACCase inhibitors like diclofop-methyl, had been reported in the past in Kentucky, but these results demonstrate Kentucky's Italian ryegrass populations has resistance to other group 1 herbicides, pinoxaden and fenoxaprop (Heap 2022).

Since the Pulaski 1 population was collected from a corn field, the resistance confirmation explains why the farmers' burndown with a glyphosate application failed to control Italian ryegrass in this field. All three Todd populations are fields managed by the same farmer which would have likely been managed historically in a similar way. That consistent management has led to the development of resistance to pinoxaden and pinoxaden plus fenoxaprop.

Some populations (Simpson 3, Simpson 4, Simpson 6, Todd 2 and Todd 3) in the dose responses had a very large LD50 but were not significantly different from the susceptible population. With these populations considerable variability was observed in their dry weights and visual control as seen in the dose response curves. This could be due to the fact only one run was completed for the pinoxaden and pinoxaden plus fenoxaprop dose responses. It also could be that the population was from a field that contains both susceptible and resistant Italian ryegrass plants. It may still be a segregating population of Italian ryegrass selecting for resistance.

The new findings along with previous herbicide resistance reports indicate that Kentucky wheat growers have very few viable postemergence herbicide options left. The lack of Italian ryegrass control by herbicides is also beginning to show up in corn and soybean fields becoming more troublesome for farmers. Other weed management options, besides chemical weed management, should be considered for farmers to continue to have productive wheat acres in Kentucky.

Table 2.1. Italian ryegrass percent control 28 days after application when subjected to 1- and 2-fold rates of glyphosate, pinoxaden, and pinoxaden plus fenoxaprop.

Population	Glyphosate (g ae/ha)		Pinoxaden (g ai/ha)		Pinoxaden + Fenoxaprop (g ai/ha)		Field Location (GPS Coordinates)
	(863)	(1737)	(61)	(121)	(60+30)	(120+59)	
SUS	88	97	85	96	75	80	
Ballard 1	85	91	72	88	78	94	
Daviess 1	68	70*	57	70	46	66	
Daviess 2	89	91	93	92	83	93	
Daviess 3	76	83	83	88	63	90	
Hickman 1	83	98	23*	47*	56	73	Corn Field
Logan 1	71	89	60	71	60	93	(36.79, -86.83)
Logan 2	77	100	84	90	70	88	(36.74, -86.99)
Logan 3	72	85	81	90	67	86	(36.74, -86.98)
Pulaski 1	13*	16*	59	75	69	70	(37.14, -84.63) Corn Field
Simpson 1	8*	7*	59	72	56	66	(36.73, -86.57) Soybean Field
Simpson 2	72	97	73	80	58	76	(36.70, -86.61)
Simpson 3	70	90	16*	14*	11*	21*	(36. 67, -86.55)
Simpson 4	75	83	22*	28*	21*	32*	(36.70, -86.46)
Simpson 5	80	94	24*	34*	42	44*	(36.70, -86.46)
Simpson 6	88	98	21*	26*	18*	27*	(36.41, -86.39)
Simpson 7	80	88	75	89	79	97	(36.45, -86. 41)
Todd 1	71	81	4*	12*	8*	6*	(36.41, -87.13)
Todd 2	70	86	6*	12*	8*	10*	(36.41, -87.13)
Todd 3	82	93	9*	12*	5*	11*	(36.41, -87.13)
Warren 1	86	84	77	91	90	92	(36.89, -86.55)
Warren 2	73	84	68	95	54	91	(36.89, -86.55)
Warren 3	76	87	70	87	73	77	(37.03, -86.15)
Wayne 1	99	96	70	91	76	85	(36.94, -84.73)

*Means with an asterisk are significantly different when compared to the susceptible population. Tukey HSD a=0.05

Table 2.2. 1/16thx to 16x rates of each herbicide tested in a dose response study.

	Glyphosate (g ae/ha)	**Pinoxaden (g ai/ha)**	**Pinoxaden + Fenoxaprop (g ai/ha)**
0x Rate	0	0	0
1/16thx Rate	58	4	4 + 2
1/8thx Rate	105	8	7 + 4
1/4thx Rate	211	15	15 + 7
1/2x Rate	420	30	30 + 15
1x Rate	841	61	60 + 30
2x Rate	1681	121	120 + 59
4x Rate	3363	241	240 + 119
8x Rate	6725	482	480 + 237
16x Rate	13,450	964	960 + 479

Figure 2.1. Italian ryegrass dry weight dose response curves 28 days after glyphosate application.

