INCREASING WEED-MANAGEMENT TOOLS WITH AN HERBICIDE SAFENER FOR CONTROL OF ANNUAL GRASSES IN WHEAT

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Authorization to Submit Thesis

This thesis of Damilola Alex Raiyemo, submitted for the degree of Master of Science with a Major in Plant Science and titled "Increasing Weed-Management Tools with an Herbicide Safener for Control of Annual Grasses in Wheat," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

The Pacific Northwest is a highly productive wheat growing region of the world. One of the challenges confronting wheat farmers in the region is control of weeds below levels that negatively impact crop yield. Annual grasses are most troublesome to wheat farmers in the region due to similarities in growth habits between wheat and annual grasses. Growers often rely on preemergence or early postemergence herbicides for control of annual grasses. Preemergence herbicides include the very-long-chain fatty acid synthesis (VLCFA) inhibitors, such as flufenacet, pyroxasulfone or their premix with other herbicides while early postemergence herbicides include (1) acetyl CoA carboxylase (ACCase) inhibitors, such as diclofop, pinoxaden, and (2) acetolactate synthase (ALS) inhibitors, such as flucarbazone, pyroxsulam, mesosulfuron. However, selection pressure from consistent herbicide use resulted in annual grasses evolving resistant populations to multiple groups of herbicides, leaving growers with few herbicide options for control.

The very-long-chain fatty acid-inhibiting herbicides are effective herbicides for control of annual grasses and small-seeded broadleaf weeds in corn, soybeans, wheat, cotton and sorghum with few reported cases of resistance. Pyroxasulfone, a VLCFA inhibitor, controls annual grass and small-seeded broadleaf weeds such as Italian ryegrass, barnyardgrass, foxtails, crabgrasses, Palmer amaranth and common waterhemp in corn, wheat and soybeans at low use rate with efficacy also against populations resistant to glyphosate, acetolactate synthase inhibitors, acetyl CoA carboxylase inhibitors and triazines. Despite the use of several VLCFA inhibitors for over six decades, potential problem associated with herbicides in the group is crop injury. Differential tolerance of crop varieties under adverse environmental conditions or soil type could hinder the selective use of some herbicides. Herbicide safeners, applied either as tank mixture with the herbicide for preemergence or postemergence use or as seed treatments in the form of seed dressing have been used to protect crops from herbicide injury. Safeners increase the expression of genes encoding enzymes involved in herbicide detoxification including cytochrome P450 monooxygenases, glycosyltransferases, glutathione *S*-transferases and ATP-binding cassette transporters. Fluxofenim (Concep III, Syngenta Crop Protection, LLC, Greensboro, NC) is a widely used seed treatment safener for protection of sorghum from *S*-metolachlor injury at the rate of 0.4 g ai kg^{-1} seed. Perhaps, fluxofenim could protect newly bred wheat cultivars of the Pacific Northwest from soil-applied preemergence herbicides.

Chapter 1 includes a general introduction to wheat production in Idaho, challenges of annual grass control in wheat, herbicide options for annual grass control in wheat, effectiveness of very-long-chain fatty acid-inhibiting herbicides in annual grass control, tolerance of crops to VLCFA inhibitors via rapid metabolism, concept of crop safening, history, mechanism of action and use. Chapter 2 provides information on series of experiments evaluating the protection of soft white wheat varieties

and a hard spring wheat variety from very-long-chain fatty acid-inhibiting herbicides using fluxofenim safener. Data analysis showed that fluxofenim protection of soft white wheat varieties from VLCFA-inhibiting herbicides was variety-dependent. Fluxofenim significantly increased dry biomass of varieties LWW 15-72223, LWW 14-75044, Bruneau and UI Sparrow for *S*-metolachlor and dimethenamid-P herbicides, and LWW 15-72458 for pyroxasulfone herbicide. Varieties 09-15702A, UI Castle CL+ and UI Palouse CL+ were however tolerant to the three herbicide treatments regardless of fluxofenim treatment at the herbicide rates evaluated. Dose-response analysis showed effective doses resulting in 10% biomass reduction due to fluxofenim-alone treatment ranged from 0.55 g ai kg^{-1} seed for UI Magic CL+ to 1.23 g ai kg^{-1} seed for UI Palouse CL+. Effective doses resulting in 90% tolerance to *S*-metolachlor due to fluxofenim ranged from 0.07 g ai kg^{-1} seed for UI Castle CL+ to 0.55 g ai kg^{-1} seed for Brundage 96 and a similar pattern of response to dimethenamid-P and pyroxasulfone herbicides were also observed for the varieties, suggesting UI Castle CL+ has some level of tolerance to the herbicides. Glutathione *S*-transferase (GST) assay revealed that variety UI Castle CL+ had a 58% increase in GST specific activity relative to UI Sparrow and Brundage 96 with 30% and 38% increase in enzyme activity respectively at 0.36 g ai kg^{-1} seed treatment. Chapter 3 describes experiments to determine fluxofenim protection of six soft white winter wheat varieties selected based on prior greenhouse experiments from *S*-metolachlor, dimethenamid-P or pyroxasulfone injury under field conditions. Wheat density, height, head count and grain yield were evaluated in response to herbicide treatments with or without fluxofenim safener. Results indicated that fluxofenim had a negative impact on parameters observed in these field studies. Fluxofenim at 0.4 g ai kg^{-1} seed sorghum label rate failed to confer protection to soft white wheat varieties in the field studies conducted in fall 2018. The results of these field studies were therefore inconclusive and efficacy of fluxofenim to protect winter wheat would need to be evaluated further under different environmental conditions and soil types.

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Dedication

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CHAPTER 1: INTRODUCTION

Idaho is a productive wheat growing region, ranked 7th among winter wheat producing states of the United States, and accounts for 4.2% of total winter wheat production in the country. Wheat is second to potato in cash revenues within Idaho and in 2017 winter wheat production was valued at about 235 million dollars (NASS 2018). Over half of Idaho's total wheat production is soft white wheat exported to Asian markets (Robertson et al. 2004, Vocke and Ali 2013). One of the challenges confronting growers in the region besides producing wheat with optimum yields is control or suppression of weeds below levels that negatively impact crop yield. Grass weeds are major concerns in different cropping systems, and 6 out of the 10 world's worst weeds documented are grasses (Holm et al. 1977). Control of grass weeds in grass crops is quite challenging due to similarities in growth habits between the weed and crop (Horton et al. 1990). More so, chemical control of weeds in botanically related crops have always been a challenge (Hatzios and Hoagland 1989). Weeds compete with crop plants for light, space, water and nutrients, resulting in crop yield losses (Zimdahl 2004). Therefore, to obtain high yields in the production of crops requires dynamic approaches to weed management.

In the Pacific Northwest, annual grass weeds such as downy brome (*Bromus tectorum* L.), wild oat (*Avena fatua* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) are some of the most serious and consistent constraints to winter wheat production (Robertson et al. 2004). Most notably in the inland Pacific Northwest is Italian ryegrass, a cool season bunchgrass and a major annual weed in different parts of the world, with occurrence along roadsides and many agronomic crops (Hashem et al. 1998, Hulting et al. 2012). Italian ryegrass is prolific, producing thousands of seeds per plant, with seeds remaining physiologically dormant for 0 to 3 years and being persistent in the soil for up to 3 years (Burke et al. 2017, Manuchehri et al. 2019). Italian ryegrass infests both winter- and spring-planted crops in the Palouse region (Rauch et al. 2010). It competes with winter wheat for nutrients, water, space and light, contributing to cereal lodging and results in low harvest grain quality (Hulting et al. 2012). Field observations near Pullman, Washington showed that Italian ryegrass germination could be just 2% to 4% of seed present in the soil with seed bank densities as high as 64,839 seeds m^{-2} (Unger et al. 2012), resulting in 1297 to 2594 seeds m^{-2}. At 93 plants m^{-2}, Italian ryegrass was found to reduce wheat yield by 70 bushel/acre and in another report 31 % wheat yield loss was reported for nine ryegrass plants growing with 100 wheat plants m^{-2} (Appleby et al. 1976, Hashem et al. 1998).

Herbicides targeting the acetyl CoA carboxylase (ACCase) and acetolactate synthase (ALS) enzymes in plants are widely used for weed control; however overdependence on these herbicide groups has led to a global widespread occurrence of resistant weed species (Kaundun 2014, Tranel and

Wright 2002). There are about 48 reported unique weed resistance cases to ACCase inhibitors and 160 reported unique weed resistance cases to ALS inhibitors (Heap, 2019). In the Pacific Northwest, wheat producers have relied on preemergence or early postemergence herbicides for control of annual grasses. Preemergence herbicides include the very-long-chain fatty acid synthesis (VLCFA) inhibitors, such as flufenacet, pyroxasulfone or their premix with other herbicides while early postemergence herbicides include (1) acetyl CoA carboxylase (ACCase) inhibitors, such as diclofop, pinoxaden, and (2) acetolactate synthase (ALS) inhibitors, such as flucarbazone, pyroxsulam, mesosulfuron (Lyon 2017, Mallory-Smith 2015). Selection pressure from consistent use of these herbicides also resulted in populations of Italian ryegrass evolving resistance to multiple groups of herbicides including acetyl CoA carboxylase inhibitors, acetolactate synthase inhibitors, photosystem II inhibitors, 5-enolpyruvylshikimate 3-phosphate synthase inhibitor (glyphosate) and very-long-chain fatty acid elongase inhibitor (flufenacet), leaving growers with few herbicide options for its control (Burke et al. 2017, Hulting et al. 2012, Liu et al. 2016, Rauch et al. 2010). Furthermore, the obligate-outcrossing nature of Italian ryegrass may have facilitated the widespread occurrence of resistant populations as a result of transfer of resistant genes within or among populations (Loureiro et al. 2016). One effective method for early season control of Italian ryegrass is tillage; however, soil erosion is a concern in the Pacific Northwest. Early season control of Italian ryegrass populations is now a challenge for wheat producers due to small selection of herbicides available. Weed management strategies would therefore have to employ effective soil-applied preemergence herbicides with residual activity for better control of annual grasses in wheat.

Very-long-chain fatty acid-inhibiting herbicides are effective herbicides for control of annual grasses and small-seeded broadleaf weeds when applied preemergence. Only seven weed species have been confirmed to be resistant to this group of herbicides and three are in the United States: Italian ryegrass (*Lolium multiflorum*), tall waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*) (Heap 2019, Strom et al. 2019). Chloroacetamide is a family of herbicides inhibiting the very-long-chain fatty acid synthesis. Herbicides in this family includes alachlor, *S*-metolachlor, acetochlor, butachlor and dimethenamid-P. Earlier development of herbicides in the chloroacetamide family began in the 1950s with the introduction of α-haloacetamide, CDAA to the market in 1956 (Hamm 1974). CDAA was effective against annual grasses in corn and soybean with low toxicity; however, it causes skin irritation and performs less on low organic sandy loam soils thereby limiting its commercial success (Hamm 1974). Further research to find herbicides with less skin irritation, improved control of broadleaf weeds and better performance in sandy soils led to the discovery of other compounds by Monsanto, the likes of alachlor in 1969 and butachlor in 1971. Similarly, Ciba-Geigy discovered the biological activity of metolachlor in 1970, with full-scale

production of over 10,000 ton/year in 1978. Metolachlor marketed as Dual contained four stereoisomers and was later discovered that the (*S*)-enantiomers had more activity against weeds at a low use rate (Blaser et al. 1999). Several years of research led to the discovery of iridium catalyst to commercially produce *S*-metolachlor in 1996 (Blaser 2002). Chloroacetamide herbicides are effective in controlling annual grasses, small-seeded broadleaves and yellow nutsedge in corn, soybeans, cotton and sorghum (Fuerst 1987). They are absorbed by both shoot and root of plants, and symptoms include failure of seedlings to emerge, unrolled leaves in emerged seedlings or inhibition of shoot elongation in grasses while broadleaves exhibit crinkled or cupped leaves (Fuerst 1987). Germination of seed is not affected however.

Very-long-chain fatty acids are main constituents of hydrophobic polymers that prevent desiccation at leaf surfaces, provide stability to pollen grains and serve as sphingolipid components in various membranes (Trenkamp et al. 2004). They are synthesized in the endoplasmic reticulum through microsomal elongation system of C2 chains with malonyl-CoA catalyzed by multiple elongases. This microsomal elongation system is a 4-step reaction involving a condensation of acyl-CoA with malonyl-CoA catalyzed by ketoacyl-CoA synthase (KCS), followed by reduction of 3-ketoacyl-CoA by 3-ketoacyl-CoA reductase (KCR), dehydration of 3-hyroxyacyl-CoA by 3-hydroxy acyl-CoA dehydratase (HCD), and reduction of enoyl-CoA by enoyl-CoA reductase (ECR) (Böger 2003, Nobusawa et al. 2013). While the chloroacetamide compounds were a huge commercial success, their mechanisms of action were not elucidated until the 1990s (Boger et al., 2000; Gotz and Boger, 2004). The FAE1 gene encoding the very-long-chain fatty acid synthase was suggested to be the specific target for the chloroacetamide herbicides (Böger 2003). Inhibition of this enzyme limits the 4-step reaction pathway of C2 elongation of long chain fatty acids to very-long-chain fatty acids. The oxyacetamide herbicide flufenacet is another very-long-chain fatty acid inhibitor and more recently in the development of herbicides targeting the very-long-chain fatty acid elongases is the discovery of pyroxasulfone by Kumia Chemical Industry Company and introduced to the United States by BASF, Valent and FMC. Pyroxasulfone belongs to the family pyrazole and is assumed to have similar mechanism of action as the chloroacetamides (Tanetani et al. 2009). Elongation steps in the biosynthesis of very-long-chain fatty acid is also inhibited, although germination of seedlings is not also affected (Tanetani et al. 2009). Pyroxasulfone controls grass and small-seeded broadleaf weeds such as Italian ryegrass, barnyardgrass, foxtails, crabgrasses, Palmer amaranth and common waterhemp in corn, wheat and soybeans at low use rate and it is also effective against populations resistant to glyphosate, acetolactate synthase inhibitors, acetyl CoA carboxylase inhibitors and triazines (Shaner 2014).

Lines of inquiry leading to the discovery of mechanisms of action of the VLCFA-inhibiting herbicides also provided additional information on possible reasons why resistance of weeds to the very-long-chain fatty acid-inhibiting herbicides rarely occurs or does so at a slow rate despite their use for decades (Busi 2014). A cysteine residue at the active SH-site of the very-long-chain fatty acid synthase enzyme was suggested to be important for enzyme activity as well as irreversible binding of VLCFAE-inhibiting herbicides, and a selection yielding a functional enzyme with reduced herbicide binding would be unlikely (Böger 2003). In addition, replacement of VLCFAs by excess normal length fatty acids which was previously demonstrated to confer high chloroacetamide resistance may not be possible in weeds (Böger 2003). Also, very-long-chain fatty acids play multifunctional roles in plants, and it may be that the functions they perform are too important that resistance evolves at a slow rate (Trenkamp et al. 2004). However, cases where resistance to VLCFA-inhibiting herbicides has been reported, complex metabolic activities of cytochrome P450 monooxygenases and glutathione *S*-transferases which endows crop tolerance to herbicides have been suggested to also confer weed resistance (Busi 2014, Busi et al. 2012).

Crops such as corn or soybean possess innate tolerance to very-long-chain fatty acid inhibitors and this is achieved through rapid metabolism by inactivation of lethal parent herbicide molecule to less toxic compounds (Breaux 1986, Jaworski 1969, Lamoureux et al. 1971). The herbicide parent molecule on getting to the cytoplasm of a tolerant plant is either hydrolyzed, oxidized or reduced to a less active form in a reaction catalyzed by cytochrome P450 monooxygenases (P450s) or carboxylesterases. The less active form is then conjugated to endogenous substrates such as glucose to form O-glycosides in a reaction catalyzed by UDP-dependent glycosyltransferases. Alternatively, the less active form could be conjugated to tripeptide molecule, glutathione, to form a herbicide-glutathione conjugate in a reaction catalyzed by glutathione *S*-transferases. In some legumes such as soybean the initial metabolite is not a glutathione conjugate but a homoglutathione conjugate (Breaux 1986). Conjugates are then transported to the vacuole by ATP-binding cassette (ABC) transporters present in the plasmalemma or tonoplast where they are further degraded through the actions of several peptidase enzymes in the vacuole. Resulting products are transported to the cell wall (Riechers et al. 2010, Zhang et al. 2007). These series of reactions involved in herbicide detoxification are classified as Phase I – IV in plant metabolism of herbicides.

VLCFA-inhibiting herbicides may be effective against several weeds; however, potential problem associated with herbicides in this group is crop injury. The ability of plants to detoxify certain herbicides by specific enzymatic reactions is not evenly distributed among crops and weed plants which has long been recognized as an important factor determining the crop selectivity of commercialized herbicides (Hatzios 1991, Hatzios and Burgos 2004a). Differential metabolism of

herbicides in crop plants has resulted in the inability of some herbicides to be used in a cropping system despite their efficacy in weed control (De Carvalho et al. 2009). Variation in tolerance of crop varieties under adverse environmental conditions or soil type could also hinder the selective use of some herbicides (Rosinger 2014). For example, pyroxasulfone could result in wheat injury at shallow depths above 2 cm or under unfavorable environmental conditions such as inadequate or excess of moisture, cool and hot temperatures, poorly drained soils or widely fluctuating temperatures (Anonymous 2017). Metabolism differences in crop plants pose a challenge to the agricultural industry and therefore, new compounds being synthesized must not only have a strong efficacy in weed control but must also be non-phytotoxic to crops. Research into tolerance or rapid detoxification of more active and broad-spectrum herbicides by crops led to the discovery and commercial success of safeners (Hatzios and Hoagland 1989, Rosinger 2014).

The concept of safening was discovered when Otto Hoffman found that tomato plants pretreated with 2,4,6-trichlorophenoxyacetic acid were not injured following an accidental exposure to 2,4-D vapor. Protection conferred to tomato by 2,4,6-T from 2,4-D injury culminated in further research that led to the commercialization of 1,8-napthalic anhydride (NA) in 1971 for safening corn from thiocarbamate herbicide injury (Hatzios and Hoagland 1989). NA applied as a seed treatment was not commercially successful and the following decades witnessed the discovery of several compounds with safening capabilities. The oxime ethers cyometrinil (Concep I), oxabetrinil (Concep II) and fluxofenim (Concep III) were discovered by Ciba-Geigy for safening sorghum from metolachlor injury and flurazole was subsequently discovered by Monsanto for also safening sorghum from alachlor injury. Further research led to the discovery of the first tank-mixed safener, dichlormid in 1972 for use in corn to prevent injury from a thiocarbamate herbicide, barban. Cloquintocet-mexyl, fenchlorazole-ethyl and mefenpyr-diethyl were later produced for postemergence tank mixtures with aryloxyphenoxypropionate herbicide for use in cereals such as wheat (Davies and Caseley 1999). Several safener products have subsequently been commercialized for protecting crops from sulfonylureas, imidazolinones, cyclohexanediones, isoxazolidinones and many other herbicides (Table E1).

Safeners protect crops from herbicide injury by increasing the expression of genes encoding enzymes involved in detoxification; cytochrome P450 monooxygenases, glycosyltransferases, glutathione *S*-transferases and ATP-binding cassette transporters (Davies and Caseley 1999). Studies on safeners have shown that several hundreds of genes encoding proteins involved in herbicide detoxification are induced within a few hours of safener application (Zhang et al. 2007). Safeners notably do not alter metabolic pathways but rather influence the speed of herbicide metabolism, which is dependent on the levels of cytochrome P450s, glycosyltransferases and GSTs, and ultimately

dependent upon the genes which encodes those enzymes (Rosinger 2014). Safeners utilize different mechanisms of action in crop plants and one such mechanism is that due to structural similarity between some safeners and their respective herbicides, safeners compete with the herbicide for a common target site (Hatzios 1991). This structure-safening activity relationship as seen with flurazole-alachlor or fluxofenim-metolachlor combinations have been validated with computer-aided molecular modeling and comparative molecular field analysis (Bordás et al. 2000, Yenne and Hatzios 1990). Another mechanism of action is the enhancement of cofactors such as glutathione and herbicide-detoxifying enzymes in crop plants, although the precise signaling pathway utilized by safeners to protect crops from herbicide injury is still poorly understood. Recent findings suggest that safener coordinately induce the expression of multiple proteins, the likes of aldo-keto reductase family, 12-oxophytodienote reductase, glutathione *S*-transferase subunits belonging to tau, phi and lambda classes, cysteine synthase, γ-glutamyltranspeptidases and multidrug resistance-associated proteins in herbicide metabolism and detoxification (Zhang et al. 2007). More recently is the hypothesis that safeners may be utilizing an oxidized lipid-mediated (oxylipins) pathway or cyclopentenone-mediated signaling pathway, which subsequently leads to the expression of GSTs and other proteins involved in detoxification and plant defense (Riechers et al. 2010). Safeners protect sensitive crop varieties from herbicides by reducing crop injury to a level which is acceptable as demonstrated with the use of isoxadifen-ethyl to protect sensitive corn varieties from tembotrione herbicide injury (Rosinger 2014). Safeners could also protect crops from potential damage of a residual herbicide during crop rotation (Davies and Caseley 1999). They could also offer increases in the spectrum of herbicides available for weed control or increase the expansion and extension of uses and marketability of generic herbicides (Jablonkai 2013).

