ABSTRACT

GRIER, LOGAN A. Winter Wheat (*Triticum aestivum*) Tolerance to and Weed Control with Pyroxasulfone (Under the direction of Dr. Wesley J. Everman).

Approximately 2 billion bushels of wheat are produced by growers in the United States every year. Among other production concerns, weed management is an important aspect of winter wheat production. With the development and spread of herbicide-resistant weeds across the Unites States, alternative solutions for weed management are needed to maintain effective control. Pyroxasulfone was recently introduced for use in wheat and is effective on many weedy broadleaf and grass species, including those resistant to commonly used herbicides. Pyroxasulfone is a preemergence herbicide categorized as a very long chain fatty acid inhibitor affecting the growth of germinating seedlings. Greenhouse studies were conducted to investigate different levels of crop tolerance to pyroxasulfone of winter wheat varieties and winter wheat classes grown in the United States. Field studies were conducted in North Carolina to investigate the effects of pyroxasulfone application rate and timing on winter wheat tolerance and weed control efficacy and to investigate weed management programs to extend residual control and broaden the spectrum of weed control in winter wheat.

Together, these studies revealed that many factors are at play in winter wheat response to and weed control efficacy with pyroxasulfone, including application rate and timing, soil type, tillage practices, and precipitation patterns. Increased weed control was demonstrated with an increase in pyroxasulfone rate and with a decrease in soil OM and clay content. In conventional tillage, increased pyroxasulfone activity was observed in contrast to lower activity in no-till fields where surface residues moderated herbicidal activity. When

rainfall occurred in adequate amounts in close proximity to application timing, winter wheat injury increased. If little rainfall occurred around the timing of application, weed control efficacy suffered. While in most cases crop safety was demonstrated, there were cases where injury occurred due to a combination of pyroxasulfone rate, precipitation, coarser soil type and tillage practices. In field studies, winter wheat injury occurred in soils with low OM (1.25%) and clay (6.0%) content under conventional tillage and a heavy rainfall event within 24 hours of application. In greenhouse studies, winter wheat height reduction was demonstrated under regular precipitation with pyroxasulfone rates of 60 – 120 g a.i ha^{-1} applied PRE in a sandy loam soil, with a notable increase in injury with a decrease in soil OM and clay content. Additionally, winter wheat classes and varieties demonstrate a wide range of responses to pyroxasulfone applications. Soft white winter (SWW) wheat exhibited the greatest tolerance while soft red winter (SRW) wheat exhibited the least tolerance to pyroxasulfone.

PPL pyroxasulfone applications of 60 to 89 g a.i. ha^{-1} provided > 95% annual bluegrass control and PRE applications of 60 to 89 g a.i. ha^{-1} provided variable henbit control depending on soil texture and precipitation. Where control was lacking of some broadleaf weeds, combining with other herbicides of different MOA with pyroxasulfone enhanced weed control efficacy. Application timing plays an important role in pyroxasulfone activity. EPP and PPL provide greater weed control than PRE applications, which in turn provide greater weed control than POST. When applications were made after wheat tillering, increasing the pyroxasulfone rate from 60 to 74 g a.i. ha^{-1} increased control of annual bluegrass.

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Winter Wheat (*Triticum aestivum*) Tolerance to and Weed Control with Pyroxasulfone

by
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DEDICATION

I dedicate this work to my wonderful family. Thank you for your never-ending wisdom, love and encouragement.

BIOGRAPHY

Logan Grier grew up in the South Carolina countryside in the small town of Blacksburg. At a young age she learned the love of plants while spending summer days at the nursery where her mother worked. Through the influence of her mother and father, she learned the value of critically analyzing ideas, asking questions and researching answers. This planted the seed for a keen interest in science. In high school, an A.P. Biology course piqued her interest in genetics, and in 2004 she began her undergraduate degree at Clemson University studying genetics and horticulture. In May 2008, she graduated *summa cum laude* in the Honors program with a Baccalaureate of Science in Genetics and a minor in Horticulture. In June 2008, she moved the Raleigh, North Carolina to begin working in the Biology group at BASF Corporation as a contract greenhouse and lab technician focusing on pre-market herbicide research in various crops. In this role, her interest in agriculture and crop protection grew. In 2010, she gained full time employment with BASF as an Agricultural Biologist in the Biology Herbicide group. In the following years her knowledge of weed science and the BASF organization developed and deepened significantly. Through this experience she came to realize the importance of agriculture and crop protection in a growing world. With the goal of developing her career in this direction, she decided to pursue a Master of Science degree in Crop Science with a focus in Weed Science. In the autumn of 2011 Logan was accepted into the Crop Science program at North Carolina State University under the direction of Dr. Wesley J. Everman.

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LITERATURE REVIEW

Wheat (*Triticum aestivum*) is an important agronomic crop in the world, in the United States, and in North Carolina. Growers around the globe produce over 26 billion bushels of wheat per year (United States Department of Agriculture [USDA] 2014). The United States produces approximately 2 billion bushels per year, of which 44 million are produced in North Carolina (USDA 2014). The USDA categorizes wheat into five main classes including hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), white wheat and durum wheat (USDA 2015). Additionally, the white wheat class includes hard and soft and spring and winter varieties. Common wheat (*Triticum aestivum*) includes HRW, HRS, SRW and white wheat while durum wheat (*Triticum durum*) is considered a different species. In the United States, wheat can be categorized by the season in which it is grown: either winter or spring. Winter wheat accounts for 75% of wheat production in the United States (Agriculture Marketing Resource Center [AMRC] 2015). HRW wheat is grown in the Great Plains, accounting for 39% of United States wheat production (AMRC 2015). SRW wheat is grown in the Southeastern, Midwestern and Mid-South regions of the United States, accounting for 23% of national production (AMRC 2015). Soft white winter (SWW) is grown in the Pacific Northwest, Michigan and New York, accounting for 12% of national production (AMRC 2015). Hard white winter (HWW) is grown in the Northern Plains, accounting for < 1% of national production (AMRC 2015).

In North Carolina, SRW wheat is planted in mid- to late- November and harvested around June (Weisz 2013). As in many agronomic cropping systems, an effective weed management program plays an important role in a successful wheat growing season. Grass weed species that are typically found in North Carolina wheat production include annual bluegrass (*Poa annua*) and Italian ryegrass (*Lolium multiflorum*). Among others, broadleaf weed species that are typically found in North Carolina wheat production include common chickweed (*Stellaria media*), henbit (*Lamium amplexicaule*), mouse-ear chickweed (*Cerastium vulgatum*), and field speedwell (*Veronica agrestis*). Some weeds are of particular concern because of their competitive nature, competing for nutrients, water and sunlight and thus creating the potential to reduce grain yield (Blackman and Templeman 1938). Italian ryegrass, a prevalent weed throughout the United States, has been shown to reduce wheat yield by 20 to 39% (Appleby et al. 1976; Scursoni et al. 2012). Hashem et al. (1998) demonstrated as little as nine Italian ryegrass plants in 100 wheat plant m^{-2} reduced wheat yield by 33%.

Historically, growers depended on weed management programs including herbicides with modes of action (MOA) inhibiting acetyl CoA carboxylase (ACCase) including pinoxaden and diclofop-methyl; inhibiting acetolactate synthase (ALS) including mesosulfuron-methyl, thifensulfuron-methyl, and pyroxsulam; or inhibiting microtubule assembly including pendimethalin (Weed Science Society of America [WSSA] 2014). Managing weed populations with these commonly used herbicides is becoming increasingly difficult due to widespread herbicide resistant weed populations. Italian ryegrass populations across the southeast have developed resistance to ACCase and/or ALS inhibiting herbicides (Heap 2015; Taylor 2013; Salas et al. 2013). Annual bluegrass populations in the southeast have developed resistance to microtubule synthesis and ALS inhibiting herbicides (Brosnan et al. 2014; Heap 2015). ALS inhibitor resistance has been reported in henbit populations in Kansas and common chickweed populations in the northeastern United States into Kentucky and Virginia (Heap 2015).

Outside of the difficulties created by herbicide resistance, each of the herbicide programs currently used by growers offers its own set of benefits and challenges. For example, flufenacet + metribuzin is labeled for control of many problematic grass and broadleaf weeds and also offers alternative modes of action of inhibiting VLCFA synthesis and photosystem II, respectively (Bayer CropScience 2007; Lechelt-Kunze et al. 2003). However, application timing in North Carolina can be a challenge. Flufenacet + metribuzin is labeled for POST applications to wheat but must be applied prior to weed emergence for effective control (Bayer CropScience 2007). For weeds that germinate concurrently with winter wheat, such as Italian ryegrass and annual bluegrass, this creates a narrow application window for effectively controlling early season weeds. Synthetic auxin herbicides, including dicamba and 2,4-D, effectively control many broadleaf weeds, but have little activity on grasses. They also must be applied after wheat is fully tillered; therefore, must be combined with an early herbicide application to control weeds germinating early in the season.

Pyroxasulfone, a recently discovered herbicide, shows promise as an effective alternative for NC wheat growers (Porpiglia 2005). It is an isoxazoline compound which inhibits very long chain fatty acid (VLCFA) synthesis, preventing shoot elongation of germinating seedlings (Tanetani et al. 2009; 2013). The Herbicide Resistance Action

Committee categorizes this MOA in group K3 (HRAC 2014) and the Weed Science Society of America categorizes it in group 15 (WSSA 2014). Registration of pyroxasulfone for use in wheat introduces an alternative MOA for delayed preemergence applications in wheat. The target application timing for pyroxasulfone ranges from delayed preemergence (PRE) applications in the fall to applications to four-tiller wheat (POST) with a target rate range between 42 and 150 g a.i. ha^{-1} depending on soil type and application timing (BASF Corporation 2013; FMC Corporation 2014; Valent U.S.A Corporation 2014).

The physical and chemical properties of pyroxasulfone, including a low soil organic carbon-water partitioning coefficient (K_{oc}) of 57-119 mL/g, a moderate to low distribution coefficient (K_d) of 0.32-9.57 mL/g, and a low water solubility (K_s = 2.9-3.5 mg/L), indicate its potential to remain readily available in the germination zone of soil, without binding tightly to soil particles or leaching downward through the soil profile (Australian Pesticides and Veterinary Medicines Authority [APVMA] 2011; Westra 2012). Additionally, researchers have demonstrated that pyroxasulfone is more persistant than other VLCFA synthesis inhibitors including acetochlor, dimethenamid-*p*, flufenacet and *S*-metolachlor, indicating the possibility for longer residual weed control activity in the soil (Mueller & Steckel 2011; Szmigielski et al. 2014; Westra et al. 2014).

While pyroxasulfone has a low sorption coefficient, it is important to note that researchers have reported effects of soil OM and clay content on herbicidal activity (Knezevic et al. 2009; Szmigielski et al. 2013; Walsh et al. 2011; Westra 2012). Westra (2012) demonstrated a direct correlation between OM content and sorption coefficients for three VLCFA synthesis inhibitors, including dimethenamid-*p*, *s*-metolachlor and pyroxasulfone. As OM increased from 0.8% to 6.25%, the sorption coefficient of pyroxasulfone increased from 0.53 to 5.91 (Westra 2012). In this study, Westra (2012) noted that clay content was not strongly correlated with sorption coefficients. However, field and greenhouse studies suggest that OM and clay content both play a role, though perhaps OM more significantly (Szmigielski et al. 2013; Walsh et al. 2011). In a bioassay investigating the effects of pH, clay content, and organic carbon content on pyroxasulfone soil bioactivity, Szmigielski et al. (2013) reported organic carbon was most closely correlated to bioactivity. Walsh et al. (2011) demonstrated in greenhouse studies that higher rates of pyroxasulfone were required to control 50% of rigid ryegrass (*Lolium rigidum*) in soils with higher OM and higher clay content. Knezevic et al. (2009) observed in field studies that an increase in OM from 1 to 2 to 3%, required an increase in pyroxasulfone rate from 143 to 165 to 202 g a.i. ha^{-1}, respectively, to control 90% of green foxtail (*Setaria viridis*).

Other factors can also have an effect on PRE herbicide activity, including tillage practices and precipitation patterns. For example, Mahoney et al. (2014) reported that significantly higher rates of a pyroxasulfone + flumioxazin were needed to control 80% of pigweed species (*Amaranthus* sp.) in no-till compared to conventional tillage. Mueller and Hayes (1997) demonstrated more complete weed control and longer residual control with herbicides, including VLCFA inhibitors among others, in no-till compared to tilled fields. Lack of timely and adequate rainfall can also interfere with PRE herbicide activity, reducing weed control efficacy (Stewart et al. 2010; Stewart et al. 2012). On the other hand, an abundance of rainfall at just the right time can increase crop injury. For example, Olson et al. (2011) reported that in most cases pyroxasulfone was safe on sunflowers; however, injury occurred in one instance when heavy rainfall occurred within a week of planting and herbicide application. Geier et al. (2009) demonstrated an increase in early season sorghum injury with PRE applications of pyroxasulfone and flufenacet when rainfall occurred within a week of planting and herbicide application. This can be attributed to herbicide made available in the soil solution during the time in which the crop is germinating, leading to injury.

