ABSTRACT

TAYLOR, ZACHARY RYAN. Distribution and Control of Herbicide Resistant Italian Ryegrass [*Lolium perenne L. ssp. multiflorum (Lam.) Husnot*] in Winter Wheat (*Triticum aestivum* L.) in North Carolina. (Under the direction of Dr. Wesley J. Everman).

Italian Ryegrass is consistently ranked as one of the most problematic weeds of winter wheat in the Southeastern United States. While resistance to herbicides used in winter wheat in North Carolina has been confirmed, a systematic survey to determine the extent of this problem has not been taken. By sampling Italian ryegrass seed collected from locations throughout the wheat producing regions of North Carolina, and screening those samples with the four postemergence herbicides currently available, resistant populations could be identified. Herbicides screened were: diclofop, pinoxaden, mesosulfuron, and pyroxsulam. Results of this research allow for the documentation of the extent and type of resistance present in North Carolina so that appropriate measures can be taken to control this problematic weed. Results show evidence of resistance to diclofop at all locations sampled throughout the state. Resistance to either mesosulfuron or pyroxsulam is less common but still found in eleven to nineteen percent of sampled locations. Italian ryegrass resistance to pinoxaden is currently limited, but was found in about five percent of sampled locations. Italian ryegrass in this area will be difficult to control however, as a few samples were resistant to all other post herbicides screened.

As herbicide resistant populations continue to become more widespread, control with chemical options alone will become more difficult. Reduced row spacing has been shown to give some crops a competitive advantage over many weeds, which can help to increase weed control. Adjusting row spacing may be able to give winter wheat a similar competitive advantage over Italian ryegrass seedlings. While this advantage alone may not give adequate

control, in conjunction with a sound herbicide program, row spacing may allow for the increased control of Italian ryegrass. This research looked at wheat planted in both systems. At the location where research was conducted, there were observable reductions in Italian ryegrass pressure in a narrow row planting system when compared to conventional row spacing over two years. While reductions were limited, this research does show the possibility of row spacing as a tool which could be further investigated for the reduction of Italian ryegrass densities over time.

Another way to combat herbicide resistance is through the use of preemergence herbicides. Pyroxasulfone is a preemergence product that has shown been shown to be effective at controlling Italian ryegrass. The effectiveness was compared to other products and how pyroxasulfone can be used as part of a complete herbicide program. When a preemergence application of pyroxasulfone is followed by a postemergence application of pinoxaden when resistance is not present, excellent control of Italian ryegrass can be achieved in North Carolina. Other programs may offer adequate control at a lower cost to growers, and should be considered depending on the density of Italian Ryegrass infestation.

Grain yield reductions correlated with increased Italian ryegrass populations in both studies, again stressing the importance effective Italian Ryegrass control. Cultural practices, such as row spacing, and the incorporation of preemergence herbicide programs will be increasingly necessary to produce sustainable wheat yields in North Carolina with the spread of herbicide resistant Italian Ryegrass. Both of these options will also have to be considered as we look for ways to slow the spread of herbicide resistance.

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

by
Zachary Ryan Taylor

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APPROVED BY:

Dr. Wesley J. Everman
Committee Chair

Dr. David Jordan

Dr. Randy Weisz

DEDICATION

I am dedicating this to my wife, family, and friends whose support has made this possible.

BIOGRAPHY

Zack is a native of North Carolina, and has roots in North Carolina agriculture. After moving to Stokes County from Northampton at a young age, Zack attended R.J. Reynolds High School in Winston-Salem, North Carolina. It was there that Zack's interest in pursuit of an agricultural career grew as he became an active member of FFA. After high school, he continued his education at North Carolina State University, receiving a Bachelor's of Science in Agronomy in 2011. Zack returned to school and began graduate studies in research in the fall of 2012, in order to advance into an agricultural career. Zack and his wife, Rachel, currently live in Sanford, North Carolina.

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Literature Review

Herbicide resistance is defined as "the evolved capacity of a previously herbicide-susceptible weed population to withstand a herbicide and complete its life cycle when the herbicide is used at its normal rate in an agricultural situation" (Heap and LeBaron 2001). Worldwide there are four hundred and forty-seven cases of species by site specific herbicide resistance. This includes two hundred and forty-four individual weed species, of which one hundred and forty-two are dicots and the remaining one hundred and two are monocots (Heap 2015). Herbicide resistance occurs naturally in a small number of individuals within a weed population. While sometimes the mutation responsible for naturally occurring resistance can be lethal, there is usually no fitness penalty for herbicide resistance mutations (Powles et al. 1996). When a herbicide with a single mode of action is used repeatedly, those individuals which are resistant are allowed to complete their life cycle and produce seed, leading to a larger resistant population in following growing seasons. This cycle is called selection pressure (Powles et al. 1996).

Selection pressure is higher in some crops than others, due to limited options for different site of action herbicides within that crop. Options for weed control in cereal crops, such as winter wheat (*Triticum aestivum* L.), are generally restricted to three herbicide site of action groups; inhibitors of acetyl CoA carboxylase (ACCase), inhibitors of acetolactate synthase (ALS), and inhibitors of photosynthesis at photosystem II (Powles et al. 1996). Italian ryegrass [*Lolium perenne L. ssp. multiflorum (Lam.) Husnot*] is a common weed of winter wheat, particularly throughout the Southern United States (Webster 2012). Effective chemical control in wheat systems has been shown to be limited to the same three site of action groups (Grey and Bridges 2003; Grey et al. 2012; Hoskins et al. 2005).

Early efforts to quantify the amount of crop interference of Italian ryegrass in wheat were done in 1970-1972. Studies conducted in Oregon showed yield losses of up to sixty percent that were attributable to increased densities of Italian ryegrass in winter wheat over the course of two years. These studies also investigated the effect of Italian ryegrass competition on wheat yield reduction by variety, however decreased yields were observed as Italian ryegrass populations increased, regardless of wheat variety (Appleby et al. 1976). Further efforts were made to quantify yield losses associated with interference from Italian ryegrass from 1981-1983 in North Carolina Liebl and Worsham (1987). They reported that for every 10 Italian ryegrass plants per meter square, wheat yield was decreased by an average of 4.2%. Liebl and Worsham (1987) also attempted to determine causes of the interference of Italian ryegrass on wheat yields. Growth rate and response to nutrients were both greater for Italian ryegrass than for winter wheat. Nitrate and potassium, both critical elements for plant growth, were taken up more rapidly from a nutrient solution in a greenhouse setting by Italian ryegrass than by wheat. It was concluded that direct competition for nutrients led to decreased tillering and yields in winter wheat when Italian ryegrass is present (Liebl and Worsham 1987).

Given the potential for yield loss due to interference by Italian ryegrass, effective control is critical for profitable yields. The herbicide diclofop was registered in the United States in 1982. It was introduced to control wild oats (*Avena fatua* L.) and annual grasses in wheat and barley cropping systems (US EPA 2000). Studies were conducted from 1980 to 1982 using diclofop alone as a postemergence herbicide to control Italian ryegrass in Arkansas; results showed control rates between 95 and 100 percent (Khodayari et al. 1983). Little to no crop injury was observed following diclofop application to the wheat crop, establishing diclofop as a good option for selective control on Italian ryegrass in winter wheat (Khodayari et al. 1983). Studies in the Great Plains in 1994 again concluded that the best option to control Italian Ryegrass was diclofop (Justice et al. 1994). Problems with the overuse of diclofop could, however, be anticipated. Diclofop was introduced in 1977 in Australia. After just four generations of exposure, populations of the ryegrass species, Rigid Ryegrass (*Lolium rigidum Gaudin*) were identified to be resistant in 1981. Researchers attributed the development of resistance to the repeated use of diclofop alone (Heap and Knight 1982). In 1987, Italian Ryegrass populations resistant to diclofop were first discovered in the United States in growers' fields in Oregon (Stanger and Appleby 1989). Since confirmation of resistance in Oregon, diclofop resistant Italian ryegrass has been confirmed in 9 other states; North Carolina, South Carolina, Idaho, Virginia, Georgia, Arkansas, Maryland, Kentucky, and Tennessee. Resistant populations of Italian ryegrass to diclofop have also been reported in five other countries; The United Kingdom, France, Italy, Chile, and Argentina (Heap 2015).

Before the introduction of diclofop, control of Italian ryegrass was difficult in wheat systems, due to similarities between the species, with both wheat and Italian ryegrass being grasses (Khodayari et al. 1983). Early studies by Appleby et al. (1976) investigating the interference of Italian ryegrass in wheat used diuron, an inhibitor of photosynthesis at photosystem two, as a preemergence herbicide to control Italian ryegrass. When resistance to diclofop was identified in Oregon in 1987, alternative products were considered to be preemergence applications of triallate, an inhibitor of lipid synthesis, plus diuron or triallate plus metribuzin, another inhibitor of photosynthesis at photosystem two. Barban is a carbamate herbicide with a mode of action which inhibits mitosis, which was identified as an alternative postemergence product; however control with barban was not as effective as control with diclofop in sensitive populations (Stanger and Appleby 1989). Since then, other ACCase POST products have been released, but it has been documented that there is the potential for cross resistance to occur between Italian ryegrass populations susceptible to these new products and the Italian ryegrass populations known to be resistant to diclofop (Bailey and Wilson 2003). Therefore, these products also have potential to become ineffective for the postemergence control of Italian ryegrass (Bailey and Wilson 2003).

According to the 2015 North Carolina Agricultural Chemical Manual, Everman (2015) indicated that only four postemergence applied herbicides are currently available for the control of Italian ryegrass in winter wheat, the ACCase inhibitors diclofop and pinoxaden, and the ALS inhibitors mesosulfuron and pyroxsulam. As mentioned previously, diclofop resistant populations of Italian ryegrass have been reported in North Carolina. The other ACCase inhibitor available, pinoxaden, was first registered in 2005 (US EPA 2005). While still an ACCase inhibitor, pinoxaden brought a new chemistry within the ACCase inhibiting herbicide group to the table. Pinoxaden falls into the phenylpyrazolin class of chemicals, where diclofop fell into the group commonly called the AOPPs, short for aryloxyphenoxyproionate. Due to this different chemistry classification, it was possible for pinoxaden to control ryegrass that was resistant to diclofop and other AOPP chemistry ACCase inhibiting herbicides. Testing for resistance levels showed that pinoxaden did in fact control many more accessions of ryegrass sampled in Arkansas and Louisiana from 1998-2005 than diclofop. However, the dose required to control Italian ryegrass with pinoxaden did show a wide amount of variability. Several accessions required significantly higher doses for effective control than the susceptible standard biotype, leading some accession to be considered resistant. This raised concerns for current levels of resistance to the new product and the likelihood for further resistance to develop (In Kuk et al. 2008). After this confirmation of resistance in Arkansas, resistant populations were also confirmed in North Carolina from seeds sampled in 2007 (Chandi et al. 2011). Currently, Italian ryegrass populations with confirmed resistance to pinoxaden have been found in Arkansas, North Carolina, and Missouri. Resistant populations have also been confirmed in Chile and Italy (Heap 2015).

The fact that the only effective POST alternatives to ACCase inhibitors are the ALS inhibitors causes some concern. Acetolactate Synthase (ALS) inhibiting herbicides were first introduced to the market in 1982 (Tranel and Wright 2002). Since their introduction, ALS inhibitors have become the most commonly used group of herbicides in the world. They also account for the most species of herbicide resistant weeds of any group of herbicides, at almost one-third of all herbicide resistant species being resistant to ALS inhibiting herbicides (Heap 2014). The first reported case of an ALS inhibitor resistant weed was reported in 1986, shortly after the release of ALS inhibiting herbicides (Heap 2014). This resistance was found in Australia, again in rigid ryegrass. While screening diclofop resistant ryegrass samples for cross-resistance to similar herbicides, researchers included two unrelated sulfonylurea-type ALS inhibiting herbicides, chlorsulfuron and metsulfuron. Samples were found to be resistant to both of the ALS inhibitors, as well as diclofop and two of the other three ACCase inhibitors tested (Heap and Knight 1986). The first case of ALS inhibitor resistance in the United States was confirmed in 1990 in Idaho, where a population of prickly lettuce (*Lactuca serriola L.)* was found to be resistant to multiple sulfonylurea-type ALS inhibiting herbicides (Mallory-Smith et al. 1990). In 1996, the first case of ALS inhibitor resistant Italian ryegrass was confirmed in the United States in the state of Mississippi. Sulfometuron was commonly used to control Italian ryegrass on roadsides. After annual applications of sulfometuron alone, it was noticed that Italian ryegrass populations on roadside right-of-ways were increasing. Testing confirmed the suspected resistance (Taylor and Coats 1996).