Annual grasses will continue to be one of the significant problems encountered by wheat producers in the Pacific Northwest. Selection pressure from consistent use of herbicides registered for wheat could further drive the evolution of resistant populations reducing the number of effective herbicides for grass control in wheat. Optimum annual grass control in wheat will therefore require novel herbicide chemistries not currently registered for use in wheat. The very-long-chain fatty acid-inhibiting herbicides are potent against several annual grasses and few weed resistance have been reported (Heap 2019). Only pyroxasulfone with low use rate and greater weed control relative to other VLCFA-inhibiting herbicides is registered for use in wheat without reported cases of weed resistance (Anonymous 2017). *S*-metolachlor and dimethenamid-P are also potent against several weeds including ryegrass (Liu et al. 2016) and are registered for use in crops such as sorghum and corn but not in wheat due to unacceptable level of injury (Chauhan et al. 2007). Similarly, pyroxasulfone could injure some wheat varieties under adverse environmental conditions (Anonymous 2017). Safeners

have been used in crops to counteract injury from herbicides (Jablonkai 2013). They are applied either as tank-mixtures with herbicides for preemergence/postemergence use or as seed treatments in the form of seed dressing. One advantage of seed treatment is that they prevent the possibility of safening weeds in the field. Fluxofenim (Concep III, Syngenta Crop Protection, LLC, Greensboro, NC) is widely used as a seed treatment safener to protect sorghum from *S*-metolachlor injury at 0.4 g ai kg^{-1} seed. Few studies have evaluated the response of wheat to fluxofenim at various rates. Riechers et al. (1997) used 0.25 g ai kg^{-1} seed of fluxofenim in a study evaluating response of wheat lines to dimethenamid. Also, response variation in the level of protection conferred to wheat cultivars against dimethenamid herbicide was reported to be dependent on variety (Riechers et al. 1996b). As a result, it is possible that safener could protect newly bred wheat cultivars of the Pacific Northwest from soil-applied herbicide injury.

Chapter 2 aims to understand the response of nineteen soft white wheat varieties and one hard white spring wheat to fluxofenim safener in the presence of *S*-metolachlor, dimethenamid-P and pyroxasulfone. Research objectives were to screen recently bred varieties for fluxofenim-enhanced tolerance to VLCFA-inhibiting herbicides, evaluate the phytotoxicity of fluxofenim-alone treatment to wheat varieties, determine rates of VLCFA-inhibiting herbicides for Italian ryegrass control, determine fluxofenim doses at which wheat varieties are protected from VLCFA-inhibiting herbicide injury and evaluate the influence of incremental doses of fluxofenim on glutathione *S*-transferase (GST) specific activity in wheat varieties.

Chapter 3 describes experiments conducted to understand the feasibility of reducing potential injury to wheat from use of VLCFA-inhibiting herbicides in the field. *S*-metolachlor and dimethenamid-P are not registered for use in wheat and although, pyroxasulfone is registered for use in wheat, since commercialization protection of wheat from potential injury using a seed-treatment safener (fluxofenim) under field conditions have not been investigated. The objective of this study was to evaluate the efficacy of fluxofenim safener in mitigating *S*-metolachlor, dimethenamid-P or pyroxasulfone injury to winter wheat varieties in the field. A field study was therefore designed to test the hypothesis that fluxofenim safener could protect winter wheat varieties evaluated previously in our greenhouse studies from *S*-metolachlor, dimethenamid-P or pyroxasulfone injury.

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CHAPTER 2: SCREENING OF WHEAT VARIETIES FOR FLUXOFENIM-ENHANCED TOLERANCE TO *S*-METOLACHLOR, DIMETHENAMID-P AND PYROXASULFONE HERBICIDES

Abstract

Annual grass weeds are consistent problems, reducing profitability to wheat farmers in the Pacific Northwest. Preemergence herbicide options for annual grass control in wheat are limited and their use may provide control. The herbicides *S*-metolachlor and dimethenamid-P control annual grasses but are not registered for use in wheat due to crop injury. The overall objective of this study was to evaluate safener protection of soft white winter wheat varieties from very-long-chain fatty acid-inhibiting herbicide injury. Response variation among wheat varieties was investigated by treating nineteen soft white winter wheat varieties with fluxofenim and then applying *S*-metolachlor, dimethenamid-P or pyroxasulfone preemergence. Aboveground response of six varieties to incremental doses of fluxofenim were also evaluated to identify any negative effects of fluxofenim on winter wheat. Additionally, a fluxofenim dose-response experiment was conducted with UI Sparrow, Brundage 96 and UI Castle CL+ in the presence of *S*-metolachlor, dimethenamid-P or pyroxasulfone. Finally, an experiment measuring glutathione *S*-transferase (GST) activity for UI Sparrow, Brundage 96 and UI Castle CL+ was performed using a spectrophotometer. Greenhouse results showed that fluxofenim-increased tolerance in winter wheat to *S*-metolachlor, dimethenamid-P and pyroxasulfone were variety-dependent. Varieties LWW 15-72223, LWW 14-75044, Bruneau and UI Sparrow had significantly high aboveground biomass with herbicide + fluxofenim seed treatments for both *S*-metolachlor and dimethenamid-P herbicides compared to their respective herbicide treatment only. Fluxofenim improved the biomass of LWW 15-72458 with pyroxasulfone treatment. Interestingly, varieties 09-15702A, UI Castle CL+ and UI Palouse CL+ were tolerant to the three herbicide treatments regardless of fluxofenim safener. Six varieties were thus selected for further study based on positive, mixed and no response to safener across both *S*-metolachlor and dimethenamid-P herbicide treatments as well as their popularity in Idaho. Crop injury from safener would limit its use and so in the absence of herbicide, we set a threshold of 10% crop injury. Dose-response analyses showed effective doses resulting in 10% biomass reduction due to fluxofenim-alone treatment ranged from 0.55 g ai kg^{-1} seed for UI Magic CL+ to 1.23 g ai kg^{-1} seed for UI Palouse CL+. The experiment evaluating the response of three varieties to safener in the presence of herbicides showed effective doses resulting in 90% fluxofenim-enhanced tolerance to *S*-metolachlor ranged from 0.07 g ai kg^{-1} seed for UI Castle CL+ to 0.55 g ai kg^{-1} seed for Brundage 96 while effective doses resulting in 90%

fluxofenim-enhanced tolerance to dimethenamid-P ranged from 0.09 g ai kg^{-1} seed for UI Sparrow to 0.73 g ai kg^{-1} seed for Brundage 96. Similar findings were observed for pyroxasulfone where effective doses resulting in 90% fluxofenim-enhanced tolerance ranged from 0.30 g ai kg^{-1} seed for UI Castle CL+ to 1.03 g ai kg^{-1} seed for Brundage 96. GST assay showed increased enzyme activity for the three varieties in the presence of safener. GST specific activity at 0.36, 0.91 and 1.96 g ai kg^{-1} seed treatments was not significantly different for Brundage 96 and UI Castle CL+ but differed for UI Sparrow. UI Castle CL+ had a 58% increase in GST specific activity relative to UI Sparrow and Brundage 96 with 30% and 38% increase in enzyme activity respectively at 0.36 g ai kg^{-1} seed treatment. Results from these series of experiments indicate that safener protects soft white winter wheat varieties (UI Sparrow, Brundage 96 and UI Castle CL+) from *S*-metolachlor, dimethenamid-P or pyroxasulfone injury at the herbicide rates tested.

Introduction

Idaho is a productive wheat growing region accounting for 4.2% of total winter wheat production in the United States (NASS 2018). One of the challenges confronting growers in the region besides producing wheat with optimum yields is control or suppression of weeds below levels that negatively impact crop yield. Annual grass weeds such as downy brome (*Bromus tectorum* L.), wild oat (*Avena fatua* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) are some of the most serious and consistent constraints to winter wheat production in the Pacific Northwest (Robertson et al. 2004). Italian ryegrass, a cool season bunchgrass and a major annual weed in different parts of the world infests both winter- and spring-planted crops in the Palouse region (Hashem et al. 1998, Hulting et al. 2012, Rauch et al. 2010). It is prolific and competes with winter wheat for nutrients, water, space and light, contributing to cereal lodging and results in low harvest grain quality (Hulting et al. 2012).

For many years, wheat producers in the Pacific Northwest relied on preemergence or early postemergence herbicides for control of annual grasses. Preemergence herbicides include the very-long-chain fatty acid synthesis (VLCFA) inhibitors, such as flufenacet, pyroxasulfone or their premix with other herbicides while early postemergence herbicides include (1) acetyl CoA carboxylase (ACCase) inhibitors, such as diclofop, pinoxaden, and (2) acetolactate synthase (ALS) inhibitors, such as flucarbazone, pyroxsulam, mesosulfuron (Lyon 2017, Mallory-Smith 2015). Selection pressure from consistent use of these herbicides have resulted in populations of Italian ryegrass evolving resistance to ACCase, ALS, EPSPS (glyphosate) and VLCFA-inhibiting herbicide (flufenacet), leaving growers with few herbicide options for early season control (Burke et al. 2017, Hulting et al. 2012, Liu et al. 2016, Rauch et al. 2010).

The very-long-chain fatty acid-inhibiting herbicides have been used for decades and are effective on annual grasses and small-seeded broadleaf weeds when applied preemergence with few reported cases of weed resistance (Heap 2019, Strom et al. 2019). Despite this effectiveness against several weeds, the ability of plants to detoxify certain VLCFA-inhibiting herbicides by specific enzymatic reactions is not evenly distributed among crops (Hatzios 1991, Hatzios and Burgos 2004). Differential tolerance of crop varieties under adverse environmental conditions or soil type could also hinder the selective use of some herbicides (Rosinger 2014). *S*-metolachlor and dimethenamid-P are potent against several weeds including Italian ryegrass (Liu et al. 2016) and are registered for use in sorghum and corn but not in wheat due to unacceptable level of injury (Chauhan et al. 2007). Pyroxasulfone is registered for use in wheat but could result in wheat injury at shallow depths above 2 cm or under unfavorable environmental conditions such as inadequate or excess of moisture, cool and hot temperatures, poorly drained soils or widely fluctuating temperatures (Anonymous 2017). Metabolism differences among crop plants or varieties therefore poses a challenge to the agricultural industry and new compounds being synthesized must not only have a strong efficacy in weed control but must also be non-phytotoxic to crops.

Safeners have been used in crops to counteract injury from herbicides (Jablonkai 2013). They are applied either to the soil as preemergence, tank-mixed with herbicides as postemergence or as seed treatments in the form of seed dressing. One advantage of seed treatment is that they prevent the possibility of also protecting weeds in the field. Safeners protect crops from herbicide injury by increasing the expression of genes encoding enzymes involved in detoxification; cytochrome P450 monooxygenases, glycosyltransferases, glutathione *S*-transferases and ATP-binding cassette transporters (Davies and Caseley 1999). They elicit their actions in varieties sensitive to herbicides by reducing crop injury to a level which is acceptable as demonstrated with the use of isoxadifen-ethyl to protect sensitive corn varieties from tembotrione herbicide injury (Rosinger 2014). Safeners could also protect crops from potential damage of a residual herbicide during crop rotation, offer increase in the spectrum of herbicides available for weed control or increase the expansion and extension of uses and marketability of generic herbicides (Davies and Caseley 1999, Jablonkai 2013).

Fluxofenim (Concep III, Syngenta Crop Protection, LLC, Greensboro, NC) is widely used to prevent *S*-metolachlor injury to sorghum at the rate of 0.4 g ai kg^{-1} seed. Few studies have evaluated the response of wheat to fluxofenim at various rates. Riechers et al. (1997) evaluated the safening of wheat lines to the herbicide dimethenamid at 0.25 g ai kg^{-1} seed of fluxofenim. Response in the level of protection conferred by fluxofenim to wheat cultivars against dimethenamid herbicide injury was reported to vary among cultivars (Riechers et al. 1996b). As a result, it is possible that safener could protect newly bred wheat cultivars of the Pacific Northwest from soil-applied herbicide injury.

The objectives of this research were to evaluate recently bred varieties for fluxofenim-enhanced tolerance to VLCFA-inhibiting herbicides, determine phytotoxicity of fluxofenim-alone treatment to wheat varieties, determine rates of VLCFA-inhibiting herbicides for Italian ryegrass control, determine fluxofenim doses at which wheat varieties are protected from VLCFA-inhibiting herbicide injury and evaluate the influence of incremental doses of fluxofenim on glutathione *S*-transferase (GST) specific activity in wheat varieties.

Materials and Methods

Response of safener treated wheat varieties to chloroacetamide and pyrazole herbicides

Plant materials and greenhouse evaluation

Nineteen soft white winter wheat varieties and one hard white spring wheat variety planted in the Pacific Northwest were evaluated for tolerance to preemergence herbicides (*S*-metolachlor, dimethenamid-P or pyroxasulfone) with or without fluxofenim seed treatment under greenhouse conditions. Fluxofenim (PESTANAL analytical standard, 34387-100MG, Sigma-Aldrich Corp, St Louis, MO) was applied to wheat seeds by seed dressing. Wheat seeds (35g) in vials were treated with either 2 mL of 80% ethanol without safener or with 0.5 g ai kg^{-1} seed fluxofenim applied as a seed treatment in 2 mL of 80% ethanol (Goodrich et al. 2018, Hatzios and Hoagland 1989, Riechers et al. 1996a). Stream of air was immediately passed into the vial with constant agitation to ensure even coverage. Seeds were dried further on blotter paper for 10 minutes under a fume hood. Seeds were then planted in 634 cm^3 plastic pots[1] containing premoisten potting mix[2] compressed 1 cm deep in the pots. Nine seeds were planted per pot at a depth of 2 cm.

S-metolachlor at 1.418 kg ai ha^{-1} (Dual Magnum, 913 g/L *S*-metolachlor EC, Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC 27409), dimethenamid-P at 1.005 kg ai ha^{-1} (Outlook, 719 g/L dimethenamid-P EC, BASF, Crop Science Division, 26 Davis Drive, Research Triangle Park, NC 27709) or pyroxasulfone at 0.118 kg ai ha^{-1} (Zidua, 500 g/L pyroxasulfone SC, BASF, Crop Science Division, 26 Davis Drive, Research Triangle Park, NC 27709) were applied 24 hours after planting with a cabinet sprayer[3] that was calibrated to deliver 121.6 L ha^{-1} at 276 kPa.

Herbicides were incorporated to approximately 0.6 cm depth by delivering 0.8 L min^{-1} of water for 5 secs via overhead irrigation for each pot. Pots were watered every two days with 83 ml of water. Pots were arranged in a randomized complete block design with 8 treatments (untreated control, safener only, and the three herbicides with or without safener seed treatment). All pots with herbicide treatments and varieties with or without safener treatment were randomized within each block (replicate) on the greenhouse benches. Greenhouse conditions were maintained at 21/10 C day/night and natural sunlight was supplemented with high pressure sodium lights at the surface of the soil to

maintain a 16/8-hour photoperiod. Each treatment including untreated control was replicated four times. The whole experiment was repeated within a period of 9 weeks from the first experiment. At 21 days after treatment (DAT), emergence counts were taken, and aboveground shoots were harvested. Wheat plants were dried at 60^0C for 72 hours and aboveground dried weight was calculated as an average of total stand counts per pot.

Statistical Analysis

Data were analyzed with SAS version 9.4[4]. Aboveground biomass was subjected to analysis of variance (ANOVA) using PROC GLIMMIX. Data from two experimental runs were analyzed separately and not pooled. Treatment means were separated with pair-wise comparisons at 95% confidence level. Normality and homogeneity of variance assumptions were determined with PROC UNIVARIATE and PROC GLIMMIX respectively. The data were analyzed using the statistical model outlined below:

$$y_{ij}=\mu + r_i + \alpha_j + \epsilon_{ij} \quad [1]$$

Where y_{ij} is the expected response for the *i*th block/replicate and *j*th herbicide-safener treatment; μ is the grand mean; r_i is the random effect of the *i*th block/replicate, NID(0, σ_r^2); α_j is the fixed effect of the *j*th herbicide-safener treatment; and ϵ_{ij} is the random error term, NID(0, σ_ϵ^2).

Fluxofenim dose-response

Response of selected wheat varieties to incremental doses of safener

Six varieties selected from previous greenhouse studies were evaluated for their response to fluxofenim-alone treatment. The commercially formulated fluxofenim safener; Concep III (Syngenta Crop Protection, Greensboro, NC) was used for this study. According to the label, treated sorghum seeds may exhibit a slight germination reduction due to differences in sorghum lines (Anonymous 2012), therefore response of wheat lines to fluxofenim-alone treatment was determined. A series of doses including 0.2, 0.4, 0.6, 0.8, 1.6 and 3.2 g ai kg^{-1} seeds were applied to wheat seeds. Preparation of the safener was done according to the label rate for sorghum seeds. Briefly, for 10 g wheat seeds with label rate of 0.4 g ai kg^{-1} seed Concep III, 4.17 µL Concep III was pipetted into a 1.5 ml Eppendorf tube, 74.07 µL distilled water was then added to make up 78.24 µL total slurry (label rate for sorghum is 118-237 ml total slurry/23 kg seeds). The tube was then agitated on a vortex mixer for 15 seconds. Ten grams of wheat seeds were then weighed, placed in a 50 ml beaker and the slurry pipetted on the seeds and mixed with a spatula to ensure even coverage. Treated seeds were planted within 24 hours in 634 cm^3 plastic pots[1] containing premoisten potting mix[2] at 2 cm depth as previously described. Experimental design was setup as a randomized complete block design with four

replications. The dose-response experiment for the six varieties was repeated within a period of 9 weeks after the first experiment. Height was recorded at 14 and 21 days after treatment (DAT), and aboveground shoots were harvested at 21 DAT. Harvested shoots were dried in the oven at 60^0C for 72 hours and aboveground dried weight was calculated as average of total stand counts per pot.

Statistical Analysis

Height and biomass data were analyzed with nonlinear regression in SAS version 9.4[4]. PROC NLMIXED was used to fit the data to a two-parameter log-logistic model. Two experimental runs were pooled for analysis (Kniss 2018, Price et al. 2012). The model was modified to incorporate a constant K as suggested by Schabenberger et al. (1999). K was calculated by taking a desired percentage response, Q and dividing it by 100 – Q, so that K = Q/(100-Q). K is therefore equal to 9 which is 90% of the control response for decreasing height or biomass variables.

$$y = K*100/(K + \exp(b(\log(x) - \log(ED_Q)))) \quad [2]$$

Where y is the response (relative) variable; x is the fluxofenim dose; ED_Q is the effective dose at which the response is 10% reduction in height or biomass; and parameter *b* describes the slope of the dose-response curve. The fluxofenim dose required to cause 10% height and biomass reduction were estimated at 14 and 21 days after treatment (DAT). We initially fit the model separately to varieties, then constructed an overall model to compare varieties. Estimates and their standard errors were obtained for 10% height reduction and 10% biomass reduction from the pooled data. Pairwise comparison was done to compare the ED_{10} values between varieties.

Italian ryegrass response to chloroacetamide and pyrazole herbicides

Greenhouse dose-response study

Seeds collected from an Italian ryegrass population near Culdesac ID in 2014 were used to characterize the response of Italian ryegrass to *S*-metolachlor, dimethenamid-P and pyroxasulfone. Plastic pots[1] (634 cm^3) were filled with growth medium containing 4:1 mixture of sieved soil (loam soil type, pH of 5.1, organic matter content of 3.3% and cation exchange capacity of 13 cmol(+) kg^{-1}) and silica sand (Lane Mountain Company, Valley Washington 99181-0127). Pots were placed individually on weigh boats to avoid soil water loss. Each pot was filled with the growth medium to 6.5 cm mark, nine seeds were then placed on the surface of the growth medium and covered with approximately 35 cm^3 of the growth medium spread evenly over the Italian ryegrass seeds**.** Within 48 hours, *S*-metolachlor, was applied at rates ranging from 66.69 to 4268 g ha^{-1}, dimethenamid-P at rates ranging from 34.47 to 2206 g ha^{-1} or pyroxasulfone at rates ranging from 14.75 to 472 g ha^{-1}. Herbicides were applied with a cabinet sprayer[5] fitted with a single 8002EVS nozzle 17 cm above the

surface of the medium and calibrated to deliver 103 L ha^{-1} at 221 kPa and a speed of 2.75 mph. Pots were subsequently irrigated with the cabinet sprayer delivering 0.3-inch water at 17 cm above the surface of the growth medium. Design for the study was a randomized complete block design with four replications and the study was repeated. Aboveground shoots were harvested at 21 DAT and harvested shoots were dried in the oven at 60^0C for 72 hours.

Biomass data were analyzed with nonlinear regression in SAS version 9.4[4]. PROC NLMIXED was used to fit the data to a two-parameter log-logistic model as described previously.

Response of wheat varieties to incremental safener doses at two herbicide rates

Greenhouse dose-response study

Three varieties were selected from the original six varieties (grouped into categories; responded, mixed response and no response) to determine safener doses at which varieties are protected from two rates of *S*-metolachlor and dimethenamid-P, and a single rate of pyroxasulfone herbicides. Seeds of varieties UI sparrow, Brundage 96 and UI Castle CL+ were treated with fluxofenim at doses ranging from 0.2 to 1.6 g ai kg^{-1} seed as previously described. Seeds were planted in 634 cm^3 plastic pots[1] filled with growth medium containing 4:1 mixture of sieved soil (loam soil type, pH of 5.1, organic matter content of 3.3% and cation exchange capacity of 13 cmol(+) kg^{-1}) and silica sand (Lane Mountain Company, Valley Washington 99181-0127). Pots were placed on weigh boats to avoid soil water loss. Nine seeds were sown at 2 cm depth. Within 48 hours, *S*-metolachlor, was applied at rates 1010 g ha^{-1} and 1782 g ha^{-1}, dimethenamid-P at rates 647 g ha^{-1} and 1005 g ha^{-1} or pyroxasulfone at 236 g ha^{-1}. Herbicides were applied with a cabinet sprayer[5] fitted with a single 8002EVS nozzle 17 cm above the surface of the medium and calibrated to deliver 103 L ha^{-1} at 221 kPa. Pots were subsequently irrigated with the cabinet sprayer delivering 0.3-inch water at 17 cm above the surface of the growth medium. Design for the study was a randomized complete block design with four replications and the study was repeated. Aboveground shoots were harvested at 21 DAT and harvested shoots were dried in an oven at 60^0C for 72 hours.