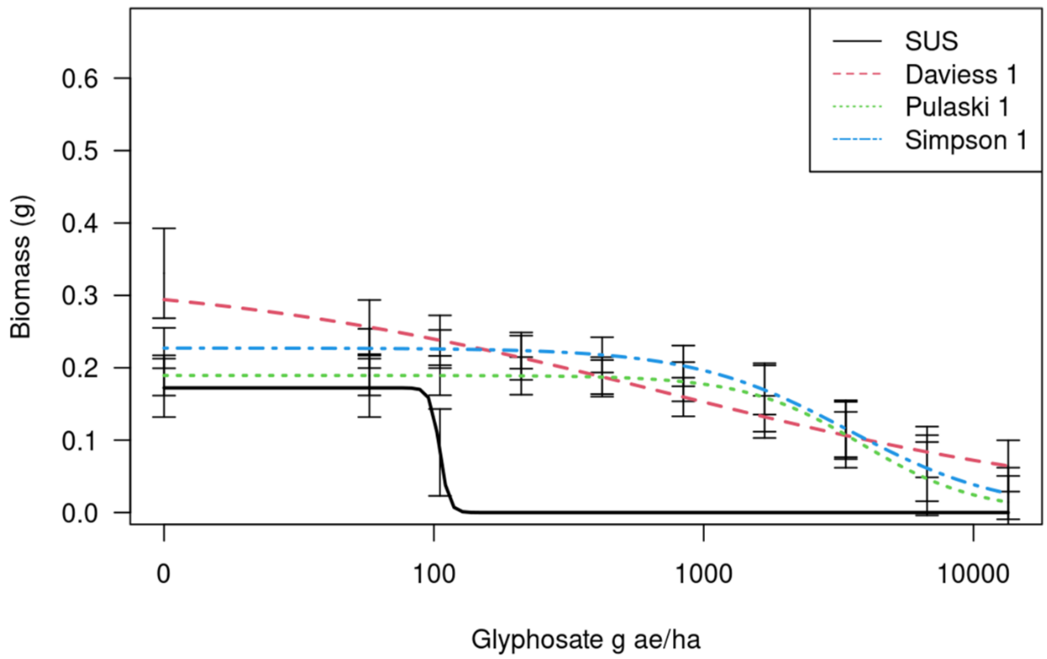


Figure 2.2. Italian ryegrass visual control dose response curves 28 days after glyphosate application.

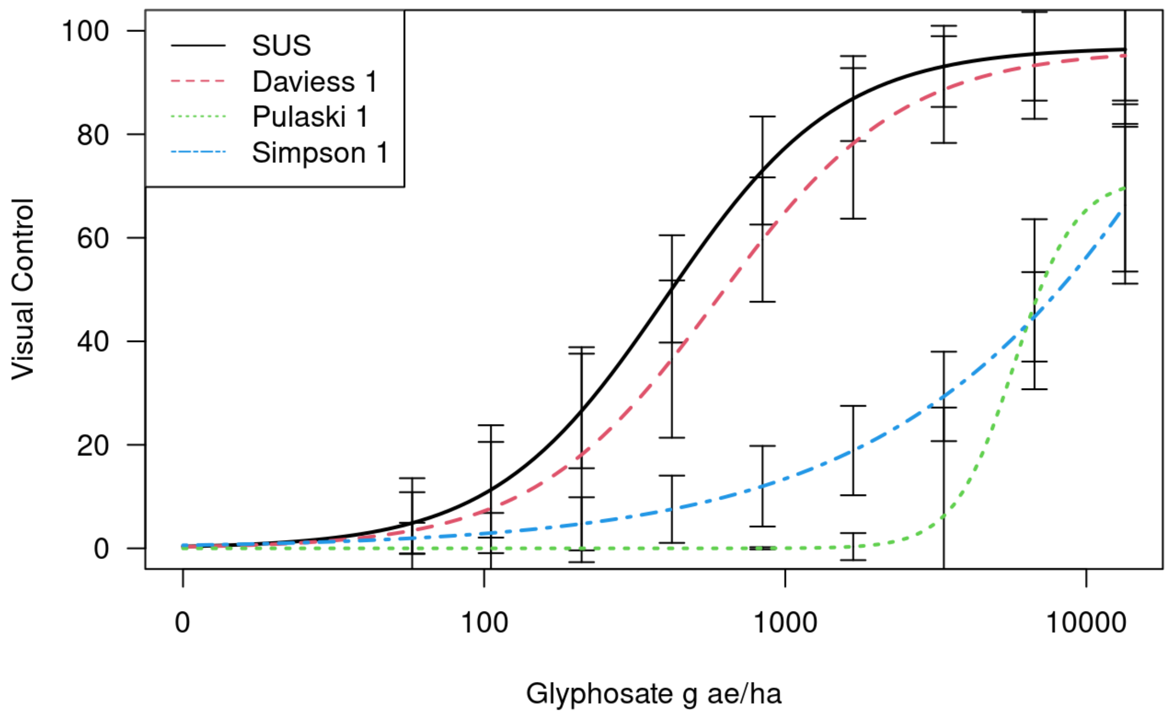


Table 2.3. Glyphosate LD50 (rate to achieve 50 percent control) of Italian ryegrass evaluated by dry weight and visual evaluation 28 days after application.

Population	g ae/ha Glyphosate	
	Dry Weight (g)	Visual Control
SUS	105	401
Daviess 1	731	596
Pulaski 1	3848*	5769*
Simpson 1	3450*	91,443

*Means with an asterisk are significantly different when compared to the susceptible population. Tukey HSD a=0.05

Figure 2.3. Italian ryegrass dry weight dose response curves 28 days after pinoxaden application.