Although the potential for resistance development was known, ALS inhibiting products began to gain more interest in the early 2000s as an option for postemergence Italian ryegrass control in winter wheat, due to the continuing increases in cases of ACCase inhibitor resistance (Bailey and Wilson 2003). The ALS inhibiting product chlorsulfuron had previously shown to be an effective PRE product, but POST applications did not give adequate control (Griffin 1986). An experimental ALS inhibiting herbicide with the active ingredient mesosulfuron was tested in Virginia in 2003. POST control with mesosulfuron was observed to be as good as control with diclofop in susceptible populations. Control was also better than POST applications of 2 other experimental ACCase inhibitors, as well as the ALS inhibitor sulfosulfuron, or ALS inhibitor chlorsulfuron when tank mixed with either metsulfuron or metribuzin (Bailey and Wilson 2003). Similar results have been observed in other studies comparing diclofop and mesosulfuron, confirming that mesosulfuron is a good alternative to diclofop (Grey et al. 2012). Mesosulfuron, in the sulfonylurea family of ALS inhibiting herbicides, gained registration in 2004 under the trade name Osprey (US EPA 2004). Shortly after the chemicals release, a study was done in Arkansas to investigate the patterns of resistance to diclofop, and also to investigate for multiple-resistance to mesosulfuron. The study was conducted with Italian ryegrass seed that had been collected from 1998-2004. There were 17 seed samples taken from different locations, sixteen in

Arkansas and one in Louisiana. Of these 17 samples, 14 were determined to be resistant to diclofop. All of the 14 samples resistant to diclofop were controlled by mesosulfuron. One sample that was found to be susceptible to diclofop exhibited observable resistance to mesosulfuron. Herbicide use history was provided to some extent for all of the samples tested, and this sample did not have a history of exposure to ALS inhibitors at the location for at least the past six years. This suggested that there were potentially naturally occurring populations of Italian ryegrass resistant to mesosulfuron, even at the time of the product registration (Kuk and Burgos 2007). Currently, mesosulfuron resistant Italian ryegrass has been confirmed in North Carolina, South Carolina, Georgia, Delaware, Missouri, and Kentucky, as well as the United Kingdom and Italy (Heap 2015).

Another ALS inhibiting herbicide that is available for ryegrass control in North Carolina is pyroxsulam. Pyroxsulam was registered under the trade name PowerFlex in 2008, and falls into the triazolopyrimidine chemical class of ALS inhibiting herbicides (US EPA 2008). The efficacy of pyroxsulam on rigid ryegrass was established in Australia in order to gain registration. Initial studies determined pyroxsulam controlled rigid ryegrass at levels comparable to mesosulfuron. The safety of the pyroxsulam was tested on winter wheat by testing fifty varieties (Wells 2008). Although visual injury was observed, it was considered to be at commercially acceptable levels, and comparable with injury by mesosulfuron (Wells 2008). Efficacy studies conducted in the United Kingdom using the pre-mix product Broadway Star, which contains pyroxsulam and florasulam (Jackson et al. 2011). Florasulam provides broadleaf weed control, so it is reasonable to believe that pyroxsulam is the component of Broadway Star providing control activity on Italian ryegrass (Anonymous 2014b). It was observed that Broadway Star provided Italian ryegrass control greater than ninety-five percent following both fall and spring applications, or through mid-tillering. This was estimated by visual control ratings observed from aerial photographs taken late in the growing season, in June (Jackson et al. 2011). Applications were also made at three different weed growth stages, BBCH11-14, 22-26, and 30-37. Significant decreases in control were observed as weed size increased, however efficacy as the largest growth stage remained over ninety percent (Jackson et al. 2011). Although proven effective, a study conducted in North Carolina to investigate Italian ryegrass resistance to ALS and ACCase herbicide revealed populations resistant to pyroxsulam in seeds collected in 2007. In this situation, the presence of resistance was observed in populations before the registration of the product, similarly to mesosulfuron. This again indicated that populations naturally resistant to ALS inhibitors exist (Chandi et al. 2011). In Arkansas, samples collected from fields with suspected resistance in eight states were tested for resistance to several products including pyroxsulam. Samples tested were collected from 2008 through 2011. Resistance to pyroxsulam was observed in eighty-one percent of the samples tested. Resistance was observed in samples collected from Arkansas, Georgia, Kansas, Mississippi, North Carolina, and South Carolina. The only states with samples tested where signs of resistance were not observed were Virginia and Washington (Salas et al. 2013).

Cross-resistance is defined as the resistance of a weed species to multiple herbicides within a mode of action (Kuk and Burgos 2007). Cross-resistance has been confirmed in Italian ryegrass to both ACCase and ALS inhibitors, as shown by studies such as the cross-resistance profile completed by Kuk and Burgos in 2007. Multiple-resistance is defined as resistance of one plant to multiple herbicides with different modes of action (Powles et al. 1996). Although not defined as multiple-resistance at the time of the study completion, multiple-resistance was found in rigid ryegrass to both ACCase and ALS inhibitors at the same time as ALS inhibitor resistance was confirmed in Australia (Heap and Knight 1986). One study looking at the resistance profiles of Italian ryegrass in the United States in 2013 showed that out of the forty-seven populations tested for resistance from multiple states, seventy-eight percent showed multiple-resistance to both ACCase and ALS inhibitors (Salas et al. 2013).

One way to prevent or delay resistance from developing has been to rotate herbicides with different modes of action in order to decrease selection pressure. This practice is known to work well when practiced proactively, however many growers do not implement resistance management practices until resistance has already developed (Beckie 2006). The practice is still encouraged for resistance management of Italian ryegrass in wheat where resistance has not developed. Many areas have developed resistance and, where cross-resistance has developed, the availability of only one effective postemergence mode of action puts pressure on developing multiple-resistance. In areas where multiple-resistance has already developed, postemergence control options are limited or non-existent (Ellis et al. 2010).

Since postemergence products for Italian ryegrass control are known to be limited, preemergence products are generally recommended to help control the weed prior to emergence in winter wheat. One product that is available is a premix product containing metribuzin and flufenacet (Koepke-Hill et al. 2011). Metribuzin is a photosystem II inhibitor that has shown both PRE and POST activity on Italian ryegrass (US EPA 1998b). Studies did however find that some wheat varieties are sensitive to this product, and that proper timing is important to prevent injury to wheat (Shaw and Wesley 1991). Flufenacet is an inhibitor of very-long-chain fatty acid biosynthesis and has been shown to control some grass species (US EPA 1998a). The premix of these products was found to control Italian ryegrass; however the level of control was highly variable (Koepke-Hill et al. 2011). It has been hypothesized that control variations were a result different biotypes, soil types, and variations in rainfall amounts and timing after application (Koepke-Hill et al. 2011). The premix of metribuzin plus flufenacet is labelled in North Carolina to control Italian ryegrass in winter wheat, but does have a short application window, from germination up to two-leaf (Anonymous 2010). Another product available after germination is pendimethalin, which is a dinitroanaline herbicide, inhibiting cell division and root growth in susceptible species. Pendimethalin is labeled for application after first leaf in wheat to suppress Italian ryegrass, but does not have postemergence activity (Anonymous 2012b). While pendimethalin is labeled, it is not recommended for control of Italian ryegrass in winter wheat by the North Carolina Agricultural Chemical Manual (Everman 2015). This may be due to previous research that has shown that pendimethalin alone does not provide a level of Italian ryegrass control through the growing season that is considered commercially acceptable (Bond et al. 2014).

Another herbicide option listed in the North Carolina Agricultural Chemical Manual is pyroxasulfone (Everman 2015). While pyroxasulfone has the same mode of action as flufenacet, it offers a longer application window (Anonymous 2013). Pyroxasulfone has been shown to control Italian Ryegrass season-long and be an acceptable alternative to metribuzin plus flufenacet. Pyroxasulfone has also been shown to have low levels of wheat injury, which did not impact yields (Hulting et al. 2012). According to the label, applications can be made after at least eighty percent of wheat has germinated and has a shoot at least one half inch long and until wheat reaches four tillers (Anonymous 2013). Pyroxasulfone does not have postemergence activity on Italian ryegrass, so the label also encourages application as soon as possible, before ryegrass germinates.

Due to the limited chemical control options, it is reasonable to consider cultural practices as an option for managing Italian ryegrass. Ryegrass is known to be competitive with wheat (Liebl and Worsham 1987) but some cultural practices may be able to give wheat a competitive advantage. One cultural practice to be considered is the seeding arrangement, or row spacing. It has been shown in many crops that reducing row spacing from common commercial practices can help to increase weed suppression (Rogers et al. 1976; Walker and Buchanan 1982; Yelverton and Coble 1991). While initial research showed that increased wheat crop densities could reduce weed biomass, the same was not observed for narrow row spacing alone (Champion et al. 1998). European research investigating the impact of row spacing on weed competition observed a reduction in weed biomass due to reduced row spacing (Olsen et al. 2005; Weiner et al. 2001). Research conducted in spring planted wheat has followed a similar trend to reduce weed density by changing planting patterns. However, instead of reducing row spacing, spring wheat was planted in a grid pattern. On average, weed biomass was reduced by 30% compared to planting in standard rows (Weiner et al. 2001). While the grid pattern was investigated in two separate studies, neither study reported results with specific weed species. The majority of weeds reported to be present in the field were broadleaves, and the studies did not report the presence of Italian ryegrass (Olsen et al. 2005; Weiner et al. 2001). Another study conducted on spring wheat in Europe did investigate the effects of row spacing on the suppression different weed species, including Italian ryegrass (Olsen et al. 2006). When low or medium densities of Italian ryegrass were present, a significant effect of suppression by row spacing was not observed. When high densities of Italian ryegrass were present however, row spacing did have a significant effect on suppression in one of two years (Olsen et al. 2006).

Few studies have been conducted in the United States investigating narrow row spacing in winter wheat as a tool for weed suppression. Two studies conducted in Oregon did focus specifically on the suppression of Italian ryegrass. Appleby and Brewster (1992), reported inconsistent results when investigating planting patterns alone, only reporting differences when Italian ryegrass were high. Therefore, it was concluded that overcoming yield losses due to Italian ryegrass by changing planting patterns alone was not feasible (Appleby and Brewster 1992). The other study showed evidence for better Italian ryegrass suppression in wide row winter wheat. Here is was observed that higher planting densities within rows when planted on conventional rows had a greater effect on Italian ryegrass suppression then narrow row wheat planted at the same population (Hashem et al. 1998). Given the inconsistency of these results combined with a lack of herbicide programs integrated into studies investigating cultural practices, results in the need for further and more current research into the possibility of narrow row spacing as a tool for managing Italian ryegrass in winter wheat.

Another cultural practice that can be considered is the tillage system used. Tillage has been shown to cause a shift in the dynamics of weed populations, specifically, studies have shown that no-till systems can cause an increase in grass weed populations and shift towards more perennial weeds (Froud-Williams et al. 1981; Sans et al. 2011; Trichard et al. 2013). As no-till gained popularity, one concern arose in Oregon, where it was determined that no-till systems required more intensive herbicide programs in order to achieve the same amount of weed control as a conventional system (Young et al. 1994). One possible reason for this is the effectiveness of PRE herbicides. It has been reported that PRE herbicides are less effective in no-till systems due to higher residue levels. One such study in Australia observed lower levels of rigid ryegrass control with a preemergence herbicide in no-till systems (Borger et al. 2013). Upon further investigation, it was observed that this reduction in control with a preemergence in a no-till system could be overcome by using higher spray carrier volumes in order to achieve better soil coverage (Borger et al. 2013).