Pyroxasulfone and other VLCFA synthesis inhibitors are active on a wide range of grasses and broadleaf weeds. Bond et al. (2014) demonstrated ≥ 93% control of glyphosate-resistant Italian ryegrass with a fall applied PRE application of 160 g a.i. ha^{-1} of pyroxasulfone or 1790 g a.i. ha^{-1} of s-metolachlor. Boutsalis et al. (2014) reported 79-96% control of trifluralin resistant rigid ryegrass (*Lolium rigidum*) with PRE applications of 150 g a.i. ha^{-1} pyroxasulfone in no till wheat, also noting that PRE applications provide significantly higher weed control than POST applications. Hulting et al. (2012) observed 65-78% Italian ryegrass control with 50 g a.i. ha^{-1} pyroxasulfone applied PRE, increasing to 90-100% control when the rate was increased to 150 g a.i. ha^{-1}. Hulting et al. (2012) also demonstrated variable ivyleaf speedwell (*Veronica hederifolia*) control from 35-86%. Lawrence and Burke (2014) showed > 74% control of rattail fescue consistently across multiple years with PRE applications of 100 g a.i. ha^{-1} of pyroxasulfone with no effect on wheat yield. Olson et al. (2011) demonstrated 95% control of large crabgrass (*Digitaria* *sanguinalis*) with a PRE application of 167 g a.i. ha^{-1} and 90% control of green foxtail with a PRE application of 333 g a.i. ha^{-1} of pyroxasulfone. Bhagirath et al. (2007) reported > 80% control of rigid ryegrass with 480 – 960 g a.i. ha^{-1} of s-metolachlor, another herbicide inhibiting VLCFA synthesis, applied pre-plant (PPL) and 71-83% control when applied early pre-plant (EPP). Ritter and Menbere (2002) observed ≥ 85% control of Italian ryegrass with 430 g a.i. ha^{-1} of s-metolachlor, reporting minor stunting at this rate; however, yield was not affected.

While researchers have demonstrated safety of pyroxasulfone and other VLCFA inhibitors in winter wheat, injury has been reported in certain cases (Boutsalis et al. 2014; Hulting et al. 2012; Bhagirath et al. 2007). Researchers shown that PRE and pre-plant incorporated (PPI) applications of 100 g a.i. ha^{-1} resulted in no phytotoxic effects on wheat and did not affect yield (Kleemann et al. 2014; Lawrence and Burke 2014). Kleemann et al. (2014) demonstrated safety on winter wheat with pyroxasulfone application of 100 g a.i. ha^{-1} applied PPI in a no-till system in a sandy loam. A significant reduction in wheat establishment was demonstrated with 150 g a.i. ha^{-1} of pyroxasulfone applied PPL and PRE; however, yield increases were reported due to reduced ryegrass competition (Boutsalis et al. 2014; Hulting et al. 2012). Bhagirath et al. (2007) demonstrated PPL applications of 480 – 960 g a.i. ha^{-1} of s-metolachlor resulted in significant reduction in wheat plant density translating to a significant yield reduction, while the EPP application had no phytotoxic effects on wheat. Ritter and Menbere (2002) reported minor stunting with 430 g a.i. ha^{-1} of s-metolachlor; however, yield was not affected.

Considering winter wheat tolerance more deeply and taking into account the methods of breeding, it is possible that different wheat varieties and wheat classes may respond differently to pyroxasulfone applications. Within each wheat class there are many varieties available. When developing new wheat varieties, breeding selection is based largely on the environmental factors of the region in which it will be grown. Wheat varieties grown within the same region of the United States are typically more closely related to each other than to those grown in other regions. This can be attributed to more commonalities in their pedigrees, adaptation, biotic and abiotic stress resistances, and end-use quality requirements. While limited research is available on differential response of wheat varieties or wheat classes to herbicides, researchers have demonstrated this in some cases (Shaw and Wesley 1991; Schroeder et al. 1986; Sikkema et al. 2007). For example, researchers observed that cultivars within SRW class responded differentially to postemergence (POST) herbicides, including metribuzin and diclofop (Shaw and Wesley 1991; Schroeder et al. 1986). Additionally, Sikkema et al. (2007) reported a differential wheat response based on wheat class to some POST herbicides, including dicamba + MCPA + mecoprop, and not others, including 2,4-D amine, dichlorprop + 2,4-D and bromoxynil + MCPA. The lack of differential response to the latter herbicides was attributed to the fact that there was no wheat injury at any rate for any wheat class from these herbicides (Sikkema et al. 2007). Additionally, in that study, more injury was demonstrated in SRW and SWW than in HRW (Sikkema et al. 2007).

Growers confronted by the challenges of controlling herbicide resistant weed species, can look to pyroxasulfone as an effective alternative to current programs. Researchers have reported safety in many crops and effective control of various weed species; however, more research is needed to better understand underlying factors that may affect herbicidal activity of pyroxasulfone on winter wheat and weed species. The effect of wheat class and wheat variety in pyroxasulfone herbicide activity has not been fully explored. Researchers have demonstrated in some cases that wheat classes and wheat varieties within a class respond differently to herbicide applications (Schroeder et al. 1986; Shaw and Wesley 1991; Sikkema et al. 2007). Because of the different genetic backgrounds of different wheat classes and of different wheat varieties, it is possible they will respond differently to pyroxasulfone applications. These studies seek to better understand the different levels of crop tolerance to pyroxasulfone across the four winter wheat classes grown in the United States and the different levels of crop tolerance to pyroxasulfone in soft red wheat varieties commonly grown in the Southeastern United States.

Another challenge with pyroxasulfone in winter wheat is application timing. Because pyroxasulfone affects germinating plants, ideal application timing is after wheat germination and prior to weed species germination in order to achieve the most effective weed control while maintaining crop safety (Tanetani et al. 2009; 2013). For weed species that germinate concurrently with wheat this can be problematic. For example, in North Carolina it is common for winter annual grasses such as annual bluegrass and Italian ryegrass to germinate concurrently with winter wheat (Everman and Jordan 2013). This creates a scenario where timely weed control applications of pyroxasulfone could increase the risk of crop injury. Researchers have demonstrated that wheat metabolizes pyroxasulfone more efficiently than rigid ryegrass, but injury in wheat has been demonstrated in field scenarios (Boutsalis et al. 2014; Hulting et al. 2012; Tanetani 2013). These studies aim to investigate the effect of application rate and timing of pyroxasulfone on winter wheat tolerance and weed control.

While pyroxasulfone is effective in controlling grasses and broadleaf weeds, there are some broadleaf weeds in North Carolina that are either not controlled or are only suppressed by pyroxasulfone. Pairing pyroxasulfone with a broadleaf herbicide may be an option for a more complete herbicide program. Additionally, some weed species have an initial flush in the fall followed by a second flush later in the season. If this later flush occurs after the residual activity of an early application of pyroxasulfone, a split application may provide better weed control. These studies were designed to compare weed control and winter wheat tolerance with pyroxasulfone herbicide programs compared to the standard POST programs.

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WINTER WHEAT VARIETY TOLERANCE TO PYROXASULFONE

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Introduction

Growers around the world produce 26 billion bushels of wheat per year, of which the United States contributes approximately 2 billion bushels (United States Department of Agriculture [USDA] 2014). The USDA categorizes wheat into five main market classes including hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), white wheat and durum wheat (USDA 2015). Additionally, the white wheat class can be broken down further into hard, soft, winter and spring varieties (USDA 2015). Common wheat (*Triticum aestivum*) includes HRW, HRS, SRW and white wheat, while durum wheat (*Triticum durum*) is considered a different species. In the United States wheat can be categorized by the season in which it is grown: either winter or spring. Winter wheat accounts for 75% of wheat production in the United States (Agriculture Marketing Resource Center [AMRC] 2015). HRW wheat is grown in the Great Plains, accounting for 39% of United States production (AMRC 2015). SRW wheat is grown in the Southeast, Midwest and Mid-South, accounting for 23% (AMRC 2015). Soft white winter (SWW) is grown in the Pacific Northwest, Michigan and New York, accounting for 12% (AMRC 2015). Hard white winter (HWW) is grown in the Northern Plains, accounting for < 1% (AMRC 2015).

Among other management concerns in winter wheat production in the United States, weed management plays an important role. Managing weed populations is becoming more challenging due to widespread resistance to commonly used herbicides (Brosnan et al. 2014; Heap 2015; Salas et al. 2013; Taylor 2013). For a number of years, growers have depended on herbicides with modes of action (MOA) inhibiting acetyl CoA carboxylase (ACCase), acetolactate synthase (ALS) or microtubule assembly. A recently introduced delayed preemergence herbicide, pyroxasulfone, shows promise as an effective alternative for wheat growers (Porpiglia et al. 2005). Pyroxasulfone inhibits very long chain fatty acid (VLCFA) synthesis, preventing shoot elongation of germinating seedlings (Tanetani et al. 2009, Tanetani et al. 2013). The Herbicide Resistance Action Committee categorizes this MOA in group K3 (HRAC 2014) and the Weed Science Society of America categorizes it in group 15 (WSSA 2014). Researchers have demonstrated the effectiveness of pyroxasulfone and other VLCFA synthesis inhibitors in controlling a wide range of grasses and broadleaf weeds including glyphosate-resistant and susceptible Italian ryegrass populations (*Lolium multiflorum*), trifluralin-resistant and susceptible rigid ryegrass populations (*Lolium rigidum*), large crabgrass (*Digitaria sanguinalis*), green foxtail (*Setaria viridis*) (Bhagirath et al. 2007; Bond et al. 2014; Boutsalis et al. 2014; Hulting et al.; 2012 Olson et al. 2011; Ritter and Menbere 2007). The target application timing for pyroxasulfone ranges from fall applications prior to wheat emergence to winter postemergence (POST) applications over the top of wheat with four tillers with a target rate between 42 and 150 g a.i. ha^{-1} depending on soil type and application timing. (BASF Corporation 2013; FMC Corporation 2014; Valent U.S.A. Corporation 2014). For coarse soils, like the one used in the study, the rate range for a delayed PRE application of pyroxasulfone is 42 – 60 g a.i. ha^{-1} (BASF Corporation 2013).

While researchers have demonstrated safety of pyroxasulfone on winter wheat, injury has been noted in some field studies with higher rates of pyroxasulfone (Bhagirath et al. 2007; Boutsalis et al. 2014; Hulting et al. 2012). Researchers demonstrated safety in winter wheat with pre-plant incorporated (PPI) and PRE applications of 100 g a.i. ha^{-1} of pyroxasulfone (Kleemann et al. 2014; Lawrence and Burke 2014). A reduction in wheat establishment was demonstrated with 150 g a.i. ha^{-1} of pyroxasulfone applied preplant (PPL) and preemergence (PRE); however, yield increases were reported due to a result of decreased ryegrass competition in these studies (Boutsalis et al. 2014; Hulting et al. 2012).

While researchers have studied the safety of pyroxasulfone in wheat, there is little research investigating differential response of wheat class or wheat variety to pyroxasulfone. Due to the genetic backgrounds of different wheat classes and varieties, it is possible they will respond differently to herbicides such as pyroxasulfone. Wheat breeding selection is based largely on environmental factors of the region in which it will be grown. Wheat varieties grown within the same region of the United States will usually be more closely related to each other than those grown in other regions due to more commonalities in their pedigrees, adaptation, biotic and abiotic stress resistances, and end-use quality requirements. These studies seek to better understand the varying levels of crop tolerance to pyroxasulfone in varieties of soft red winter wheat commonly grown in the Southeast United States and to investigate different levels of crop tolerance to pyroxasulfone in wheat classes grown across the United States. The findings from this study will provide a basis for more effective use of pyroxasulfone in different wheat classes and regions.

Materials and Methods

Two separate studies were established to investigate the response to pyroxasulfone of commonly grown soft red winter wheat varieties in the Southeast U.S. (Single-class Study) and also to determine if tolerance to pyroxasulfone varies in hard or soft, red and white wheat varieties from around the country (Multi-class Study).

Single-class Study

A single-class study was conducted utilizing a factorial treatment arrangement of herbicide rates and wheat varieties. This initial study was conducted to compare crop response of ten SRW wheat varieties to pyroxasulfone[1]. Treatments were arranged in a 3 x 10 factorial randomized complete block experimental design including four replications, consisting of three pyroxasulfone rates and ten SRW wheat varieties. To optimize conditions for observing differences in injury between treatments, 1x and 2x rates (60 and 120 g a.i ha^{-1}, respectively) of pyroxasulfone for medium soil textures were applied PRE, including non-treated control pots for comparison. Varieties commonly planted in North Carolina were chosen for the study (Table 1). Eight winter wheat seeds of each variety were individually planted 1.3 cm deep in 7.6 x 7.6 cm plastic pots[2] filled with North Carolina dark sand[3], a Candor sandy loam containing 82% sand, 13% silt, 5% clay, and 3.6% organic matter (OM). Herbicides were applied after seeding with a spray chamber[5] calibrated to deliver 140 L ha^{-1} with an 80015E nozzle at 207 kPa. The study was conducted twice over different months. Similar environmental conditions in the greenhouse were maintained by using supplemental light and temperature control to ensure a minimum light intensity of 21,500 lumens m^{-2}, a minimum day-length of 14 hours, and average day and night temperatures of 27^{o}C and 24^{o}C, respectively. Pots were watered overhead twice daily totaling 0.7 cm per day. Percent germination was assessed after wheat emergence, and height reduction was assessed at 7, 14, 21 and 28 days after planting. Effects of pyroxasulfone rate on percent SRW wheat variety germination and height reduction at 7, 14, 21 and 28 DAT were analyzed.

Data were subjected to analysis of variance (ANOVA) by using PROC GLM procedure in SAS 9.4[6]. Mean comparisons were performed using Fisher's Protected LSD test when F values were statistically significant ($P \leq 0.05$). Fixed effects included herbicide treatments, SRW wheat varieties, and the subsequent interaction. Replications over time and within each trial run, amounting to 8 replications per treatment, were considered random effects. Germination and height reduction were measured as a percentage of the non-treated; therefore, statistical analysis omits data from non-treated plots.