Biomass data were analyzed with nonlinear regression in SAS version 9.4[4]. PROC NLMIXED was used to fit the data to a two-parameter exponential function (Kniss et al. 2011). Two runs of the experiment were pooled for analysis.

$$y = C + A*(1 - \exp(-b*\text{rate})) \quad [3]$$

Where y is the response (relative) variable; C is the lower asymptote; *b* is the slope of the dose-response curve; and A is the increase from C to the maximum asymptote, A+C. An estimated fluxofenim dose, ED_{90} required to cause 90% increased tolerance (biomass) and their standard errors 21 days after treatment (DAT) were obtained using the equation:

$$\log (0.1*(A+C)/A)/-b \quad [4]$$

Glutathione *S*-transferase enzyme assay

Plant material

Seeds of varieties UI Sparrow, Brundage 96 or UI Castle CL+ were treated with the commercial fluxofenim safener at 0.36, 0.91 or 1.96 g ai kg^{-1} seed as previously described. Fluxofenim doses were obtained from previous study evaluating fluxofenim-alone injury to six varieties. The doses were pooled estimates of effective doses resulting in 4%, 10% and 20% biomass reduction to wheat varieties. Nine seeds of each variety were treated and planted 2 cm deep in potting mix[6] within 24 hours of seed treatment. Pots were randomized on the greenhouse bench and watered every two days. Plants were grown at 21/10 C day/night temperature and natural sunlight was supplemented with high pressure sodium lights at the surface of the soil to maintain a 16/8-hour photoperiod. After 7 days, plants were harvested mid-morning to lower the impact of the environment or circadian rhythm on protein levels (Burns et al. 2017).

Enzyme Assay

All tissue homogenization and extraction steps were carried out at 4 C. Protein extraction was performed as previously described (Riechers et al. 1997, Taylor et al. 2013). Seven-day-old wheat shoots weighing 0.4 to 0.8 g were ground with a pestle and mortar under liquid nitrogen and then extracted in 1 ml extraction buffer containing 0.1 M Tris HCl (pH 7.5), 2 mM ethylenediaminetetraacetic acid (EDTA), 1 mM DTT and 10% polyvinylpolypyrrolidone (PVPP). The homogenates were vortexed and centrifuged at 10,000g for 20 min at 4 C, followed by straining through filter columns (Thermo Scientific™ Disposable Filter Columns). Filtrates were then re-centrifuged at 10,000g for 5 min at 4 C. Supernatants were decanted and total protein concentrations determined using Quick Start™ Bradford Protein Assay (Bio-Rad). A concentration of 5000 µg ml^{-1} total protein was then used in each assay sample. Crude extracts were held on ice and subjected to GST enzyme activity measurement using a GST assay kit (Sigma CS0410) according to the manufacturer's instructions. Enzyme activities were determined spectrophotometrically (Nanodrop 2000C, Thermo Fisher Scientific) by measuring the conjugation of L-glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm over 5 min at 21 C. Enzyme activity were calculated as GST specific activities. The experiment evaluating the influence of fluxofenim on GST specific activities was conducted twice, with four replications per experiment. Analysis of variance followed by pairwise-comparison test using PROC GLIMMIX in SAS version 9.4[4] was conducted to determine

significant differences in GST specific activity among seed treatment rates for each variety. Results are reported as means +/- SE.

Results

Response of safener treated wheat varieties to chloroacetamide and pyrazole herbicides

Plant materials and greenhouse evaluation

Greenhouse results indicated that fluxofenim significantly increased the biomass of varieties LWW 15-72223, LWW 14-75044, Bruneau, UI Sparrow, Bobtail and a spring wheat UI Platinum across two experimental runs when fluxofenim-treated seeds were compared to untreated seeds for *S*-metolachlor herbicide treatment (Table 2.1). Similarly, fluxofenim significantly improved the biomass of varieties LWW 15-72223, LWW 14-75044, Bruneau, UI Sparrow, 10-08606A, LCS Artdeco and UI Platinum when fluxofenim seed treatment was compared to their respective without-fluxofenim seed treatment for dimethenamid-P herbicide across two experimental runs (Table 2.2). Thirteen varieties including eight released varieties did not respond to safener with pyroxasulfone treatment (Table 2.3). Only variety LWW 15-72458 had a significantly higher biomass with fluxofenim treatment for pyroxasulfone herbicide. Pyroxasulfone is registered for use in winter wheat and although maximum label rate was used for screening of response to safener, innate tolerance of the varieties to this herbicide may be masking any protection due to safener.

Overall, five varieties responded to safener for *S*-metolachlor and dimethenamid-P herbicide treatment across both experimental run 1 and 2 while five varieties did not respond to safener for the three herbicide treatments across both experimental runs. Responses of ten varieties were inconsistent for the herbicides across two experimental runs (Table 2.4). Fluxofenim did not significantly increase the biomass of varieties 09-15702A, UI Castle CL+ and UI Palouse CL+ across two experimental runs for *S*-metolachlor, dimethenamid-P or pyroxasulfone, suggesting that the varieties could possess some level of innate tolerance to the herbicides (Table 2.4). Untreated controls of most varieties compared with their safener only treatments were not statistically different at 5% significance level suggesting that there was no safener effect on the varieties at 0.5 g ai kg^{-1} seed used for varietal screening. Untreated control of varieties 09-18702A, LCS Artdeco and UI Platinum were however significantly higher than their respective safener-only treatments (data not shown). UI Platinum, a hard-white spring wheat responded significantly to fluxofenim safener across *S*-metolachlor and dimethenamid-P herbicide treatments (Table 2.2, Table 2.3). However, attention was focused on soft white winter wheat varieties which are mostly grown in Idaho. Based on the patterns of responses to safener, varieties were grouped into three categories; (1) varieties responding to safener in both experimental runs, (2) varieties with mixed response in both experimental runs and (3) varieties with no response to

safener i.e. safener did not significantly increase the biomass of the variety relative to a no-safener treatment in the presence of a herbicide (Table 2.5). These categories were also corroborated by visual assessment following 21 days of herbicide treatments (Figure 2.1). A smaller set of varieties were thus selected from these categories for further study.

Fluxofenim dose-response

Response of selected wheat varieties to incremental doses of safener

Fluxofenim is only registered for use in sorghum at the rate of 0.4 g ai kg^{-1} seed, therefore it was important to ascertain winter wheat varietal responses to potential injury from fluxofenim treatment. Fluxofenim dose values resulting in 10% biomass reduction (ED_{10}) for six varieties selected were calculated. Response of winter wheat to increasing fluxofenim treatment was variable across varieties, with ED_{10} values ranging from 0.55 to 1.23 g ai kg^{-1} seed (0.55, 0.67, 0.85, 0.87, 1.15 and 1.23 g ai kg^{-1} seed for UI Magic CL+, Brundage 96, UI Sparrow, UI Castle CL+, LWW 15-72223 and UI Palouse CL+ respectively) for pooled analysis across two experimental runs (Table 2.6). These estimates were 1.375- to 3.075- times higher than recommended label rate for use in sorghum. Variety UI Palouse CL+ (ED_{10} value = 1.23 g ai kg^{-1}) and a yet to be released wheat line LWW 15-72223 (ED_{10} value = 1.15 g ai kg^{-1}) were more tolerant to fluxofenim compared to variety UI Magic CL+ (ED_{10} value = 0.55 g ai kg^{-1}). The tolerance levels for UI Palouse CL+ and LWW 15-72223 were 2-3 times higher than UI Magic CL+. The rate or slope parameter, *b* describes the steepness of the dose-response curve. Varieties LWW 15-72223 (*b* = 1.442), UI Castle CL+ (*b* = 1.306) and UI Palouse CL+ (*b* = 1.244) were much steeper in biomass response compared to variety UI Magic CL+ (*b* = 0.613) (Table 2.6). Pairwise comparison between varieties with respect to effective doses causing 10% biomass reduction showed no significant differences among varieties except variety LWW 15-72223 with significantly higher ED_{10} value than UI Magic CL+ ($p = 0.0202$), and variety UI Palouse CL+ also with significantly higher ED_{10} value than UI Magic CL+ ($p = 0.0171$) (Table 2.7).

Visual observation of wheat response to increasing doses of fluxofenim at 7 days following seed treatment showed 1.6 and 3.2 g ai kg^{-1} seed caused observable inhibition of seedling emergence across the six varieties (Figure 2.4). A follow-up assessment of visual injury 14 days following seed treatment showed recovery of varieties from this initial delay in emergence (Figure 2.5) and by 21 days following seed treatment, injury was less noticeable across the varieties (figure not shown). Fluxofenim dose values resulting in 10% height reduction for the six varieties at 14 days following fluxofenim seed treatment were estimated (Table 2.8). Response of wheat height to increasing fluxofenim treatment was also variable among varieties, with ED_{10} values (10% height reduction) ranging from 0.28 to 0.77 g ai kg^{-1} seed (0.28, 0.33, 0.36, 0.40, 0.51 and 0.77 g ai kg^{-1} seed for UI

Sparrow, UI Magic CL+, UI Palouse CL+, LWW 15-72223 and Brundage 96 respectively) for pooled analysis across two experimental runs. Varieties Brundage 96 (ED_{10} value = 0.77) and LWW 15-72223 (ED_{10} value = 0.51) were more tolerant to fluxofenim treatment compared to variety UI Sparrow (ED_{10} = 0.28) (Table 2.9). Both UI Sparrow and Brundage 96 are semi dwarf cultivars and therefore other factors may underlie their reduced plant height in response to fluxofenim. Estimates of effective doses causing 10% height reduction at 21 days following seed treatment ranged from 0.63 to 0.83 g ai kg^{-1} seed, however, pairwise comparison showed no significant differences in height between varieties at 21 days following seed treatment (data not shown). Similarly, the rate or slope parameter, *b* did not differ between varieties (data not shown). The series of dose-response studies evaluating the impact of fluxofenim to winter wheat varieties suggests that although fluxofenim delays emergence at doses above 1.6 g ai kg^{-1} seed in winter wheat, this form of injury is transient.

Italian ryegrass response to chloroacetamide and pyrazole herbicides

Greenhouse dose-response study

S-metolachlor and dimethenamid-P herbicides are effective for control of annual grass weeds and small-seeded broadleaf weeds. Control of a ryegrass population was evaluated in the greenhouse. Effective doses of *S*-metolachlor or dimethenamid-P resulting in 50% and 90% biomass reduction were calculated. Pyroxasulfone at the lowest dose of 14.75 g ai ha^{-1} resulted in mortality of the Italian ryegrass population and data were not available to fit a dose response analysis. The complete mortality observed in this study is consistent with another study reported in Oregon where 7.5 g ai ha^{-1} controlled both susceptible and resistant ryegrass populations (Liu et al. 2016). Dose-response analyses showed that dimethenamid-P or *S*-metolachlor at a dose of 22.97 g ai ha^{-1} or 71.06 g ai ha^{-1} respectively reduced the biomass of the Italian ryegrass population by 50%, and at a dose of 213.04 g ai ha^{-1} or 558.12 g ai ha^{-1} for dimethenamid-P or *S*-metolachlor respectively, the biomass of the Italian ryegrass population was reduced by 90% (Table 2.10). The dose-response curves showing the relationship between increasing herbicide doses and biomass reduction 21 days following herbicide treatments are presented in Figure 2.6.

Response of wheat varieties to incremental safener doses at two herbicide rates

Greenhouse dose-response study

Based on the level of tolerance of varieties to fluxofenim treatment from our previous dose-response experiments and 2019 end-use quality ranking of wheat varieties, UI Sparrow, Brundage 96 and UI Castle CL+ were further evaluated for response to incremental doses of fluxofenim at two doses of *S*-metolachlor, dimethenamid-P and twice the recommended label rate of pyroxasulfone for

use in winter wheat. Varieties exhibited a pattern of increase in biomass with fluxofenim seed treatments relative to herbicide treatments only across two experimental runs for *S*-metolachlor, dimethenamid-P or pyroxasulfone (Figures 2.7, 2.8, 2.9. 2.10, 2.11, 2.12, 2.13). This increase in biomass peaked at an upper asymptote for two varieties and further increase in fluxofenim seed treatment did not result in additional increase in biomass. Brundage 96 on the other hand showed a continuous increase in biomass up to the highest fluxofenim rate of 1.6 g ai kg^{-1} seed evaluated. Estimates of effective doses of fluxofenim resulting in 90% protection against herbicide injury were calculated. Protection (ED_{90}) in this case we defined as a minimum dose of fluxofenim conferring tolerance to a variety in the presence of the herbicide. UI Castle CL+ was most tolerant requiring 0.07 g ai kg^{-1} seed of fluxofenim for 90% tolerance to *S*-metolachlor at 1010 g ha^{-1} while Brundage 96 was least tolerant requiring 0.55 g ai kg^{-1} seed of fluxofenim for 90% protection from *S*-metolachlor at a rate of 1010 g ha^{-1}. Brundage 96 therefore requires more safener to be protected from *S*-metolachlor injury at 1010 g ha^{-1} compared to UI Castle CL+ and UI Sparrow. Similar pattern was observed when the rate of *S*-metolachlor was increased to 1782 g ha^{-1}. Brundage 96 was also least tolerant requiring 0.44 g ai kg^{-1} seed of fluxofenim for 90% protection from *S*-metolachlor at 1782 g ha^{-1} while UI Sparrow and UI Castle CL+ were more tolerant requiring 0.20 g ai kg^{-1} seed and 0.17 g ai kg^{-1} seed fluxofenim for 90% protection respectively (Table 2.11 Figures 2.7, 2.8, 2.9).

Effective doses of fluxofenim resulting in 90% protection against dimethenamid-P at 647 g ha^{-1} for Brundage 96 was higher (0.86 g ai kg^{-1} seed) while UI Castle CL+ and UI Sparrow were lower (0.29 g ai kg^{-1} seed and 0.15 g ai kg^{-1} seed respectively). Same trend of estimates was observed when the rate of dimethenamid-P was increased to 1005 g ha^{-1} with Brundage 96 having a higher fluxofenim ED_{90} value (0.73 g ai kg^{-1} seed) while UI Castle CL+ (0.10 g ai kg^{-1} seed) and UI Sparrow (0.09 g ai kg^{-1} seed) had lesser fluxofenim ED_{90} values. This pattern of response to dimethenamid-P herbicide suggests that Brundage 96 would once again require a high amount of safener to confer 90% protection against injury from dimethenamid-P at 1005 g ha^{-1} (Table 2.12, Figures 2.10, 2.11, 2.12). Estimates of effective doses of fluxofenim resulting in 90% protection from pyroxasulfone injury at 236 g ha^{-1} was similar to patterns obtained with *S*-metolachlor or dimethenamid-P whereby Brundage 96 had fluxofenim dose value for 90% tolerance to pyroxasulfone at 1.03 g ai kg^{-1} seed while UI Sparrow and UI Castle CL+ had effective dose values for 90% protection from pyroxasulfone injury at 0.49 g ai kg^{-1} seed and 0.30 g ai kg^{-1} seed respectively. UI Castle CL+ requiring a less amount of safener for 90% protection from pyroxasulfone injury at 236 g ha^{-1} suggests some level of tolerance to pyroxasulfone at twice the label rate (Table 2.13, Figures 2.13, 2.14, 2.15). In general, increasing the rate of fluxofenim beyond 0.5 g ai kg^{-1} seed for *S*-metolachlor, 0.9 g ai kg^{-1} seed for dimethenamid-P

and 1.0 g ai kg^{-1} seed for pyroxasulfone herbicide treatments does not confer additional visually detectable protection to the three varieties.

Glutathione *S*-transferase enzyme assay

Plant material and Enzyme Assay

Glutathione *S*-transferase (GST) enzyme activities in crops such as maize, sorghum and wheat have been reported to be induced by safeners. Therefore, we evaluated the activity of GST in varieties UI Sparrow, Brundage 96 and UI Castle CL+ at three incremental doses of fluxofenim seed treatment. Seven-day-old wheat shoots were harvested, protein extracted, and reduced glutathione analyzed for conjugation with a standard substrate, 1-chloro-2,4-dinitrobenzene (CDNB). In the absence of fluxofenim, GST specific activity was similar for the three varieties as observed in untreated controls, however, in the presence of fluxofenim seed treatments, GST specific activity increased for the three varieties (Table 2.14). Pairwise comparison showed significant differences in GST specific activity between control and the three fluxofenim doses (0.36, 0.91 or 1.96 g ai kg^{-1} seed) for each variety. There were however no significant differences in GST specific activity between fluxofenim doses for Brundage 96 and UI Castle CL+ ($\alpha = 0.05$). GST specific activity for UI Sparrow seed treatment at 0.91 g ai kg^{-1} seed was significantly higher than seed treatment at 0.36 g ai kg^{-1} seed. Similarly, UI Sparrow seed treatment at 1.96 g ai kg^{-1} seed had significantly higher GST specific activity than seed treatment at 0.36 g ai kg^{-1} seed. However, there was no significant difference in GST specific activity between seed treatment at 0.91 g ai kg^{-1} seed and 1.96 g ai kg^{-1} seed. Fluxofenim at 0.36 g ai kg^{-1} seed increased GST specific activity of UI Sparrow by 30%, which increased by 53% at 0.91 g ai kg^{-1} seed treatment and further increased by 64% at 1.96 g ai kg^{-1} seed treatment. Brundage 96 had a 38%, 43% and 38% increase in GST specific activity at 0.36, 0.91 and 1.96 g ai kg^{-1} seed respectively. UI Castle CL+ also had a similar pattern of GST specific activity as Brundage 96, with 58%, 52% and 53% increase at 0.36, 0.91 and 1.96 g ai kg^{-1} seed respectively (Figure 2.16).

Discussion

Response of safener treated soft-white winter wheat varieties to chloroacetamide and pyrazole herbicides

Varieties differed in their responses to fluxofenim safener in the presence of *S*-metolachlor, dimethenamid-P or pyroxasulfone herbicide treatments. Varieties either had increased biomass or no significant change in biomass between treatments with or without fluxofenim seed treatment. A previous study evaluating the response of fluxofenim-treated wheat lines to dimethenamid also showed variability in the response of the lines to dimethenamid (Riechers et al. 1996b). Variation in

response may be attributed to inherent differences in growth rate and time of emergence of varieties, thus allowing some varieties to escape herbicide treated zones faster than others (Riechers et al. 1996b). The consistency in positive response of varieties LWW 15-72223, LWW 14-75044, Bruneau and UI Sparrow to fluxofenim safener across two experimental runs in contrast to the consistency in no response of varieties 09-15702A, UI Castle CL+ and UI Palouse CL+ to fluxofenim safener across two experimental runs suggests a possible difference in growth rate of the varieties or herbicide metabolism differences mediated by several detoxification enzymes. UI Castle CL+ and UI Palouse CL+ are part of the Clearfield production system with tolerance to imidazolinone herbicides. Clearfield cultivars were produced with ethyl methanesulfonate (EMS) mutagenesis with mutations occurring in the ALS gene on the long arm of chromosome 6D. Cultivars with "+" are two-gene cultivars with ALS mutations on both the A and D, or B and D, genomes (Anderson et al. 2004, Nakka et al. 2019, Pozniak and Hucl 2004). The advanced breeding techniques that produced UI Castle CL+ and UI Palouse CL+ may have resulted in the addition of other traits such as increased growth rate or some tolerance mechanisms to other herbicides such as the very-long-chain fatty acid-inhibiting herbicides from this study. Metabolic resistance and cross resistance to herbicides have been reported in several weeds and such mechanisms may be responsible for cross-protection of crops to different herbicides (Powles and Yu 2010, Yu and Powles 2014).

Tolerance of crops to herbicides occurs via rapid metabolism resulting from activities of detoxification enzymes such as cytochrome P450 monooxygenases, glutathione *S*-transferases and glucosyl transferases, and safeners have been shown to induce the expression of several of these enzymes (Hatzios and Burgos 2004, Riechers et al. 2010). Riechers et al. (2003) showed that safener dramatically induced the expression of GST proteins in *Triticum tauschii* (Coss.) Schmal, a diploid wheat species considered a progenitor and D-genome donor of hexaploid bread wheat *Triticum aestivum* L. Safener may therefore be conferring protection to varieties with significant increase in biomass by inducing herbicide detoxification enzymes in these varieties. In varieties with no response to safener, it may be that the varieties already have either higher enzyme activities, increased levels of glutathione or other underling mechanism conferring tolerance (Farago et al. 1994). The differences in phenotypic responses observed among wheat varieties in this study further explains the complex herbicide-safener-crop-environment interaction.

Fluxofenim dose-response

Response of selected wheat varieties to incremental doses of safener

Wheat injury in the form of delayed emergence was noticeable at 4 or 8 times fluxofenim sorghum label rate 7 days after safener treatment. The most tolerant variety, UI Palouse CL+ from our

dose-response studies required an estimated dose of 1.23 g ai kg^{-1} seed fluxofenim to cause 10% biomass reduction. Increasing the dose of fluxofenim treatment more than 1.6 g ai kg^{-1} seed caused stunted or delayed growth in wheat. Overall, tolerance in wheat to fluxofenim is dependent on variety. Fluxofenim injury to sorghum in the form of slight germination inhibition could occur as a result of differences in sorghum lines (Anonymous 2012). In addition, performance of safener is influenced by environmental factors such as temperature, soil moisture, soil structure and the rate of application of the safener which could contribute to delayed emergence seen in this study. Naphthalic anhydride for example at commercial rate was reported to cause injury such as stunting and chlorosis to corn and sorghum with similar reports of reduction in germination rate with earlier oxime ethers; oxabetrinil and cyometrinil (Abu-Qare and Duncan 2002, Yenne and Hatzios 1990). Phytotoxicity of 1,8-naphthalic anhydride (NA) seed treatment to crops was reported to increase as time of exposure of the safener to seeds increases (Jablonkai 2013). Delayed emergence of crop seedlings due to high safener dose could result in more time the crop seedlings spends in a herbicide-treated zone. Although, a higher safener dose was reported to provide a longer activity of the GST herbicide detoxifying enzymes (Taylor et al. 2013).

Recovery of varieties from this delayed emergence at 14 days after safener treatment and beyond is an indication of a transient form of injury which may not impact yield. Corn seedlings have been observed to recover from minor injury resulting from lower rates of metolachlor or when metolachlor was applied with a safener (Bernards et al. 2006). Similarly, initial corn injury observed with metolachlor under wet conditions known to favor injury was reported not to have impact on grain yield and a safener applied in combination with metolachlor at high rates of metolachlor protected corn from injury with no reduction in yield (Viger et al. 1991). Overall, varieties from this study were tolerant to fluxofenim at rates higher than the label rate for use in sorghum.