One theory has shown that tillage can have an effect on Italian ryegrass control specifically. A study was conducted in Japan comparing conventional tillage to reduced tillage in two different fields. One field had been historically managed with reduced tillage while the other had been historically managed with conventional tillage. In the historically reduced tillage field, the use of conventional tillage led to a large reduction in the biomass of Italian ryegrass compared to continuing reduced tillage. The same effect was seen when the historically conventional tillage field was suddenly managed with reduced tillage. This suggested a field's dominant weed species could be adapting to the tillage system that is being repeatedly used. While not conclusive, this evidence does suggests that changing tillage systems could help to suppress the growth of the dominant weed species (Nakamoto et al. 2006).

It has become evident that Italian ryegrass is a problem for wheat producers worldwide. In order to assess this problem it is important to first understand what control options are available. While it is known that there is resistance to postemergence herbicide products in North Carolina, it is not clear how wide spread the problem is. Understanding this can help growers make important management decisions depending on what products are known to be effective in their area. With reduced postemergence control options, it is also important to think about new approaches to weed control, which may extend the effectiveness of current chemistries and give farmers new options to consider. Understanding the effectiveness of cultural practices can be crucial. If a cultural practice can suppress Italian ryegrass densities, or increase the effectiveness of a herbicide program, then that practice can become a valuable recommendation to wheat producers in helping to reduce weed seed banks and increase yields.

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Distribution of Herbicide Resistant Italian Ryegrass [*Lolium perenne L. ssp. multiflorum (Lam.) Husnot*] in North Carolina

Italian Ryegrass is consistently ranked as one of the most problematic weeds of winter wheat (*Triticum aestivum L.)* in the southeastern United States. While resistance to herbicides used in winter wheat in North Carolina has been confirmed, a systematic survey to determine the extent of this problem has not been taken. By sampling Italian ryegrass seed collected from locations throughout the wheat producing regions of North Carolina, and screening those samples with the four postemergence herbicides currently available, resistant populations can be identified. Herbicides screened were: diclofop, pinoxaden, mesosulfuron, and pyroxsulam. Results show evidence of resistance to diclofop at all locations sampled throughout the state. Resistance to either mesosulfuron or pyroxsulam was found in eleven to nineteen percent of sampled locations. Italian ryegrass resistance to pinoxaden was confirmed in five percent of sampled locations. Italian ryegrass in four locations was found to be resistant to all screened herbicides.

Introduction

Herbicide resistance is defined as "the evolved capacity of a previously herbicide-susceptible weed population to withstand a herbicide and complete its life cycle when the herbicide is used at its normal rate in an agricultural situation" (Heap and LeBaron 2001). Worldwide there are four hundred and forty-seven cases of species by site specific herbicide resistance. This includes two hundred and forty-four individual weed species, of which one hundred and forty-two are dicots and the remaining one hundred and two are monocots (Heap 2015). Herbicide resistance occurs naturally in a small number of individuals within a weed population. While sometimes the mutation responsible for naturally occurring resistance can be lethal, there is usually no fitness penalty for herbicide resistance mutations (Powles et al. 1996). When a herbicide with a single mode of action is used repeatedly, those individuals which are resistant are allowed to complete their life cycle and produce seed, leading to a larger resistant population in following growing seasons. This cycle is called selection pressure (Powles et al. 1996).

Selection pressure is higher in some crops than others, often due to limited options for different site of action herbicides within that crop. Options for weed control in cereal crops, such as winter wheat are generally restricted to three herbicide site of action groups; Inhibitors of acetyl CoA carboxylase (ACCase), Inhibitors of acetolactate synthase (ALS), and inhibitors of photosynthesis at photosystem II (Powles et al. 1996). Italian ryegrass is a common weed of winter wheat, particularly throughout the Southern United States (Webster 2012). Efforts to quantify the amount of crop interference of Italian ryegrass in wheat have shown yield losses of up to sixty percent with increased densities of Italian ryegrass in winter wheat (Appleby et al. 1976). Further efforts were made to quantify yield losses associated with interference from Italian ryegrass from 1981-1983 in North Carolina where it was reported that for every 10 Italian ryegrass plants per m^2, wheat yield was decreased by an average of 4.2% (Liebl and Worsham 1987). Everman (2015) reported that the only postemergence applied herbicides currently available in North Carolina for the control of Italian ryegrass in winter wheat are the ACCase inhibitors diclofop and pinoxaden, and the ALS inhibitors mesosulfuron and pyroxsulam.

The herbicide diclofop was registered in the United States in 1982. It was introduced to control wild oats (*Avena fatua* L.) and annual grasses in wheat and barley cropping systems (US EPA 2000). Studies were conducted from 1980 to 1982 using diclofop alone as a postemergence product to control Italian ryegrass in Arkansas; results showed control rates between 95 and 100 percent (Khodayari et al. 1983). Little to no crop injury was observed following diclofop application to the wheat crop, establishing diclofop as a good option for selective control on Italian ryegrass in winter wheat (Khodayari et al. 1983). Problems with the overuse of diclofop could, however, be anticipated. Diclofop was introduced in 1977 in Australia. After four generations of exposure, populations of a ryegrass species, Rigid ryegrass (*Lolium rigidum Gaudin*) were identified to be resistant in 1981. Researchers attributed the development of resistance to the repeated use of diclofop alone (Heap and Knight 1982). In 1987, Italian ryegrass populations resistant to diclofop were first discovered in the United States in growers' fields in Oregon (Stanger and Appleby 1989). Since confirmation of resistance in Oregon, diclofop resistant Italian ryegrass has been confirmed in 9 other states; North Carolina, South Carolina, Idaho, Virginia, Georgia, Arkansas, Maryland, Kentucky, and Tennessee. Resistant populations of Italian ryegrass to diclofop have also been reported in five other countries; The United Kingdom, France, Italy, Chile, and Argentina (Heap 2015).

The other ACCase inhibitor available, pinoxaden, was first registered in 2005 (US EPA 2005). Testing for resistance levels showed that pinoxaden did control many more accessions of ryegrass sampled in Arkansas and Louisiana from 1998-2005 than diclofop. However, the dose required to control Italian ryegrass with pinoxaden did show a wide amount of variability. Several accessions required significantly higher doses for effective control than the susceptible standard biotype, leading some accessions to be considered resistant. This raised concerns for current levels of resistance to the new product and the likelihood for further resistance to develop (In Kuk et al. 2008). After this confirmation of resistance in Arkansas, resistant populations were also confirmed in North Carolina from seeds sampled in 2007 (Chandi et al. 2011). Currently, Italian ryegrass populations with confirmed resistance to pinoxaden have been found in Arkansas, North Carolina, and Missouri. Resistant populations have also been confirmed in Chile and Italy (Heap 2015).

Acetolactate synthase inhibiting herbicides began to gain more interest in the early 2000s as an option for postemergence Italian ryegrass control in winter wheat, due to the continuing increases in cases of ACCase inhibitor resistance (Bailey and Wilson 2003). An experimental ALS inhibiting herbicide with the active ingredient mesosulfuron was tested in Virginia in 2003. POST control with mesosulfuron was shown to be as good as control with diclofop in susceptible populations (Bailey and Wilson 2003). Mesosulfuron, in the sulfonyl urea family of ALS inhibiting herbicides, gained registration in 2004 under the trade name Osprey (US EPA 2004). Shortly after the chemicals release, a study was done in Arkansas to investigate the patterns of resistance diclofop, and also to look for multiple-resistance to mesosulfuron. The study was conducted with Italian ryegrass seed that had been collected from 1998-2004. There were seventeen seed samples taken from different locations, sixteen in Arkansas and one in Louisiana. Of these 17 samples, fourteen were determined to be resistant to diclofop. All of the fourteen samples resistant to diclofop were controlled by mesosulfuron. One sample that was found to be susceptible to diclofop showed resistance to mesosulfuron. Herbicide use history was provided to some extent for all of the samples tested, and this sample did not have a history of exposure to ALS inhibitors at the location for at least the past six years. This suggested that there were potentially naturally occurring populations of Italian ryegrass resistant to mesosulfuron, even at the time of the product registration (Kuk and Burgos 2007). Currently, mesosulfuron resistant Italian ryegrass has been confirmed in North Carolina, South Carolina, Georgia, Delaware, Missouri, and Kentucky, as well as the United Kingdom and Italy (Heap 2015).

Another ALS inhibiting herbicide that is available for ryegrass control in North Carolina is pyroxsulam. Pyroxsulam was registered under the trade name PowerFlex in 2008, and falls into the triazolopyrimidine chemical class of ALS inhibiting herbicides (US EPA 2008). The efficacy of pyroxsulam on rigid ryegrass was established in Australia in order to gain registration (Wells 2008). Further efficacy studies conducted in the United Kingdom using the pre-mix product Broadway Star, which contains pyroxsulam and florasulam (Jackson et al. 2011). Florasulam provides broadleaf weed control, so it is reasonable to believe that pyroxsulam is the component of Broadway Star providing control activity on Italian ryegrass (Anonymous 2014b). It was observed that Broadway Star provided Italian ryegrass control greater than ninety-five percent following both fall and spring applications, or through mid-tillering (Jackson et al. 2011). Although proven effective, a study conducted in North Carolina to investigate Italian ryegrass resistance to ALS and ACCase herbicide revealed populations resistant to pyroxsulam in seeds collected in 2007. This again indicated the presence of resistance in populations before the registration of the herbicide, similar to results observed with mesosulfuron, indicating that naturally resistant populations exist (Chandi et al. 2011). In Arkansas, samples collected from fields with suspected resistance in eight states were tested for resistance to several products including pyroxsulam. Samples tested were collected from 2008 through 2011. Resistance to pyroxsulam was observed in eighty-one percent of the samples tested. Detectable levels of resistance were observed in samples from Arkansas, Georgia, Kansas, Mississippi, North

Carolina, and South Carolina. The only states with samples tested where signs of resistance were not observed were collected from Virginia and Washington (Salas et al. 2013).

Cross-resistance is defined as the resistance of a weed species to multiple herbicides within a mode of action (Kuk and Burgos 2007). Cross-resistance has been confirmed in Italian ryegrass to both ACCase and ALS inhibitors, as shown by studies such as the cross-resistance profile completed by Kuk and Burgos (2007). Multiple-resistance is defined as resistance of one plant to multiple herbicides with different modes of action (Powles et al. 1996). Although not defined as multiple-resistance at the time of the study completion, multiple-resistance was found in rigid ryegrass to both ACCase and ALS inhibitors at the same time as ALS inhibitor resistance was confirmed in Australia (Heap and Knight 1986). One study investigating the resistance profile of Italian ryegrass in the United States in 2013 showed that out of the forty-seven populations tested for resistance from multiple states, seventy-eight percent had signs of multiple-resistance to both ACCase and ALS products (Salas et al. 2013).

It is known that biotypes of Italian ryegrass which are resistant to the postemergence products available for control in winter wheat production are present in North Carolina. In order to determine the extent and distribution of this resistance, a survey was conducted using seed collected in the spring of 2012 and 2013. Surveys such as this are useful tools in determining weed control recommendations. The objective of this study was to determine the extent and distribution of Italian ryegrass in North Carolina resistant to applications of diclofop, pinoxaden, pyroxsulam, and mesosulfuron.

Materials and Methods

Italian ryegrass seeds were collected from wheat fields in North Carolina in the spring of 2012 and 2013. Sample locations were chosen using the 2010 ninth edition DeLorme Atlas and Gazetteer of North Carolina. Sample locations were chosen on a longitudinal spacing of every thirteen degree minutes, and a latitude spacing of every ten degree minutes, leading to a grid spacing of just less than 20 km. Using this grid, a total of 239 locations were selected for sampling (Figure 1). Some sites were wooded, residential, urban, or military sites, and no sample was collected. If the site was in an agricultural area, an effort was made to locate ryegrass within approximately 3.22 kilometers of the central grid point. Once ryegrass was found, seed heads from that location were collected, bagged, and marked with the GPS coordinate of the location, the location identifier, which was made up of the atlas page number and grid location on that page, and the crop system from which the sample was taken. Of the 239 locations visited, viable Italian ryegrass seed was collected from 155 locations (Figure 2). One hundred and thirty-six of these locations were sampled in 2012, with the other 20 being sampled in 2013.