Multi-class Study

A multi-class study was conducted utilizing a factorial treatment arrangement of 3 pryoxasulfone[1] rates applied on 25 varieties of wheat representing four winter wheat classes grown in the U.S.: soft red, soft white, hard red, and hard white. Treatments were arranged in a 3 x 25 factorial randomized complete block experimental design including four replications. To optimize conditions for observing differences in injury between treatments, 1x and 2x rates (60 and 120 g a.i ha^{-1}, respectively) of pyroxasulfone in medium soil texture were applied PRE, including non-treated control pots for comparison. Varieties were chosen based on those commonly planted in the region in which they are grown (Table 1). Eight seeds of each variety were planted 1.3 cm deep in individual 7.6 x 7.6 cm plastic pots[2] filled with North Carolina light sand[4], a Candor loamy sand containing 84% sand, 14% silt, 2% clay and 2% OM. Herbicides were applied in a spray chamber[5] calibrated to deliver 140 L ha^{-1} with an 80015E nozzle at 30 PSI. The study was conducted twice over different months. Similar environmental conditions in the greenhouse were maintained by using supplemental light and temperature control to ensure a minimum light intensity of 21,500 lumens m^{-2}, a minimum day-length of 14 hours, and average day and night temperatures of $27^{o}C$ and $24^{o}C$, respectively. Pots were watered overhead twice daily totaling 0.7 cm per day. Percent germination was assessed after wheat emergence, and height reduction was assessed at 7, 14, 21 and 28 days after planting. Winter wheat germination and height reduction were analyzed at 7, 14, 21 and 28 DAT.

Data were subjected to ANOVA by using PROC GLM procedure in SAS 9.4[6]. Mean comparisons were performed using Fisher's Protected LSD test when F values were statistically significant ($P \leq 0.05$). Fixed effects included herbicide treatment, winter wheat class, winter wheat variety, pyroxasulfone rate, and the subsequent interactions. Wheat class and wheat variety were analyzed in different models to avoid confounding the two variables. Replications over time and within each trial run, amounting to 8 replications per treatment, were considered random effects. Germination and height reduction were measured as a percentage of the non-treated; therefore, statistical analysis omits data from non-treated plots.

Results and Discussion

Single-class Study

No significant differences in germination were observed for pyroxasulfone rate or winter wheat variety (data not shown). While pyroxasulfone prevents shoot elongation of germinating seedlings, generally the seedlings in this study at least cracked the surface of the soil, reaching spike to two-leaf stage over the course of 28 days with height reduction as the predominant injury symptom (Tanetani et al. 2009, 2013).

Analysis of height reduction indicated an interaction between pyroxasulfone rate and SRW wheat variety for all timings; therefore, data are presented by rate and variety ($P \leq 0.05$). Additionally, the F value for rate effect was > 20x higher than the F value for the rate-by-variety interaction; therefore, the rate main effect is also presented, pooled across varieties.

Across all varieties, height reduction resulting from 60 g a.i. ha^{-1} of pyroxasulfone was minor and transient with ≤ 11% height reduction observed across all varieties at 14 DAT and declining to ≤ 4% at 28 DAT (Table 2). The two Southern States varieties, SS 8340 and SS 8404, responded similarly to 60 and 120 g a.i. ha^{-1} pyroxasulfone applications over time, likely an indication of their similar genetic pedigrees (Table 2). At 14 DAT, height reduction resulting from 120 g a.i. ha^{-1} of pyroxasulfone ranged from 11% to 43% declining to a range of 0% to 32% at 28 DAT (Table 2). Other researchers have also reported differential SRW varietal responses to herbicides, including metribuzin and diclofop (Shaw and Wesley 1991; Schroeder et al. 1986).

Pooled across SRW wheat varieties, pyroxasulfone rate had a significant main effect on height reduction at all rating timings (Figure 1). From 7 to 28 DAT, SRW wheat height reduction declined from 12% to 1% with PRE applications of 60 g a.i. ha^{-1} and 36% to 14% with 120 g a.i. ha^{-1} of pyroxasulfone (Figure 1). As demonstrated in this greenhouse study and by researchers in field studies: an increase in pyroxasulfone rate can result in an increase in winter wheat injury (Hulting et al. 2012; Walsh et al. 2011).

Multi-class Study

No significant differences in germination were observed between pyroxasulfone rates, winter wheat classes or winter wheat varieties (data not shown). The predominant injury symptom observed in this study was height reduction. Even seedlings more affected by pyroxasulfone cracked the surface of the soil at least reaching spike stage.

At 7, 14, 21 and 28 DAT, height reduction was affected by the main effects of pyroxasulfone rate, winter wheat class and winter wheat variety with no rate-by-class or rate-by-variety interactions observed; therefore, data are presented by rate pooled across all varieties, by variety pooled across pyroxasulfone rates, and by class pooled across pyroxasulfone rates and varieties within each class ($P \leq 0.05$).

From 7 to 28 DAT, winter wheat height reduction declined from 61% to 31% with PRE applications of 60 g a.i. ha^{-1} and 77 to 48% with 120 g a.i. ha^{-1} of pyroxasulfone (Figure 2). Walsh et al. (2011) reported crop safety when 42 g a.i. ha^{-1} pyroxasulfone was applied PRE, while increasing to 100 g a.i. ha^{-1} resulted in a 39% plant biomass reduction in a loam soil with 2.3% OM. Researchers have also demonstrated a reduction in wheat establishment with 150 g a.i. ha^{-1} of pyroxasulfone applied PPL and PRE (Boutsalis et al. 2014; Hulting et al. 2012). Boutsalis et al. (2014) reported no wheat injury where pyroxasulfone was applied at 60 and 120 g a.i. ha^{-1} in Chromosol and Calcarosol soils in Australia.

In general, the SWW wheat class was more tolerant to pyroxasulfone than other classes evaluated in this study (Figure 3). Pooled across rates, SWW wheat was least affected by pyroxasulfone applications, resulting in 58% height reduction at 7 DAT and declining to 24% by 28 DAT (Figure 3). By contrast, SRW wheat had 75% height reduction at 7 DAT which declined to 47% by 28 DAT (Figure 3). HRW wheat injury, 71 and 44% at 7 and 28 DAT, respectively, was not statistically different from SRW wheat injury. HWW wheat injury was not statistically different from other classes (Figure 3), but with only one variety representing this class, further study is required to confirm this.

Across wheat classes and within wheat classes considerable differences in variety tolerance to pyroxasulfone were demonstrated (Figure 4). The least affected SRW wheat varieties responded similarly to the SWW wheat varieties. Across all classes, winter wheat injury at 28 DAT ranged from 12% to 66% height reduction (Figure 4). Within SRW wheat class, the height reduction at 28 DAT ranged from 22% to 60% (Figure 4). The wide range of response within a wheat class points to the unlikeliness that alleles encoding tolerance are associated with primary agronomic traits currently selected during the breeding process and suggests there may have been no selective advantage for these alleles within breeding nurseries prior to the use of pyroxasulfone (Ma et al. 2012). The wide range in tolerance levels between varieties shows the importance of variety selection for pyroxasulfone.

It is important to note that while these studies were analyzed separately, the height reduction of SRW wheat in the previously discussed single-class study is lower overall than observed in the SRW wheat in the multi-class study (Figures 1 & 3). In both studies, conditions were favorable to crop injury: applying pyroxasulfone at planting, doubling the labeled rate, and watering thoroughly and regularly to ensure herbicide activation. While these soils were from the same source, soil testing revealed that the soil in the multi-class study contained lower OM (2% instead of 3.6%) and clay content (2% instead of 5%) than the soil in the single-class study. While this change in soil texture was unintentional, it proved to aid in understanding the nature of pyroxasulfone activity. At least one common variety was included in both studies: AGS 2035. In the multi-class study, 86% height reduction of AGS 2035 was observed with 120 g a.i. ha^{-1} at 7 DAT, while in the single-class study only 48% injury was observed (data not shown). The watering patterns, light regiments, and seed depth were consistent across the trials; therefore, these differences may be attributed to differences in soil properties. Researchers have demonstrated a reduction in pyroxasulfone activity in finer texture soils with higher OM and/or clay content (Knezevic et al. 2009; Szmigielski et al. 2013; Walsh et al. 2011; Westra 2012).

Conclusion

These studies demonstrate that many factors are at play in winter wheat response to pyroxasulfone, including winter wheat class and variety, pyroxasulfone rate, and potentially soil properties. An increase in winter wheat injury was observed with an increase in pyroxasulfone rate and with a decrease in soil OM and clay content. In this study, SWW wheat exhibited the greatest tolerance, indicating the possibility for more flexibility in pyroxasulfone applications in the PNW region of the United States. On the other hand, SRW wheat exhibited the least tolerance to pyroxasulfone applications, potentially indicating less flexibility in pyroxasulfone applications made in the Southeast and Mid-South regions of the United States. These results indicate the possibility that different guidelines may be appropriate for pyroxasulfone applications across growing regions. Because this study was based on a small number of varieties within classes ($1 \leq n \leq 12$), and because these data demonstrate the large variability in tolerance across varieties within classes, future studies with larger sample sizes are needed.

Differences among wheat classes may be attributed to the differences in pedigrees. Furthermore, differences within wheat classes may be attributed to the unlikeliness that alleles encoding tolerance are associated with primary agronomic traits selected during the breeding process. Future studies investigating the tolerance level of a more robust sample size of wheat varieties with known pedigrees would aid breeders to map tolerance to pyroxasulfone and to target those genes in their breeding programs. In the future, a more robust field study could also be conducted to see if and when height reduction gives rise to yield reduction.

Sources of Materials

[1]Pyroxasulfone Herbicide, BASF Corporation, Research Triangle Park, NC 27709

[2]Black Plastic Pots, Hummer International, Topeka, KS 66618

[3]North Carolina Dark Sand, Sands and Soils, Durham, NC 27703

[4]North Carolina Light Sand, Sands and Soils, Durham, NC 27703

[5]Spray Chamber, New-Tech™, Midland, MI 48641

[6]SAS Statistical Software, Version 9.4, Cary, NC 27513

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Table 1. Winter wheat varieties used in single-class study and multi-class study, categorized by wheat class.

Single-class Study	Multi-class Study			
Soft Red	**Soft Red**	**Soft White**	**Hard Red**	**Hard White**
113[a]	AGS 2020[c]	Brundage 96[m]	Armour[r]	Danby[t]
9223[b]	AGS 2035[c]	Eltan[n]	Hatcher[s]	
AGS 2035[c]	AGS 2060[c]	Madsen[o]	Jagalene[f]	
NC Yadkin[d]	Coker 9553[f]	ORCF 102[p]	Jagger[t]	
P26R41[e]	DG 9171[i]	Tubbs[q]	OK Bullet[u]	
Oakes[f]	Fleming[j]	Xerpha[o]		
Shirely[b]	GA Gore[k]			
SS 8340[g]	LA 821[l]			
SS 8404[g]	Magnolia[f]			
USG 3120[h]	P25R32[e]			
	P26R31[e]			
	SS 8641[g]			

[a]113, Beck’s Hybrids, Atlanta, IN 46031

[b]9223 and Shirley, Dyna-Gro, Richmond, CA 94806

[c]AGS 2020, AGS 2035, AGS 2060, AGSouth Genetics, Albany, GA 31708

[d]NC Yadkin, North Carolina State University, Raleigh, NC 27695

[e]P25R32, P26R31, P26R41, Pioneer Hi-Bred International, Inc.

[f]Coker 9553, Magnolia, Jagalene, Oakes, AgriPro, Greensboro, NC 27409

Table 1 continued.

[g]SS 8641, SS 8340, SS 8404, Southern States Cooperative, Richmond, VA23260

[h]USG 3120, UniSouth Genetics, Inc., Dickson, TN 37055

[i]DG 9171, Dyna-Gro, Richmond, CA 94806

[j]Fleming, Georgia AES, Griffin, GA 30223

[k]GA Gore, Georgia AES, Griffin, GA 30223

[l]LA 821, Terral Seed, Rayville, LA 71269

[m]Brundage 96, Idaho AES, USDA-ARS, Moscow, ID 83844

[n]Eltan, Washington AES, USDA-ARS, Prosser, WA 99350

[o]Madsen, Xerpha, Washington ARC, Prosser, WA 99350; Oregon AES, USDA-ARS, Hermiston, OR 97838; Idaho AES, USDA-ARS, Moscow, ID 83844

[p]ORCF 102, Oregon AES, USDA-ARS, Hermiston, OR 97838; BASF Corporation, Research Triangle Park, NC 27709

[q]Tubbs, Oregon AES, USDA-ARS, Hermiston, OR 97838

[r]Armour, WestBred, St. Louis, MO 63167

[s]Hatcher, Colorado AES, Fort Collins, CO 80523

[t]Danby, Jagger, Kansas AES, Manhattan, KS 66506

[u]OK Bullet, Oklahoma Genetics Inc., Stillwater, OK 74076

Table 2: Percent height reduction of ten soft red winter wheat varieties at 14 and 28 days after preemergence application of pyroxasulfone in single-class study.[ab]

	14 DAT		28 DAT	
	Pyroxasulfone rate (g a.i. ha-1)			
Variety	60	120	60	120
	----------------------------------%----------------------------------			
P26R41	3 f	11 def	0 e	0 e
Oakes	8 ef	12 def	4 de	8 cde
SS 8340	3 f	14 de	0 e	13 bcd
SS 8404	4 ef	14 de	0 e	13 bcd
USG 3120	4 ef	19 cd	0 e	14 bcd
9223	4 ef	30 bc	0 e	19 b
Shirley	11 def	30 bc	0 e	8 cde
NC Yadkin	11 def	31 b	0 e	22 ab
113	8 ef	34 ab	0 e	16 bc
AGS 2035	6 ef	43 a	0 e	32 a

[a] Abbreviations: DAT, days after treatment.