Italian ryegrass response to chloroacetamide and pyrazole herbicides

Greenhouse dose-response study

Pyroxasulfone resulted in the mortality of Italian ryegrass population used in this study and so no dose response curve could be fit. Mortality of Italian ryegrass observed in this study even at the lowest rate of 14.75 g ha^{-1} is consistent with a study reported in Oregon where 7.5 g ha^{-1} controlled both susceptible and resistant ryegrass populations (Liu et al. 2016). Dimethenamid-P herbicide caused more injury to the population of Italian ryegrass tested than *S*-metolachlor. Dimethenamid-P and *S*-metolachlor at the rate of 550 g ai ha^{-1} and 1069 g ai ha^{-1} respectively were reported to control populations of Italian ryegrass suspected to be resistant to flufenacet (Liu et al. 2016). Estimated rates in the study are higher than rates estimated to cause 90% mortality of a population of Italian ryegrass

evaluated in our study (213 g ai ha^{-1} for dimethenamid-P and 558.12 g ai ha^{-1} for *S*-metolachlor), suggesting that different populations of Italian ryegrass respond differently to very-long-chain fatty acid-inhibiting herbicides. A previous study of ten corn hybrids by Rowe and Penner (1990) found that several factors including hybrid type, herbicide, herbicide application rate and soil moisture content at the time of plant emergence also play a role in the extent of chloroacetamide injury. Several populations of Italian ryegrass would therefore need to be continually evaluated to determine the levels of resistance to very-long-chain fatty acid inhibitors.

Response of wheat varieties to incremental safener doses at two herbicide rates

Greenhouse dose-response study

In weed science, researchers are often interested in herbicide effective doses to access crop tolerance to a herbicide or efficacy of a herbicide in weed control (Ritz et al. 2015). Many studies have used nonlinear regression to estimate weed-crop interaction (Cousens 1985) or herbicide absorption in weeds (Kniss et al. 2011). In this study, we were interested in estimates of fluxofenim doses conferring 90% protection to selected wheat varieties in the presence of a herbicide. We defined protection as a low dose of safener required to attain the desired level of protection (90%) against herbicide injury. Therefore, a variety having a low effective dose of fluxofenim (ED_{90}) implies better protection from herbicide injury compared to a variety with high fluxofenim dose in the presence of the herbicide. Variety UI Palouse CL+ was most tolerant to potential phytotoxicity from our previous study evaluating the impact of fluxofenim-alone to six wheat varieties; however, UI Castle CL+ was used in further evaluation because it is planted on more acres in Northern Idaho than UI Palouse CL+ and also ranked ‘most desirable’ based on 2019 end-use quality ranking (Kurt Schroeder, personal communication).

Similar to our varietal screening in which varieties UI Castle CL+ and UI Palouse CL+ showed innate tolerance to *S*-metolachlor and dimethenamid-P herbicides and also tolerance to fluxofenim-alone treatment, variety UI Castle CL+ had a low estimated fluxofenim dose for 90% protection against *S*-metolachlor rate at 1010 g ha^{-1}. Brundage 96 on the other hand had a higher estimated fluxofenim dose for 90% protection from *S*-metolachlor injury at 1010 g ha^{-1}. Same trend was observed with the herbicide dimethenamid-P in which Brundage 96 had a higher safener dose value resulting in 90% protection from dimethenamid-P injury, and when *S*-metolachlor and dimethenamid-P herbicides were increased to 1782 g ha^{-1} and 1005 g ha^{-1} respectively, similar pattern of responses were also observed with the varieties. Pyroxasulfone applied at twice the recommended rate for use in winter wheat also followed similar pattern as *S*-metolachlor and dimethenamid-P herbicides. Brundage 96 required a high fluxofenim dose to elicit 90% tolerance to pyroxasulfone

compared to UI Castle CL+. Overall, wheat varieties differed in the amount of safener required for similar level of protection against VLCFA-inhibiting herbicide injury. Increasing the dose of fluxofenim beyond 0.5 g ai kg^{-1} seed does not confer additional protection against for UI Castle CL+ and UI Sparrow. Taylor et al. (2013) in a study with another safener used postemergence in wheat found that higher concentrations of the safener cloquintocet-mexyl only provides a longer induction of glutathione *S*-transferase activity, an enzyme with role in herbicide metabolism rather than a larger induction of glutathione *S*-transferase activity suggesting that a minimal concentration of the active safening agent is required to sustain a response. Findings from Taylor et al. (2013) may therefore explain why a small dose of fluxofenim elicits similar response as high dose of fluxofenim in our study.

Innate tolerance of wheat varieties to VLCFA-inhibiting herbicide injury may bring into question the need for a safener such as the use of safener seed treatment for pyroxasulfone herbicide which is registered for use in wheat. However, as noted by Viger et al. (1991) in their study, corn subjected to severe levels of environmental stresses such as temperature extremes, drought, poor nutrition, hail or insect damage may limit the ability of the corn to recover from metolachlor injury without a yield loss. UI Castle CL+ was somewhat tolerant at the lower rates of *S*-metolachlor or dimethenamid-P herbicides used in this study, however, that tolerance slightly reduced when the herbicide rates were increased, meanwhile similar response in protection whereby fluxofenim reduced injury to the varieties at low and increased rate of both *S*-metolachlor and dimethenamid-P herbicides were still observed. Therefore, the use of a safener is justifiable under conditions for which wheat varieties will suffer from herbicide injury. Also, the protection conferred by safener to a crop have been suggested to be only relevant under certain environmental conditions that favors reduced enzyme activity or lower rate of herbicide metabolism in the crop (Paporisch and Rubin 2017). Varieties UI Castle CL+ and UI Palouse CL+ would therefore need to be evaluated further under field conditions based on their consistency in tolerance from these studies.

Glutathione *S*-transferase enzyme assay

Plant material and Enzyme Assay

Previous research have shown that safeners induce the expression of herbicide detoxification enzymes in crops such as rice, corn, sorghum and wheat (Davies 2001, Hatzios and Burgos 2004, Jablonkai 2013). Widely studied of the herbicide detoxification enzymes induced by safeners are the glutathione *S*-transferases (Baek et al. 2019, Cummins et al. 2013, Hatzios 1991, Riechers et al. 2010). This study therefore focused on measuring glutathione *S*-transferase specific activity in selected wheat varieties and at incremental safener doses. GST specific activity towards the standard substrate

(CDNB) increased for the three varieties. This activity increased further for variety UI Sparrow as the dose of fluxofenim seed treatment increased; however, increasing the dose of fluxofenim for Brundage 96 and UI Castle CL+ did not result in significant change in GST activity. A previous study from Taylor et al. (2013) showed that higher concentrations of cloquintocet-mexyl did not result in larger induction of glutathione *S*-transferase activity. Hirase and Molin (2002) however demonstrated that increasing the concentration of flurazole, fluxofenim, napthalic anhydride, benoxacor and dichlormid safeners protected sorghum from growth inhibition by alachlor and increased extractable cysteine synthase activity in sorghum shoots, an enzyme indirectly involved in glutathione biosynthesis. It is worth mentioning that safening efficacy of the safeners were not clearly correlated with the increase in cysteine activity reported in the study.

UI Castle CL+ had a 58% increase in GST enzyme activity at 0.36 g ai kg^{-1} seed treatment relative to Brundage 96 (38% increase) and UI Sparrow (30% increase). Scarponi et al. (2006) reported a 75.4% increase in GST enzyme activity in wheat 72 h after treatment. GST proteins have been found to be located in the coleoptile of safener-treated shoots which is also the site for chloroacetamide herbicide uptake (Riechers et al. 2003). Safeners therefore protect grass crops from herbicide injury by dramatically inducing the expression of GST proteins in the outer cell layers of grass coleoptile, preventing the herbicide from reaching sensitive new leaves of etiolated shoots as they emerge from the soil (Riechers et al. 2003). Although, GSTs have been reported as major group of proteins induced by safeners in phase II herbicide detoxification-related proteins, the expression of other proteins such as aldo-keto reductase family, 12-oxophytodienoate reductase (OPR), cysteine synthase, γ-glutamyltranspeptidase, or multidrug resistance-associated proteins (MRP) shows a complex cascade of events utilized by safener in herbicide detoxification (Riechers et al. 2003, 2010). These proteins in addition to GSTs could therefore be involved in the tolerance of varieties observed in these studies to VLCFA-inhibiting herbicides.

Overall, the consistency in tolerance of UI Castle CL+ to *S*-metolachlor, dimethenamid-P and pyroxasulfone herbicides which was also corroborated by the GST assay implies that GST assay could be a valuable tool for screening existing wheat varieties for tolerance to VLCFA-inhibiting herbicides. Further research would be to investigate gene expression profiles of VLCFA elongases between wheat varieties such as Brundage 96 and UI Castle CL+ using real time RT-PCR or microarrays with or without safener treatments.

Table 2.1: Estimated mean biomass of fluxofenim-treated and untreated wheat varieties to *S*-metolachlor herbicide 21 DAT for two experimental runs.

Variety	Run 1 Treatment				Run 2			
	Untreated control	M	MS	M vs MS	Untreated control	M	MS	M vs MS
	g plant^{-1}			p-value	g plant^{-1}			p-value
LWW 15-72223*	0.153	0.067	0.121	<0.001	0.141	0.070	0.129	0.001
LWW 14-75044*	0.159	0.076	0.130	<0.001	0.152	0.111	0.164	0.007
Bruneau	0.143	0.102	0.132	0.013	0.161	0.128	0.191	0.001
UI Sparrow	0.176	0.062	0.127	<0.001	0.151	0.092	0.145	0.001
Bobtail	0.140	0.077	0.117	0.025	0.149	0.050	0.095	0.013
UI Magic CL+	0.145	0.085	0.135	<0.001	0.141	0.101	0.115	0.252
LWW 15-72234*	0.165	0.121	0.148	0.031	0.159	0.147	0.158	0.296
UI WSU Huffman	0.136	0.091	0.121	0.017	0.128	0.071	0.077	0.476
SY Ovation	0.157	0.128	0.158	0.012	0.115	0.095	0.123	0.056
10-08606A*	0.149	0.093	0.124	<0.001	0.119	0.074	0.077	0.662
Brundage 96	0.155	0.135	0.144	0.342	0.164	0.147	0.186	0.006
LWW 15-72458*	0.151	0.133	0.140	0.185	0.156	0.144	0.184	0.030
07-28017B*	0.153	0.134	0.147	0.169	0.114	0.099	0.117	0.030
LCS Artdeco	0.140	0.117	0.126	0.208	0.112	0.094	0.100	0.540
LWW 15-72138*	0.169	0.132	0.139	0.438	0.127	0.105	0.116	0.195
10-20604A*	0.162	0.136	0.146	0.403	0.145	0.129	0.136	0.505
09-15702A*	0.164	0.130	0.129	0.893	0.136	0.136	0.171	0.053
UI Castle CL+	0.149	0.129	0.127	0.783	0.110	0.114	0.128	0.211
UI Palouse CL+	0.140	0.124	0.131	0.391	0.113	0.128	0.116	0.398
UI Platinum[a]	0.196	0.127	0.162	0.010	0.151	0.089	0.112	0.015

M, *S*-metolachlor; MS, *S*-metolachlor + fluxofenim treated seeds.

*Advanced breeding lines.

[a]Hard white spring wheat.

Table 2.2: Estimated mean biomass of fluxofenim-treated and untreated wheat varieties to dimethenamid-P herbicide 21 DAT for two experimental runs.

Variety	Run 1 Treatment				Run 2			
	Untreated	D	DS	D vs DS	Untreated	D	DS	D vs DS
	g plant^{-1}			p-value	g plant^{-1}			p-value
LWW 15-72223*	0.153	0.031	0.064	<0.001	0.141	0.041	0.104	0.001
LWW 14-75044*	0.159	0.050	0.095	0.003	0.152	0.072	0.138	0.001
Bruneau	0.143	0.076	0.100	0.049	0.161	0.089	0.133	0.015
UI Sparrow	0.176	0.033	0.075	0.003	0.151	0.060	0.099	0.004
Bobtail	0.140	0.057	0.100	0.017	0.149	0.047	0.068	0.222
UI Magic CL+	0.145	0.083	0.099	0.042	0.141	0.054	0.090	0.005
LWW 15-72234*	0.165	0.069	0.124	<0.001	0.159	0.123	0.134	0.332
UI WSU Huffman	0.136	0.071	0.088	0.153	0.128	0.048	0.080	0.001
SY Ovation	0.157	0.099	0.118	0.087	0.115	0.094	0.107	0.891
10-08606A*	0.149	0.066	0.098	<0.001	0.119	0.038	0.079	<0.001
Brundage 96	0.155	0.105	0.109	0.652	0.164	0.125	0.144	0.145
LWW 15-72458*	0.151	0.096	0.126	<0.001	0.156	0.113	0.096	0.344
07-28017B*	0.153	0.110	0.126	0.080	0.114	0.079	0.089	0.236
LCS Artdeco	0.140	0.071	0.097	0.002	0.112	0.058	0.075	0.091
LWW 15-72138*	0.169	0.103	0.121	0.063	0.127	0.089	0.101	0.183
10-20604A*	0.162	0.097	0.112	0.202	0.145	0.108	0.140	0.005
09-15702A*	0.164	0.087	0.107	0.051	0.136	0.103	0.127	0.181
UI Castle CL+	0.149	0.109	0.119	0.239	0.110	0.090	0.117	0.025
UI Palouse CL+	0.140	0.109	0.123	0.119	0.113	0.094	0.116	0.117
UI Platinum[a]	0.196	0.096	0.121	0.048	0.151	0.074	0.098	0.012

D, Dimethenamid-P; DS, Dimethenamid-P + fluxofenim treated seeds.

*Advanced breeding lines.

[a]Hard white spring wheat.

Table 2.3: Estimated mean biomass of fluxofenim-treated and untreated wheat varieties to pyroxasulfone herbicide 21 DAT for two experimental runs.

Variety	Run 1 Treatment				Run 2			
	Untreated	P	PS	P vs PS	Untreated	P	PS	P vs PS
	g plant^{-1}			p-value	g plant^{-1}			p-value
LWW 15-72223*	0.153	0.118	0.139	0.096	0.141	0.107	0.114	0.647
LWW 14-75044*	0.159	0.131	0.122	0.498	0.152	0.114	0.149	0.059
Bruneau	0.143	0.127	0.141	0.204	0.161	0.108	0.132	0.158
UI Sparrow	0.176	0.123	0.144	0.108	0.151	0.108	0.174	<0.001
Bobtail	0.140	0.110	0.126	0.351	0.149	0.126	0.141	0.377
UI Magic CL+	0.145	0.110	0.125	0.051	0.141	0.106	0.110	0.747
LWW 15-72234*	0.165	0.106	0.141	0.006	0.159	0.120	0.131	0.302
UI WSU Huffman	0.136	0.115	0.122	0.518	0.128	0.092	0.089	0.734
SY Ovation	0.157	0.122	0.135	0.249	0.115	0.110	0.103	0.607
10-08606A*	0.149	0.119	0.124	0.581	0.119	0.099	0.103	0.631
Brundage 96	0.155	0.134	0.132	0.867	0.164	0.135	0.164	0.037
LWW 15-72458*	0.151	0.136	0.155	0.019	0.156	0.120	0.161	0.027
07-28017B*	0.153	0.115	0.120	0.594	0.114	0.111	0.116	0.548
LCS Artdeco	0.140	0.109	0.113	0.557	0.112	0.080	0.091	0.259
LWW 15-72138*	0.169	0.126	0.146	0.040	0.127	0.096	0.103	0.385
10-20604A*	0.162	0.111	0.119	0.477	0.145	0.090	0.122	0.005
09-15702A*	0.164	0.109	0.114	0.578	0.136	0.108	0.133	0.152
UI Castle CL+	0.149	0.131	0.128	0.700	0.110	0.115	0.119	0.739
UI Palouse CL+	0.140	0.116	0.120	0.626	0.113	0.113	0.129	0.268
UI Platinum[a]	0.196	0.142	0.152	0.440	0.151	0.113	0.131	0.046

P, Pyroxasulfone; PS, Pyroxasulfone + fluxofenim treated seeds.

*Advanced breeding lines.

[a]Hard white spring wheat.

Table 2.4: Varietal response to fluxofenim safener in the presence of three herbicides across two experimental runs.

Variety	*S*-metolachlor		Dimethenamid-P		Pyroxasulfone	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
LWW 15-72223*	+	+	+	+	-	-
LWW 14-75044*	+	+	+	+	-	-
Bruneau	+	+	+	+	-	-
UI Sparrow	+	+	+	+	-	+
Bobtail	+	+	+	-	-	-
UI Magic CL+	+	-	+	-	-	-
LWW 15-72234*	+	-	+	-	+	-
UI WSU Huffman	+	-	-	+	-	-
SY Ovation	+	-	-	-	-	-
10-08606A*	+	-	+	+	-	-
Brundage 96	-	+	-	-	-	+
LWW 15-72458*	-	+	+	-	+	+
07-28017B*	-	+	-	-	-	-
LCS Artdeco	-	-	+	+	-	-
LWW 15-72138*	-	-	-	-	+	-
10-20604A*	-	-	-	-	-	+
09-15702A*	-	-	-	-	-	-
UI Castle CL+	-	-	-	-	-	-
UI Palouse CL+	-	-	-	-	-	-
UI Platinum[a]	+	+	+	+	-	+

+ Significant difference in aboveground biomass between herbicide and herbicide with safener treated seeds ($\alpha = 0.05$).

- No significant difference in aboveground biomass between herbicide and herbicide with safener treated seeds ($\alpha = 0.05$).

*Advanced breeding lines.

[a]Hard white spring wheat.

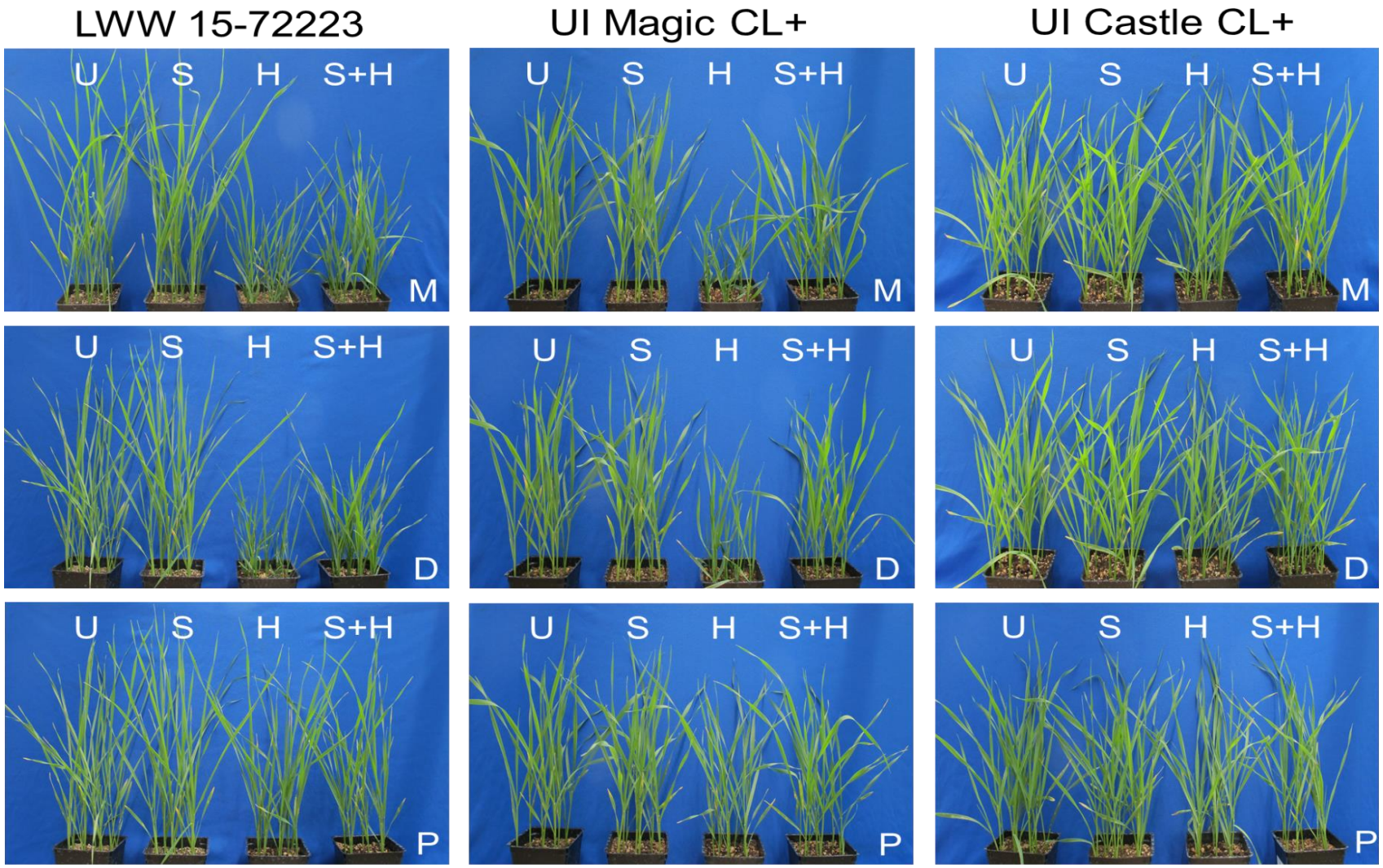

Figure 2.1: Visual assessment of safener effects on three wheat varieties at 21 DAT. U: Untreated control; S: Safener only; H: herbicides; S+H: Safener + Herbicide. M: *S*-metolachlor; D: Dimethenamid-P; P: Pyroxasulfone.

Table 2.5: Varieties selected for further study.

	Responded	**Mixed**	**No response**
Category/variety	LWW 15-72223	UI Magic CL+	UI Castle CL+
	UI Sparrow	Brundage 96	UI Palouse CL+

*Responded- safener significantly increased dried biomass across *S*-metolachlor and dimethenamid-P treatments in both experiment 1 and 2; Mixed response- Safener activity was inconsistent in both experiment 1 and 2; No response- Safener did not significantly increase dried biomass across *S*-metolachlor and dimethenamid-P treatments in both experiment 1 and 2.