Collected seeds were planted into Fafard 2B potting mix in 8.9 by 12.7 cm flat inserts in the greenhouse, with each insert containing one sample location of Italian ryegrass. Once the first leaf had emerged, approximately three to four seedlings were transferred into 10.2 cm square pots filled with Fafard 2B potting mix. Overhead irrigation was supplied and light was supplemented by 1000 watt metal halide bulbs for 12 hours per day. Average daytime temperatures were 25 C, and average nighttime temperatures were 15.5 C. Once the plants were at the three to five leaf stages, averaging four leaves, they were treated with one of four herbicide treatments. Treatments consisted of: a non-treated check, pyroxsulam at 18.4 g ai/ha plus non-ionic surfactant at 0.5% v/v, diclofop-methyl at 1077 g ai/ha, pinoxaden at 61 g ai/ha, and mesosulfuron-methyl at 15 g ai/ha plus methylated seed oil at 1750 g ai/ha. Treatments were applied in a spray chamber calibrated to deliver 140 L/ha of solution at 206.8 kPa through a TT 8002 EVS TeeJet nozzle. Rates were selected based on labeled field rate for each herbicide used, and adjuvants were added based on label recommendations. Treatments were replicated four times and were arranged in a randomized complete block design with all treatments repeated in time. Statistical analysis was performed using SAS 9.3, with analysis of variance being conducted using PROC GLM. Variability between runs was not significant, therefore runs were combined for analysis.

Visual injury ratings were taken at 14, 21, and 28 days after herbicide treatment. Injury was estimated as a sum of total chlorosis, necrosis, and stunting. A susceptible location was identified based on injury ratings and was defined as a location sample where there was complete control observed across all replications and in both runs. There were multiple locations which could have been identified by this criterion as susceptible to both pinoxaden and mesosulfuron. One site was chosen for both of these which is located in a remote area of Stokes County, North Carolina. This site was chosen because there were few other row crop fields in that area, and the field which the sample came from was being managed with a strip crop technique, which likely results in a variety of herbicides being used and reducing the chances of selection pressure for resistance. Only one location met the criterion of susceptible to pyroxsulam. This was a remote site in Carteret County, North Carolina which was largely surrounded by the Croatan National Forest. This could be the reason for this site being susceptible, as there is limited Italian ryegrass germplasm in the area. There was no identifiable location susceptible to diclofop, which was not surprising due to known widespread resistance to this product. Once a susceptible biotype was determined PROC GLIMMIX was used with Dunnet's Procedure ($P \leq 0.05$) to separate locations which showed control lower than the susceptible biotype. Outliers were not removed as they represent the variability of resistance within sampled locations most likely due to segregation (Poirier et. al 2014).

Results and Discussion

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

ACCase-inhibitors

Two of the herbcides screened for resistance were ACCase-inhibitors, diclofop and pinoxaden. Italian ryegrass resistance to diclofop was first observed in North Carolina in 1990 (Heap 2015), so resistance was expected to be common and widespread. While there was some variability in control, no single site could be identified as susceptible to diclofop. It is likely that there was at least some level of diclofop resistance at all sampled locations.

Resistance to pinoxaden was not confirmed in North Carolina until 2007 (Heap 2015). While pinoxaden has the same mode of action as diclofop, the chemistry of pinoxaden is different, and resistance has not been reported to be as common in similar surveys (Salas et al. 2013). While there were samples from many locations in which complete control was observed across all replications, one location was chosen to represent a susceptible location for comparison due to the proximity to other wheat fields and agronomic practice used. Out of the 155 sampled locations in North Carolina, plants from eight locations showed injury levels below those of the susceptible sample and are therefore considered to be resistant (Figure 3). The distribution of resistance was restricted to the Piedmont and Sandhill regions of North Carolina. Since all locations showed signs of resistance to diclofop, these four locations are cross resistant to ACCase-inhibitors.

ALS-Inhibitors

Two ALS-inhibiting herbicides were screened for resistance as well, mesosulfuron and pyroxsulam. Resistance to both of these products was reported in North Carolina in 2007 (Heap 2015). Similar to pinoxaden, there were many sites which met the criterion to be identified as susceptible to mesosulfuron. The same site was used to represent the susceptible population for statistical analysis. Eighteen locations were observed to be different than the susceptible and identified as resistant to mesosulfuron (Figure 4). Resistance appears to be more common in the southern and western part of North Carolina's wheat growing region, however there were two sites on the Virginia border which were identified as resistant as well.

There was only one location which met the criterion to be determined susceptible to pyroxsulam. Resistance to pyroxsulam was more common than resistance to mesosulfuron. Twenty-nine locations were determined to be resistant to pyroxsulam and resistance appeared to be fairly widespread throughout the state (Figure 5).

Out of these locations, seventeen were cross resistant to both ALS-inhibitors (Figure 6). Along with the high levels of cross-resistance to ALS-inhibiting products, there was also a high level variability in injury levels across locations, as demonstrated by the difficulty in identifying a pyroxsulam susceptible location. This variability suggests the potential for more resistance to develop. In further research, a dose response study would be recommended to further pinpoint the level and distribution of resistance. It is also important to note that frequency of resistance within individual populations may vary; therefore, locations with a low frequency of resistance may not have been identified in this survey.

Four locations were found to show evidence of multiple-resistance, being resistant to both ALS and ACCase inhibiting herbicides (Figure 7). These four locations were identified in Richmond, Stanly, and Union counties. In these locations, chemical control of Italian Ryegrass in winter wheat would likely be limited or impossible with postemergence herbicides.

Findings from this survey can be a valuable resource to growers when making decisions concerning Italian ryegrass control in winter wheat. While resistance may not be present on every farm near one of the sample locations, the possibility should be taken into account when designing a herbicide program.

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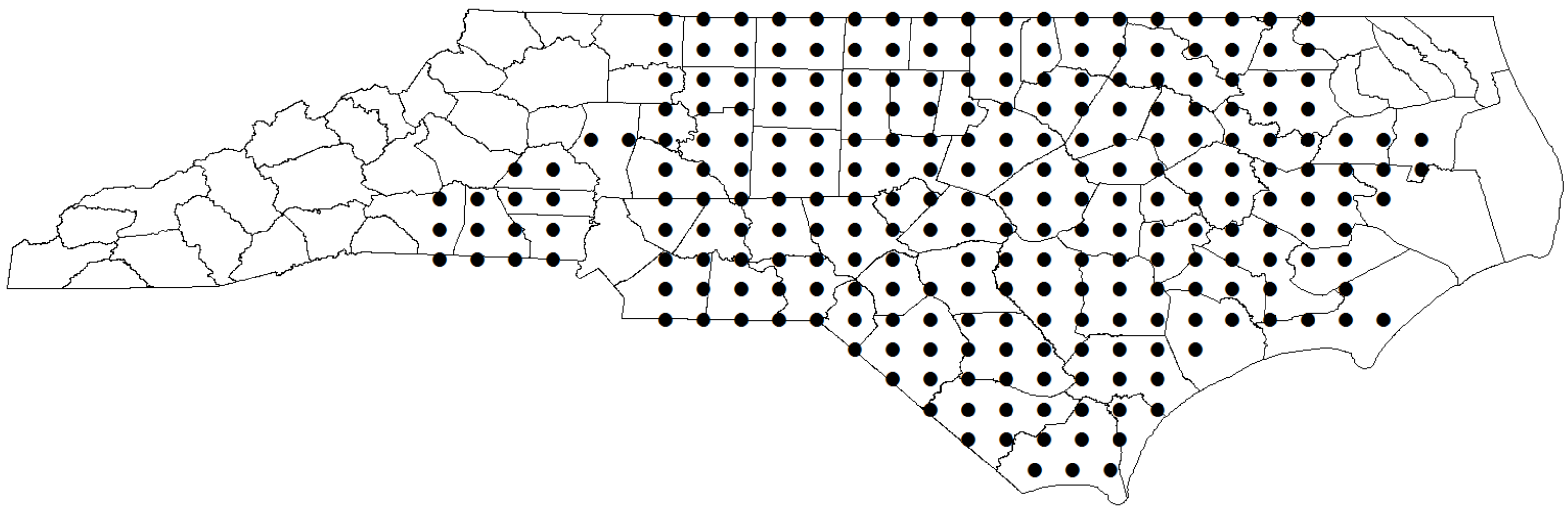

Figure 1. Location of 239 pre-determined sites selected to be visited in the spring of 2012 and 2013 to determine if Italian ryegrass was present.

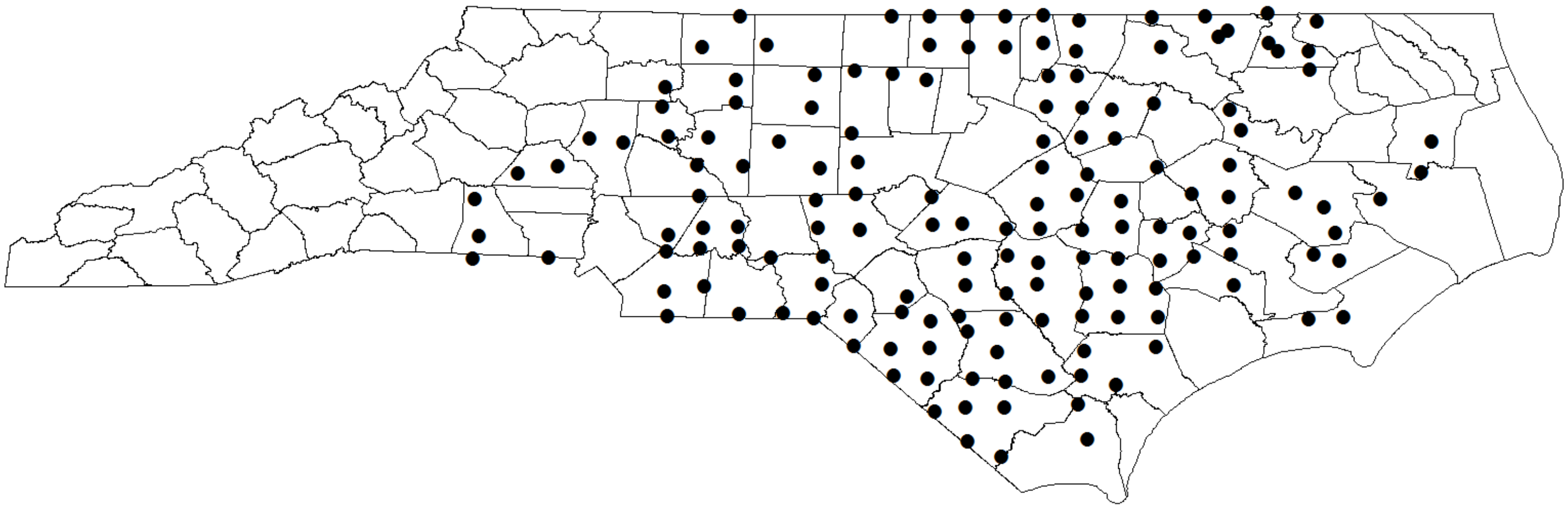

Figure 2. Location of 155 sites where viable Italian ryegrass seeds were collected in the spring of 2012 and 2013.