[b] Means within a single rating timing followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

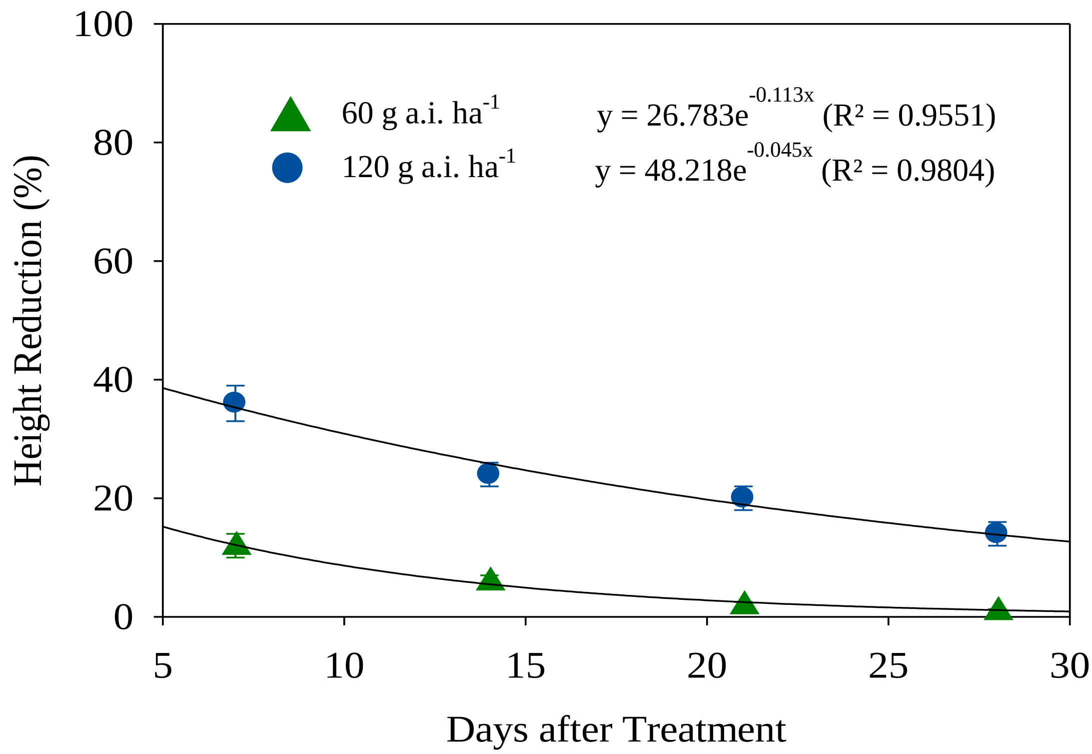

Figure 1. Height reduction of soft red winter wheat as affected by pyroxasulfone rate pooled across varieties in single-class study.[ab]

[a] Abbreviations: g a.i. ha^{-1}, grams of active ingredient per hectare.

[b] Error bars represent 95% confidence intervals for percent height reduction

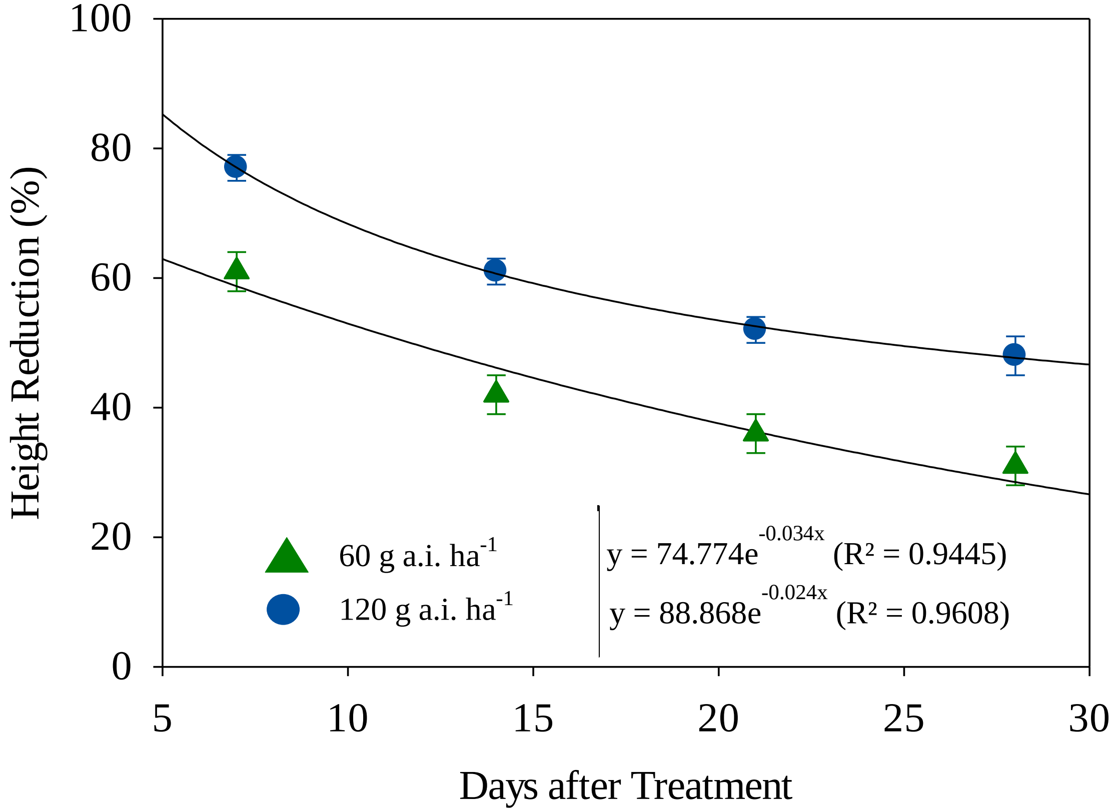

Figure 2. Height reduction of winter wheat as affected by pyroxasulfone rate pooled across varieties in multi-class study.[ab]

[a] Abbreviations: g a.i. ha^{-1}, grams of active ingredient per hectare.

[b] Error bars represent 95% confidence intervals for percent height reduction.

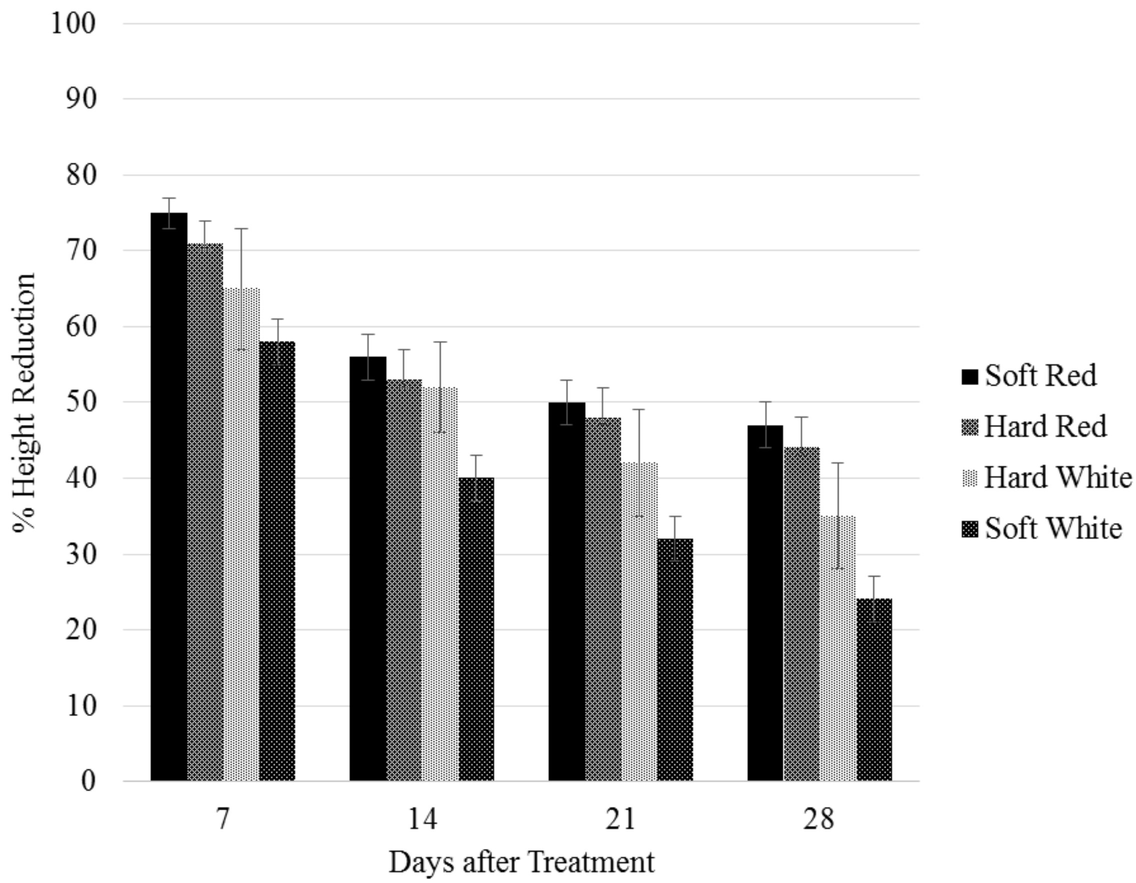

Figure 3. Height reduction of winter wheat treated with pyroxasulfone as affected by winter wheat class pooled across pyroxasulfone rates and varieties in multi-class study.[a]

[a] Error bars represent 95% confidence intervals for percent height reduction.

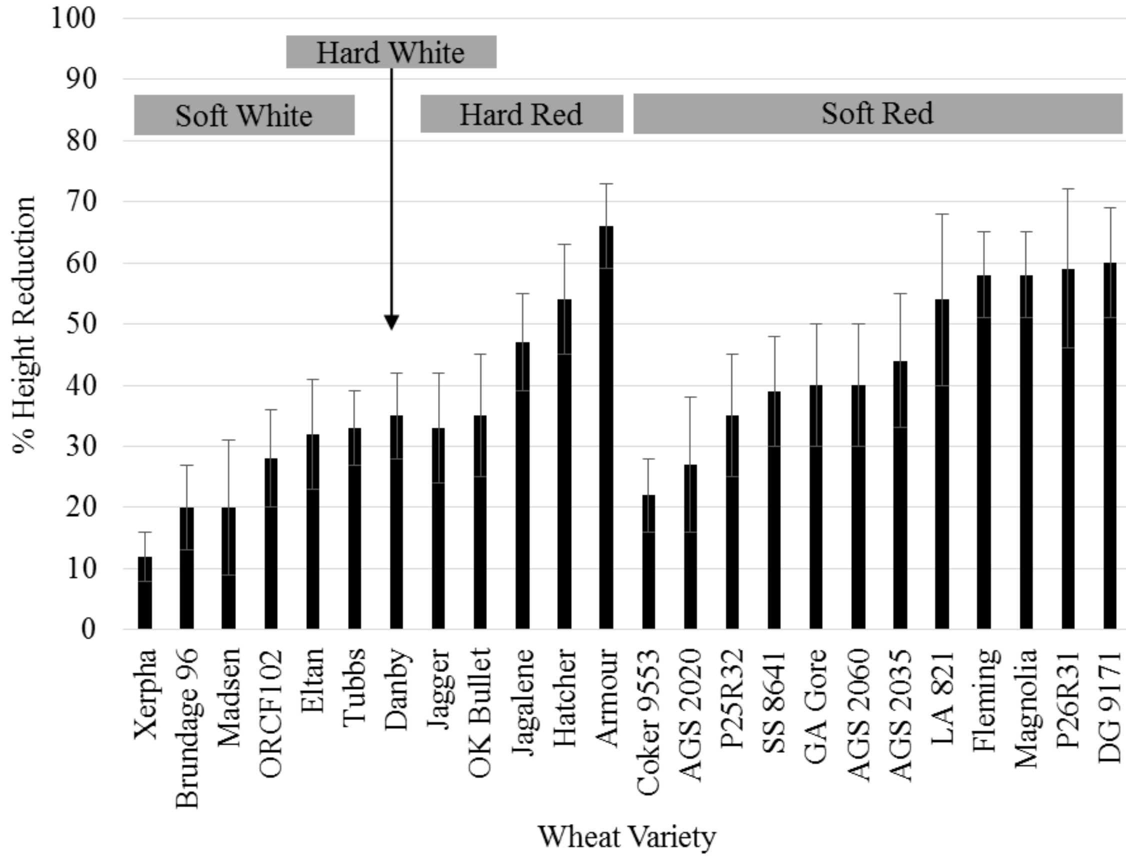

Figure 4. Height reduction of winter wheat treated with pyroxasulfone as affected by variety at 28 days after treatment pooled across pyroxasulfone rates in multi-class study.[a]

[a] Error bars represent 95% confidence intervals for percent height reduction.