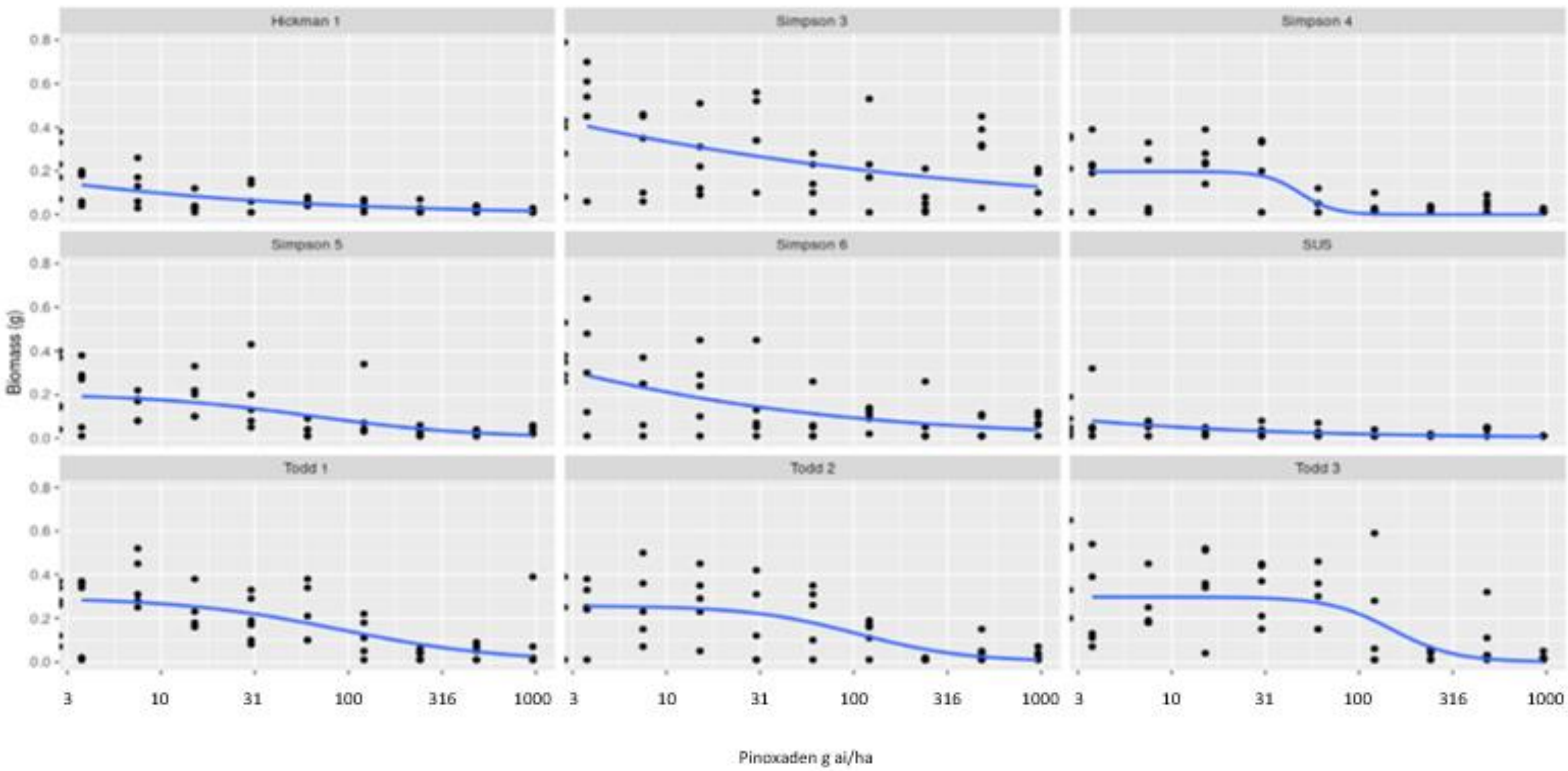


Figure 2.4. Italian ryegrass visual control dose response curves 28 days after pinoxaden application.

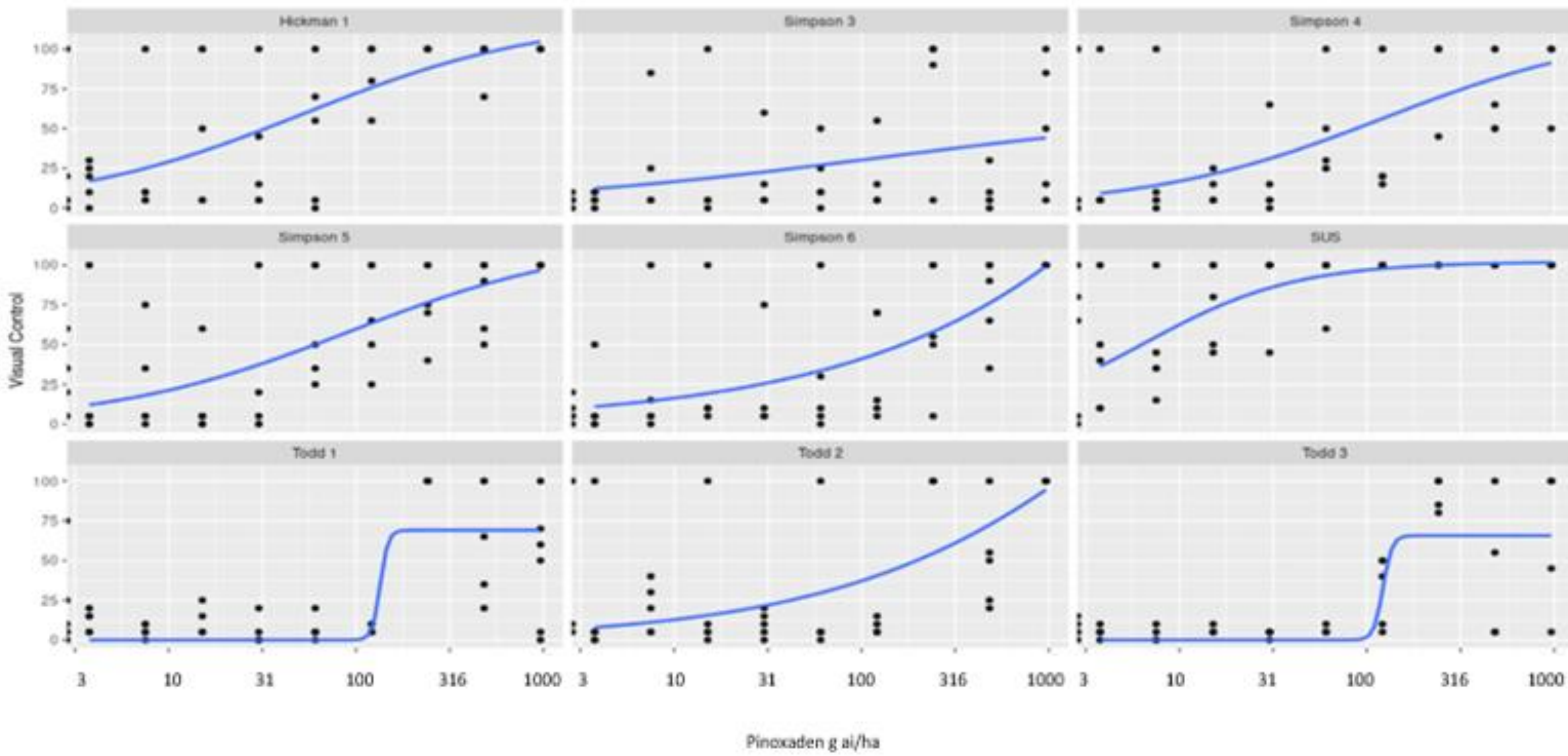


Figure 2.5. Italian ryegrass dry weight dose response curves 28 days after pinoxaden plus fenoxaprop application.