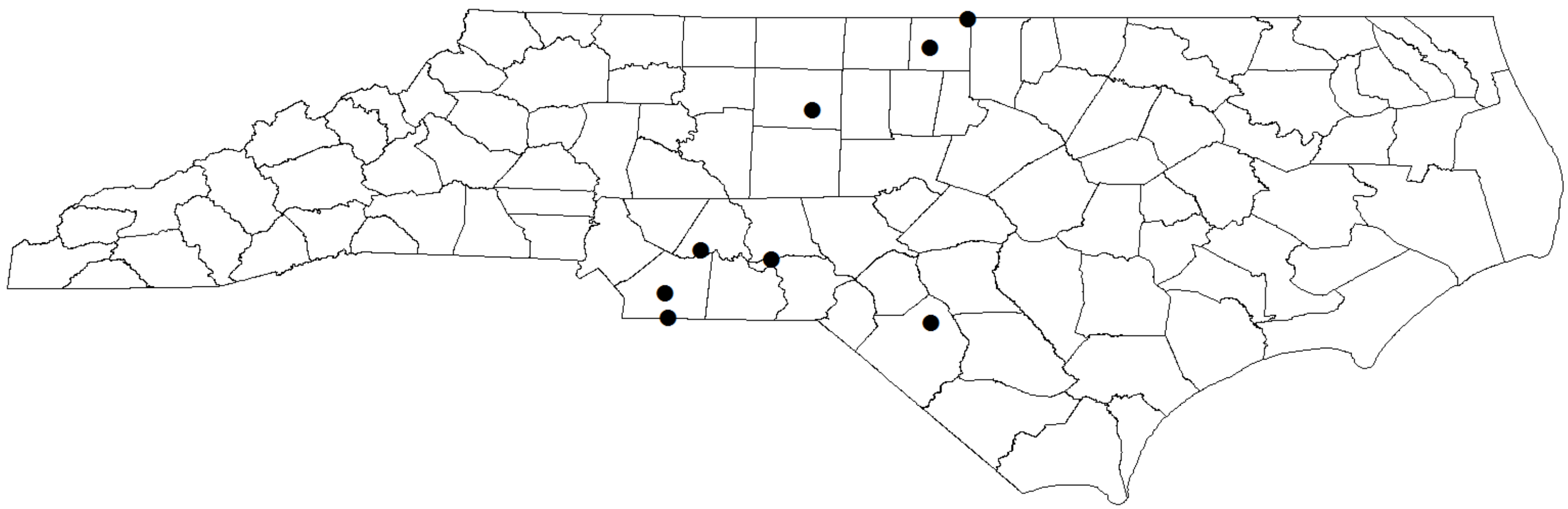

Figure 3. Location of eight sites where Italian ryegrass samples were collected which were observed to be resistant to pinoxaden.

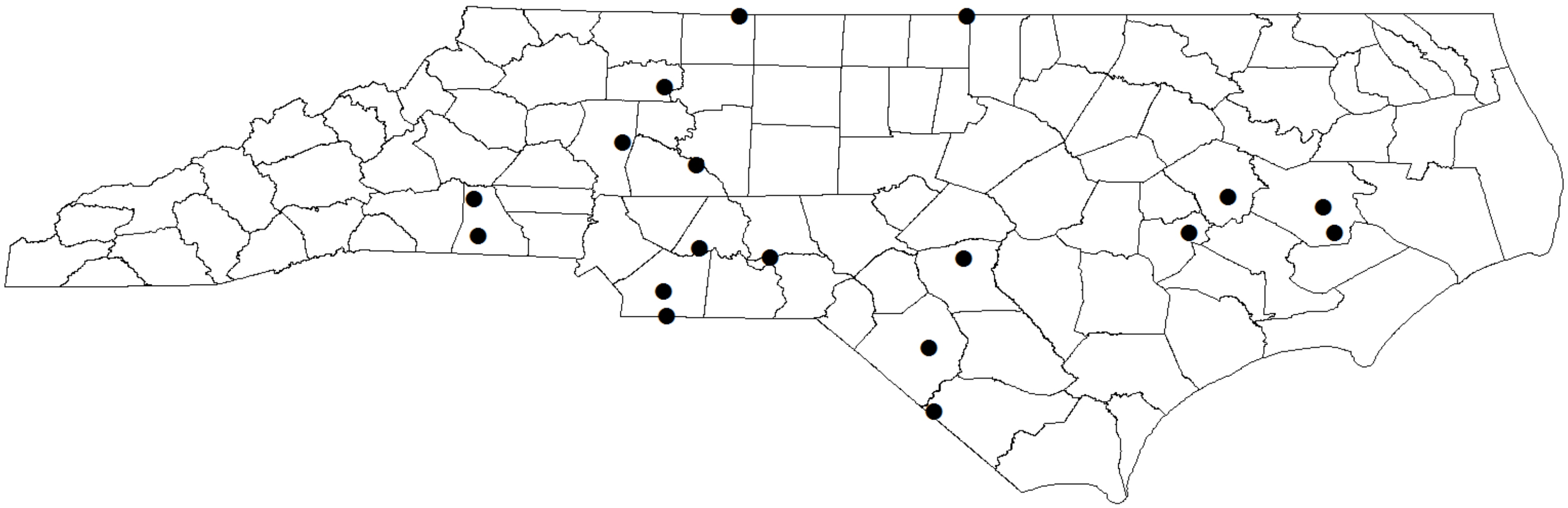

Figure 4. Location of eighteen sites where Italian ryegrass samples were collected which were observed to be resistant to mesosulfuron.

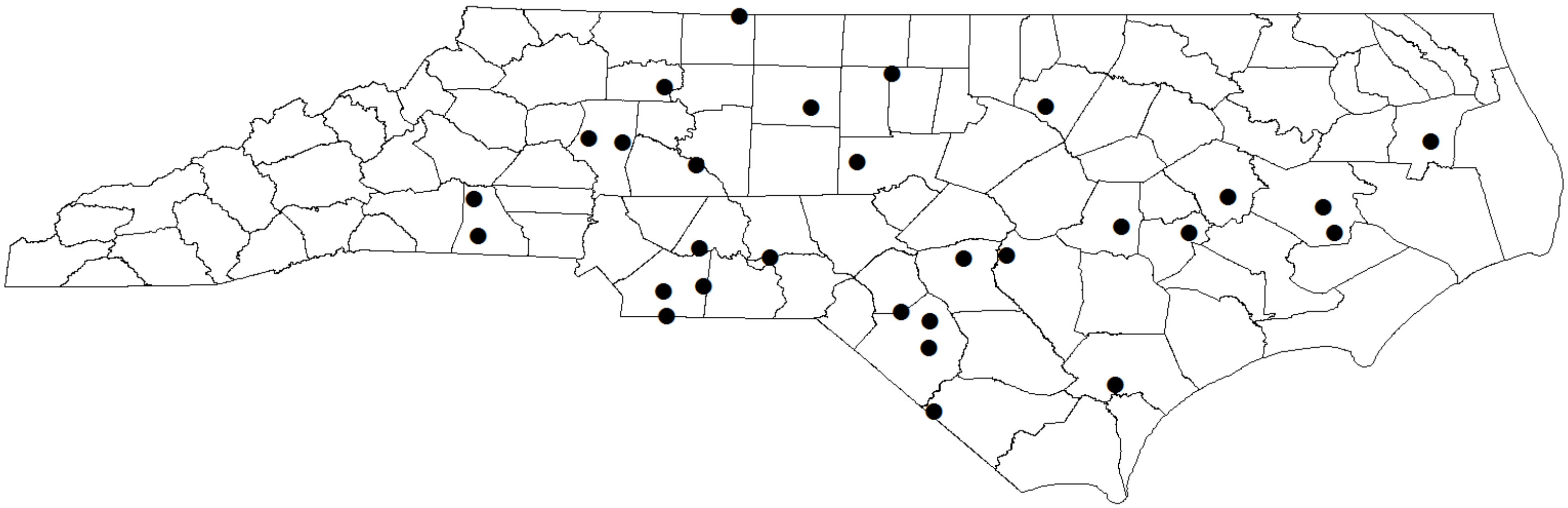

Figure 5. Location of twenty-nine sites where Italian ryegrass samples were collected which were observed to be resistant to pyroxsulam.

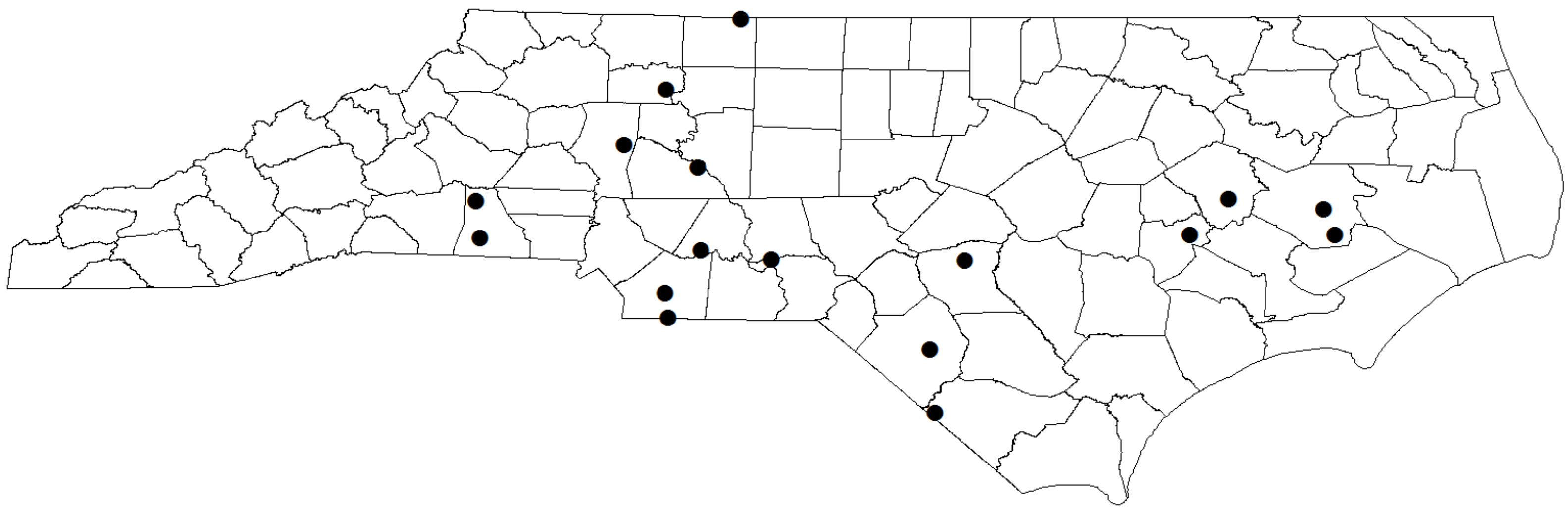

Figure 6. Location of seventeen sites where Italian ryegrass samples were collected which were observed to be resistant to both pyroxsulam and mesosulfuron.