EFFECT OF PYROXASULFONE APPLICATION TIMING AND RATE ON WEED CONTROL AND WINTER WHEAT TOLERANCE

Logan A. Grier, Randy Weisz, Travis W. Gannon, Wesley J. Everman

Introduction

Over 26 billion bushels of wheat (*Triticum aestivum*) per year are produced around the world (United States Department of Agriculture [USDA] 2014). The United States produces approximately 2 billion bushels per year, of which 44 million are produced in North Carolina (USDA 2014). Many factors contribute to successful wheat production, including variety selection, pathogen control, insect control, and weed management. Managing weeds has become particularly difficult in the southeast, among other regions of the United States and the world, because common problematic weeds have developed resistance to herbicides regularly used in wheat production, including acetyl CoA carboxylase (ACCase) inhibitors, acetolactate synthase (ALS) inhibitors and microtubule assembly inhibitors. Annual bluegrass populations in the southeast have developed resistance to microtubule synthesis and ALS inhibiting herbicides (Brosnan et al. 2014; Heap 2015). ALS inhibitor resistance has also been reported in henbit populations in Kansas and common chickweed populations in the northeastern United States into Kentucky and Virginia (Heap 2015). Italian ryegrass populations across the southeast have developed resistance to ACCase and/or ALS inhibiting herbicides (Heap 2015; Salas et al. 2013; Taylor 2013).

Pyroxasulfone, a recently introduced herbicide, offers an alternative for wheat growers (Porpiglia 2005). Characterized as a very long chain fatty acid (VLCFA) synthesis inhibitor, pyroxasulfone prevents shoot elongation of germinating seedlings (Tanetani et al. 2009; 2013). The Herbicide Resistance Action Committee categorizes this mode of action MOA in group K3 (HRAC 2014) and the Weed Science Society of America categorizes it in group 15 (WSSA 2014).

Pyroxasulfone and other VLCFA synthesis inhibitors such as *S*-metolachlor are active on a wide range of grasses and broadleaf weeds. Researchers have demonstrated ≥ 85% control of Italian ryegrass and rigid ryegrass (*Lolium rigidum*), including those resistant to glyphosate and trifluralin, with PRE pyroxasulfone applications of 42 – 160 g a.i. ha^{-1} and PRE *S*-metolachlor applications of 430 – 1790 g a.i. ha^{-1} (Bond et al 2014; Boutsalis et al. 2014; Hulting et al. 2012; Ritter and Menbere 2002; Walsh et al. 2011). Researchers have reported > 90 % control of large crabgrass (*Digitaria sanguinalis*) with PRE pyroxasulfone applications of 141 – 167 g a.i. ha^{-1} (Knezevic et al. 2009; Olson et al. 2011). Lawrence and Burke (2014) observed > 74% control of rattail fescue consistently across multiple years with PRE a pyroxasulfone application of 100 g a.i. ha^{-1} and Olson et al. (2011) demonstrated 90% control of green foxtail (*Setaria viridis*) with a PRE pyroxasulfone application of 333 g a.i. ha^{-1}. Application timing and rate of these herbicides can also affect weed control efficacy. Boutsalis et al. (2014) noted that PRE pyroxasulfone applications provided significantly higher weed control that POST applications. Bhagirath et al. (2007) demonstrated > 80% control of rigid ryegrass with 480 – 960 g a.i. ha^{-1} of *S*-metolachlor applied pre-plant (PPL) and 71-83% control when applied early pre-plant (EPP). Hulting et al. (2012) demonstrated 65-78% control of Italian ryegrass with 50 g a.i. ha^{-1} pyroxasulfone applied PRE, increasing to 90-100% control when the rate was increased to 150 g a.i. ha^{-1}.

While pyroxasulfone has strong graminicidal activity, researchers have demonstrated that wheat metabolizes pyroxasulfone more efficiently than weed species such as rigid ryegrass (Tanetani et al. 2013). However, in some cases injury has been observed in field scenarios with pyroxasulfone and other VLCFA inhibitors such as *S*-metolachlor (Bhagirath et al. 2007; Boutsalis et al. 2014; Hulting et al. 2012). A significant reduction in wheat establishment was demonstrated with 150 g a.i. ha^{-1} of pyroxasulfone applied PPL and PRE; however, yield increases were reported due to reduced ryegrass competition (Boutsalis et al. 2014; Hulting et al. 2012). Bhagirath et al. (2007) demonstrated PPL applications of 480 – 960 g a.i. ha^{-1} of *S*-metolachlor resulted in significant reduction in wheat plant density translating to a significant yield reduction, while the EPP application had no phytotoxic effects on wheat. Ritter and Menbere (2002) reported minor stunting with 430 g a.i. ha^{-1} of *S*-metolachlor; however, yield was not affected.

The target application timing for pyroxasulfone ranges from delayed preemergence applications in the fall to postemergence (POST) applications to four-tiller wheat with a target rate range between 42 and 150 g a.i. ha^{-1} depending on soil type and application timing (BASF Corporation 2013; FMC Corporation 2014; Valent U.S.A Corporation 2014). Because pyroxasulfone primarily affects germinating seedlings it is important that application occurs prior to weed germination to ensure maximum weed control but after wheat germination to ensure minimum crop injury (Tanetani et al. 2009). However, it is common in North Carolina for winter annual grasses, such as, annual bluegrass and Italian ryegrass, to germinate when wheat is planted or concurrently with wheat (Everman and Jordan 2013). This creates a scenario where timely weed control applications of pyroxasulfone could increase the risk of crop injury, which has been demonstrated in field scenarios (Boutsalis et al. 2014; Hulting et al. 2012). These studies aim to investigate the effect of application rate and timing of pyroxasulfone on winter wheat tolerance and weed control.

Materials and Methods

Two studies were conducted to investigate the effects of pyroxasulfone rate and timing on winter wheat and weed control.

In Study 1, research was conducted in North Carolina at the Central Crops Research Station in Clayton, the Tidewater Research Station in Plymouth in the 2011-12 field season (35°40'27.43"N, 78°30'50.78"W; 35°52'8.57"N, 76°39'52.63"W) and at the Piedmont Research Station in Salisbury in 2011-12 and 2012-13 field seasons (35°41'41.51"N, 80°37'48.35"W; 35°41'38.48"N, 80°37'29.15"W). Winter wheat (*Triticum aestivum*)[1] was planted on 19 cm rows. The field descriptions including tillage, soil types, and soil properties are outlined in Table 1 (Web Soil Survey 2015). Precipitation of each location during the study months are listed in Table 2. Treatments were arranged in a randomized complete block experimental design including four replications with plots dimensions of 1.83 m by 3.05 m. Treatments consisted of pyroxasulfone[2] rates of 74, 149 and 298 g a.i. ha^{-1} applied 7 days pre-plant (PPL), at planting (PRE) or postemergence to 2-4 tiller wheat (POST). Treatments also included a split pyroxasulfone application of 60 g a.i. ha^{-1} applied PRE followed by 89 g a.i. ha^{-1} applied POST. Non-treated control plots were included for comparison. Herbicides were applied with a CO_2 backpack sprayer[4] calibrated to deliver 140 L ha^{-1} at 30 PSI.

In Study 2, research was conducted in North Carolina at the Caswell Research Station in Kinston and the Tidewater Research Station in Plymouth in the 2011-12 field season (35°16'37.47"N, 77°40'4.98"W; 35°52'8.57"N, 76°39'52.63"W) and at the Piedmont Research Station in Salisbury in 2011-12 and 2012-13 field seasons (35°41'38.48"N, 80°37'29.15"W). Winter wheat (*Triticum aestivum*)[1] was planted on 19 cm rows. The field descriptions including tillage, soil types, and soil properties are outlined in Table 1 (Web Soil Survey 2015). Precipitation of each location during the study months are listed in Table 2. Treatments were arranged in a 3x6 factorial randomized complete block experimental design including four replications with plot dimensions of 1.83 m by 3.05 m. Treatments consisted of pyroxasulfone[2] rates of 60, 74 and 89 g a.i. ha^{-1} applied 14 days pre-plant (EPP), 7 days pre-plant (PPL), at planting (PRE), at spike, at initial wheat tillering (POST). Non-treated control plots were included for comparison. Herbicides were applied with a CO_2 backpack sprayer[3] calibrated to deliver 140 L ha^{-1} at 30 PSI.

In Study 1, visual weed control ratings of henbit (*Lamium amplexicaule*) were recorded at 14 days after PRE application (DAPRE) and 35 days after POST application (DAPOST) (Salisbury 2011, Salisbury 2012). Visual weed control ratings of annual bluegrass (*Poa annua*) were recorded at 77 DAPOST (Plymouth 2012). Visual weed control ratings for field speedwell (*Veronica agrestis*) were recorded at 14 DAPRE and 35 DAPOST (Salisbury 2011). Visual ratings for wheat phytotoxicity, height reduction and chlorosis were recorded at 14 and 35 DAPRE (Salisbury 2011, Plymouth 2011) and at 7, 35 and 84 DAPOST at all site-years. Wheat yield was evaluated at all site-years.

In Study 2, visual weed control ratings of henbit (*Lamium amplexicaule*) were recorded at 14 and 77 days after spike application (DASpike) (Plymouth 2011, Salisbury 2011, Salisbury 2012) and 105 DASpike (Kinston 2011, Salisbury 2012). Visual weed control ratings of annual bluegrass (*Poa annua*) were recorded at 14 DASpike (Plymouth 2011) and 105 DASpike (Plymouth 2011). Visual weed control ratings for field speedwell (*Veronica agrestis*) were recorded at 14 and 77 DASpike (Salisbury 2011, 2012). Visual weed control ratings of common chickweed (*Stellaria media*) and mouse-ear chickweed (*Cerastium vulgatum*) were recorded at 105 DASpike (Salisbury 2012). Visual ratings for wheat phytotoxicity, height reduction and chlorosis were recorded 14 DASpike (Kinston 2011, Plymouth 2011, Salisbury 2011), 105 DASpike (Kinston 2011, Plymouth 2011, Salisbury 2011) and 28 days after spring application (Kinston 2011, Salisbury 2012). Wheat yield was evaluated at all site-years.

In these studies, data were subjected to analysis of variance (ANOVA) by using PROC GLM procedure in SAS 9.4[4]. Year and location were combined as proposed by Carmer et al. (1989) and hereafter referred to as 'environment.' Mean comparisons were performed using Fisher's Protected LSD test when F values were statistically significant ($P \leq 0.05$). Winter wheat injury and weed control ratings were measured as a percentage of the non-treated; therefore, statistical analysis omits data from non-treated plots. In Study 1, fixed effects included environment, herbicide treatment, and the subsequent interaction. Additionally in Study 1, where a factorial existed between rate and timing of pyroxasulfone, other treatments were removed to analyze this factorial; however, due to a significant rate-by-timing interaction, data are presented by herbicide treatment ($P \leq 0.05$). In Study 2, fixed effects included environment, pyroxasulfone rate, pyroxasulfone timing and the subsequent interactions. In both studies, replications (nested within environment) were considered random effects. If no interaction was observed between main factors, data are pooled to show main effect; otherwise, data are presented by the interaction.

Results and Discussion

Winter Wheat Tolerance

Winter wheat injury is presented by environment due to a significant environment-by-treatment interaction ($P \leq 0.05$). This is due to the fact that the only wheat injury observed in these studies occurred in Study 1 in Clayton 2011. In Clayton, significant differences in injury were observed between treatments at 14, 35, and 84 DAPOST (Table 3). Minor chlorosis (< 15%) was observed early in the season in Clayton, but this injury was transient (data not shown). Stunting was the primary form of injury observed at this location. The split pyroxasulfone application and all POST applications resulted in ≤ 11% stunting at 14 DAPOST declining to 0% at 84 DAPOST (Table 3). The greatest injury, 74 and 79%, was observed at 35 DAPOST where pyroxasulfone was applied PPL at 149 g a.i. ha^{-1} and 298 g a.i. ha^{-1}, respectively (Table 3). At 84 DAPOST, wheat stunting was not as great, with 50% observed where pyroxasulfone was applied PPL at 298 g a.i. ha^{-1} (Table 3). Less wheat stunting (30%) was observed 84 DAPOST where pyroxasulfone was applied at a lower rate, 149 g a.i. ha^{-1}, PPL (Table 3). Similarly, at 84 DAPOST, wheat injury observed with PRE applications declined to < 20% (Table 3). Other research has demonstrated crop safety of pyroxasulfone on wheat treated with rates up to 160 g a.i ha^{-1} (Lawrence and Burke 2014; Kleemann et al. 2014; Walsh et al. 2011). However, in some cases researchers demonstrated reduced wheat establishment with 150 g a.i. ha^{-1} of pyroxasulfone applied PRE; however, yield increased due to a reduction in weed competition (Boutsalis et al. 2014; Hulting et al. 2012). The injury observed in Clayton can be attributed to precipitation, soil type, and tillage practices. During the end of October and middle of November, the months in which PPL and PRE applications were made in Clayton, precipitation occurred, incorporating the herbicide into the germination zone of the soil and (Table 2). Within two weeks of the PPL application 5.5 cm of precipitation occurred, and within two weeks of the PRE application (or four weeks of the PPL application) an additional 5.1 cm of precipitation occurred (State Climate Office of North Carolina 2015). Also, the soil in Clayton has a combination of low OM, low clay content and conventional tillage, resulting in increased herbicide activity (Table 1). Researchers have demonstrated an increase in pyroxasulfone activity in soils with lower OM and/or clay content (Knezevic et al. 2009; Szmigielski et al. 2013; Walsh et al. 2011; Westra 2012). Additionally, researchers have shown that efficacy of PRE herbicides can vary between conventional and no-till sites, demonstrating that fourfold rate increases were required to control 80% of weed species in no-till compared to conventional tillage (Geier et al. 2006; Mahoney et al. 2014; Mueller and Hayes 1997).