Table 2.6: Parameter estimates for 10% biomass reduction of six winter wheat varieties.

	Fluxofenim[b]	
Variety	ED_{10}[a]	Rate parameter, *b*
	g ai kg^{-1} seed	
LWW 15-72223	1.148 ± 0.186	1.442 ± 0.261
UI Sparrow	0.852 ± 0.197	0.908 ± 0.204
UI Magic CL+	0.547 ± 0.175	0.613 ± 0.155
Brundage 96	0.674 ± 0.232	0.773 ± 0.225
UI Castle CL+	0.868 ± 0.145	1.306 ± 0.206
UI Palouse CL+	1.234 ± 0.224	1.244 ± 0.271

[a] ED_{10}, effective dose of fluxofenim resulting in 10% biomass reduction at 21 days after treatment.
[b] Values represent mean ± SE in g ai kg^{-1} seed.

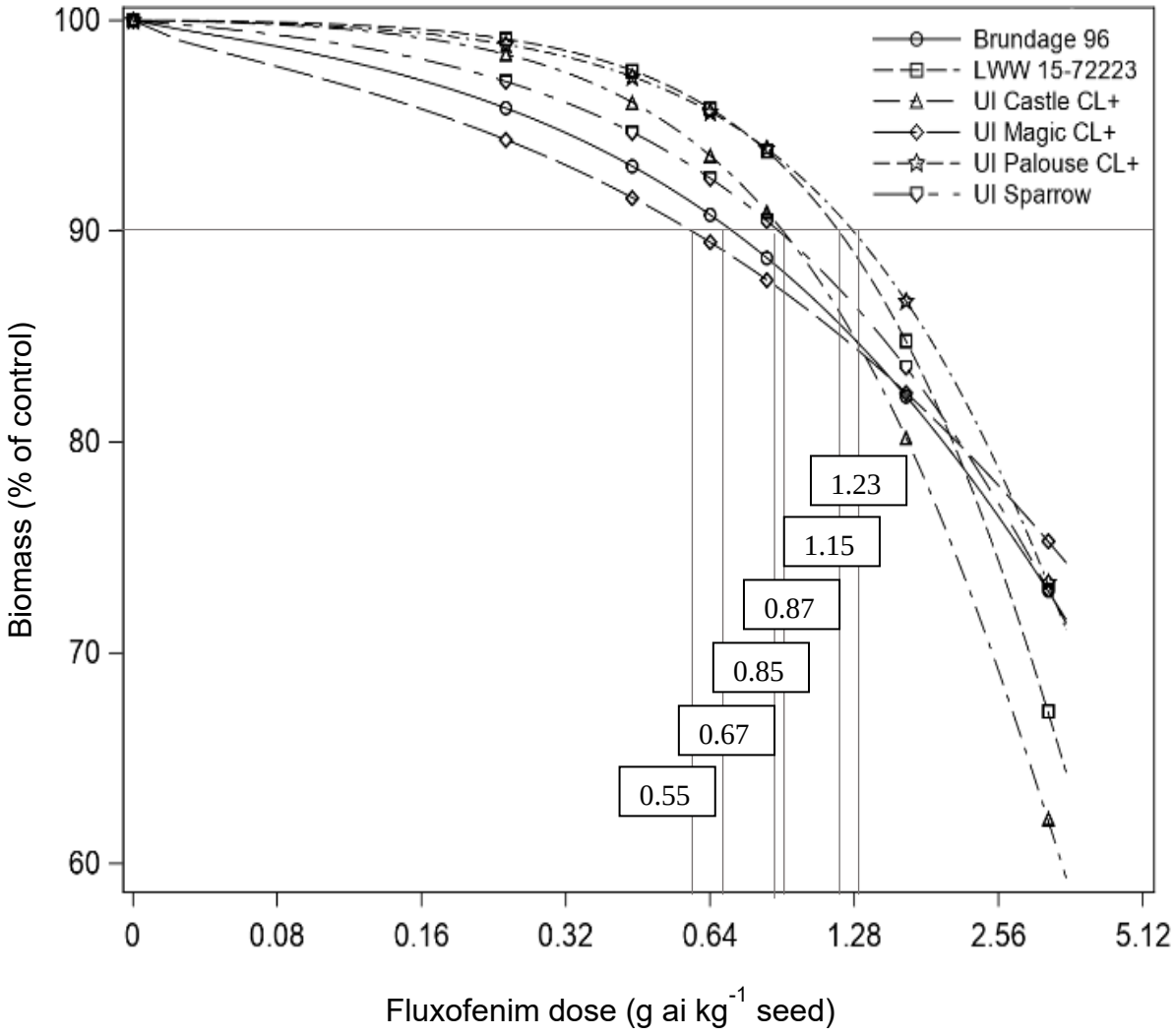

Figure 2.2: Fluxofenim dose-response on biomass reduction of six winter wheat varieties of the Pacific Northwest at 21 DAT. Dose axis is on a log scale.

Table 2.7: Pairwise comparison between fluxofenim doses resulting in 10% (ED_{10}) biomass reduction for six soft white winter wheat varieties of the Pacific Northwest.

Contrast[a]	p-value[b]
LWW 15-72223 vs UI Sparrow	0.2779
UI Sparrow vs Brundage 96	0.5650
UI Sparrow vs UI Magic CL+	0.2526
UI Palouse CL+ vs UI Sparrow	0.2042
UI Castle CL+ vs UI Sparrow	0.9437
LWW 15-72223 vs Brundage 96	0.1163
LWW 15-72223 vs UI Magic CL+	0.0202*
UI Palouse CL+ vs LWW 15-72223	0.7704
LWW 15-72223 vs UI Castle CL+	0.2418
Brundage 96 vs UI Magic CL+	0.6669
UI Palouse CL+ vs Brundage 96	0.0872
UI Castle CL+ vs Brundage 96	0.4832
UI Palouse CL+ vs UI Magic CL+	0.0171*
UI Castle CL+ vs UI Magic CL+	0.1619
UI Palouse CL+ vs UI Castle CL+	0.1764

[a] Varieties to the left of the contrast have higher estimates than varieties to the right.
[b] p-value representing pairwise comparison of doses causing 10% height reduction between varieties.
* Significant difference between parameter estimate of varieties being compared ($\alpha = 0.05$).

Table 2.8: Parameter estimates for 10% height reduction of six winter wheat varieties.

	Fluxofenim[b]	
Variety	ED_{10}[a]	Rate parameter, *b*
	g ai kg^{-1} seed	
LWW 15-72223	0.506 ± 0.089	0.821 ± 0.105
UI Sparrow	0.279 ± 0.060	0.676 ± 0.081
UI Magic CL+	0.327 ± 0.075	0.635 ± 0.088
Brundage 96	0.770 ± 0.140	0.752 ± 0.126
UI Castle CL+	0.402 ± 0.069	0.825 ± 0.092
UI Palouse CL+	0.362 ± 0.075	0.665 ± 0.086

[a] ED_{10}, effective dose of fluxofenim resulting in 10% height reduction at 14 days after treatment.
[b] Values represent mean ± SE in g ai kg^{-1} seed.

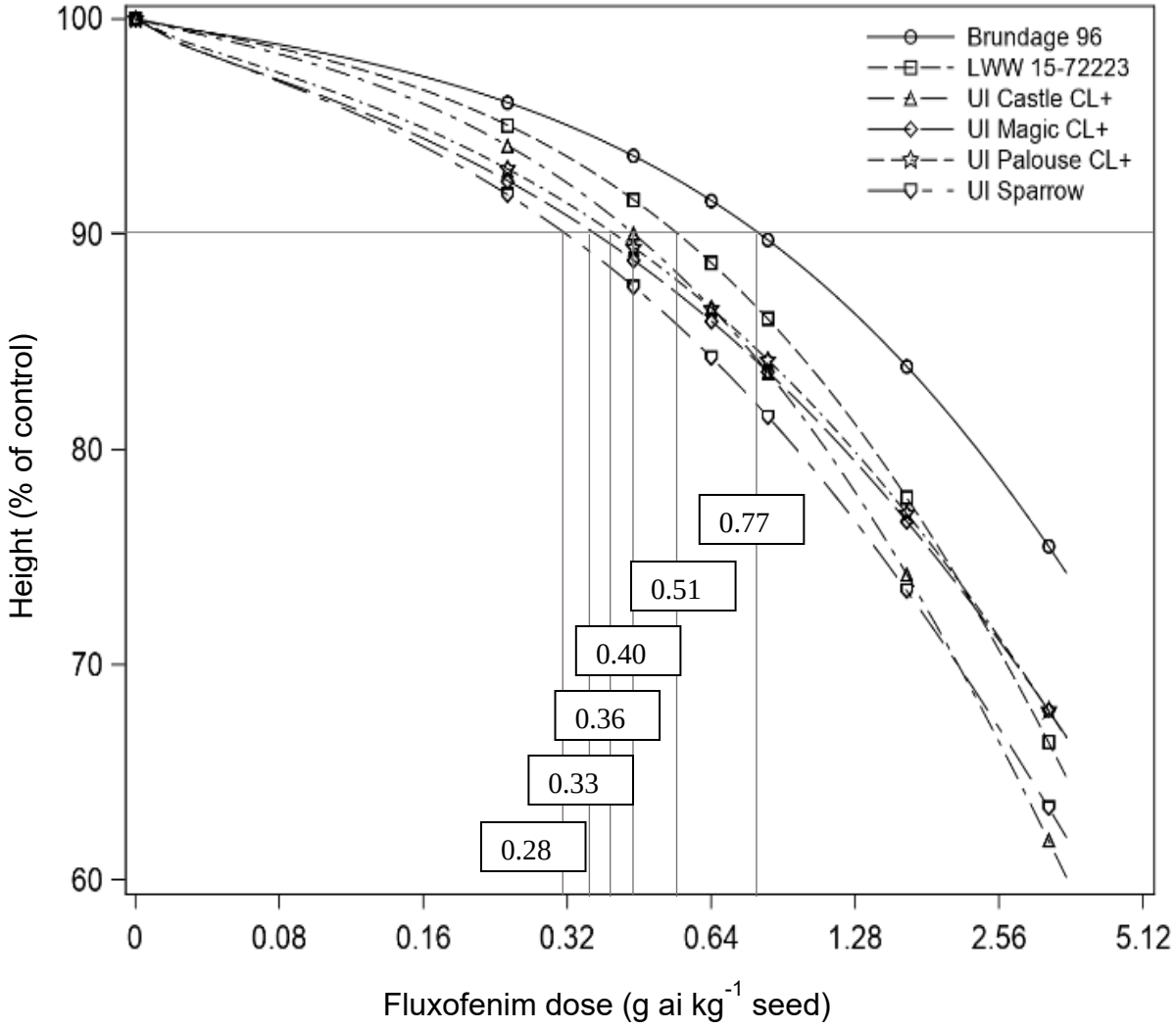

Figure 2.3: Fluxofenim dose-response on height reduction of six winter wheat varieties of the Pacific Northwest at 14 DAT. Dose axis is on a log scale.

Table 2.9: Pairwise comparison of fluxofenim doses resulting in 10% (ED_{10}) height reduction for six soft white winter wheat varieties of the Pacific Northwest at 14 days after treatment.

Contrast[a]	p-value [b]
LWW 15-72223 vs UI Sparrow	0.0341*
Brundage 96 vs UI Sparrow	0.0013*
UI Magic CL+ vs UI Sparrow	0.6110
UI Palouse CL+ vs UI Sparrow	0.3836
UI Castle CL+ vs UI Sparrow	0.1794
Brundage 96 vs LWW 15-72223	0.1114
LWW 15-72223 vs UI Magic CL+	0.1244
LWW 15-72223 vs UI Palouse CL+	0.2166
LWW 15-72223 vs UI Castle CL+	0.3537
Brundage 96 vs UI Magic CL+	0.0055*
Brundage 96 vs UI Palouse CL+	0.0105*
Brundage 96 vs UI Castle CL+	0.0186*
UI Palouse CL+ vs UI Magic CL+	0.7419
UI Castle CL+ vs UI Magic CL+	0.4668
UI Castle CL+ vs UI Palouse CL+	0.7003

[a] Varieties to the left of the contrast have higher estimates than varieties to the right.
[b] p-value representing pairwise comparison of doses causing 10% height reduction between varieties.
* Significant difference between parameter estimate of varieties being compared ($\alpha = 0.05$).

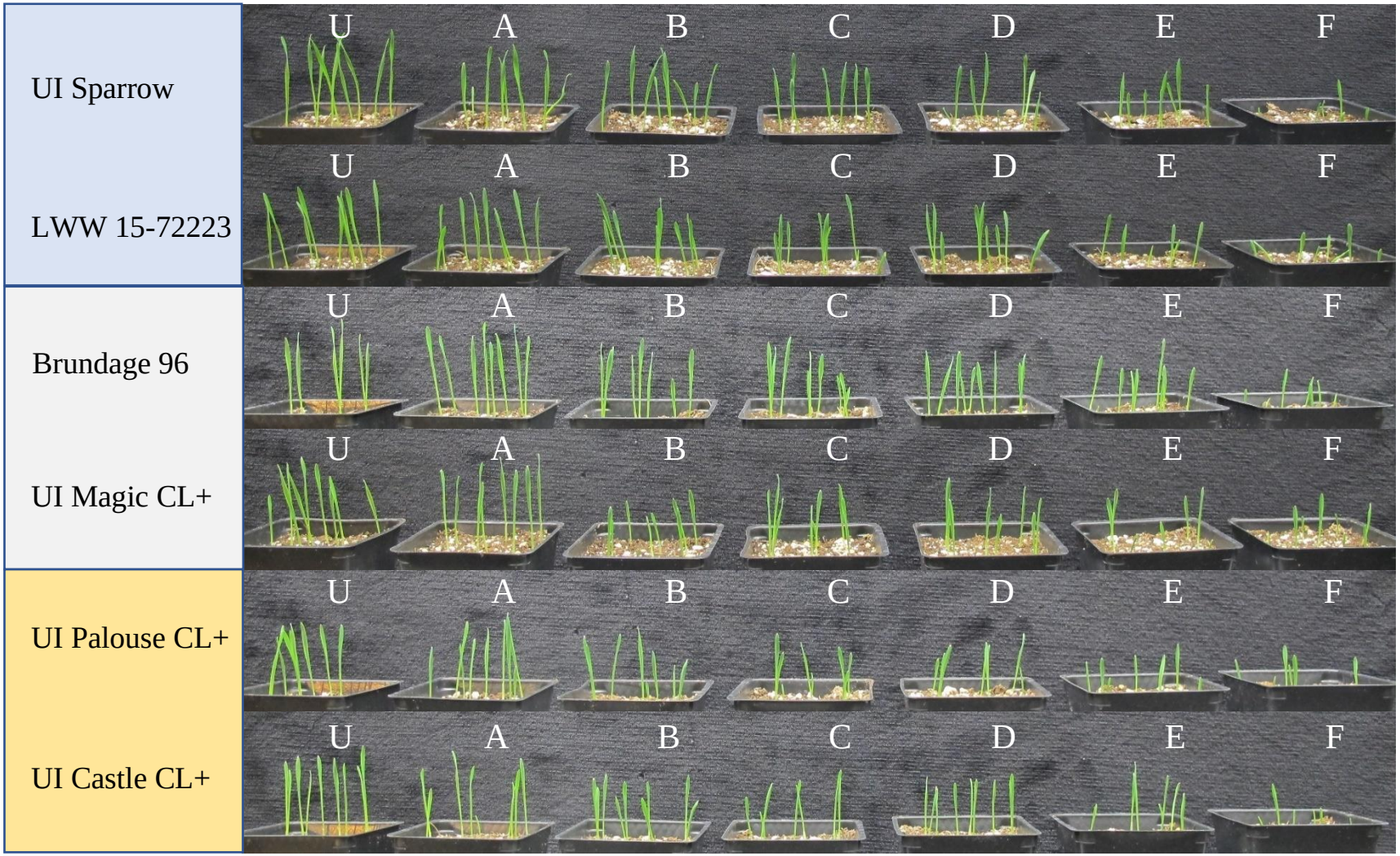

Figure 2.4: Response of six wheat varieties to incremental doses of safener (U) untreated control, (A) 0.2 g ai kg^{-1} seed, (B) 0.4 g ai kg^{-1} seed, (C) 0.6 g ai kg^{-1} seed, (D) 0.8 g ai kg-1 seed, (E) 1.6 g ai kg^{-1} seed, and (F) 3.2 g ai kg^{-1} seed at 7 DAT.

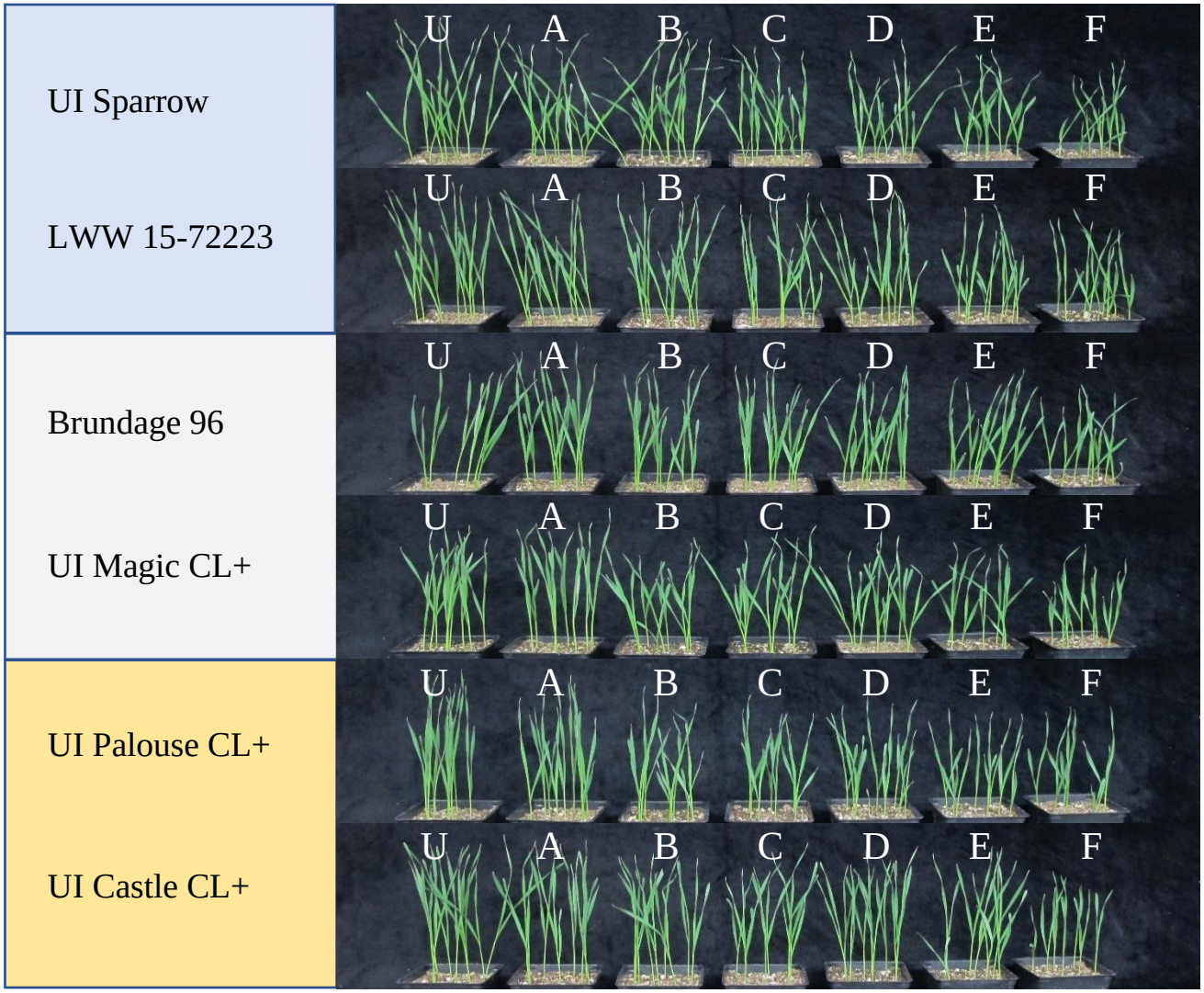

Figure 2.5: Response of six wheat varieties to incremental doses of safener (U) untreated control, (A) 0.2 g ai kg^{-1} seed, (B) 0.4 g ai kg^{-1} seed, (C) 0.6 g ai kg^{-1} seed, (D) 0.8 g ai kg-1 seed, (E) 1.6 g ai kg^{-1} seed, and (F) 3.2 g ai kg^{-1} seed at 14 DAT.

Table 2.10: Parameter estimates of herbicide doses resulting in 50% (ED_{50}) and 90% (ED_{90}) biomass reduction for a population of Italian ryegrass. Values represent mean ± SE in g ai ha^{-1}.

Herbicide	ED_{50}[a]	ED_{90}[a]
	g ai ha^{-1}	
Dimethenamid-P	22.965 ± 6.700	213.04 ± 67.296
S-metolachlor	71.059 ± 12.977	558.12 ± 187.62

[a] ED_{50}, effective dose of herbicide resulting in 50% biomass reduction at 21 days after treatment; ED_{90}, effective dose of herbicide resulting in 90% biomass reduction at 21 days after treatment.