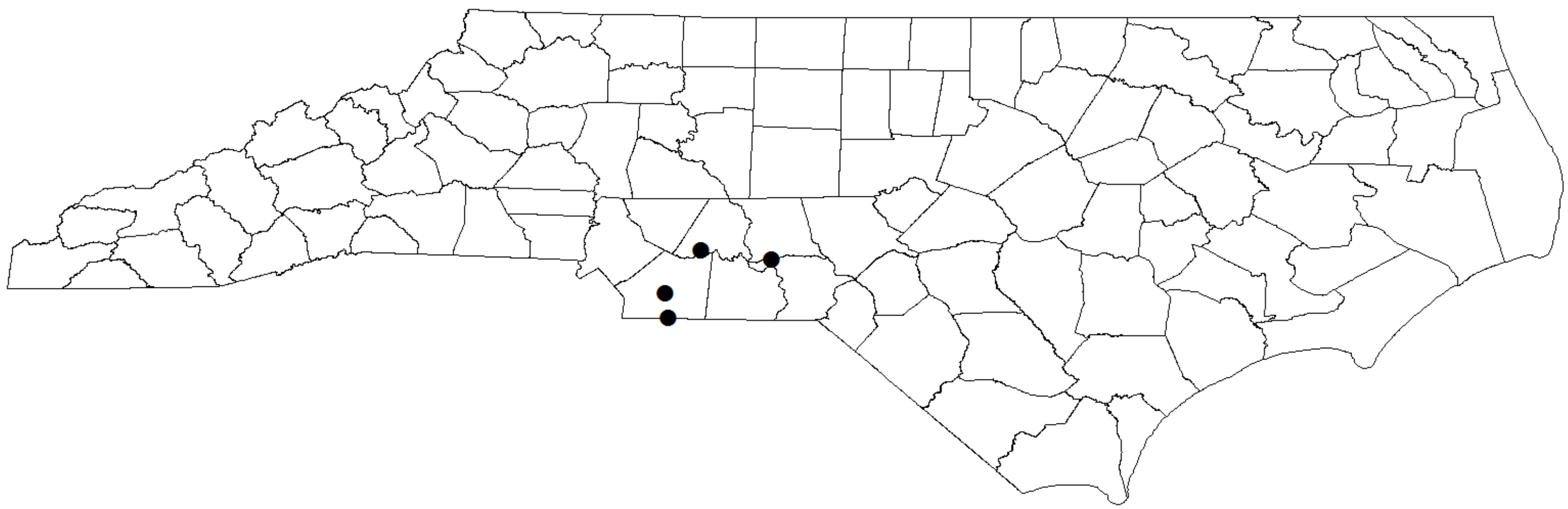

Figure 7. Location of four sites where Italian ryegrass samples were collected which were observed to be resistant to diclofop, pyroxsulam, pinoxaden, and mesosulfuron.

NARROW ROW SPACING IN WINTER WHEAT AS A TOOL FOR MANAGING ITALIAN RYEGRASS [*Lolium perenne L. ssp. multiflorum (Lam.) Husnot*]

As herbicide resistant populations continue to become more widespread, control with chemical options alone will become more difficult. Reduced row spacing has been shown to give some crops a competitive advantage over many weeds, which can help to increase weed control. Adjusting row spacing may be able to give winter wheat a similar competitive advantage over Italian ryegrass seedlings. While this advantage alone may not give adequate control, this advantage in conjunction with a sound herbicide program may allow for the increased control of Italian ryegrass. This research looked at wheat planted in both systems. At the location where research was conducted, there were observable reductions in Italian ryegrass pressure in a narrow row planting system when compared to conventional row spacing over two years. While reductions were limited, this research does show the possibility of row spacing as a tool which could be further investigated for the reduction of Italian ryegrass densities over time.

Introduction

Italian ryegrass [*Lolium perenne L. ssp. multiflorum (Lam.) Husnot*] is a common weed of winter wheat, particularly throughout the Southern United States (Grey and Bridges 2003; Grey et al. 2012; Hoskins et al. 2005; Webster 2012). Early efforts to quantify the amount of crop interference of Italian ryegrass in wheat were done in 1970-1972. Studies conducted in Oregon showed yield losses of up to sixty percent that were attributable to increased densities of Italian ryegrass in winter wheat over the course of two years. These studies also investigated the effect of Italian ryegrass competition on wheat yield reduction by variety; however decreased yields were observed as Italian ryegrass populations increased, regardless of wheat variety (Appleby et al. 1976). Further efforts were made to quantify yield losses associated with interference from Italian ryegrass from 1981-1983 in North Carolina (Liebl and Worsham 1987). They reported that for every 10 Italian ryegrass plants per meter square, wheat yield was decreased by an average of 4.2%. Liebl and Worsham (1987) also attempted to determine causes of the interference of Italian ryegrass on wheat yields. Growth rate and response to nutrients were both greater for Italian ryegrass than for winter wheat. Nitrate and potassium, both critical elements for plant growth, were taken up more rapidly from a nutrient solution in a greenhouse setting by Italian ryegrass than by wheat. It was concluded that direct competition for nutrients led to decreased tillering and yields in winter wheat when Italian ryegrass is present (Liebl and Worsham 1987).

Effective chemical control of Italian ryegrass in wheat systems has been shown to be limited (Grey and Bridges 2003; Grey et al. 2012; Hoskins et al. 2005). According to the 2015 North Carolina Agricultural Chemical Manual (Everman 2015), the only four postemergence applied herbicides are currently available for the control of Italian ryegrass in winter wheat, the ACCase inhibitors diclofop and pinoxaden, and the ALS inhibitors mesosulfuron and pyroxsulam. Populations of Italian ryegrass which are resistant to each of these products have been identified in North Carolina (Heap 2015; Taylor 2014).

Since postemergence products for Italian ryegrass control are known to be limited, preemergence products are generally recommended to help control the weed prior to emergence in winter wheat. One product that is available is a premix product containing metribuzin and flufenacet (Koepke-Hill et al. 2011). Metribuzin is a photosystem II inhibitor that has shown both PRE and POST activity on Italian ryegrass (US EPA 1998b). Studies did however find that some wheat varieties are sensitive to this product, and that proper timing is important to prevent injury to wheat (Shaw and Wesley 1991). Flufenacet is an inhibitor of very-long-chain fatty acid biosynthesis and has been shown to control some grass species (US EPA 1998a). The premix of these products was found to control Italian ryegrass; however the level of control was highly variable (Koepke-Hill et al. 2011). It has been hypothesized that control variations were a result different biotypes, soil types, and variations in rainfall amounts and timing after application (Koepke-Hill et al. 2011). The premix of metribuzin plus flufenacet is labelled in North Carolina to control Italian ryegrass in winter wheat, but does have a short application window, from germination up to two-leaf (Anonymous 2010). Another product available after germination is pendimethalin, which is a dinitroanaline herbicide, inhibiting cell division and root growth in susceptible species. Pendimethalin is labeled for application after first leaf in wheat to suppress Italian ryegrass, but does not have postemergence activity (Anonymous 2012b). While pendimethalin is labeled, it is not recommended for control of Italian ryegrass in winter wheat by the North Carolina Agricultural Chemical Manual (Everman 2015). This may be due to previous research that has shown that pendimethalin alone does not provide a level of Italian ryegrass control through the growing season that is considered commercially acceptable (Bond et al. 2014).

Another herbicide option listed in the North Carolina Agricultural Chemical Manual is pyroxasulfone (Everman 2015). While pyroxasulfone has the same mode of action as flufenacet, it offers a longer application window (Anonymous 2013). Pyroxasulfone has been shown to control Italian Ryegrass season-long and be an acceptable alternative to metribuzin plus flufenacet. Pyroxasulfone has also been shown to have low levels of wheat injury, which did not impact yields (Hulting et al. 2012). According to the label, applications can be made after at least eighty percent of wheat has germinated and has a shoot at least one half inch long and until wheat reaches four tillers (Anonymous 2013). Pyroxasulfone does not have postemergence activity on Italian ryegrass, so the label also encourages application as soon as possible, before ryegrass germinates.

Due to the limited chemical control options and occurrence of herbicide resistance, it is reasonable to consider cultural practices as an option for managing Italian ryegrass. Ryegrass is known to be competitive with wheat (Liebl and Worsham 1987) but some cultural practices may be able to give wheat a competitive advantage. One cultural practice to be considered is the seeding arrangement, or row spacing. It has been shown in many crops that reducing row spacing from common commercial practices can help to increase weed suppression (Rogers et al. 1976; Walker and Buchanan 1982; Yelverton and Coble 1991). While initial research showed that increased wheat crop densities could reduce weed biomass, the same was not observed for narrow row spacing alone (Champion et al. 1998). European research investigating the impact of row spacing on weed competition observed a reduction in weed biomass due to reduced row spacing (Olsen et al. 2005; Weiner et al. 2001). Research conducted in spring planted wheat has followed a similar trend to reduce weed density by changing planting patterns. However, instead of reducing row spacing, spring wheat was planted in a grid pattern. On average, weed biomass was reduced by 30% compared to planting in standard rows (Weiner et al. 2001). While the grid pattern was investigated in two separate studies, neither study reported results with specific weed species. The majority of weeds reported to be present in the field were broadleaves, and the studies did not report the presence of Italian ryegrass (Olsen et al. 2005; Weiner et al. 2001).

Another study conducted on spring wheat in Europe did investigate the effects of row spacing on the suppression of different weed species, including Italian ryegrass (Olsen et al. 2006). When low or medium densities of Italian ryegrass were present, a significant effect of suppression by row spacing was not observed. When high densities of Italian ryegrass were present however, row spacing did have a significant effect on suppression in one of two years (Olsen et al. 2006).

Few studies have been conducted in the United States investigating narrow row spacing in winter wheat as a tool for weed suppression. Two studies conducted in Oregon did focus specifically on the suppression of Italian ryegrass. Appleby and Brewster (1992) reported inconsistent results when investigating planting patterns alone, only reporting differences when Italian ryegrass densities were high. Therefore, it was concluded that overcoming yield losses due to Italian ryegrass by changing planting patterns alone was not feasible (Appleby and Brewster 1992). The other study showed evidence for better Italian ryegrass suppression in wide row winter wheat. Here it was observed that higher planting densities within rows when planted on conventional rows had a greater effect on Italian ryegrass suppression then narrow row wheat planted at the same population (Hashem et al. 1998).

The inconsistency of previous results combined with a lack of herbicide programs integrated into studies investigating cultural practices, results in the need for further and more current research into the possibility of narrow row spacing as a tool for managing Italian ryegrass in winter wheat. Therefore a study was initiated to investigate the effectiveness of different chemical weed control programs in a narrow row system compared to a wide row system typically used by most producers.

Materials and Methods

Field research studies were conducted at two separate locations in North Carolina in the 2012 through 2013 growing season, and in only one location the following two growing seasons. The locations in the fall of 2012 were planted on a private farm approximately five kilometers west of the town of Pinetops along NC highway 42 and at the Central Crops Research Station in Clayton. During the 2013-14 and 2014-15 growing seasons, research was conducted at the Central Crops Research Station in Clayton. In the 2012-13 growing season, the soil of the field location near Pinetops was identified as Aycock very fine sandy loam which are fine-silty, siliceous, subactive, thermic Typic Paleudults. The field used in the 2012-13 growing season at the Central Crops Research Station was Wagram loamy sand, which are loamy, kaolinitic, thermic Arenic Kandiudults. In the 2013-14 and 2014-15 growing seasons, the soils at the Central Crops Research Station consisted predominately of a Rains sandy loam. Soil types were identified using the USDA web soil survey (2013).

Planting at Pinetops and Clayton in 2012 was done using an 8 row Tye small plot drill with 19 cm row spacing. Planting at Clayton in 2013 was done with a three point Great Plains 606NT small plot drill with 19 cm row spacing. In 2014, planting was done using a 3 meter wide commercial grain drill with 19 cm row spacing. Wheat was seeded at a rate of 72 seed per meter of row in the standard 19 cm row spacing study. Narrow rows were achieved by first planting one pass through the plot at a seeding rate of 36 seed per meter of row, then making a second pass at approximately a 20 degree angle to the first at the same seeding rate of 36 seed per meter of row, resulting in the same seeding rate per hectare as the standard 19 cm row spacing. The narrow row planting arrangement was meant to represent an average row spacing of 9.5 cm.

Twelve herbicide treatments were applied in a randomized complete block design with four replications within each row spacing block. Treatments consisted of PRE, SPIKE, PRE fb POST, or POST only applications (Table 1). The presence or absence of residual herbicides was an additional factor in POST applications. Herbicide rates and timings are outlined in Table 1. POST applications of thifensulfuron, formulated as Harmony SG (Anonymous 2012a), were applied over all treatments as needed to control annual broadleaf weeds. Treatments were applied using a CO_2 pressurized backpack sprayer calibrated to a pressure of 207 kPa, delivering 140 liters per hectare with TeeJet XR11002 nozzles.

During the season visual estimations of weed control were recorded and Italian ryegrass densities were estimated prior to harvest by averaging the frequency of seed heads recorded in three 0.5 by 0.5 meter square areas per plot.

Statistical analysis was performed using SAS 9.3. Analysis of Italian ryegrass density counts and yield was conducted under the PROC GLM procedure and means were separated using Fisher's Protected LSD ($p \leq 0.05$). Data was transformed using a log plus one transformation where applicable.