Weed Control

In both studies, field speedwell control was variable. In both studies, no environment-by-treatment interaction was observed; therefore, data are pooled across environments ($P \leq$

0.05). At 14 DAPRE in Study 1, no significant differences were observed in field speedwell control; however, significant differences were observed between treatments at 35 DAPOST (Table 4). The greatest field speedwell control (94%) was observed with pyroxasulfone applied PRE at 298 g a.i. ha^{-1}; however, there was considerable variability as this was not statistically different than PPL applications of pyroxasulfone at 149 to 298 g a.i. ha^{-1} or the PRE application of pyroxasulfone at 149 g a.i. ha^{-1}, ranging from 67% to 79% control (Table 4). The split application of pyroxasulfone and all POST applications achieved ≤ 35% control (Table 4). In Study 2, field speedwell control was evaluated at 14 and 63 DASpike, and no significant differences were observed for pyroxasulfone rate or application timing (data not shown). The variability in control observed with pyroxasulfone was expected as *Veronica* species are not labeled for control with pyroxasulfone and variable *Veronica* control with pyroxasulfone has been demonstrated previously (BASF Corporation 2013; Hulting et al. 2012).

In Study 1, henbit control is pooled across environments because of a lack of significant environment-by-treatment interaction ($P \leq 0.05$). At 14 and 70 DAPRE, while henbit control with herbicide treatments was significantly higher than the non-treated plot, no significant differences were observed among treatments (data not shown). However, significant differences were observed between treatments at 35 DAPOST (Table 4). The greatest henbit control, 85%, was observed with 298 g a.i. ha^{-1} applied PRE; however, there was considerable variability as this was not statistically different than PPL applications of 298 g a.i. ha^{-1} which achieved 68% control at 35 DAPOST (Table 4). All other applications resulted in < 70% control of henbit (Table 4). In Study 2, application timing had a significant effect on henbit control at 14 DASpike (Table 5). These data are pooled over environment and pyroxasulfone rate because of the lack of rate-by-timing, rate-by-environment, timing-by-environment, and rate-by-timing-by-environment interactions ($P \leq 0.05$). At 14 DASpike EPP, PPL and PRE applications of pyroxasulfone resulted in 79 to 84% henbit control; while the spike application only resulted in 41% control. At 105 DASpike, no significant differences in henbit control were observed at any pyroxasulfone rate or timing (data not shown).

In Study 2, application timing had a significant effect on control of chickweed species and because of the lack of significant rate-by-timing, rate-by-environment, timing-by-environment, and rate-by-timing-by-environment interactions, these data are pooled over environment and pyroxasulfone rate ($P \leq 0.05$) (Table 5). PPL and PRE pyroxasulfone applications provided significantly higher control of chickweed species than POST applications at 105 DASpike in Study 2; however, the highest control was only 71% for common chickweed and 49% for mouse-ear chickweed with EPP application timing (Table 5). These results were expected as pyroxasulfone is only labeled for suppression of chickweed species (BASF Corporation 2013). Additionally, mouse-ear chickweed is a perennial weed species in North Carolina. Because pyroxasulfone affects germinating seedlings, control of existing plants is not expected resulting in lower overall control of mouse-ear chickweed.

Effective control of annual bluegrass was demonstrated in both studies. In Study 1, all pyroxasulfone treatments provided > 90 % control of annual bluegrass except the lowest rate of 74 g a.i. ha^{-1} applied POST, which resulted in 71% control (Table 4). In Study 2 at 14

DASpike, application timing had a significant effect on annual bluegrass control (Table 5). Due to a lack of rate-by-timing interaction, data are pooled across pyroxasulfone rate ($P \leq 0.05$). In Study 2, at 105 DASpike, an interaction between pyroxasulfone rate and application timing on annual bluegrass control was observed; therefore, data are presented by rate and timing (Figure 1). At 14 DASpike and 105 DASpike, PPL pyroxasulfone applications achieved ≥ 90% control of annual bluegrass; whereas the PRE applications resulted in slightly lower control ranging from 80 to 85% (Table 5; Figure 1). Across all pyroxasulfone rates, applications made prior to wheat emergence controlled annual bluegrass ≥ 80% (Figure 1). Other researchers have demonstrated ≥ 74% control of grass species with PRE application rates between 50-80 g a.i. ha^{-1} and ≥ 85% control with PRE application rates between 100-160 g a.i. ha^{-1} (Bond et al. 2014; Boutsalis et al. 2014; Hulting et al. 2014; Lawrence and Burke 2014; Olson et al. 2011). For POST applications, 60 g a.i. ha^{-1} provided significantly less control than 74 and 89 g a.i. ha^{-1}; however, all provided < 70% control indicating POST applications do not provide adequate control when applied without a product with POST activity (Figure 1). Boutsalis et al. (2014) also demonstrated that POST applications of pyroxasulfone alone did not provide effective control of rigid ryegrass where PRE applications provided 96% control.

Winter Wheat Yield

In Study 1, winter wheat yield data are presented by environment due to a significant environment-by-treatment interaction ($P \leq 0.05$). At Clayton in 2011, significant differences in yield due to herbicide treatments were demonstrated (Table 6). An approximate 50% yield reduction from the non-treated control was observed where pyroxasulfone was applied at 298 g a.i. ha^{-1} PPL in comparison to the non-treated control (Table 6). Yield differences can be attributed to herbicide treatments which resulted in the greatest injury observed at 84 DAPOST. Although only the highest rate of pyroxasulfone applied PPL resulted in significant yield reductions, other PPL and PRE pyroxasulfone treatments applied at 149 and 298 g a.i. ha-1 led to a lower yield than the non-treated (Table 6).

In Study 2, herbicide treatments did not affect wheat yield (data not shown). However, there were significant differences by location with an average of 64 bu $acre^{-1}$ harvested in Salisbury, 42 bu $acre^{-1}$ in Plymouth, and 33 bu $acre^{-1}$ in Kinston. This can be attributed to the normal yield potentials in these soils. The lack of yield differences could be attributed to the overall crop safety observed at these locations and the non-competitive nature of the weeds present (Conley and Bradley 2005; Scott et al. 1995).

Conclusion

These studies demonstrate effective control of annual bluegrass with pyroxasulfone and variable to little control of henbit, common chickweed, mouse-ear chickweed and field speedwell. PPL and PRE applications are more effective than POST applications at providing residual grass control. Weed control with different rates of pyroxasulfone did not differ significantly at PPL, PRE and spike applications for annual bluegrass control; however, increased control was observed where rate was increased for applications made after tillering. Additionally, increased variability in annual bluegrass control was observed with POST applications.

These studies also demonstrate that in most cases applications of pyroxasulfone applied EPP to POST are safe on wheat. High rates applied PPL to PRE resulted in injury in soil with lower OM/clay content when followed by heavy rainfall. Height reduction up to 30% did not translate to a significant yield reduction, but when stunting reached 50% a significant reduction in yield was observed.

Sources of Materials

[1]Winter Wheat Seed, Pioneer Hi-Bred International, Inc., Johnston, IA 50131

[2]Pyroxasulfone Herbicide, BASF Corporation, Research Triangle Park, NC 27709

[3]CO_2 Pressurized Backpack Sprayer, Spraying Systems Co., Wheaton, IL 60189

[4]SAS Statistical Software, Version 9.4, Cary, NC 27513

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Table 1: Field locations, soil types, tillage types, and crop seasons for research trials.[a]

			Soil Properties					Crop Season	
Field Location	Tillage	Soil Type(s)	pH	OM	Sand	Silt	Clay	Study 1	Study 2
				--------------- % ---------------					
Clayton	Conventional	Wagram loamy sand	5.3	1.25	77.7	16.3	6.0	2011-12	
Kinston	Conventional	Torhunta loam	4.5	12.0	67.5	21.0	11.5		2011-12
Plymouth	Conventional	Cape fear loam	5.5	10.0	45.7	41.8	12.5	2011-12	2011-12
Salisbury	No Tillage	Lloyd clay loam	6.4	1.25	33.5	36.5	30.0	2012-13	2011-12 2012-13
Salisbury	No Tillage	Pacolet sandy clay loam	6.2	0.75	55.1	17.4	27.5	2011-12	

[a] Abbreviations: OM, organic matter.

Table 2. Precipitation data during 2011-2013 for field locations.

Month	Clayton	Kinston	Plymouth	Salisbury	
	2011-12	2011-12	2011-12	2011-12	2012-13
	----------------------------- cm -----------------------------				
October	5.59	16.10	5.44	11.25	3.20
November	10.70	9.98	9.26	12.63	0.48
December	4.04	1.95	2.27	7.90	6.33
January	6.04	8.39	6.72	4.72	12.85
February	7.02	6.26	8.91	4.92	9.26
March	13.22	10.97	11.47	8.23	6.76
April	10.55	7.88	6.21	3.65	12.46
May	14.32	22.48	15.52	16.67	6.20
June	4.28	5.31	6.65	9.84	18.42

Table 3. Winter wheat injury in Study 1 in Clayton as affected by herbicide rate and timing.[ab]

Pyroxasulfone Rate (g a.i. ha^{-1})	Timing	Days after POST Application 14	35	84
		---------------%--------------		
74	PPL	43 b	26 bc	0 d
149	PPL	65 a	74 a	30 b
298	PPL	64 a	79 a	50 a
74	PRE	11 c	3 d	0 d
149	PRE	18 c	11 cd	6 d
298	PRE	35 b	36 b	19 c
60 + 89	PRE + POST	9 c	5 d	0 d
74	POST	6 c	9 d	0 d
149	POST	6 c	5 d	0 d
298	POST	11 c	0 d	0 d

[a] Abbreviations: g a.i. ha^{-1}, grams active ingredient per hectare; PPL, treatments applied 7 days pre-plant; PRE, treatments applied prior to crop emergence; POST, treatments applied after crop emergence.

[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 4. Henbit, field speedwell and annual bluegrass control in Study 1 as affected by herbicide rate and timing.[ab]

Pyroxasulfone Rate (g a.i. ha^{-1})	Timing	35 DAPOST Henbit	35 DAPOST Field speedwell	84 DAPOST Annual bluegrass
		---------------------%---------------------		
74	PPL	33 ed	48 bcd	100 a
149	PPL	53 bcd	67 abc	100 a
298	PPL	68 ab	79 ab	100 a
74	PRE	36 cde	24 cd	92 a
149	PRE	58 bc	75 ab	98 a
298	PRE	85 a	94 a	98 a
60 + 89	PRE + POST	24 ef	34 bcd	92 a
74	POST	4 f	0 d	71 b
149	POST	23 ef	15 d	92 a
298	POST	23 ef	35 bcd	92 a

[a] Abbreviations: g a.i. ha^{-1}, grams active ingredient per hectare; PPL, treatments applied 7 days pre-plant; PRE, treatments applied prior to crop emergence; POST, treatments applied after crop emergence.

[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 5: Weed control as affected by application timing in Study 2 at 14 and 105 DASpike pooled over pyroxasulfone rates (60, 74, 89 g a.i. ha^{-1}).[ab]

Application Timing	14 DASpike		105 DASpike	
	Henbit	Annual bluegrass	Mouse-ear chickweed	Common chickweed
	----------------------------------	----------%	----------	----------------------------------
EPP	84 a	90 ab	71 a	49 a
PPL	83 a	92 a	69 a	34 a
PRE	79 a	81 b	54 ab	22 ab
Spike	41 b	45 c	38 b	22 ab
POST	-	-	37 b	2 c

[a] Abbreviations: DASpike, days spike application; EPP, 14 days pre-plant; PPL, 7 days pre-plant; PRE, pre-emergence; POST, postemergence at tillering.

[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 6. Yield in Study 1 in Clayton 2011-12 as affected by pyroxasulfone rate and timing.[ab]

Pyroxasulfone Rate ($g\ a.i.\ ha^{-1}$)	Timing	bushels/acre
Non-treated	-	41 abcd
74	PPL	47 abc
149	PPL	35 cde
298	PPL	24 e
74	PRE	44 abcd
149	PRE	39 cd
298	PRE	34 de
60 + 89	PRE + POST	52 ab
74	POST	39 cd
149	POST	45 abcd
298	POST	54 a

[a]Abbreviations: $g\ a.i.\ ha^{-1}$, grams active ingredient per hectare; PPL, treatments applied 7 days pre-plant; PRE, treatments applied prior to crop emergence; POST, treatments applied after crop emergence.

[b] Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD at $p \leq 0.05$.