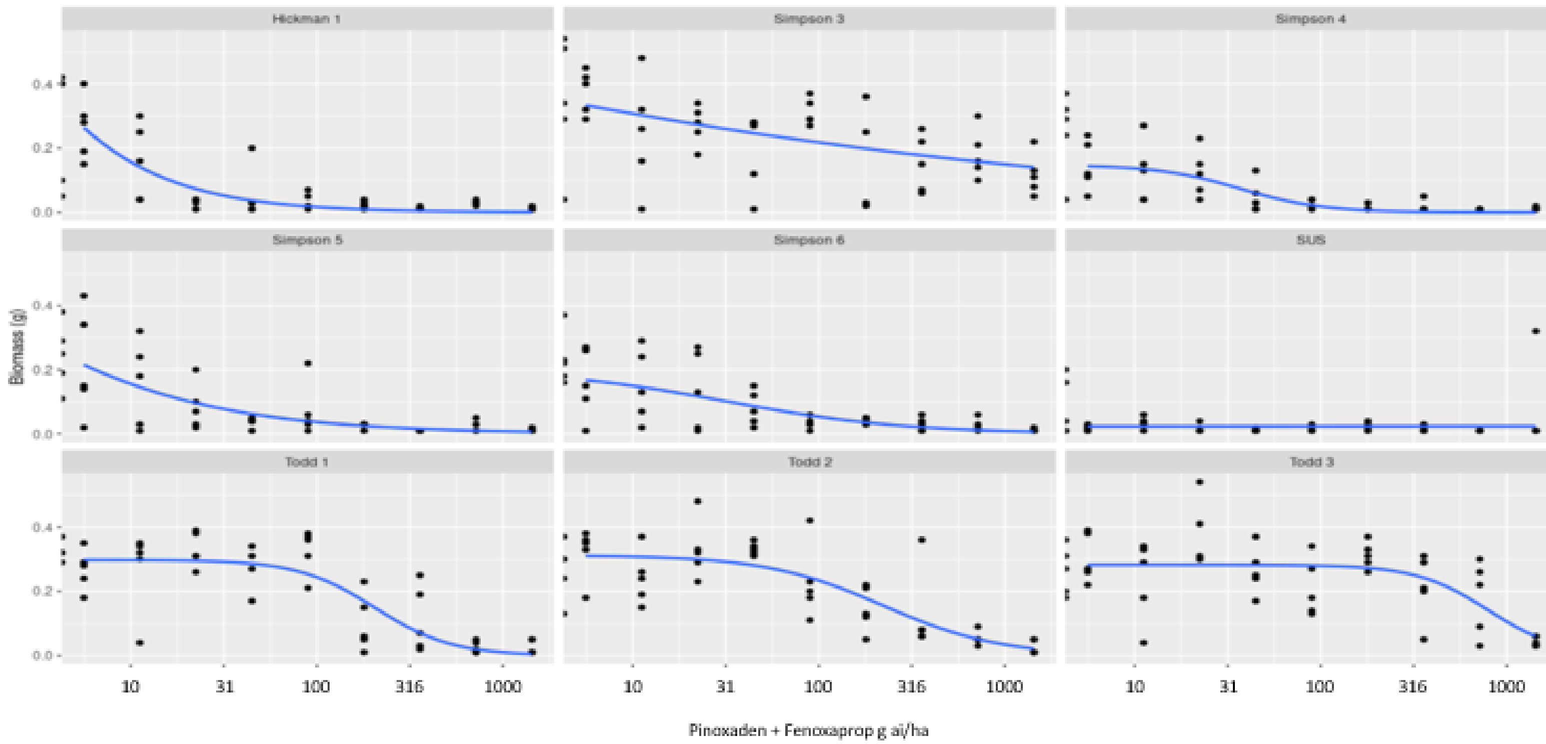


Figure 2.6. Italian ryegrass visual control dose response curves 28 days after pinoxaden plus fenoxaprop application.