Results and Discussion

The initial study planted in 2012 in both the Pinetops and Clayton location yielded little data due to low natural populations of Italian ryegrass. Due to this, there were no differences observed by herbicide treatment in either the wide row or narrow row system (data not shown). It is important to note that at the Pinetops location, average yields in the narrow row system were 3.97 tons per hectare (t/ha) while yields in the wide row system were 3.23 t/ha (data not shown). Though it is unknown if this difference of 0.74 t/ha is caused by the planting system alone, it is important to note as a possibility of a future area of research, as narrow row spacing system have previously been shown to lead to increases in yield (Johnson et al. 1988).

At the Central Crops Research Station in 2013, a different field was used than in 2012, in search of higher natural Italian ryegrass densities. This same field was used again in 2014. There was not a significant effect of year on Italian ryegrass density counts, so data was combined across years. There were two replications of row spacing each year, so year was also analyzed as a replication to give a total of four row spacing replications. Lower densities of Italian ryegrass were observed in the narrow row spacing system, when averaged across all treatments (Table 2). This provides evidence that the cultural practice of using narrow row spacing was effective at reducing Italian ryegrass populations at this location regardless of the herbicide program used.

Differences in Italian ryegrass densities were also observed among herbicide treatments, as shown in Table 6. All treatments were effective at reducing Italian ryegrass densities below those observed in the non-treated check. There was evidence of ALS-resistance at this location, which can be noted due to the higher Italian ryegrass densities observed with treatments of mesosulfuron applied POST only, either alone or in combination with pyroxasulfone. The lowest Italian ryegrass densities were observed when a PRE application of pyroxasulfone was followed by a POST application containing either mesosulfuron or pinoxaden. This was true despite the presence of mesosulfuron resistance. Lower Italian ryegrass densities in PRE fb POST application compared to POST only treatments shows the importance of a two-pass system for enhanced control.

While Italian ryegrass densities were able to be combined across years, this was not true for yield results. There was an effect of year on yield, so years are analyzed separately. This leaves us with only two replications of row spacing in each year, which caused strong replication effects, therefore the narrow and wide row spacing systems are analyzed independently of one another.

In the narrow row system in 2013 planted wheat, there was no effect of herbicide program on yield results (data not shown). In the wide row system in 2013 planted wheat, there were differences in yield depending on the herbicide treatment. Results are shown in Table 7 and were subject to a log plus one transformation. Yields were reduced when no herbicide was applied or when a POST only application of mesosulfuron with or without pyroxasulfone or a POST application of pinoxaden alone was applied. This indicates that despite a POST only application that is effective at controlling Italian ryegrass, yields are still reduced due to early season competition in a low yielding environment. When a POST only application of pinoxaden was applied with pyroxasulfone, there was a yield improvement over the untreated check, which is likely due to prevention of Italian ryegrass germination and competition during the spring.

The 2014 growing season at the Central Crops Research Station was compromised by heavy rains following planting, which resulted in poor wheat populations. This is likely the reason for low yields, which resulted in no observable differences by herbicide treatment.

There was not a significant correlation of yield reduction as a result of Italian ryegrass densities during the two years of this study. As a result, whether or not narrow row spacing planting or the most beneficial herbicide programs would be economically viable options cannot be determined. It can however be determined that narrow row spacing and a two-pass herbicide system would have the greatest effectiveness on reducing Italian Ryegrass seed in the overall seed bank.

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Table 1. Herbicide treatments applied in both 19.05 and 9.525 cm row spacings.

Treatment No.	Herbicide	Rate	Application Timing[a]
1	non-treated	n/a	n/a
2	pyroxasulfone	74.4 g ai/ha	PRE
3	pyroxasulfone	74.4 g ai/ha	PRE
	pyroxasulfone	74.4 g ai/ha	POST
4	pyroxasulfone	74.4 g ai/ha	PRE
	mesosulfuron	15 g ai/ha	POST
	non-ionic surfactant	0.25 % v/v	POST
5	pyroxasulfone	74.4 g ai/ha	PRE
	pinoxaden	60.5 g ai/ha	POST
6	pyroxasulfone	74.4 g ai/ha	PRE
	pyroxasulfone	74.4 g ai/ha	POST
	mesosulfuron	15 g ai/ha	POST
	non-ionic surfactant	0.25 % v/v	POST
7	pyroxasulfone	74.4 g ai/ha	PRE
	pyroxasulfone	74.4 g ai/ha	POST
	pinoxaden	60.5 g ai/ha	POST
8	mesosulfuron	15 g ai/ha	POST
	non-ionic surfactant	0.25 % v/v	POST
9	pinoxaden	60.5 g ai/ha	POST
10	pyroxasulfone	74.4 g ai/ha	POST
	mesosulfuron	15 g ai/ha	POST
	non-ionic surfactant	0.25 % v/v	POST
11	pyroxasulfone	74.4 g ai/ha	POST
	pinoxaden	60.5 g ai/ha	POST
12	flufenacet + metribuzin[b]	304.6 g ai/ha + 76.2 g ai/ha	Spike

[a] Abbreviations: PRE, preemergence; POST, postemergence; SPIKE, radical to 1 leaf crop emergence.

[b] Axiom Herbicide (Anonymous 2010).

Table 2. Italian ryegrass densities observed in wide row and narrow row planting system averaged over herbicide treatment and year at Central Crops Research Station [a].

Planting system	Ryegrass density (per .5 m meter square)
Wide rows	7.15 a
Narrow rows	4.71 b

[a] Means followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$.

Table 3. Italian ryegrass densities as affected by herbicide treatment averaged over row spacing and year at Central Crops Research Station [a].

Treatment No.	Treatment[b]	Application Timing[c]	Ryegrass density (per meter square)[d]
1	non-treated	n/a	50 a
2	pyroxasulfone	PRE	21 b
3	pyroxasulfone pyroxasulfone	PRE POST	10 cd
4	pyroxasulfone mesosulfuron	PRE POST	2 efg
5	pyroxasulfone pinoxaden	PRE POST	2 fg
6	pyroxasulfone pyroxasulfone mesosulfuron	PRE POST	1 fg
7	pyroxasulfone pyroxasulfone pinoxaden	PRE POST	1 g
8	mesosulfuron	POST	21 b
9	pinoxaden	POST	7 de
10	pyroxasulfone mesosulfuron	POST	14 bc
11	pyroxasulfone pinoxaden	POST	9 e
12	flufenacet + metribuzin[c]	SPIKE	2 ef

[a] Means followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$.

[b] Treatments including mesosulfuron also include non-ionic surfactant.

[c] Abbreviations: PRE, preemergence; POST, postemergence; SPIKE, radical to 1 leaf crop emergence.

[c] Axiom Herbicide (Anonymous 2010).

Table 4. Yield of winter wheat planted in 2013 wide rows at Central Crops Research Station [a].

Treatment No.	Treatment[b]	Application Timing[c]	Yield (kg/ha)
1	non-treated	n/a	2230 c
2	pyroxasulfone	PRE	2880 ab
3	pyroxasulfone pyroxasulfone	PRE POST	3150 a
4	pyroxasulfone mesosulfuron	PRE POST	2830 ab
5	pyroxasulfone pinoxaden	PRE POST	3050 a
6	pyroxasulfone pyroxasulfone mesosulfuron	PRE POST	2930 ab
7	pyroxasulfone pyroxasulfone pinoxaden	PRE POST	2730 ab
8	mesosulfuron	POST	2450 bc
9	pinoxaden	POST	2480 bc
10	pyroxasulfone mesosulfuron	POST	2200 c
11	pyroxasulfone pinoxaden	POST	2800 ab
12	flufenacet + metribuzin[c]	SPIKE	3030 a

[a] Means followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$.

[b] Treatments including mesosulfuron also include non-ionic surfactant.

[c] Abbreviations: PRE, preemergence; POST, postemergence; SPIKE, radical to 1 leaf crop emergence.

[c] Axiom Herbicide (Anonymous 2010).

Italian Ryegrass [*Lolium perenne L. ssp. multiflorum (Lam.) Husnot*] Control with Pyroxasulfone Based Systems in Winter Wheat in North Carolina

As herbicide resistant populations continue to become more widespread, postemergence control with chemical options alone will become more difficult. This will increase the need for preemergence herbicides will increase. Pyroxasulfone is a preemergence product that has shown been shown to be effective at controlling Italian ryegrass. The effectiveness was compared to other products and how pyroxasulfone can be used as part of a complete herbicide program. When a preemergence application of pyroxasulfone is followed by a postemergence application of pinoxaden when resistance is not present, excellent control of Italian ryegrass can be achieved in North Carolina. Other programs may offer adequate control at a lower cost to growers, and should be considered depending on the density of Italian Ryegrass infestation.

Introduction

Italian ryegrass [*Lolium perenne L. ssp. multiflorum (Lam.) Husnot*] is a common weed of winter wheat, particularly throughout the Southern United States (Grey and Bridges 2003; Grey et al. 2012; Hoskins et al. 2005; Webster 2012). Early efforts to quantify the amount of crop interference of Italian ryegrass in wheat were done in 1970-1972. Studies conducted in Oregon showed yield losses of up to sixty percent that were attributable to increased densities of Italian ryegrass in winter wheat over the course of two years. These studies also investigated the effect of Italian ryegrass competition on wheat yield reduction by variety; however decreased yields were observed as Italian ryegrass populations increased, regardless of wheat variety (Appleby et al. 1976). Further efforts were made to quantify yield losses associated with interference from Italian ryegrass from 1981-1983 in

North Carolina Liebl and Worsham (1987). They reported that for every 10 Italian ryegrass plants per meter square, wheat yield was decreased by an average of 4.2%. Liebl and Worsham (1987) also attempted to determine causes of the interference of Italian ryegrass on wheat yields. Growth rate and response to nutrients were both greater for Italian ryegrass than for winter wheat. Nitrate and potassium, both critical elements for plant growth, were taken up more rapidly from a nutrient solution in a greenhouse setting by Italian ryegrass than by wheat. It was concluded that direct competition for nutrients led to decreased tillering and yields in winter wheat when Italian ryegrass is present (Liebl and Worsham 1987).

Effective chemical control of Italian ryegrass in wheat systems has been shown to be limited (Grey and Bridges 2003; Grey et al. 2012; Hoskins et al. 2005). According to the 2015 North Carolina Agricultural Chemical Manual (Everman 2015), the only three postemergence applied herbicides are currently available for the control of Italian ryegrass in winter wheat, the ACCase inhibitors diclofop and pinoxaden, and the ALS inhibitors mesosulfuron and pyroxsulam. Populations of Italian ryegrass which are resistant to each of these products have been identified in North Carolina (Heap 2015; Taylor 2014).

Since postemergence products for Italian ryegrass control are known to be limited, preemergence products are generally recommended to help control the weed prior to emergence in winter wheat. One product that is available is a premix product containing metribuzin and flufenacet (Koepke-Hill et al. 2011). Metribuzin is a photosystem II inhibitor that has shown both PRE and POST activity on Italian ryegrass (US EPA 1998b). Studies did however find that some wheat varieties are sensitive to this product, and that proper timing is important to prevent injury to wheat (Shaw and Wesley 1991). Flufenacet is an inhibitor of very-long-chain fatty acid biosynthesis and has been shown to control some grass species (US EPA 1998a). The premix of these products was found to control Italian ryegrass; however the level of control was highly variable (Koepke-Hill et al. 2011). It has been hypothesized that control variations were a result different biotypes, soil types, and variations in rainfall amounts and timing after application (Koepke-Hill et al. 2011). The premix of metribuzin plus flufenacet is labelled in North Carolina to control Italian ryegrass in winter wheat, but does have a short application window, from germination up to two-leaf (Anonymous 2010). Another product available after germination is pendimethalin, which is a dinitroanaline herbicide, inhibiting cell division and root growth in susceptible species. Pendimethalin is labeled for application after first leaf in wheat to suppress Italian ryegrass, but does not have postemergence activity (Anonymous 2012b). While pendimethalin is labeled, it is not recommended for control of Italian ryegrass in winter wheat by the North Carolina Agricultural Chemical Manual (Everman 2015). This may be due to previous research that has shown that pendimethalin alone does not provide a level of Italian ryegrass control through the growing season that is considered commercially acceptable (Bond et al. 2014).