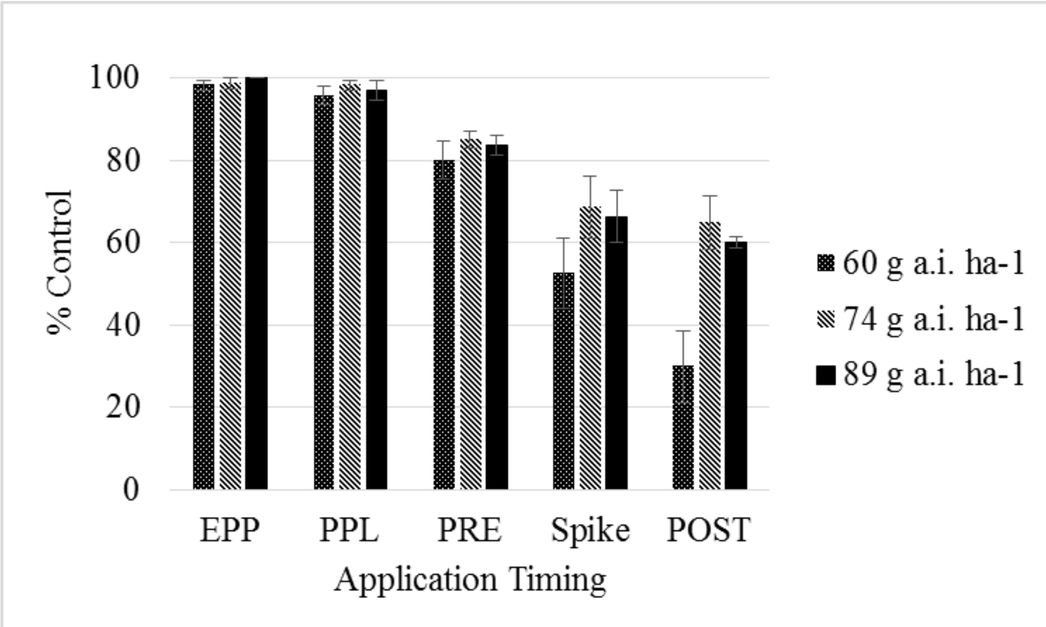

Figure 1: Annual bluegrass control 105 days after wheat spike in Study 2 as affected by pyroxasulfone rate and application timing at Plymouth in 2011.[ab]

[a] Abbreviations: EPP, 14 days pre-plant; PPL, 7 days pre-plant; PRE, pre-emergence.; POST, at wheat tillering.

[b] Error bars represent 95% confidence intervals for percent control.

WEED CONTROL AND WINTER WHEAT RESPONSE TO PYROXASULFONE

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Introduction

In United States agricultural production, wheat acreage ranks third behind corn and soybean, producing 2 billion bushels per year (United States Department of Agriculture [USDA] 2014). In North Carolina alone, 44 million bushels of soft red winter wheat are produced each year (USDA 2014). Weed management plays an important role because weeds compete with the crop for nutrients, water and sunlight, potentially reducing grain yield (Blackman & Templeman 1938). Italian ryegrass (*Lolium multiflorum*), a common problematic weed in North Carolina winter wheat production, has been shown to reduce wheat yield by > 30% with as little as nine ryegrass plants m^{-2} present (Appleby et al. 1976; Hashem et al. 1998; Scursoni et al. 2012). In North Carolina, other problematic winter annual grass and broadleaf weeds include annual bluegrass (*Poa annua*), henbit (*Lamium amplexicaule*), and common chickweed (*Stellaria media*) (Everman & Jordan 2013).

In recent years, growers have depended on herbicides with modes of action (MOA) inhibiting acetyl CoA carboxylase (ACCase), acetolactate synthase (ALS), or microtubule assembly. However, managing weed populations with these commonly used herbicides is becoming increasingly difficult due to widespread herbicide resistant weed populations. Italian ryegrass populations across the southeast have developed resistance to herbicides inhibiting ACCase and/or ALS (Heap 2015; Salas et al. 2013; Taylor 2013). Annual bluegrass populations in the southeast have developed resistance to herbicides inhibiting microtubule synthesis and ALS (Brosnan et al. 2014; Heap 2015). ALS inhibitor resistance has also been reported in henbit populations in Kansas and common chickweed populations in the northeastern United States into Kentucky and Virginia (Heap 2015).

A recently discovered herbicide, pyroxasulfone, offers North Carolina wheat growers an effective solution for residual weed control in winter wheat (Porpiglia 2005; Tanetani et al. 2009). Pyroxasulfone inhibits very long chain fatty acid (VLCFA) synthesis, preventing shoot elongation of germinating seedlings (Tanetani et al. 2009, 2013). The Herbicide Resistance Action Committee categorizes this MOA in group K3 (HRAC 2014) and the Weed Science Society of America categorizes it in group 15 (WSSA 2014). The target application timing for pyroxasulfone ranges from fall delayed preemergence applications to winter postemergence (POST) applications over to top of wheat with four tillers. The target rate is 42 – 150 g a.i. ha^{-1} depending on soil type and application timing. (BASF Corporation 2013; FMC Corporation 2014; Valent U.S.A 2014).

The physical and chemical properties of pyroxasulfone, including a low soil organic carbon-water partitioning coefficient (K_{oc} = of 57-119 mL/g), a moderate to low distribution coefficient (K_d = 0.32-9.57 mL/g), and a low water solubility (K_s = 2.9-3.5 mg/L), indicate its potential to remain readily available in the germination zone of soil, without binding tightly to soil particles or leaching downward through the soil profile (Australian Pesticides and Veterinary Medicines Authority [APVMA] 2011; Westra 2012). Researchers have demonstrated that pyroxasulfone is more persistant than other VLCFA synthesis inhibitors including acetochlor, dimethenamid-*p*, flufenacet and *S*-metolachlor, indicating the possibility for longer residual weed control activity in the soil (Mueller & Steckel 2011; Szmigielski et al. 2014; Westra et al. 2014).

Control of many grass and broadleaf weeds has been demonstrated with pyroxasulfone and other VLCFA inhibitors such as *S*-metolachlor; however, winter wheat tolerance varied depending on application timing and experimental conditions (Boutsalis et al. 2014; Bhagirath et al. 2007; Hulting et al. 2012). Bond et al. (2014) demonstrated ≥ 93% control of glyphosate-resistant Italian ryegrass with a fall applied PRE application of 160 g a.i. ha^{-1} of pyroxasulfone or 1790 g a.i. ha^{-1} of *S*-metolachlor. Boutsalis et al. (2014) showed 79-96% control of trifluralin resistant rigid ryegrass (*Lolium rigidum*) with PRE applications of 150 g a.i. ha^{-1} pyroxasulfone in no till wheat, also noting that PRE applications provide significantly higher weed control that POST applications. While a statistically significant reduction in wheat establishment has been shown with this rate, yield increases were demonstrated with PPL and PRE applications, likely due to a result of decreased ryegrass competition in these studies (Boutsalis et al. 2014; Hulting et al. 2012). Hulting et al. (2012) reported 65-78% control of Italian ryegrass with 50 g a.i. ha^{-1} pyroxasulfone applied PRE, increasing to 90-100% control when the rate was increased to 150 g a.i. ha^{-1}. Olson et al. (2011) reported 95% control of large crabgrass (*Digitaria sanguinalis*) with a PRE application of 167 g a.i. ha^{-1} and 90% control of green foxtail (*Setaria viridis*) with a PRE application of 333 g a.i. ha^{-1} of pyroxasulfone. Bhagirath et al. (2007) showed > 80% control of rigid ryegrass with 480 – 960 g a.i. ha^{-1} of *S*-metolachlor, another herbicide inhibiting VLCFA synthesis, applied pre-plant (PPL) and 71-83% control when applied early pre-plant (EPP). In this study the PPL application resulted in significant reduction in wheat plant density translating to a significant yield reduction, while the EPP application had no phytotoxic effects on wheat (Bhagirath et al. 2007). Ritter & Menbere (2002) demonstrated ≥ 85% control of Italian ryegrass with 430 g a.i. ha^{-1} of *S*-metolachlor, reporting minor stunting at this rate; however, yield was not affected.

Each of the current herbicide programs used by growers offers its own set of benefits and challenges. Flufenacet + metribuzin is labeled for control of many problematic grass and broadleaf weeds and also offers alternative modes of action of inhibiting VLCFA synthesis and photosystem II, respectively (Lechelt-Kunze 2003). However, application timing in North Carolina can be a challenge. Flufenacet + metribuzin is labeled for POST applications to wheat but must be applied prior to weed emergence for effective control (Bayer CropScience 2007). For weeds that germinate concurrently with winter wheat, such as Italian ryegrass and annual bluegrass, this creates a narrow application window for effectively controlling early season weeds. Pyroxasulfone is strong on grass species; however, it will not control many problematic broadleaf weeds in North Carolina. Adding saflufenacil as a PRE mixture could help control those broadleaf weeds. Additionally, splitting the pyroxasulfone application may provide a benefit for those grass species which flush later in the season. This study was designed to compare weed control and winter wheat tolerance with these residual herbicide programs compared to the standard POST program, aiming at positioning pyroxasulfone for optimal weed control and crop selectivity for winter wheat.

Materials and Methods

Research was conducted in North Carolina at the Central Crops Research Station in Clayton and the Piedmont Research Station in Salisbury in 2011-2014 (35°40'27.43"N, 78°30'50.78"W ; 35°41'38.48"N, 80°37'29.15"W, respectively); at the Tidewater Research Station in Plymouth in 2011-2012 (35°52'8.57"N, 76°39'52.63"W); and on a cooperator farm in Edgecombe County in 2012-2013 (35°46’16.91”N, 77°43’16.87”W) (Table 1). Winter wheat (*Triticum aestivum*)[1] was planted on 19 cm rows. The soil types and tillage for each site are listed in Table 1 (Web Soil Survey 2015). Herbicide treatments (Table 2) were arranged in a randomized complete block design including four replications with plot dimensions of 1.83 m by 3.05 m. Non-treated control plots were included for comparison. Herbicides were applied with a CO_2 backpack sprayer[8] calibrated to deliver 140 L ha^{-1} at 30 PSI.

Visual weed control ratings of henbit (*Lamium amplexicaule*) were recorded 21 days after wheat spike (DASpike) in Clayton 2011, Plymouth 2011, Salisbury 2011, and Salisbury 2012; and 35, 49 and 105 DASpike in Salisbury 2011 and 2012. Visual weed control ratings for annual bluegrass (*Poa annua*) were recorded at 14 DASpike and 140 DASpike in Plymouth 2011. Visual weed control ratings of wild radish (*Raphanus raphanistrum*) were recorded at 14 DASpike and 105 DASpike in Clayton 2012 and 2013. Visual ratings for wheat phytotoxicity, height reduction and chlorosis were recorded at 21 DASpike (Clayton 2012, Edgecombe 2012, Plymouth 2011, Salisbury 2011 and 2012), 49 DASpike (Clayton 2011, Salisbury 2011 and 2012), and 105 DASpike (Clayton 2011, Edgecombe 2012, Salisbury 2011 and 2012). Wheat yield was evaluated at all site-years.

Winter wheat injury and weed control ratings were measured as a percentage of the non-treated; therefore, statistical analysis omits data from non-treated plots. Yield was measured and is presented by bushels/acre harvested per plot; therefore, the non-treated plots were included in the statistical analysis. Data were subjected to analysis of variance (ANOVA) by using PROC GLM procedure in SAS 9.4[9]. Year and location were combined as proposed by Carmer et al. (1989) and here after referred to as ‘environment.’ Environment, herbicide treatment, and the interaction between environment and herbicide treatment were considered fixed effects. Replications (nested within environment) were considered random effects. Mean comparisons were performed using Fisher’s Protected LSD test when F values were statistically significant ($P \leq 0.05$).

Results and Discussion

Winter Wheat Tolerance

All herbicide treatments resulted in similar levels of injury at Clayton in 2011 and virtually no injury at any other environment. Therefore, winter wheat injury data are pooled over herbicide treatments and presented by environment due to a significant environment effect and lack of a significant interaction between herbicide treatment and environment ($P \leq 0.05$). At 35 DASpike, 24% injury was observed at Clayton in 2011 compared to ≤ 3% injury at all other locations (Table 3). Injury at Clayton in 2011 was transient, decreasing to 10% at 105 DASpike (Table 3). This can be attributed to the course soil texture, conventional tillage practices, and a rainfall event (2.9 cm) within 24 hours of planting and application in Clayton (State Climate Office of North Carolina 2015; Table 1). In field, greenhouse and lab studies, researchers have demonstrated an increase in pyroxasulfone activity with increased OM and/or clay content (Knezevic et al. 2009; Szmigielski et al. 2013; Walsh et al. 2011; Westra 2012). Also, researchers have demonstrated a decrease in herbicide activity with no-till compared to conventional tillage due to the physical barrier created by the surface residue and/or the binding to the OM in the decaying residue (Geier et al. 2006; Mahoney et al. 2014). It is also important to note that injury did not occur in Clayton in 2012 or 2013. This can be attributed to more time between the herbicide application and a rainfall event and also highlights that multiple factors play a role in pyroxasulfone activity.

Weed Control

Henbit control data at 21 DASpike as affected by herbicide treatment were presented by environment due to a significant treatment-by-environment interaction ($P \leq 0.05$). All other weed control data are pooled over environment because of the lack of significant interaction.

At 21 DASpike in Clayton, 100% henbit control was observed with all PRE and spike herbicide applications (Table 4). Hulting et al. (2012) also demonstrated cases where a rate increase did not affect weed control due to high herbicide activity in the soil resulting in high efficacy at all rates. An overall trend of decreasing henbit control was observed from Clayton 2011 to Plymouth 2011 to Salisbury 2011 to Salisbury 2012 (Table 4). This can be attributed to multiple factors including differences in soil type, tillage practices and precipitation amounts, all of which affect herbicide activity. More specifically, researchers have demonstrated a reduction in pyroxasulfone activity in finer texture soils with higher OM and/or clay content, decreased herbicide activity when reducing tillage, and decreased herbicide activity with low amounts of rainfall (Geier et al. 2006; Knezevic et al. 2009; Mahoney et al. 2014; Mueller & Hayes 1997; Stewart et al. 2010; Stewart et al. 2012; Szmigielski et al. 2013; Walsh et al. 2011; Westra 2012). In Clayton, the combination of a loamy sand soil containing low OM (1.25%) and clay content (6.0%), conventional tillage practice, and adequate activating rainfall after application (10.70 cm) contributed to increased herbicide activity (Table 1; Table 4-5). Plymouth is also under conventional tillage practice and received adequate activating rainfall (9.26 cm); however has a loam soil containing higher OM (10.0%) and clay content (12.5%) than Clayton, resulting in less herbicide activity (Table 1; Table 4-5). Salisbury has sandy clay loam soil with low OM (0.75%) and high clay content (27.5%), and is under no-till practice. Pyroxasulfone activity in Salisbury 2011 was slightly reduced compared to Plymouth (Table 4). In Salisbury, a significant reduction in weed control was observed from the 2011-12 to the 2012-13, which can be attributed to the differences in precipitation where 12.63 cm were received in November 2011 compared to 0.48 cm in November 2012 (Table 5).