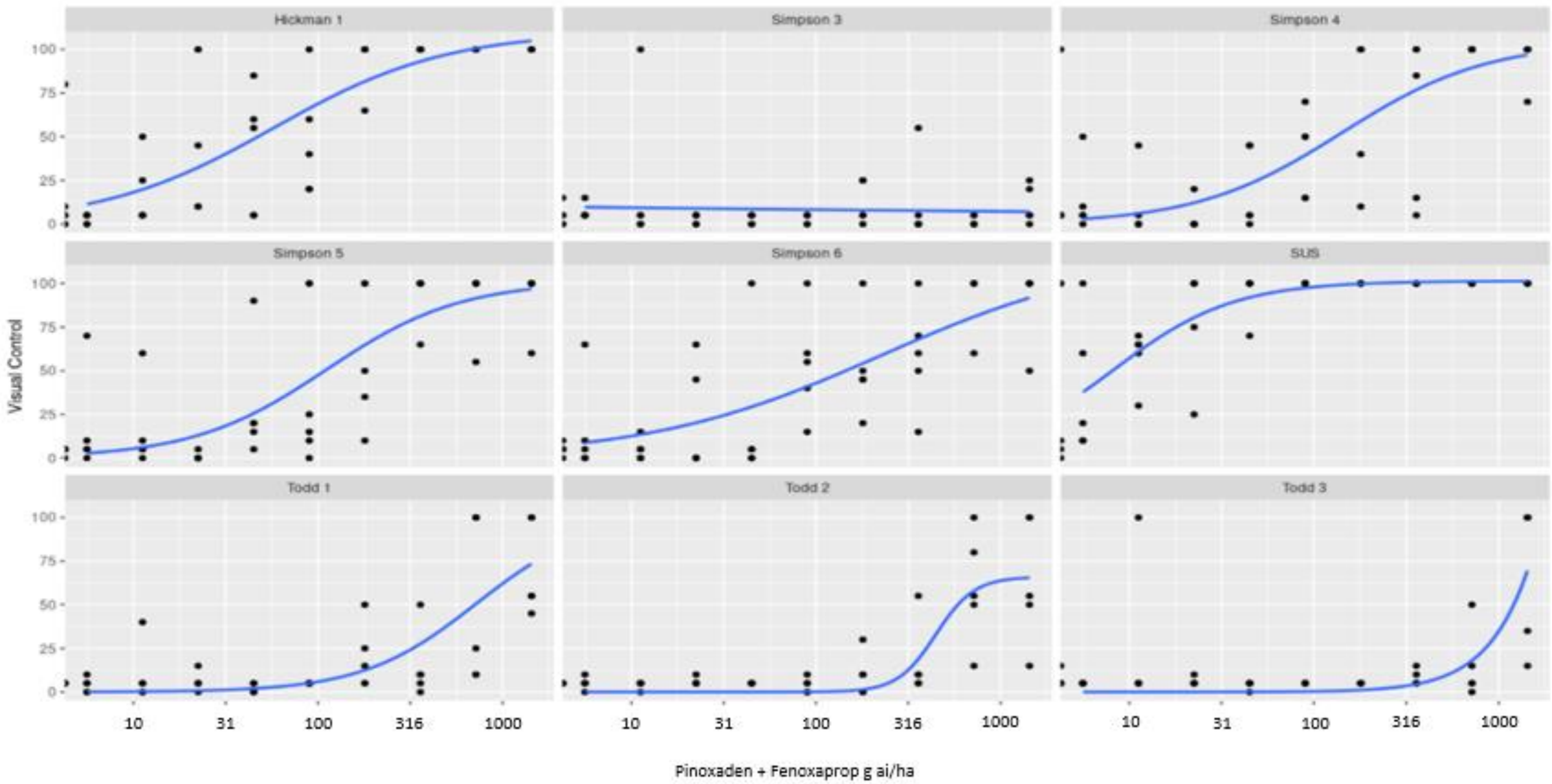


Table 2.4. Pinoxaden and pinoxaden plus fenoxaprop LD50 (rate to achieve 50 percent control) of Italian ryegrass evaluated by dry weight and visual evaluation 28 days after application.

Population	g ai/ha Pinoxaden		g ai/ha Pinoxaden+Fenoxaprop
	Dry Weight (g)	% Visual Control	% Visual Control
SUS	23	6.5	8
Hickman 1	5	53	58
Simpson 3	130	81	
Simpson 4	49	122	138
Simpson 5	54	93	111*
Simpson 6	17	6688	226
Todd 1	112	130*	1201*
Todd 2	113	132	443*
Todd 3	137*	122*	2201*

*Means with an asterisk are significantly different when compared to the susceptible population. Tukey HSD a=0.05

CHAPTER 3.

Introduction

Herbicide resistance cases continue to increase, and farmers are looking for alternative methods of weed control. Harvest weed seed control, originated in Australia, is one non-chemical weed control option that is being evaluated for introduction in North America (Walsh et al., 2013). One specific method of harvest weed seed control is the use of high impact cage mills acting as a crushing device to destroy weed seed in the chaff exiting the combine (Walsh et al., 2012). Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) is a problematic weed in the southern United States that is closely related to rigid ryegrass (*Lolium rigidum*), which is the primary species targeted with harvest weed seed control in Australia. Since 1987 there have been 35 reported cases of herbicide resistance in the United States with Italian ryegrass (Heap 2022). Kentucky has documented cases of ACCase inhibitors and ALS inhibitors resistance in Italian ryegrass (Heap 2022). Soft red winter wheat (*Triticum aestivum*) fields in Kentucky have infestations of Italian ryegrass with possible cases of herbicide resistance increasing. Harvest weed seed control is one possible new strategy for farmers who are struggling to control Italian ryegrass in Kentucky.

Harvest weed seed control is not new to agriculture, but it is a newer concept in the United States (Shergill et al., 2020). The goal of harvest weed seed control is only effective if the targeted weed retains its seed up to harvest and the goal is to prevent weed seed at harvest from adding to the seed bank (Walsh et al., 2013). Harvest weed seed control options include the use of chaff carts at harvest, narrow windrow burning, bale direct, or a mechanical seed destructor (Walsh et al., 2013). Chaff carts remove the weed seed from the field, preventing it from being added to the field's weed seed bank by attaching a cart to the combine and collecting all the chaff exiting the combine. The chaff is either deposited in piles in the field and burned to destroy any weed seed within or the piles are grazed to help remove weed seed (Walsh and Powles, 2007). Narrow windrow burning is conducted using a chute mounted to the combine to deposit the chaff exiting the combine in narrow rows that are then burned when conditions are ideal (Walsh and Powles, 2007). Bale direct harvest weed seed control is the method baling the chaff that exits the combine (Walsh and Powles, 2007). Lastly, a seed destructor is a mechanical system attached to a combine that uses cage mills to destroy any weed seed in the chaff as it exits the combine (Walsh et al., 2012). The use of some method of harvest weed seed control, even if the efficacy of the strategy is as low as 20 percent, can reduce the amount of seed entering the seed bank and maintain the seed bank at its current level (Shergill et al., 2020).

Harvest weed seed control can only be effective against Italian ryegrass in Kentucky's soft red winter wheat if the Italian ryegrass retains its seed up to the time of wheat harvest. It was found that 95 percent of rigid ryegrass seed will enter the combine at harvest, which is potentially why harvest weed seed control has worked so effectively in Australia (Walsh and Powles, 2007). However, in the Pacific Northwest, specifically Washington and Oregon, it was found that less than 50 percent of Italian ryegrass seeds will be retained on the seed head up to the time of harvest (San Martín et al., 2021). This may indicate that the location where the Italian ryegrass grows could affect seed retention at wheat harvest.