Another herbicide option listed in the North Carolina Agricultural Chemical Manual is pyroxasulfone (Everman 2015). While pyroxasulfone has the same mode of action as flufenacet, it offers a longer application window (Anonymous 2013). Pyroxasulfone has been shown to control Italian Ryegrass season-long and be an acceptable alternative to metribuzin plus flufenacet. Pyroxasulfone has also been shown to have low levels of wheat injury, which did not impact yields (Hulting et al. 2012). According to the label, applications can be made after at least eighty percent of wheat has germinated and has a shoot at least one half inch long and until wheat reaches four tillers (Anonymous 2013). Pyroxasulfone does not have postemergence activity on Italian ryegrass, so the label also encourages application as soon as possible, before ryegrass germinates.

Materials and Methods

Field research studies were conducted at two locations in the 2013-14 growing season. One location was near the town of Hertford, North Carolina and the other was at the Piedmont Research Station near Salisbury, North Carolina. In the 2014-15 growing season, only the location at the Piedmont research station was used. The field used near Hertford, North Carolina location is composed of Roanoke silt loam, which is a fine, mixed, semiactive, thermic Typic Endoaquults. Soils at the Piedmont Research Station consisted of Lloyd clay loam, which is a fine, kaolinitic, thermic Rhodic Kanhapludults. Soil types were identified using the USDA web soil survey (2013).

Planting at each location was done using a commercial grain drill. Wheat was seeded at a rate of 72 seed per meter of row, with a row spacing of 19 cm. The study was planted twice in each location and year, with one planted in a no-till environment and one in a tilled environment. Tillage at the Hertford, North Carolina location was done using a chisel plow, resulting in moderate incorporation of previous crop residue. At the Piedmont research station, a Great Plains turbo-till was the tillage tool used in the tilled study. This resulted in light incorporation of previous crop residues, while still complying with minimum tillage regulations. Fertility and non-weed management practices were performed by station personnel at the Piedmont Research Station and by the land owner at the Hertford site, in accordance with standard commercial production practices (Weisz et al. 2013).

Nine herbicide treatments were applied in a randomized complete block design with four replications. Treatments consisted of a non-treated, PRE only, SPIKE only, PRE followed by POST, and POST only applications (Table 12). A single POST application of thifensulfuron, formulated as Harmony SG herbicide (Anonymous 2012a), was applied over all treatments at the Piedmont Research Station during the 2014-15 growing season to control annual broadleaf weeds. Treatments were applied using a CO_2 pressurized backpack sprayer calibrated to a pressure of 207 kPa, delivering 140 liters per hectare with TeeJet XR11002 nozzles.

Italian ryegrass densities were estimated prior to harvest by averaging the frequency of seed heads recorded in three 0.5 by 0.5 meter square areas per plot. Italian ryegrass was not planted at any location to supplement natural populations, so all densities are a result of the natural seed bank.

Statistical analysis was performed using SAS 9.3. ANOVA was conducted under the PROC GLM procedure and means were separated using Fisher's Protected LSD ($p \geq 0.05$). PROC GLM was also used to conduct orthogonal contrast comparisons and PROC CORR was used to determine the impact of Italian Ryegrass densities on grain yield.

Results and Discussion

Environments at each location were not different from one another, and were therefore combined at each location. There was not a significant interaction of environment by herbicide on the Italian ryegrass densities, however there was an interaction effect of environment by yield. Therefore, all locations were combined to compare herbicides for Italian ryegrass control, but yield was analyzed independently by location.

Italian ryegrass densities were used to determine the effectiveness of herbicide programs implemented. The herbicide treatment of pyroxasulfone PRE followed by a POST application of pinoxaden resulted in the lowest observed Italian ryegrass densities pre-harvest across all environments and years (Table 5). The application of pinoxaden tank mixed with pyroxasulfone applied POST was not as effective as the two pass system of these herbicides, but was more effective than all other treatments at reducing Italian ryegrass densities. All other treatments were more effective at reducing Italian ryegrass densities than the non-treated check, but were not different from one another. It is important to note that POST applications of ALS-inhibiting herbicides did not reduce Italian ryegrass densities any more than PRE or SPIKE treatments. This is an indication of ALS resistance which is suspected at these locations, and explains why there was limited control with a POST application. Some of the control was likely as result of pyroxasulfone which was applied along with mesosulfuron post. While pyroxasulfone does not control Italian ryegrass that has emerged, this application prevented further emergence in the spring after the POST application.

Herbicide programs were also compared by application timing. Again, as expected all programs were more effective than the non-treated check (Table 6). This makes it clear that PRE followed by POST systems provided increased control of Italian ryegrass; however there was no difference in control between PRE and SPIKE only treatments. No significant difference between PRE or SPIKE only treatments and POST only treatments was observed, however this is due to the limited control seen with mesosulfuron resulting from the presence of ALS-inhibitor resistant Italian ryegrass populations.

Yields were analyzed separately by location. At the location near Hertford, the highest yields were observed when a two-pass herbicide program was used with a PRE application of pyroxasulfone followed by a POST application of pinoxaden (Table 7). This was the same treatment which led to the lowest observed Italian ryegrass densities. All other treatments yielded higher than the non-treated check with the exception of a POST only treatment of mesosulfuron tank mixed with pyroxasulfone. While this treatment did reduce Italian ryegrass densities, as stated previously, that reduction was likely a result of the pyroxasulfone preventing further Italian ryegrass emergence after the POST application was applied. Yield reductions had already occurred due to previous crop interference before the POST application was applied. It was also noted Italian ryegrass control was greater with a POST only treatment of pinoxaden than with PRE or SPIKE only programs. Yield however was not greater than PRE or SPIKE treatments with this POST only treatment. Again this is most likely due to early season competition between Italian ryegrass and wheat. Wet growing conditions in the spring of 2014 impacted yields throughout the state, and resulted in yields which were somewhat lower than expected.

Yield results were not as clear at the Piedmont Research Station, where overall yields were higher. At this location, the POST only treatment of mesosulfuron tank mixed with pyroxasulfone was the only treatment which resulted in yields which were no different from the untreated check (Table 7). This location overall had less Italian ryegrass pressure, which proved to have less of an impact on yields. There were also favorable growing conditions, which resulted in overall high yields.

While the treatment of a PRE application of pyroxasulfone followed by a POST application of pinoxaden resulted in the lowest density of Italian ryegrass observed, this can be a costly treatment for growers. Both of the PRE followed by POST and POST only treatments cost just under $60 per hectare based on quotes from chemical dealers in North

Carolina. The SPIKE treatment of flufenacet + metribuzin cost approximately $33 per hectare for the low rate and $41 per hectare for the high rate. The PRE only treatment of pyroxasulfone herbicide cost approximately $23 and $28 per hectare for the low rate and high rate, respectively. While the pyroxasulfone followed by pinoxaden treatment is costly, at our Hertford location, this treatment yielded 925 kg per hectare higher than all other treatments. With current wheat prices in North Carolina at around $4.50 per bushel, or approximately 27 kilograms, this would result in a $154 profit increase, which justifies the cost of a two-pass system.

A linear correlation was found between decreased yields and increased Italian ryegrass densities, and plotted in Figure 8. Yields are not shown on this graph below 1,000 kilograms per hectare, as yields below this level were not observed. This shows a decrease of ninety-one kilograms per hectare in yield for every ten Italian ryegrass heads counted in one square meter. At current grain prices, this equates to a loss of just over $15 per hectare for every ten Italian ryegrass heads per square meter. Understanding correlation between Italian ryegrass densities and yield losses can help to determine if a treatment can be economically justified. In some cases it may still be advisable to make an application in order to prevent additional seed from accumulating in the seed bank.

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Table 5. Herbicide programs implemented in both tilled and no-till systems.

Treatment No.	Herbicide	Rate	Application Timing[a]
1	non-treated	N/A	n/a
2	pyroxasulfone	74.4 g ai/ha	PRE
3	pyroxasulfone	89.3 g ai/ha	PRE
4	flufenacet + metribuzin[b]	304.6 g ai/ha + 76.2 g ai/ha	SPIKE
5	flufenacet + metribuzin[b]	308.8 g ai.ha + 95.2 g ai/ha	SPIKE
6	pyroxasulfone	74.4 g ai/ha	PRE
	mesosulfuron	15 g ai/ha	POST
	non-ionic surfactant	0.25 % v/v	POST
7	pyroxasulfone	74.4 g ai/ha	PRE
	pinoxaden	60.5 g ai/ha	POST
8	pyroxasulfone	59.5 g ai/ha	POST
	mesosulfuron	15 g ai/ha	POST
	non-ionic surfactant	0.25 % v/v	POST
9	pyroxasulfone	59.5 g ai/ha	POST
	pinoxaden	60.5 g ai/ha	POST

[a] Abbreviations: PRE, preemergence; POST, postemergence; SPIKE, radical to 1 leaf crop emergence.

[b] Axiom Herbicide (Anonymous 2010).

Table 6. Pre-harvest average Italian Ryegrass densities per square meter in all environments[a].

Treatment No.	Treatment[b]	Application Timing[c]	Ryegrass density (per .5 m meter square)
1	non-treated	n/a	187 a
2	pyroxasulfone (Low Rate)	PRE	86 bc
3	pyroxasulfone (High Rate)	PRE	92 b
4	flufenacet + metribuzin[d] (Low Rate)	SPIKE	79 bc
5	flufenacet + metribuzin[d] (High Rate)	SPIKE	63 c
6	pyroxasulfone mesosulfuron	PRE POST	62 c
7	pyroxasulfone pinoxaden	PRE POST	6 e
8	pyroxasulfone mesosulfuron	POST POST	150 b
9	pyroxasulfone pinoxaden	POST POST	16 d

[a] Means followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$.

[b] Treatments including mesosulfuron also include non-ionic surfactant.

[c] Abbreviations: PRE, preemergence; POST, postemergence; SPIKE, radical to 1 leaf crop emergence.

[d] Axiom Herbicide (Anonymous 2010).

Table 7. Comparison of herbicide programs.

Comparison	Significance[a]
Treated vs. Untreated	***
PRE vs. SPIKE	NS
PRE fb POST vs. PRE	***
POST vs. PRE	NS
PRE fb POST vs. SPIKE	**
SPIKE vs. POST	NS
PRE fb POST vs. POST	**

[a] NS = Not Significant, ** = $p < 0.05$, *** = $p < 0.001$

Table 8. Yields of wheat planted in Hertford, North Carolina and at the Piedmont Research Station near Salisbury, North Carolina[a].

Treatment No.	Treatment[b]	Application Timing[c]	Yield (kg/ha)	
			Hertford	Salisbury
1	non-treated	n/a	1425 c	4563 c
2	pyroxasulfone (Low Rate)	PRE	2475 b	5234 ab
3	pyroxasulfone (High Rate)	PRE	2675 b	5030 ab
7	flufenacet + metribuzin[d] (Low Rate)	SPIKE	2675 b	5291 ab
8	flufenacet + metribuzin[d] (High Rate)	SPIKE	2888 b	5362 a
9	pyroxasulfone mesosulfuron	PRE POST	3075 b	5199 ab
11	pyroxasulfone pinoxaden	PRE POST	4000 a	5460 a
12	pyroxasulfone mesosulfuron	POST POST	1463 c	4856 bc
14	pyroxasulfone pinoxaden	POST POST	2586 b	5493 a

[a] Means within a location followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$.

[b] Treatments including mesosulfuron also include non-ionic surfactant.

[c] Abbreviations: PRE, preemergence; POST, postemergence; SPIKE, radical to 1 leaf crop emergence.

[d] Axiom Herbicide (Anonymous 2010).

Figure 8. Regression line showing effect of Italian Ryegrass on yield of winter wheat.