In Plymouth 2011 and Salisbury 2011, a rate response was observed with PRE pyroxasulfone applications (Table 4). Olson et al. (2011) and Hulting et al. (2012) have also demonstrated an increase in weed control with increasing pyroxasulfone rates. Adding saflufenacil, a broadleaf herbicide, to the 60 g a.i. ha^{-1} pyroxasulfone treatment increased henbit control significantly from 56% to 95% in Plymouth and 43% to 86% in Salisbury 2011 (Table 4). Because saflufenacil and pyroxasulfone are only labeled for suppression of henbit, consistently high control is not expected; however, it can be attributed to the high rate of saflufenacil and an additive effect of the two herbicides (BASF Corporation 2012, 2013).

Knezevic et al. (2010) demonstrated 90% control of henbit with 35 g a.i. ha^{-1} with saflufenacil and Price et al. (2002) demonstrated 80% control with flumioxazin, another protoporphyrinogen oxidase (PPO) inhibiting herbicide. Flufenacet + metribuzin applied at spike stage consistently provided the highest henbit control 21 DASpike (Table 4). In 2011, ≥ 95% henbit control was observed in Clayton, Plymouth, and Salisbury with flufenacet + metribuzin applied at wheat spike provided (Table 4). This result is expected as flufenacet + metribuzin is labeled for control of henbit with burndown applications while saflufenacil and pyroxasulfone are only labeled for suppression of henbit (BASF Corporation 2012, 2013; Bayer CropScience 2007). Additionally, researchers have demonstrated effective henbit control with metribuzin alone and in combination with flufenacet (Grey et al. 2012; Hasty et al. 2004; King et al. 2003; Krausz et al. 2003). In Salisbury 2012 at 21 DASpike, all PRE and spike herbicide applications provided < 50% control, which can be attributed to low precipitation amounts during applications in October and November (Tables 4-5).

At 35 DASpike, 49 DASpike and 105 DASpike a significant treatment effect on henbit control was observed (Table 6). No treatment-by-environment interaction was observed; therefore, data are pooled over environment ($P \leq 0.05$). After post applications, no significant differences were observed between pyroxasulfone PRE applications and pyroxasulfone split applications (Table 6). Adding saflufenacil to the 60 g a.i. ha^{-1} rate of pyroxasulfone applied PRE increased henbit control significantly across all rating timings; however, < 60% control was achieved at 105 DASpike (Table 6). Henbit is labeled for suppression only with PRE pyroxasulfone applications and with saflufenacil burndown applications; therefore, consistently effective control of henbit was not expected with these applications (BASF Corporation 2012, 2013). Additionally, henbit pressure normally occurs in a single flush in the fall in North Carolina; therefore, splitting the application is not expected to provide a benefit for this particular weed. Flufenacet + metribuzin consistently provided the highest control of henbit at 87% at 42 DASpike, declining to 62% at 105 DASpike (Table 6).

At 14 DASpike, 93% control of wild radish was observed with the pyroxasulfone + saflufenacil applied PRE and 80% control was observed with flufenacet + metribuzin applied at spike (Table 6). Adding saflufenacil to the low rate of pyroxasulfone provided a significant increase in wild radish control from 5 to 93% at 14 DASpike (Table 6). This is expected as wild radish is labeled for control with burndown applications of saflufenacil but not labeled for control with pyroxasulfone (BASF Corporation 2012, 2013).

At 14 DASpike, ≥ 70% control of annual bluegrass was observed with all PRE applications containing pyroxasulfone (Table 6). At 140 DASpike, the highest control, 98%, was observed with the POST application of mesosulfuron + thifensulfuron (Table 6). This demonstrates that ALS resistance is not present in the annual bluegrass population in this field in Plymouth, NC. No significant differences were observed with PRE pyroxasulfone applications or split applications, all achieving 78-85% control (Table 6).

Winter Wheat Yield

Wheat yields associated with herbicide applications did not significantly differ from the non-treated control; however, a significant environmental effect was observed (Table 7). Therefore, winter wheat yield data are pooled over herbicide treatments and presented by environment due to a significant environment effect and lack of a significant interaction (P ≤

0.05). The absence of treatment effect may be due to the fact that the primary weed population was henbit. Researchers have demonstrated that a high population of henbit must be present to interfere with crop yield (Conley & Bradley 2005). The highest yields across all cropping seasons were observed in Salisbury ranging from 69 – 83 bushels per acre (Table 7). In Plymouth 2011 the yield was 25 bushels/acre (Table 7). This is lower than expected and is due to wildlife damage in this field. The lowest yield of 7 bushels/acre was observed in Clayton 2012 (Table 7). While low yields are expected in this sandier soil, this was much lower than normal. This is the result of a late harvest at the end of July due to heavy rainfall during harvest season leading to wet field conditions (Table 5).

Conclusion

This study shows that pyroxasulfone and its combination with other wheat herbicides can provide effective control or suppression of annual bluegrass and henbit while maintaining good winter wheat safety. For broadleaf weeds such as henbit and wild radish, combining pyroxasulfone with other herbicides of different MOA tends to enhance weed control efficacy by providing complimentary spectrum of control or suppression. Besides pyroxasulfone rate, other environmental factors including precipitation, OM content, and tillage practices can affect weed control efficacy and crop selectivity. Pyroxasulfone requires timely and adequate precipitation for activation, without which weed control efficacy suffers. Also, as soil OM, clay content and/or surface residue increases, herbicide efficacy decreases and winter wheat safety increases.

Sources of Materials

[1]Winter Wheat Seed, Pioneer Hi-Bred International, Inc., Johnston, IA 50131

[2]Pyroxasulfone Herbicide, BASF Corporation, Research Triangle Park, NC 27709

[3]Saflufenacil Herbicide, BASF Corporation, Research Triangle Park, NC 27709

[4]Flufenacet Herbicide, Bayer CropScience LP, Research Triangle Park, NC 27709

[5]Metribuzin Herbicide, Bayer CropScience LP, Research Triangle Park, NC 27709

[6]Mesosulfuron Herbicide, Bayer Cropscience LP, Research Triangle Park, NC 27709

[7]Thifensulfuron, DuPont, Wilmington, DE 19898

[8]CO_2 Pressurized Backpack Sprayer, Spraying Systems Co., Wheaton, IL 60189

[9]SAS Statistical Software, Version 9.4, Cary, NC 27513

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Table 1. Field locations, soil types, tillage types and crop seasons for research trials. [a]

			Soil Properties					
Location	Soil Type(s)	Tillage	pH	OM	Sand	Silt	Clay	Crop Season
				--------------- % ---------------				
Clayton	Wagram loamy sand	Conventional	5.3	1.25	77.7	16.3	6.0	2011-12
Clayton	Wagram loamy sand	Conventional	5.3	1.25	77.7	16.3	6.0	2012-13
	Wedowee sandy loam		6.4	1.25	67.9	19.6	12.5	2013-14
Edgecombe	Aycock very fine sandy loam	Conventional	5.3	2.50	64.1	26.4	9.5	2012-13
Plymouth	Cape fear loam	Conventional	5.5	10.0	45.7	41.8	12.5	2011-12
Salisbury	Pacolet sandy clay loam	No-Tillage	6.2	0.75	55.1	17.4	27.5	2011-12
								2012-13
								2013-14

[a] Abbreviations: OM, organic matter.

Table 2. Herbicide treatment list.[a]

Herbicide	Rate (g a.i. ha^{-1})	Timing
pyroxasulfone	60	PRE
pyroxasulfone	74	PRE
pyroxasulfone	89	PRE
pyroxasulfone + saflufenacil[b]	60 + 50	PRE
flufenacet + metribuzin	305 + 76	Spike
mesosulfuron + thifensulfuron[c]	15 + 21	POST
pyroxasulfone	60 fb 30	PRE fb POST
pyroxasulfone	60 fb 60	PRE fb POST

[a] Abbreviations: g a.i. ha^{-1}, grams active ingredient per hectare; PRE, treatments applied prior to crop emergence; Spike, treatments applied at wheat spike stage; POST, treatments applied after crop emergence; fb, followed by.

[b] Treatment included 1% V/V methylated seed oil.

[c] Treatment included 0.25% W/V nonionic surfactant

Table 3. Winter wheat injury as affected by environment.[a]

Location	Days after Wheat Spike					
	35			105		
	Phytotoxicity	Chlorosis	Stunting	Phytotoxicity	Chlorosis	Stunting
	----------%----------			----------%----------		
Clayton 2011	24 a	17 a	23 a	10 a	10 a	0 a
Clayton 2012	0 b	0 b	0 b	0 b	0 b	0 a
Clayton 2013	0 b	0 b	0 b	0 b	0 b	0 a
Edgecombe 2012	-	-	-	3 b	3 b	0 a
Plymouth 2011	0 b	0 b	0 b	0 b	0 a	0 a
Salisbury 2011	0 b	0 b	0 b	1 b	0 b	1 a
Salisbury 2012	0 b	0 b	0 b	2 b	2 b	0 a
Salisbury 2013	0 b	0 b	0 b	0 b	0 b	0 a

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

Table 4. Henbit (*Lamium amplexicaule*) control 21 days after wheat spike.[ab]

Treatment			Environment			
Herbicide	Rate ($g\ a.i.\ ha^{-1}$)	Application Timing	Clayton 2011	Plymouth 2011	Salisbury 2011	Salisbury 2012
			------------------------------%	-----------------------		
pyroxasulfone	60	PRE	100 a	56 ef	43 fg	3 h
pyroxasulfone	74	PRE	100 a	75 cd	66 de	14 h
pyroxasulfone	89	PRE	100 a	93 ab	76 bcd	13 h
pyroxasulfone + saflufenacil	60 + 50	PRE	100 a	95 a	86 abc	33 g
flufenacet + metribuzin	305 + 76	Spike	100 a	95 a	98 a	46 fg

[a] Abbreviations: $g\ a.i.\ ha^{-1}$, grams active ingredient per hectare; PRE, treatments applied 7 days prior to crop emergence; Spike, treatments applied at wheat spike stage.

[b] Means within table followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 5. Precipitation data during 2011-2014 field seasons for Clayton, Edgecombe, Plymouth, and Salisbury.

Month	Clayton			Edgecombe	Plymouth	Salisbury		
	2011-12	2012-13	2013-14	2012-13	2011-12	2011-12	2012-13	2013-14
	---------- cm ----------							
October	5.59	7.16	4.12	6.38	5.44	11.25	3.20	3.61
November	10.70	2.23	9.17	0.98	9.26	12.63	0.48	10.35
December	4.04	11.60	13.84	10.85	2.27	7.90	6.33	13.75
January	6.04	5.71	18.87	6.83	6.72	4.72	12.85	9.01
February	7.02	10.90	9.22	11.59	8.91	4.92	9.26	5.48
March	13.22	4.88	15.25	3.88	11.47	8.23	6.76	13.50
April	10.55	9.87	11.88	7.76	6.21	3.65	12.46	9.83
May	14.32	10.83	11.74	6.23	15.52	16.67	6.20	1.00
June	4.28	33.00	10.33	17.69	6.65	9.84	18.42	10.09

Table 6. Henbit, annual bluegrass, and wild radish control pooled over environments.[ab]

Treatment			Henbit			Annual bluegrass		Wild radish
			Days after Wheat Spike					
Herbicide	Rate (g a.i. ha^{-1})	Application Timing	35	49	105	14	140	14
			%					
pyroxasulfone	60	PRE	19 b	29 c	22 b	70 b	78 b	5 b
pyroxasulfone	74	PRE	26 b	38 bc	25 b	75 ab	79 b	8 b
pyroxasulfone	89	PRE	34 b	37 bc	26 b	89 a	87 ab	15 b
pyroxasulfone + saflufenacil	60 + 50	PRE	65 a	49 b	59 a	89 a	80 b	93 a
flufenacet + metribuzin	305 + 76	Spike	87 a	64 a	62 a	70 b	83 b	80 a
mesosulfuron + thifensulfuron	15 + 21	POST	-	34 bc	65 a	-	98 a	-
pyroxasulfone	60 fb 30	PRE fb POST	-	41 bc	26 b	-	85 ab	-
pyroxasulfone	60 fb 60	PRE fb POST	-	37 bc	26 b	-	83 b	-

Table 6 continued.

[a] Abbreviations: g a.i. ha^{-1}, grams active ingredient per hectare; fb, followed by; PRE, treatments applied prior to crop emergence; POST, treatments applied after crop emergence; Spike, treatments applied at wheat spike stage.

[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 7. Winter wheat yield as affected by environment pooled over herbicide treatments.[a]

	Crop Season		
Field Location	2011-12	2012-13	2013-14
	----------bushels/acre----------		
Clayton	43 d	7 h	30 f
Edgecombe	-	39 e	-
Plymouth	25 g	-	-
Salisbury	83 a	77 b	69 c

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