The goals of this study was to evaluate the potential use of harvest weed seed control as an additional tool for Italian ryegrass control in soft red winter wheat in Kentucky. The first goal was to evaluate Italian ryegrass seed retention prior to wheat harvest. The second goal was to evaluate the dispersal of Italian ryegrass seed during wheat harvest.

Materials and Methods

Field Selections

Wheat fields with heavy Italian ryegrass infestations (> 40 seedheads per m^2) were evaluated and selected for use two to three weeks prior to wheat harvest for the preharvest seed retention study. For both 2020 and 2021, three commercial wheat fields and one field at the UKREC (University of Kentucky Research and Education Center) were selected for their heavy Italian ryegrass infestations to be included in the preharvest study (Table 3.1). The preharvest study fields in 2020 were located in Caldwell, Warren and Simpson County, Kentucky. Preharvest study fields in 2021 were located in Caldwell, Logan, Warren and Simpson County. The UKREC field, located in Caldwell County, was used in both the preharvest and harvest studies in both 2020 and 2021. Overall, eight fields in 5 counties over 2 years were used in the preharvest study, and two fields at the UKREC over 2 years were included in the harvest study (Table 3.1).

Preharvest Seed Retention and Seed Rain

Preharvest samples were collected from a few days prior to the harvest up to the same day as harvest of the selected field. Samples were collected from one m^2 area for each 0.2 ha of Italian ryegrass infestations. Each sampled area was selected randomly within the Italian ryegrass infested sections of each field. All of the Italian ryegrass seed heads within each sampled area were clipped, counted, and collected as the seed retained on the seed head sample. As soon as the seedheads were clipped, they were placed into a bag to prevent any seeds from falling to the ground. These are the seeds that would possibly enter the combine at harvest. Once all seed heads from within each sampled area had been collected, a battery powered vacuum was used to collect any debris on the soil surface within the m^2 area. Any seeds collected from the ground are the seeds that are lost prior to wheat harvest and would not enter the combine at harvest. The debris collected in the vacuum was deposited into a separate paper bag as the seed rain or seed not retained on seed head sample. All the samples were transported to the UKREC lab for further cleaning and processing as described below.

Harvest Seed Dispersal

Italian ryegrass's seed dispersal at harvest was studied at the time of Kentucky's soft red winter wheat harvest at the UKREC site in both 2020 and 2021. A John Deere 9650 combine with a 7.62 m head was used for the seed dispersal study. In 2020 the wheat was harvested on June 26th at 16 percent moisture and in 2021 the wheat was harvested on June 25th at 14.5 percent moisture. There was an average of 125 seed heads m^{-2} in 2020 while in 2021 there was only an average of 80 seed heads per m^2. The harvest settings on the combine were not adjusted from the settings used for commercial wheat harvest at the UKREC. In 2020 the combine had a fan speed of 1280 rpm, a cylinder speed of 830 rpm, and a concave setting of 1. In 2021, the fan speed was 1050 rpm, the cylinder speed was 850 rpm, and the concave setting again was 1.

The fields selected were divided into four 0.4-ha blocks. Samples were collected to observe Italian ryegrass seed shattering at the combine head, seed within the chaff exiting the combine, and seed being deposited in the grain tank with the wheat grain. Within each 0.4 ha block, four head shatter samples, four chaff samples, and one grain tank sample were collected. The head shatter and chaff collection samples were taken randomly within the block.

Head shatter samples were collected using 24 trays, each measuring 25 cm wide by 17 cm long with a depth of 2.5 cm. The 24 trays were placed within the unharvested wheat row perpendicular to the combines harvest path. The combine was allowed to start harvesting and come to full harvesting speed prior to approaching the head shatter trays. The combine head was allowed to pass over the trays and stopped once the head had cleared the tray, prior to running over the trays with the combine drive tires. The combine was then reversed allowing for exposure of the trays for collection. Contents within each of the 24 trays were carefully collected and placed in a paper bag as the head shatter collection.

Chaff collection samples were collected using four trays, each measuring 66 cm long by 46 cm wide with a depth of 2.5 cm. The combine was allowed to start harvesting and come to full harvesting speed prior to approaching where the chaff collection would take place. The chaff spreader was on and operating during the collection of chaff samples. Once the header was past, the trays were laid down to collect any chaff exiting the combine. The combine continued harvesting until no more chaff was being deposited into the trays. The combine then stopped allowing the chaff samples to be collected. All contents of all four trays were combined within a paper bag as the chaff collection sample.

Samples from the grain tanks were taken with a chambered grain probe. The sample was collected after an entire block was harvested. The chambered grain probe was lowered into the grain tank of the combine until four chambers were submerged in the wheat and a sample was pulled. This was done twice for each block to achieve a grain tank sample. After the grain tank sample was collected, the grain tank was emptied.

Sample Processing and Cleaning

All samples, whether preharvest or at harvest samples, were cleaned using similar methods. The seed head retention samples were hand threshed, then cleaned using an air column blower (Oregon Seed Blower) to remove any fine debris and chaff from the ryegrass seed. The soil surface samples, head shatter samples, and chaff collection samples were sieved to remove large sized debris. The samples were then further cleaned of fine debris using the air column seed blower. The grain tank samples were processed in the air column seed blower to separate the wheat from the Italian ryegrass seed. Samples were in the air column for 2 minutes to separate chaff and non-viable seed from seed with a viable embryo as non-viable seed is lighter than seeds with a viable embryo. Following the cleaning of all samples, 10 samples from each category and site were randomly selected. A subsample of 0.5 grams was taken from each of the 10 selected samples and all the Italian ryegrass seed in the subsample counted. All samples were then weighed and the counts from the subsamples used to create an estimate of the number of seed per sample.