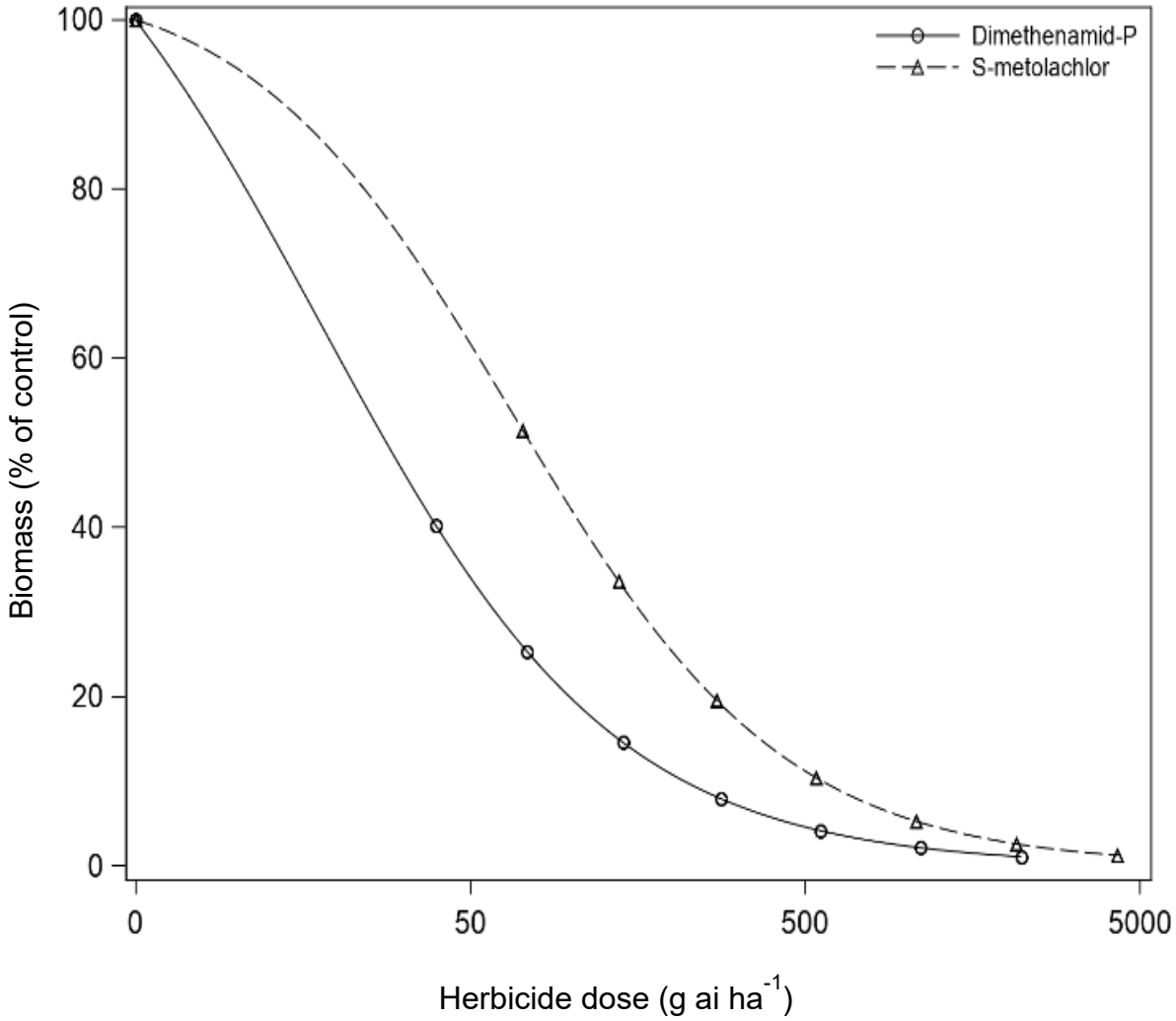

Figure 2.6: Response of an Italian ryegrass population to increasing doses of Dimethenamid-P and *S*-metolachlor herbicides at 21 DAT. Dose axis is on a log scale.

Table 2.11: Estimates from pooled analysis of fluxofenim doses resulting in 90% (ED_{90}) increased tolerance (biomass) to *S*-metolachlor for three winter wheat varieties. Values are presented as means ± SE.

Variety	*S*-metolachlor (g ai ha^{-1})	
	1010	1782
	ED_{90} (g ai kg^{-1} seed)	
UI Sparrow	0.256 ± 0.175	0.196 ± 0.117
Brundage 96	0.547 ± 0.778	0.436 ± 0.366
UI Castle CL+	0.069 ± 0.281	0.169 ± 0.120

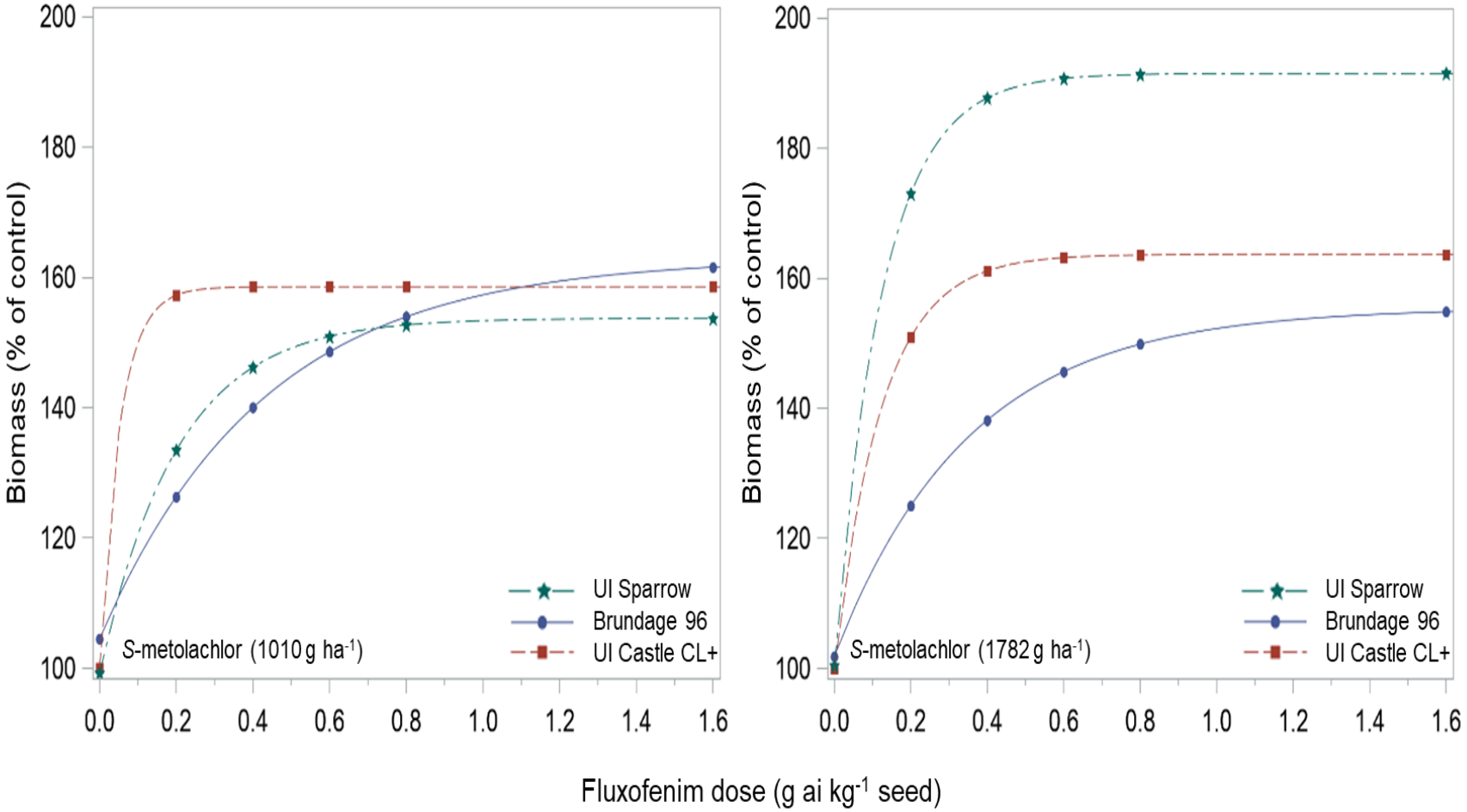

Figure 2.7: Response of three winter wheat varieties to incremental doses of fluxofenim safener at two *S*-metolachlor rates 21 days after herbicide treatment. Biomass (g plant^{-1}) is represented as percentage of herbicide treated control at the respective rates. Biomass (g plant^{-1}) for each variety at 1010 g ha^{-1} *S*-metolachlor are 0.0281 g plant^{-1} (UI Sparrow), 0.0342 g plant^{-1} (Brundage 96) and 0.0425 g plant^{-1} (UI Castle CL+). Biomass (g plant^{-1}) for each variety at 1782 g ha^{-1} *S*-metolachlor are 0.0228 g plant^{-1} (UI Sparrow), 0.0354 g plant^{-1} (Brundage 96) and 0.0378 g plant^{-1} (UI Castle CL+).

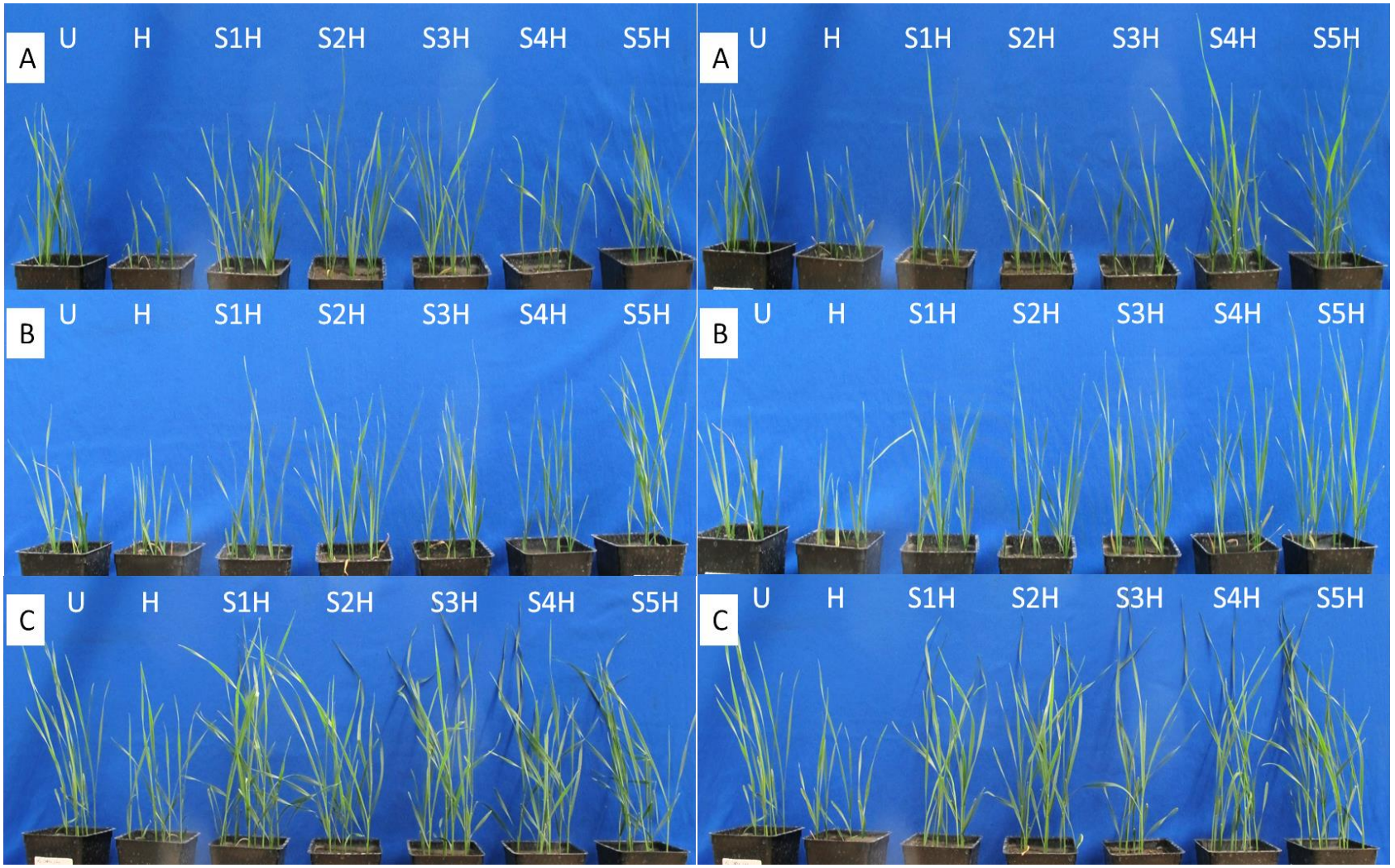

Figure 2.8: Response of wheat varieties (A) UI Sparrow, (B) Brundage 96, and (C) UI Castle CL+ to incremental doses of safener (S) at two *S*-metolachlor rate (H) 21 DAT. (U) untreated control without herbicide or safener treatment, (H) *S*-metolachlor rate at 1010 g ha^{-1} (left) or 1782 g ha^{-1} (right), (S1H) 0.2 g ai kg^{-1} seed, (S2H) 0.4 g ai kg^{-1} seed, (S3H) 0.6 g ai kg^{-1} seed, (S4H) 0.8 g ai kg^{-1} seed, (S5H) 1.6 g ai kg^{-1} seed. Photograph from first run of the experiment.

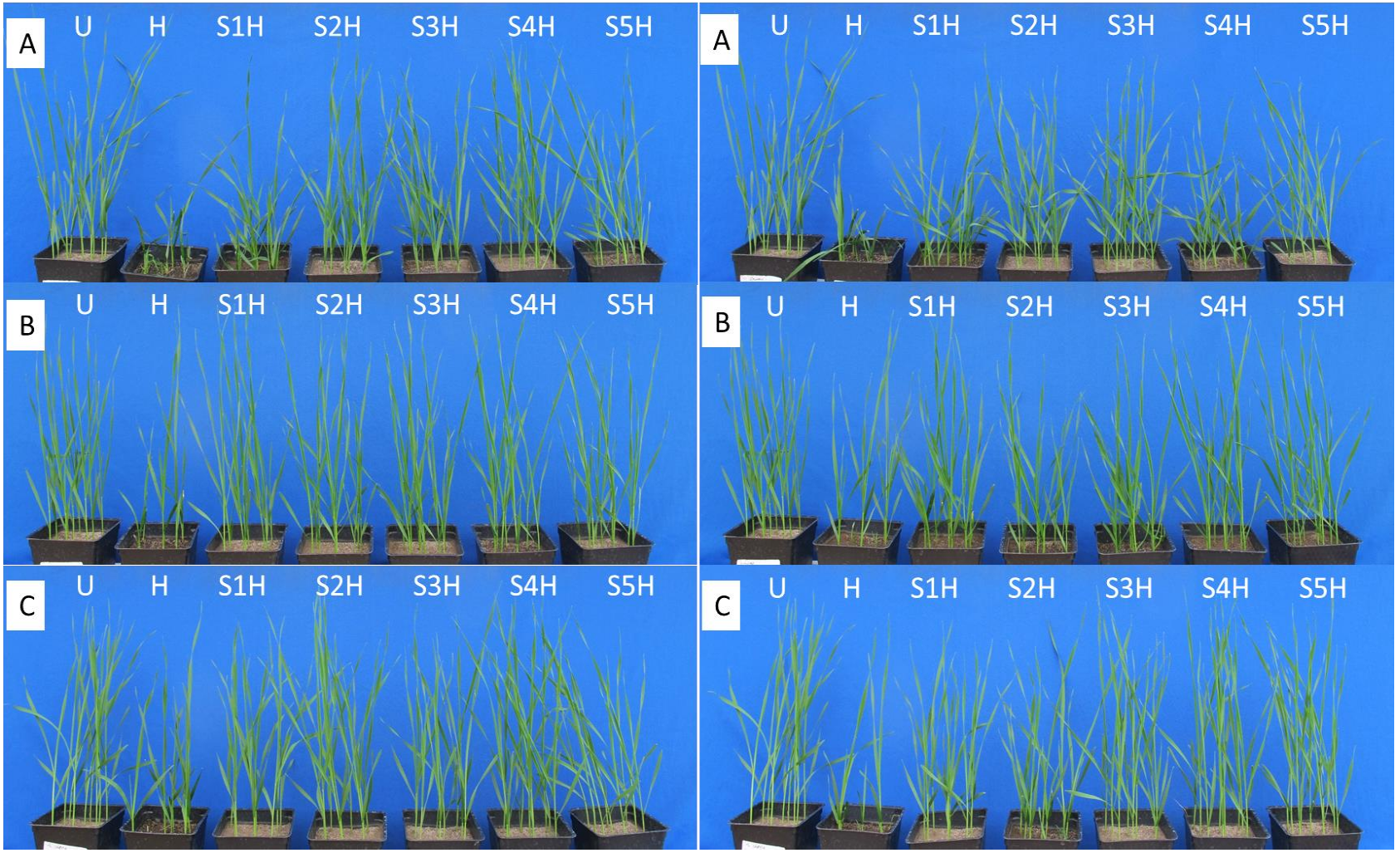

Figure 2.9: Response of wheat varieties (A) UI Sparrow, (B) Brundage 96, and (C) UI Castle CL+ to incremental doses of safener (S) at two *S*-metolachlor rate (H) 21 DAT. (U) untreated control without herbicide or safener treatment, (H) *S*-metolachlor rate at 1010 g ha^{-1} (left) or 1782 g ha^{-1} (right), (S1H) 0.2 g ai kg^{-1} seed, (S2H) 0.4 g ai kg^{-1} seed, (S3H) 0.6 g ai kg^{-1} seed, (S4H) 0.8 g ai kg^{-1} seed, (S5H) 1.6 g ai kg^{-1} seed. Photograph from second run of the experiment.

Table 2.12: Estimates from pooled analysis of fluxofenim doses resulting in 90% (ED_{90}) increased tolerance (biomass) to dimethenamid-P for three winter wheat varieties. Values are presented as means ± SE.

Variety	Dimethenamid-P (g ai ha^{-1})	
	647	1005
	ED_{90} (g ai kg^{-1} seed)	
UI Sparrow	0.146 ± 0.114	0.091 ± 0.130
Brundage 96	0.856 ± 1.192	0.729 ± 0.605
UI Castle CL+	0.292 ± 0.270	0.095 ± 0.149

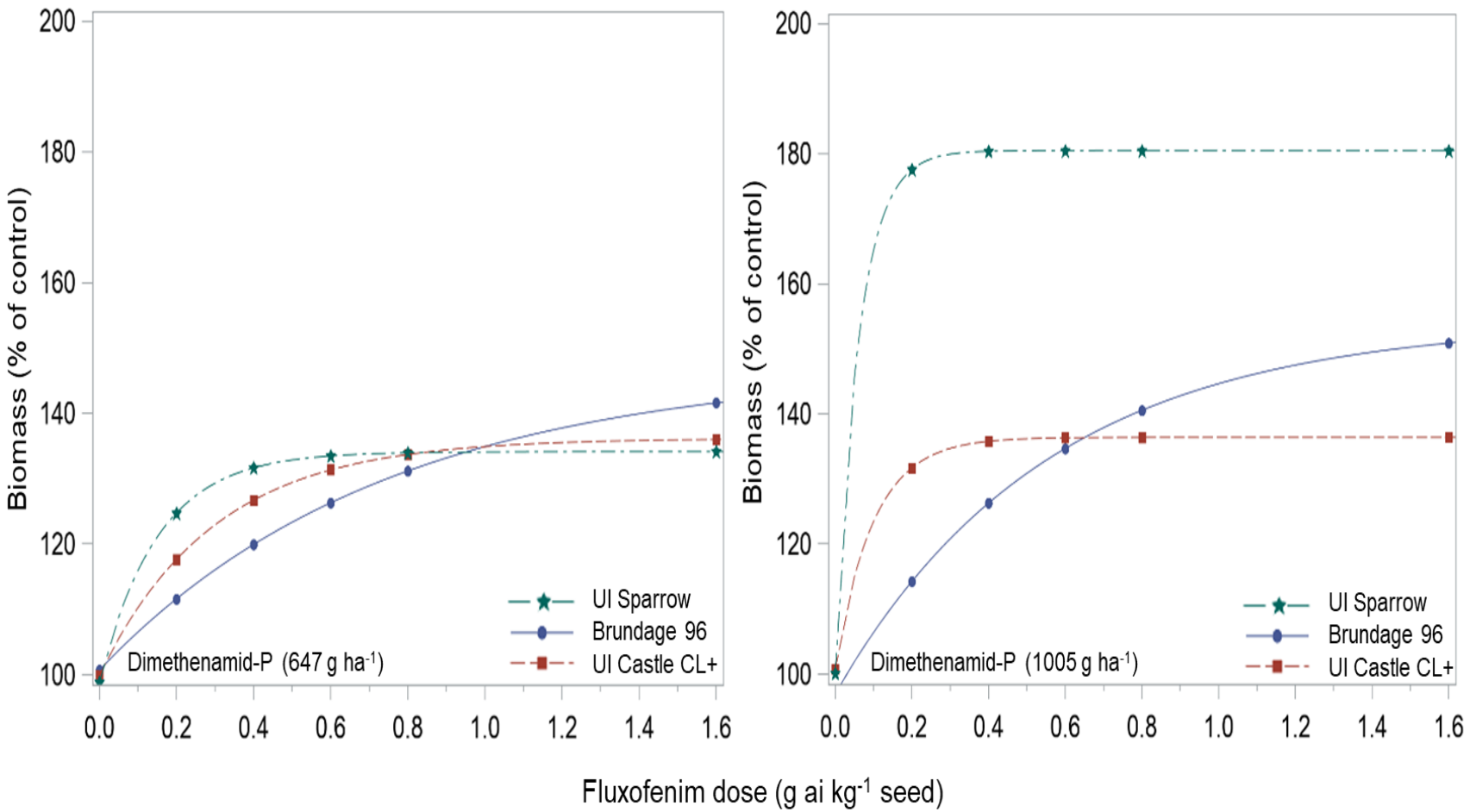

Figure 2.10: Response of three winter wheat varieties to incremental doses of fluxofenim safener at two dimethenamid-P rates 21 days after herbicide treatment. Biomass (g plant^{-1}) is represented as percentage of herbicide treated control at the respective rates. Biomass (g plant^{-1}) for each variety at 647 g ha^{-1} dimethenamid-P are 0.0314 g plant^{-1} (UI Sparrow), 0.0344 g plant^{-1} (Brundage 96) and 0.0411 g plant^{-1} (UI Castle CL+). Biomass (g plant^{-1}) for each variety at 1782 g ha^{-1} dimethenamid-P are 0.0188 g plant^{-1} (UI Sparrow), 0.0289 g plant^{-1} (Brundage 96) and 0.0341 g plant^{-1} (UI Castle CL+).

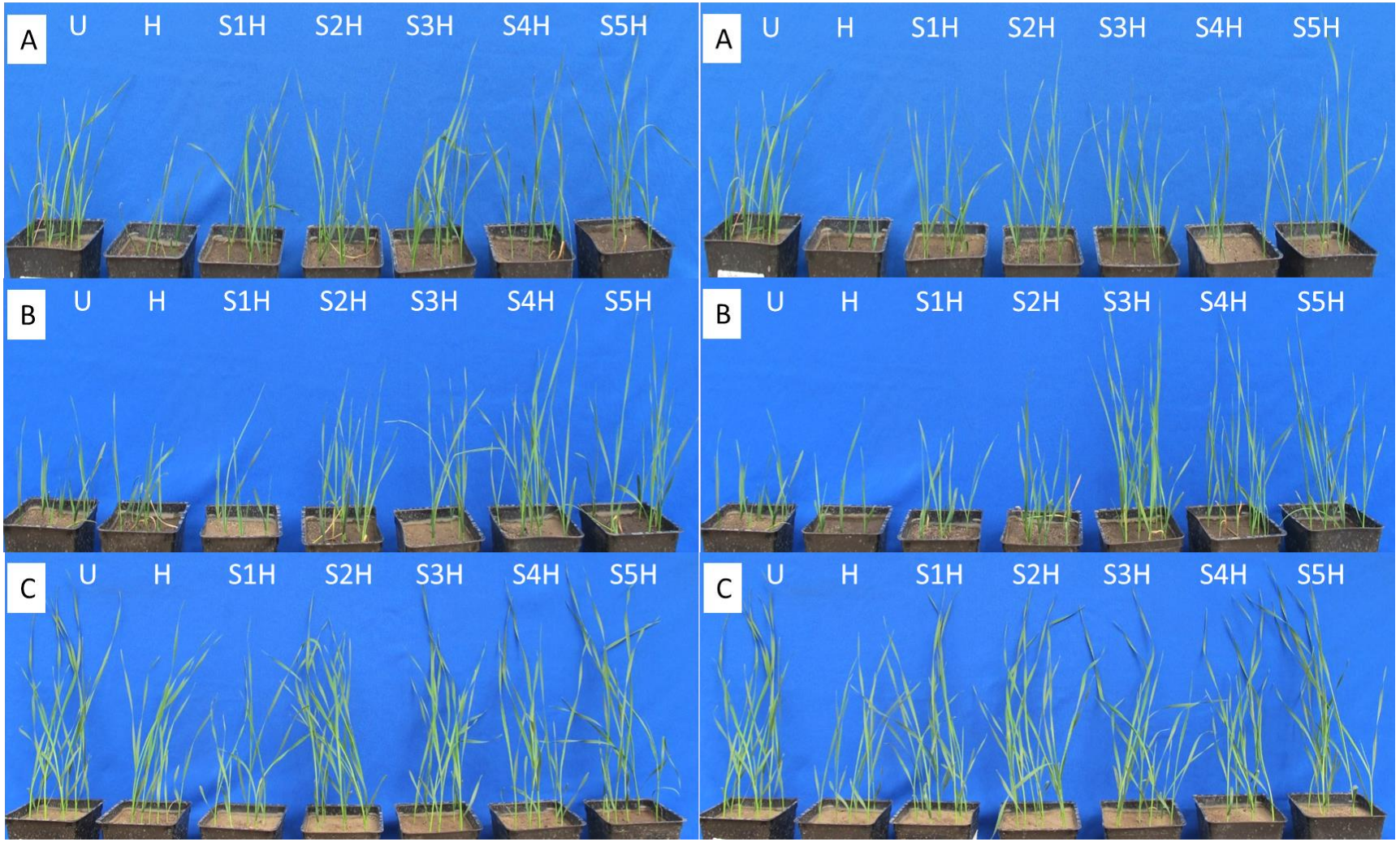

Figure 2.11: Response of wheat varieties (A) UI Sparrow, (B) Brundage 96, and (C) UI Castle CL+ to incremental doses of safener (S) at two dimethenamid-P rate (H) 21 DAT. (U) untreated control without herbicide or safener treatment, (H) dimethenamid-P rate at 647 g ha^{-1} (left) or 1005 g ha^{-1} (right), (S1H) 0.2 g ai kg^{-1} seed, (S2H) 0.4 g ai kg^{-1} seed, (S3H) 0.6 g ai kg^{-1} seed, (S4H) 0.8 g ai kg^{-1} seed, (S5H) 1.6 g ai kg^{-1} seed. Photograph from first run of the experiment.

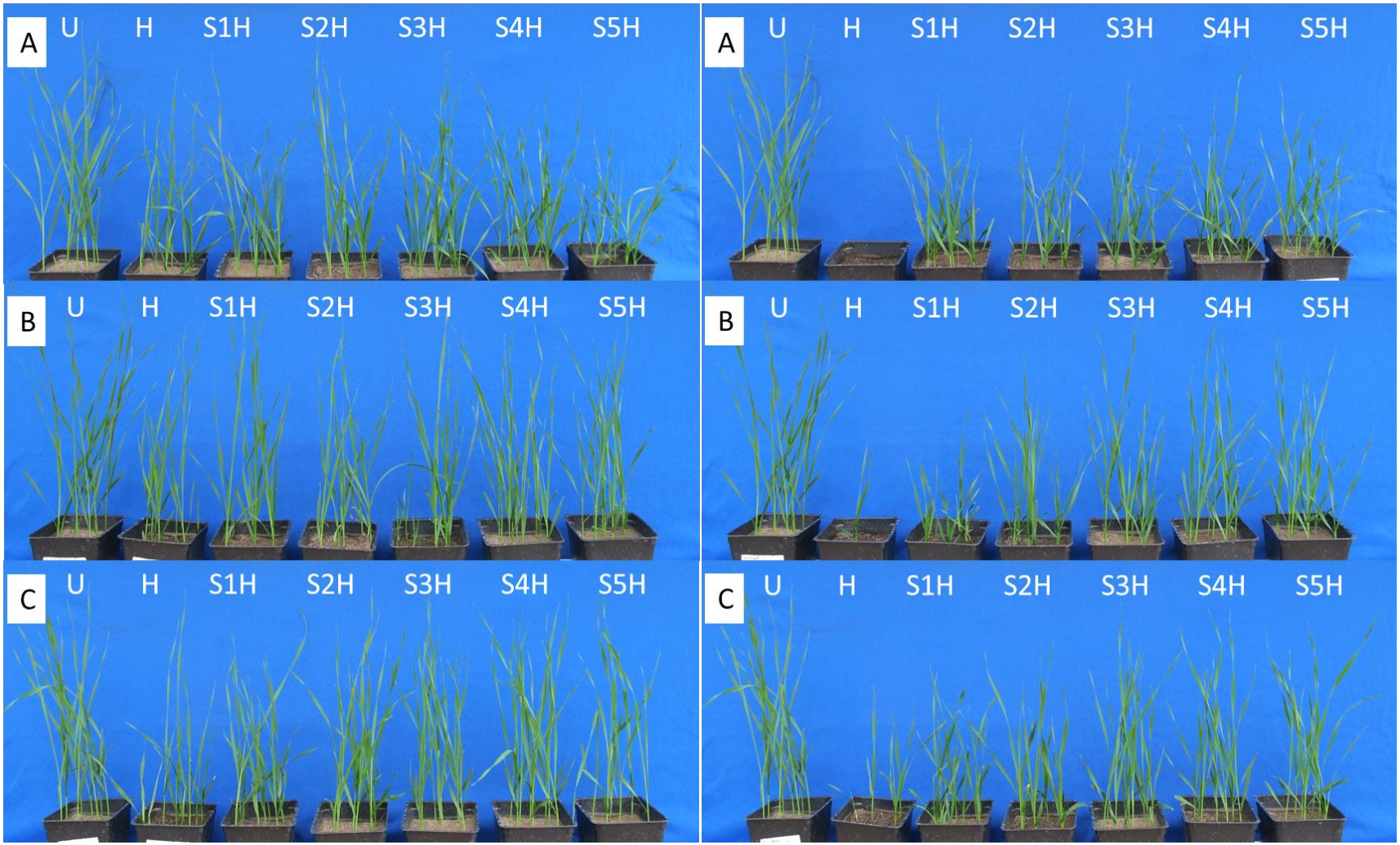

Figure 2.12: Response of wheat varieties (A) UI Sparrow, (B) Brundage 96, and (C) UI Castle CL+ to incremental doses of safener (S) at two dimethenamid-P rate (H) 21 DAT. (U) untreated control without herbicide or safener treatment, (H) dimethenamid-P rate at 647 g ha^{-1} (left) or 1005 g ha^{-1} (right), (S1H) 0.2 g ai kg^{-1} seed, (S2H) 0.4 g ai kg^{-1} seed, (S3H) 0.6 g ai kg^{-1} seed, (S4H) 0.8 g ai kg^{-1} seed, (S5H) 1.6 g ai kg^{-1} seed. Photograph from second run of the experiment.

Table 2.13: Estimates from pooled analysis of fluxofenim doses resulting in 90% (ED_{90}) increased tolerance (biomass) to pyroxasulfone for three winter wheat varieties. Values are presented as means ± SE.

Variety	Pyroxasulfone (g ai ha^{-1})
	236
	ED_{90} (g ai kg^{-1} seed)
UI Sparrow	0.486 ± 0.171
Brundage 96	1.034 ± 1.391
UI Castle CL+	0.295 ± 0.396

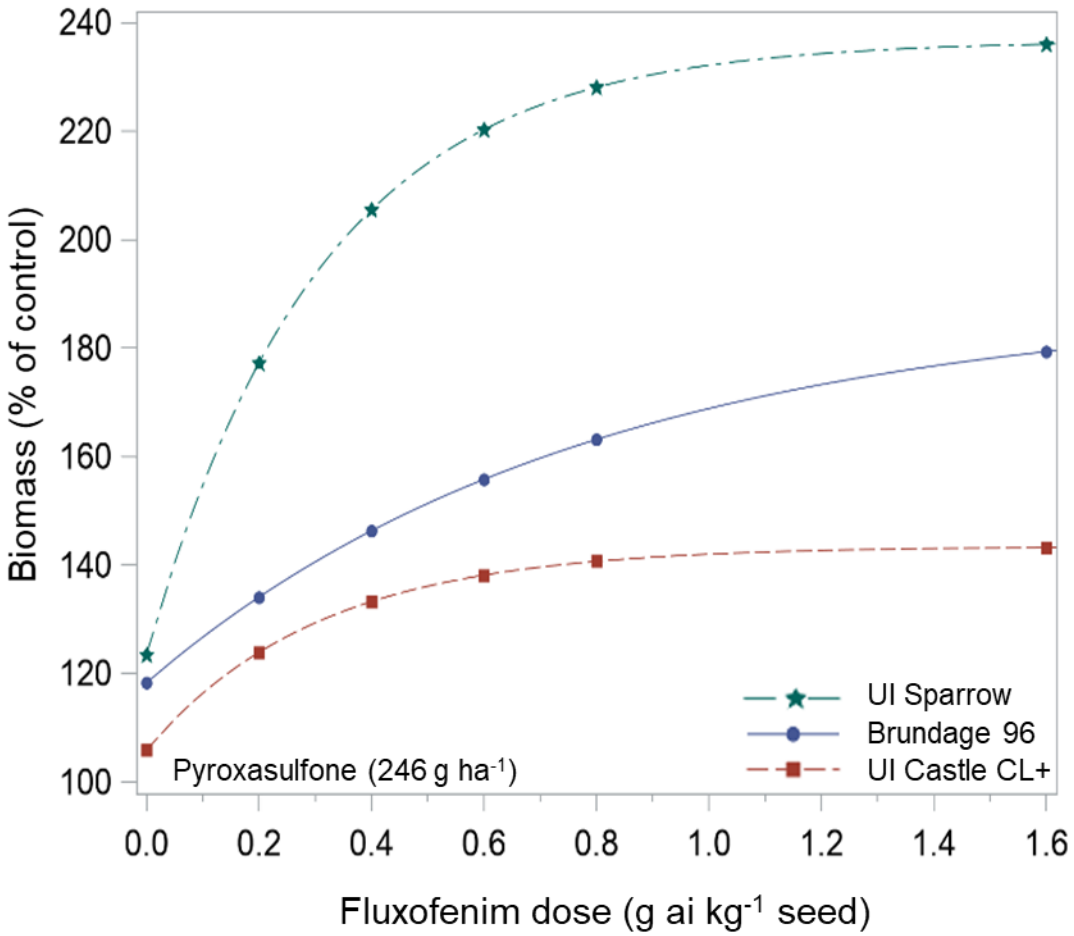

Figure 2.13: Response of three winter wheat varieties to incremental doses of fluxofenim safener at single pyroxasulfone rate 21 days after herbicide treatment. Biomass (g plant^{-1}) is represented as percentage of herbicide treated control at the respective rates. Biomass (g plant^{-1}) for each variety at 236 g ha^{-1} pyroxasulfone are 0.0320 g plant^{-1} (UI Sparrow), 0.0542 g plant^{-1} (Brundage 96) and 0.0603 g plant^{-1} (UI Castle CL+).

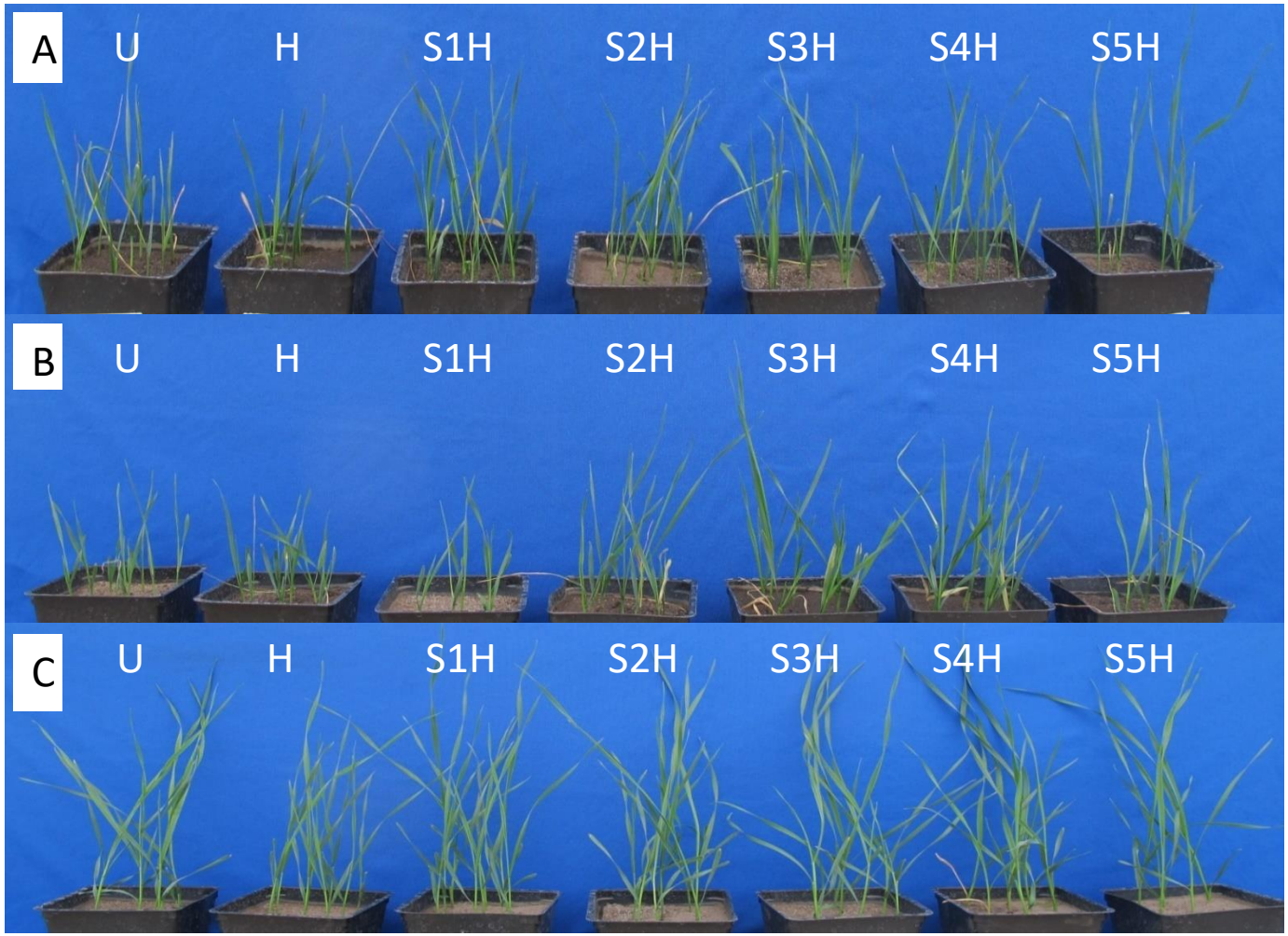

Figure 2.14: Response of wheat varieties (A) UI Sparrow, (B) Brundage 96, and (C) UI Castle CL+ to incremental doses of safener (S) at single pyroxasulfone rate (H) 21 DAT. (U) untreated control without herbicide or safener treatment, (H) 236 g ai ha^{-1}, (S1H) 0.2 g ai kg^{-1} seed, (S2H) 0.4 g ai kg^{-1} seed, (S3H) 0.6 g ai kg^{-1} seed, (S4H) 0.8 g ai kg^{-1} seed, (S5H) 1.6 g ai kg^{-1} seed. Photograph from first run of the experiment.

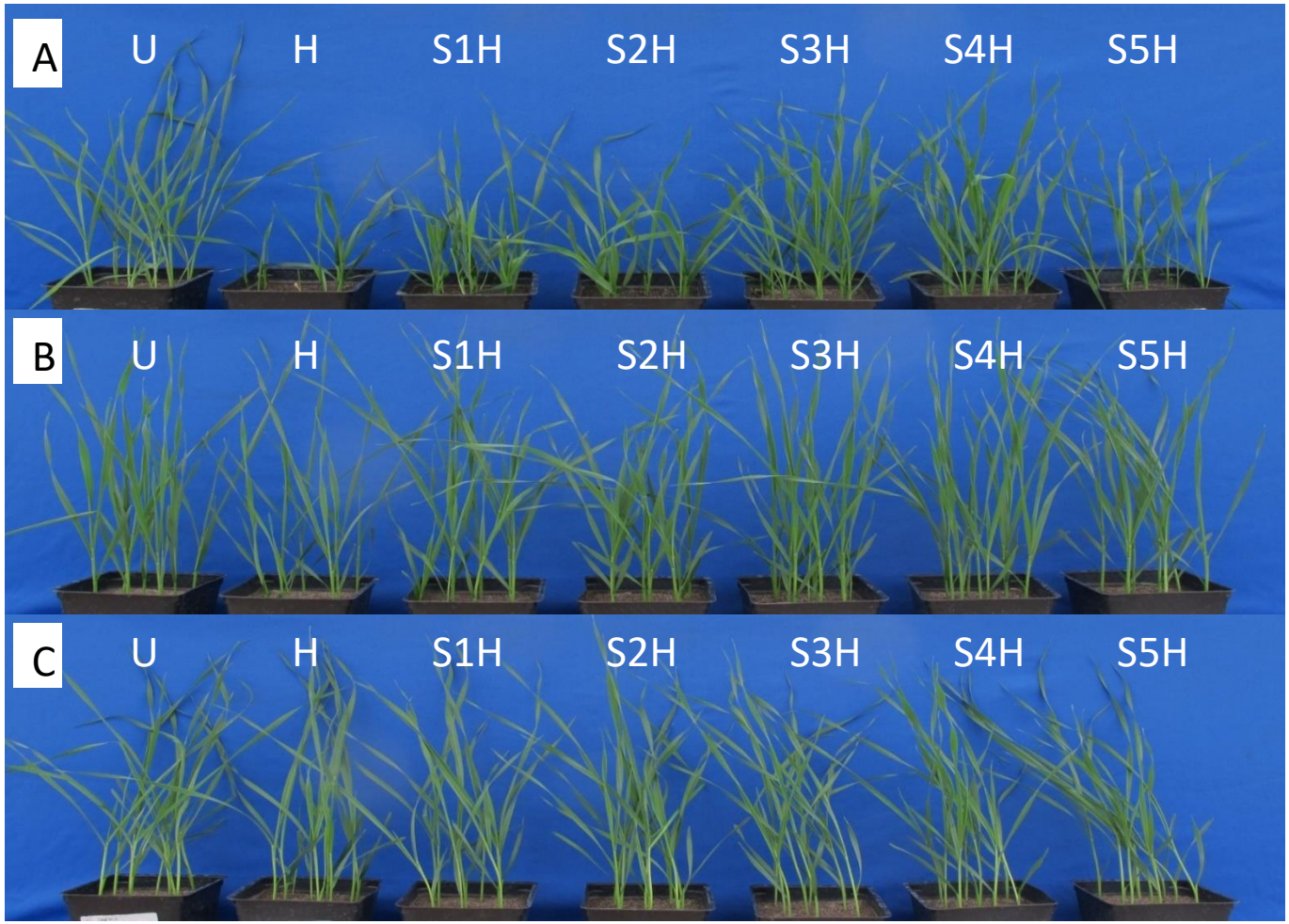

Figure 2.15: Response of wheat varieties (A) UI Sparrow, (B) Brundage 96, and (C) UI Castle CL+ to incremental doses of safener (S) at single pyroxasulfone rate (H) 21 DAT. (U) untreated control without herbicide or safener treatment, (H) 236 g ai ha^{-1}, (S1H) 0.2 g ai kg^{-1} seed, (S2H) 0.4 g ai kg^{-1} seed, (S3H) 0.6 g ai kg^{-1} seed, (S4H) 0.8 g ai kg^{-1} seed, (S5H) 1.6 g ai kg^{-1} seed. Photograph from second run of the experiment.