The grain tank samples were converted to the number of Italian ryegrass seeds per m^2 so that all samples could be compared. The number of Italian ryegrass seeds per 0.5 g subsample from each grain tank was averaged to estimate the number of Italian ryegrass seeds within each grain tank sample. The number of Italian ryegrass seeds per gram of wheat grain was determined using the known weight of each of the grain samples. The total weight of the wheat grain in the grain tank was calculated using the wheat yield of the field and the test weight (Table 3.2). The number of Italian ryegrass seeds per gram of wheat in the sample was multiped by the wheat grain weight (g) in the grain tank to determine the number of Italian ryegrass seeds per acre using the known area of each harvested block. The number of Italian ryegrass seeds was then converted from the number of Italian ryegrass seeds per acre to the number of Italian ryegrass seeds per m^2.

Data Analysis

The Italian ryegrass preharvest seed retention and harvest seed distribution data were checked for normality and equal variances prior to analysis using the Conditional Pearson Residuals. Once all assumptions were met, the analysis for both the preharvest and harvest seed distribution were evaluated using SAS 9.4 (SAS Institute Inc.) PROC GLIMMIX for analysis of variance. The fixed effects in the preharvest study were where the Italian ryegrass seed was prior to wheat harvest and in the harvest study where the Italian ryegrass seed ends up at wheat harvest. The random affects were the replicates in both studies. The preharvest seed retention data were pooled across sites and year as there were no interactions between sample types and year. The harvest seed distribution had an interaction between sample types and year, so the 2020 data and 2021 analysis were conducted separately. The means for the preharvest seed retention and harvest seed distribution were separated using Tukey’s HSD with alpha at 0.05.

Results and Discussion

Preharvest Seed Retention and Seed Rain

Significantly more seed was retained on the seed head as compared to the amount of seed found on the soil surface ($P<0.0001$) (Figure 3.1). The preharvest samples showed that 11,506 Italian ryegrass seeds m^{-2} remained on the seed heads prior to wheat harvest as compared to 1,481 Italian ryegrass seeds m^{-2} found in the soil surface. This study conducted across eight site years indicates that Italian ryegrass will retain 89 percent of its seeds up to the time of soft red winter wheat harvest. This is similar to what was found for rigid ryegrass in Australia (Walsh and Powles, 2007). However, in a cooler climate like the Pacific Northwest, Italian ryegrass shed more than 50 percent of its seed prior to harvest (San Martín et al., 2021). This likely indicates that Italian ryegrass seed retention is dependent on location and environment. Harvest weed seed control could be a viable option, for Italian ryegrass control in Kentucky as 89 percent of the seed was retained on the seed head at wheat harvest.

Harvest Seed Dispersal

Italian ryegrass seed distribution during the 2020 wheat harvest was 7100 seeds per m^2 within the chaff, 4992 seeds per m^2 within the grain tank, and 4461 seeds per m^2 shattered at the combine header (Figure 3.2). The Italian ryegrass seed distribution in 2021 was 771 seeds per m^2 passed through the combine and exited with the chaff, 761 seeds per m^2 in the grain tank, and 64 seeds per m^2 lost at the combine header. In 2020 Italian ryegrass seed distribution among the three collection areas at harvest was not significantly different ($p=0.1965$). Conversely, in 2021 differences in Italian ryegrass seed distribution between three collection points was found ($p=0.0398$). The amount of Italian ryegrass seed that shattered at the combine header was significantly lower as compared to amount of seed found in the chaff collection and the grain tank in 2021 (Figure 3.2).

Because the potential efficacy of harvest weed seed control was being evaluated, the Italian ryegrass seed entering into the combine versus seed shattering at the head or being deposited bank into the soil seed bank 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. When comparing the amount of seed shattered at the head to the seed entering the combine, differences between the two proportions were found in both 2020 and 2021 ($P<0.0001$) (Figure 3.3). In 2020, 4461 Italian ryegrass seeds per m^2 shattered at the header and was significantly less than the 12,092 Italian ryegrass seeds per m^2 that entered the combine. In 2021, 64 Italian ryegrass seeds per m^2 shattered at the header and was significantly less as compared to the 1532 Italian ryegrass seeds per m^2 that entered the combine. 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 results support the concept that harvest weed seed control may be a viable option for Italian ryegrass in Kentucky wheat.

The two site years for the harvest study had different Italian ryegrass infestations levels, which resulted in differences found between years. This is also likely why the amount of seed shatter at the combine header differed between years. The 64 Italian ryegrass seeds per m^2 lost at the combine header in 2021, is likely an acceptable loss by farmers, whereas the 4461 Italian ryegrass seeds per m^2 observed in 2020 would not be considered acceptable by farmers.

Summary

The preharvest data along with the combine distribution data during harvest suggest the possible use of harvest weed seed control methods in Kentucky wheat fields could increase control of Italian ryegrass infestations. The majority of Italian ryegrass seed was retained on the seed head up to the time of harvest. During harvest, most of the seed that was retained on the plant entered the combine where it could be destroyed or removed using a harvest weed seed control method. In this study the combine's settings were not changed from the settings used for commercial wheat harvest at the UKREC, although there could be minor adjustments in combine setup that might divert some of the seed shatter proportion to the proportion entering the combine. The combine settings could also be adjusted to relocate some of the Italian ryegrass seed from the grain tank by exiting the combine in the chaff where it could exit through a seed destructor. Further evaluation of the efficiency of harvest weed control methods in Kentucky wheat fields should be conducted to determine which harvest weed seed control methods might be an effective tool for weed management. The observations from this study confirm that most of the Italian ryegrass seed will be on the seed heads at harvest and most of that seed will enter the farmer's combine.

The use of harvest weed seed control will not be the only method of control a producer will need to effectively manage Italian ryegrass in winter wheat. Although the results show that Kentucky Italian ryegrass is a good candidate for harvest weed seed control practices, it must be combined with additional weed management tools. The use of effective herbicides and crop rotation in addition to harvest weed seed control is a more ideal strategy to keep infestations lower at the time of harvest. These other weed control strategies will help reduce the number of plants maturing to produce seed in the field and reduce the pressure on the harvest weed seed control to manage the targeted weed species.

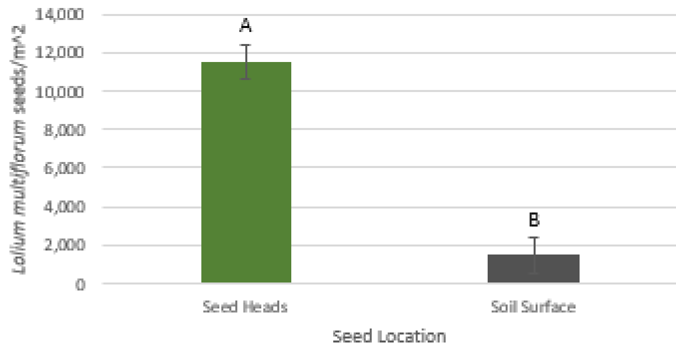
Table 3.1. Ryegrass seed retention collection parameters for 2020 and 2021 sites.

Location (GPS Coordinates)	Size of Field (ha)	Number of Samples	Year of Study	Date Collected	Italian Ryegrass (Seedheads/m^2)
Young Road (36.45, -86. 41)	4.9	24	2020	6/15/20 and 6/20/20	78
UKREC (37.10, -87.83)	1.6	16	2020	6/24/20 to 6/25/20	125
Warren County (36.89, -86.55)	12.1	20	2020	6/18/20	91
Russellville-Gallatin Road (36.41, -86.39)	12.1	16	2020	6/19/20	104
Armstead Road (36.68, -86.76)	2.8	14	2021	6/15/21	67
Sulphur Springs Church Road (36.70, -86.64)	4.0	20	2021	6/15/21 and 6/17/21	40
Dillard Road (36.54, -86.28)	4.5	22	2021	6/16/21	128
UKREC (37.10, -87.86)	1.6	16	2021	6/21/21	80

Table 3.2. The test weights and yields of the wheat in the harvest studies in 2020 and 2021 at the UKREC.

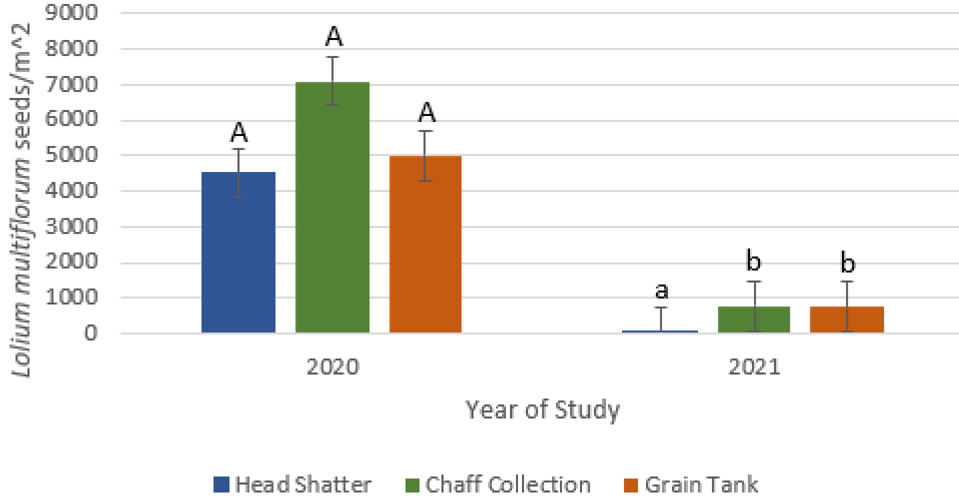
	2020	2021
Test Weight (lb/bu)	49	57
Yield (bu)	65	62

Figure 3.1. Italian ryegrass seed distribution prior to Kentucky's soft red winter wheat harvest.



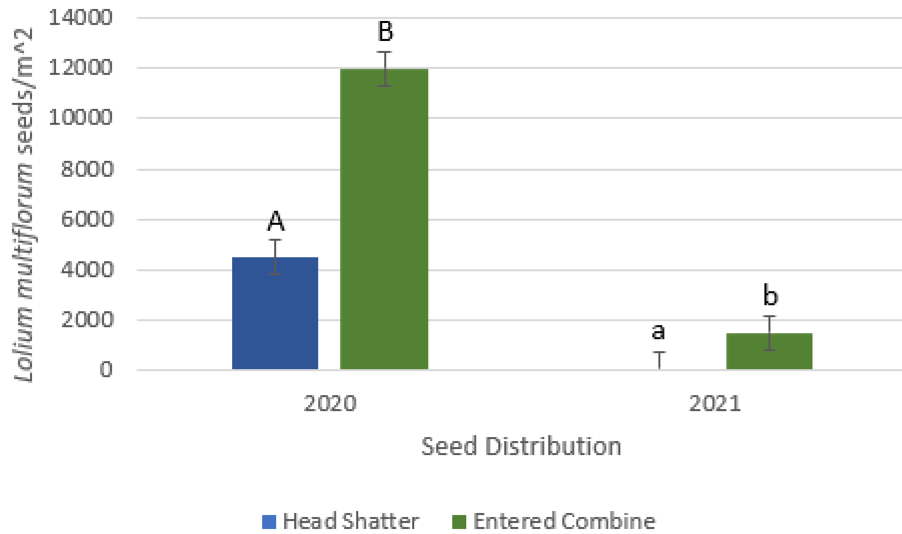
*Means with a different letter are significantly different. Tukey HSD a=0.05

Figure 3.2. The Italian ryegrass seed distribution at harvest of soft red winter wheat in Kentucky.



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

Figure 3.3. Distribution of the Italian ryegrass seed at harvest comparing head shatter versus ryegrass seed entering the combine.



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

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