Table 2.14: Specific glutathione *S*-transferase (GST) activity towards 1-chloro-2,4-dinitrobenzene (CDNB) in wheat shoots with or without fluxofenim seed treatment at 7 DAT. Values are presented as mean ± SE.

Variety	Specific GST (CDNB) Activity			
	μmol min^{-1} ml^{-1} protein			
	Untreated control	0.36 g ai kg^{-1} seed	0.91 g ai kg^{-1} seed	1.96 g ai kg^{-1} seed
UI Sparrow	0.0244 ± 0.0021	0.0318 ± 0.0021	0.0374 ± 0.0021	0.0410 ± 0.0021
Brundage 96	0.0258 ± 0.0021	0.0357 ± 0.0021	0.0368 ± 0.0021	0.0357 ± 0.0021
UI Castle CL+	0.0257 ± 0.0021	0.0406 ± 0.0021	0.0391 ± 0.0021	0.0393 ± 0.0021

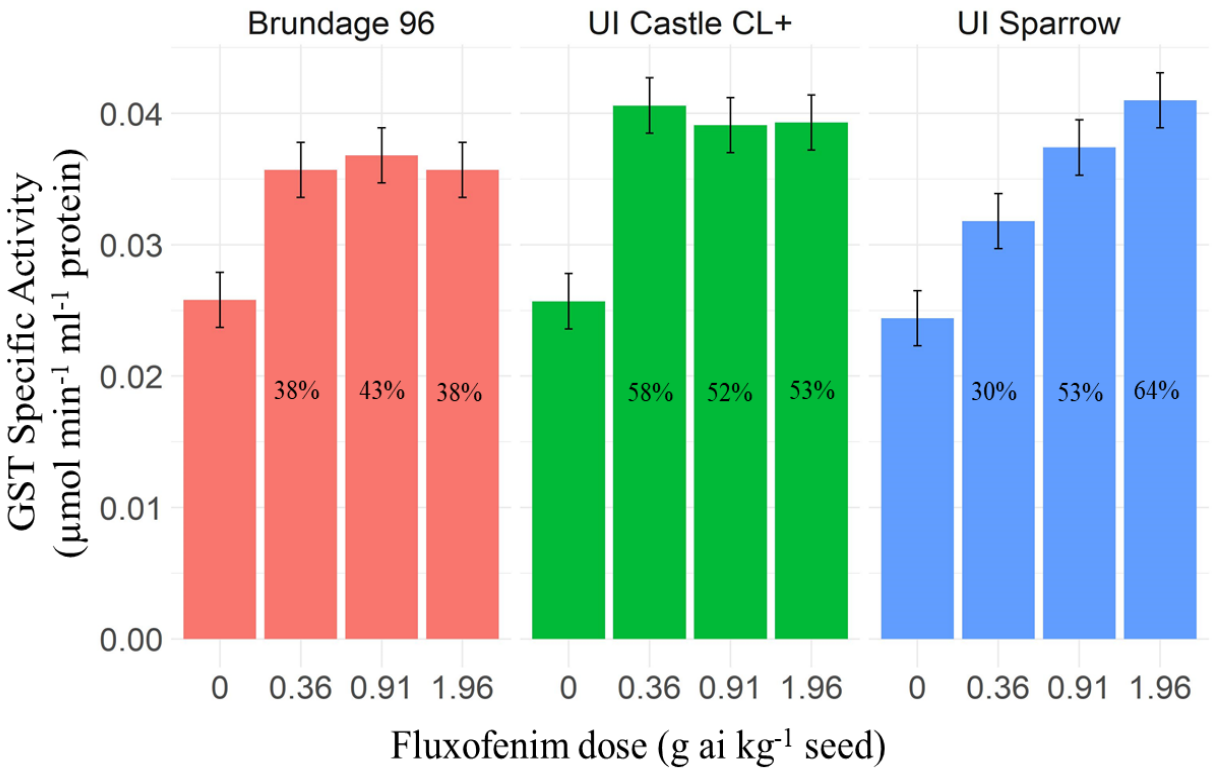

Figure 2.16: Specific glutathione *S*-transferase (GST) activity towards 1-chloro-2,4-dinitrobenzene (CDNB) in wheat shoots with or without fluxofenim seed treatment at 7 DAT. Values are presented as mean ± SE (n=4). Percentage increase in GST specific activity were calculated based on average GST specific activity of nontreated control.

Source of Materials

[1]McConkey Grower Products. 8.76 cm X 8.76 cm X 8.255 cm Tech Square Pot JMCTS35. 1615 Puyallop St. P. O Box 1690 Summer, WA 98390.

[2]Sungro Horticulture. Sunshine Professional Growing Mix #1 with 75 to 85% Canadian sphagnum peat moss, Perlite, dolomite limestone and 0.0001% silicon dioxide. 770 Silver Street, Agawam MA 01001.

[3]Allen Track Sprayer. Allen Machine Works. 607 E. Miller Road Midland, Michigan 48640.

[4]Statistical Analysis Software (SAS) 9.4. SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513.

[5]Generation III Research Sprayer, DeVries Manufacturing, 86956 State Highway 251, Hollandale, MN 56045.

[6]Premier Tech Horticulture. Pro-Mix BX Mycorrhizae general purpose with 75-85% Canadian sphagnum peat moss, perlite, vermiculite, dolomite and calcitic limestone, wetting agent and endomycorrhizal fungi. 200 Kelly Rd. Unit E-1 Quakertown, PA 18951.

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CHAPTER 3: FLUXOFENIM-ENHANCED WINTER WHEAT TOLERANCE TO *S*-METOLACHLOR, DIMETHENAMID-P AND PYROXASULFONE HERBICIDES UNDER FIELD CONDITIONS

Abstract

Field studies were conducted in Moscow and Genesee, Idaho during 2018 fall growing season to determine the impact of fluxofenim safener in mitigating wheat injury from very-long-chain fatty acid-inhibiting herbicides. Seeds of six soft white winter wheat varieties were treated with the commercial fluxofenim (Concep III) at a rate of 0.4 g ai kg^{-1} seed and the herbicides; *S*-metolachlor, dimethenamid-P or pyroxasulfone were applied preemergence at a rate of 1.782 kg ha^{-1}, 1.005 kg ha^{-1} or 0.236 kg ha^{-1} respectively. Response of varieties to fluxofenim safener in both field locations were inconsistent. Reduction in grain yield due to fluxofenim treatment was observed for Genesee field location with a similar trend at Moscow field location. Herbicides had a negative impact on wheat height 7 weeks after planting for Moscow location but not for Genesee. Wheat head count varied across locations and varieties. Test weight of the varieties was not however affected by safener or herbicide treatments. Injury to wheat varieties observed in this study was due to safener treatment and not a phytotoxic effect of herbicides. A greenhouse experiment to determine if the mixture of fluxofenim with a commonly used fungicide-insecticide premix in winter wheat resulted in negative impacts caused by fluxofenim to wheat failed to show any incompatibility of the chemical mixtures resulted in the injury. Results of these field studies are therefore inconclusive for efficacy of fluxofenim to protect winter wheat. Further study under different environmental conditions may clarify efficacy of fluxofenim for annual grass control in wheat with *S*-metolachlor or dimethenamid-P herbicides.

Introduction

Wheat is one of the three most important food crops in the world. In the United States, wheat ranks third among field crops in planted acreage and gross farm receipts, behind corn and soybeans (USDA-ERS 2019). In Idaho, wheat is second to potato in cash revenues (NASS 2018). Soft white winter wheat is an important crop that is exported to Asian markets (Robertson et al. 2004). A problem confronting wheat farmers in the Inland Pacific Northwest is selective control of winter annual grass weeds such as downy brome, wild oat and Italian ryegrass, that reduce wheat yield (Lyon 2017, Robertson et al. 2004). Wheat farmers have relied on preemergence and early postemergence herbicides such as diclofop, pinoxaden, flucarbazone, pyroxsulam, flufenacet and pyroxasulfone for

annual grass control in wheat (Lyon 2017, Mallory-Smith 2015). However, occurrence of resistant grass weed populations across the region has limited preemergence options to pyroxasulfone as the sole option for grass control without reported cases of resistance (Heap 2019, Hulting et al. 2012, Liu et al. 2016, Rauch et al. 2010).

The very-long-chain fatty acid-inhibiting herbicides have been used for decades and are effective in control of annual grasses and small-seeded broadleaf weeds (Böger 2003, Fuerst 1987). Pyroxasulfone, a recent addition to the VLCFAE inhibitors, controls grass and small-seeded broadleaf weeds such as Italian ryegrass, barnyardgrass, foxtails, crabgrasses, Palmer amaranth and common waterhemp in corn, wheat and soybeans at low use rate and it is also effective against populations resistant to glyphosate, acetolactate synthase inhibitors, acetyl CoA carboxylase inhibitors and triazines (Shaner 2014, Tanetani et al. 2009). Chauhan et al. (2007) reported that metolachlor applied preplant (PP) or early preplant (EPP 20 days before crop sowing) at 0.48 or 0.96 kg ha^{-1} controlled rigid ryegrass by 71 to 90%, and no injury to wheat density or grain yield when applied EPP. In another study evaluating control of Italian ryegrass in winter wheat, Ritter and Menbere (2002), reported *S*-metolachlor at 1.12 kg ha^{-1} provided 85% Italian ryegrass control with no impacts to wheat yields despite initial stunting. VLCFAE inhibitors are effective in control of several weeds, however, tolerance of grass crops to herbicides in the group is not evenly distributed, thus resulting in injury to some species of grass crops (Hatzios 1991). Environmental factors such as inadequate or excess of moisture, cool and hot temperatures, poorly drained soils or widely fluctuating temperatures could also favor herbicide injury (Anonymous 2017).

Safeners applied either as seed treatment or as a tank-mixture with herbicides have been used to protect crops from herbicide injury without compromising the efficacy of the herbicide in weed control (Hatzios and Burgos 2004, Hatzios and Hoagland 1989, Riechers et al. 2010). *S*-metolachlor and alachlor are two herbicides used along with a seed-treatment safener such as fluxofenim or flurazole for selective weed control in grain sorghum (Goodrich et al. 2018, Rosinger 2014). Although, flufenacet and pyroxasulfone are registered for use in wheat without the use of safener, few studies have evaluated the use of other effective VLCFAE inhibitors such as *S*-metolachlor, dimethenamid-P or alachlor for weed control in wheat, not to mention the use of safener to counteract possible injury from these herbicides. Greenhouse studies have shown that fluxofenim protects wheat varieties from dimethenamid injury Riechers et al. (1996) and in another study evaluating safener protection and weed control with pyroxasulfone in grain sorghum under field conditions, fluxofenim was found to reduce stand count injury at a high rate of pyroxasulfone of 210 g ha^{-1}, although split application of 90/120 g ha^{-1} PRE was reported to cause less injury without compromising weed control (Goodrich et al. 2018).

Since commercialization of pyroxasulfone for use in wheat, protection of wheat from potential injury using a seed-treatment safener (fluxofenim) under field conditions have not been investigated. The objective of this study was to evaluate the efficacy of fluxofenim safener in mitigating very-long-chain fatty acid-inhibiting herbicide injury to winter wheat varieties in the field. A field study was therefore designed to test the hypothesis that fluxofenim safener could protect winter wheat varieties from *S*-metolachlor, dimethenamid-P or pyroxasulfone injury.

Materials and Methods

Response of wheat varieties to fluxofenim safener and preemergence herbicides in the field

Field studies were conducted at two locations during Fall 2018 winter wheat growing season in Northern Idaho. One location was situated in Genesee ID (Kambitsch Research Farm) and had silt loam soil type, pH of 5.0, organic matter content of 5% and cation exchange capacity of 18.3 meq/100g. The other location was situated in Moscow ID with silt loam soil type, pH of 4.7, organic matter content of 4% and cation exchange capacity of 18.1 meq/100g (Soiltest Farm Consultants, Inc, 2925 Driggs Dr., Moses Lake, WA 98837). Land preparation included fall chisel plowing and spring field cultivation with harrow prior to planting to eliminate weeds that may have emerged. Spring anhydrous ammonia was shanked in for fertilization.

Previously, we reported response variation in 19 soft white winter wheat varieties to *S*-metolachlor and dimethenamid-P herbicides and varieties were placed in one of three categories with respect to safener response: 1) variety responded, 2) mixed response 3) no response. Six soft white winter varieties, two from each response category were selected: 1) UI Sparrow, LWW 15-72223, 2) Brundage 96, UI Magic CL+ 3), UI Palouse CL+, UI Castle CL+. Varieties were released lines except LWW 15-72223 which is an advanced breeding line. UI Sparrow is commonly planted in Southern Idaho in irrigated farmland while the rest of the varieties are planted in the non-irrigated cropping regions of Northern Idaho. Fluxofenim (Concep III) was applied to seed at the 0.4 g ai kg^{-1} seed recommended label rate for use in grain sorghum (Anonymous 2012) along with a fungicide-insecticide premix [Vibrance Extreme at 1.83 ml kg^{-1} seed containing the active ingredients; sedaxane, difenoconazole and mefenoxam, Sharda Imidacloprid 5SC at 0.33 ml kg^{-1} seed containing the active ingredient Imidacloprid, and water at 7.17 ml kg^{-1} seed] while another 4.2 kg of same variety had a fungicide-insecticide premix [Vibrance Extreme at 1.83 ml kg^{-1} seed, Sharda at 0.33 ml kg^{-1} seed, and water at 7.17 ml kg^{-1} seed] without Concep III. Treated seeds were planted at Genesee on 08 October 2018, and Moscow on 12 October 2018. Seeds were planted at a targeted seeding depth of 3.8 cm and seeding rate of 112 kg ha^{-1} for both locations using a Hege small plot cone seeder drill with double disc openers. Size of each plot was 6.1 m long by 1.5 m wide, and each plot has 7 rows.

Preemergence herbicide application at Genesee was done the same day as planting, and herbicide application at Moscow was done two days after planting (14 October 2018). The preemergence herbicide treatments include: i) *S*-metolachlor at 1.782 kg ha^{-1} (Dual Magnum, 913 g L^{-1} *S*-metolachlor EC, Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC 27409), dimethenamid-P at 1.005 kg ha^{-1} (Outlook, 719 g L^{-1} dimethenamid-P EC, BASF, Crop Science Division, 26 Davis Drive, Research Triangle Park, NC 27709) or pyroxasulfone at 0.236 kg ha^{-1} (Zidua, 500 g L^{-1} pyroxasulfone SC, BASF, Crop Science Division, 26 Davis Drive, Research Triangle Park, NC 27709) applied using a pressurized CO_2 backpack sprayer equipped with Teejet flat fan nozzles 110015 spaced 61 cm apart on a 2.44 m long boom calibrated to deliver 94 L ha^{-1} at 221 kPa. *S*-metolachlor and dimethenamid-P herbicides were applied within recommended label rate while pyroxasulfone was applied at twice the label rate.

Experimental design was a blocked split-plot design. The whole plot factor was the herbicide treatment (*S*-metolachlor, dimethenamid-P or pyroxasulfone), and the subplot factor was the variety with or without safener treatment. Both field locations were sprayed with pyrosulfatole/bromoxynil at 212.96 g ae ha^{-1}, florasulam/fluroxypyr at 44.83 g ae ha^{-1}, and florasulam/MCPA at 358.67 g ae ha^{-1} on 13 May 2019 for postemergence control of broadleaf weeds.

Wheat density was evaluated in April 2019 (24 WAP), height data were collected in May 2019 (28 WAP), wheat head counts were conducted in June 2019 (32 WAP) and grains were harvested in August 2019 (40 WAP). Plant density was counted along one-meter length in the center of rows 2 and 3. Heads were counted along one-meter length in the center of row 2. Height was measured by averaging 8 height measurements of wheat plants in a meter length at the center of row 2. Grain yields were estimated by harvesting each plot and final yield converted to kg ha^{-1} for each of the plots.

Response of wheat variety to mixture of fluxofenim safener and fungicide-insecticide premix

In a previous greenhouse experiment evaluating the response of wheat varieties to safener doses at two herbicide rates, additional seeds of variety UI Castle CL+ were treated with a mixture of 0.4 g ai kg^{-1} seed Concep III (containing active ingredient fluxofenim) and 9.32 ml kg^{-1} seed of the fungicide-insecticide premix (Vibrance Extreme with active ingredients sedaxane, difenoconazole and mefenoxam, and Sharda Imidacloprid 5SC with active ingredient imidacloprid) as previously described to determine if there is a negative effect due to mixture of fluxofenim safener and the fungicide-insecticide premix.

Statistical Analysis

Data were analyzed with SAS version 9.4 (Statistical Analysis Software (SAS) 9.4. SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC). Grain yield at harvest, wheat height, test weight, wheat density and head counts were subjected to analysis of variance (ANOVA) using a generalized linear mixed model (PROC GLIMMIX). Data from both locations were analyzed separately. Treatment means were separated with pair-wise comparisons at 95% confidence level. Normality and homogeneity of variance assumptions were determined with PROC UNIVARIATE and PROC GLIMMIX respectively. The data were analyzed using the statistical model outlined below:

$$y_{ijkl} = \mu + r_i + \alpha_j + w_{ij} + \beta_k + (\alpha\beta)_{jk} + \gamma_l + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} + s_{ijkl} \quad [5]$$

Where y_{ijkl} is the expected response for the *i*th block/replicate, the *j*th herbicide treatment, the *k*th variety and the *l*th safener treatment; μ is the grand mean; α_j and β_k are the fixed effects of the *j*th herbicide treatment and the *k*th variety respectively; r_i is the random effect of the *i*th block/replicate, NID(0, σ_r^2); w_{ij} is the random whole plot error term, NID(0, σ_w^2); $(\alpha\beta)_{jk}$ is the fixed interaction between herbicide treatment and variety; γ_l is the fixed effect of the *l*th safener treatment; $(\alpha\gamma)_{jl}$ is the fixed interaction between herbicide and safener treatments; $(\beta\gamma)_{kl}$ is the fixed interaction between variety and safener treatment; $(\alpha\beta\gamma)_{jkl}$ is the random three-way interaction, NID(0, $\sigma_{\alpha\beta\gamma}^2$); and s_{ijkl} is the random split plot error term, NID(0, σ_s^2).

Results

Response of wheat varieties to fluxofenim safener and preemergence herbicides in the field

Fluxofenim safener treatment reduced grain yield of varieties at Moscow field location (Table 3.1a), with a similar trend in yield reduction of varieties at Genesee field location (Table 3.1b). There was a significant interaction of variety by herbicide treatments, as well as safener by herbicide treatments at Moscow location on wheat height. *S*-metolachlor, dimethenamid-P or pyroxasulfone had a negative impact on height of varieties at Moscow location (Table 3.2a). Fluxofenim seed treatment reduced wheat height by 10%, 4%, 6% and 5% for untreated control, *S*-metolachlor, dimethenamid-P and pyroxasulfone herbicides respectively (Table 3.2b). There was no significant interaction of variety, herbicide and safener treatment for wheat height at Genesee location. Neither safener treatment nor herbicide treatment had any impact on varieties. There was also no impact of safener and herbicide treatments on test weight at both locations (Data not shown).

Safener significantly increased the density of UI Sparrow by 19%, but density of UI Palouse CL+ and UI Castle CL+ were significantly reduced by 27% and 19% respectively at Moscow location (Table 3.3a). There was a significant interaction of herbicide by safener treatment for wheat density at Moscow location. Fluxofenim seed treatment reduced wheat density by 25% and 12% for untreated

control and pyroxasulfone treatments respectively (Table 3.3b). Safener significantly reduced wheat density of only variety UI Castle CL+ at Genesee location (Table 3.3c). There was also a significant interaction of herbicide by safener treatment for head count at Moscow location. Untreated control had 20% reduction in head count with safener treatment (Table 3.4a). There was a significant interaction of variety, herbicide and safener treatments for head count at Genesee location. Fluxofenim seed treatment reduced head count of UI Sparrow by 21% for untreated control. In the presence of *S*-metolachlor herbicide, fluxofenim significantly increased head counts of UI Sparrow and LWW 15-72223 by 38% and 34% respectively. Fluxofenim seed treatment also significantly increased head counts of varieties LWW 15-72223 and UI Palouse CL+ for dimethenamid-P herbicides by 32% and 25% respectively. Safener however reduced head count of UI Sparrow for pyroxasulfone herbicide by 21% (Table 3.4b).

Overall, yield at Moscow across treatments and varieties were higher than at Genesee. Air temperature was similar with high/low of 16/2 C in Moscow and 13/-0.3 C in Genesee at the time of planting in October. Precipitation was also similar at the time of planting; however, Moscow had more precipitation than Genesee for the next 8 months. Soil temperature also had similar pattern for both locations, with high/low temperature at 13/7 C at the time of planting which subsequently fell to 1 C from December to March before rising again in April.

Response of wheat variety to mixture of fluxofenim safener and fungicide-insecticide premix

A greenhouse experiment to determine if a mixture of fluxofenim and the fungicide-insecticide premix (Vibrance Extreme with active ingredients sedaxane, difenoconazole and mefenoxam, and Sharda Imidacloprid 5SC with active ingredient imidacloprid) used in winter wheat could result in the injury observed in this study did not show that the mixture of both chemicals caused the injury observed.

Discussion

Fluxofenim reduced grain yield and wheat height in both research locations while wheat density and head count varied across location and varieties. Test weight was not however affected by safener or herbicide treatments. Overall, injury to wheat varieties observed in this study was due to the safener treatment. This finding is contradictory to our previous results in which fluxofenim safener did not cause injury to winter wheat under greenhouse conditions. Several researchers have shown that the extent of protection of crops by safener is influenced by interactions between the safener and environment (Bernards et al. 2006). Environmental conditions such as cool soil temperatures and high than average moisture have been shown to compromise the efficacy of some safeners (Ketchersid et al.

1981, Leif et al. 1987). Hatzios and Hoagland (1989) reported that antidotes that protect sorghum from injury by acetanilide herbicides sometimes lose their protective ability under cool and/or wet conditions. In addition, phytotoxicity of seed safener have been shown to increase as the time the safener is exposed to the seed increases; an observation made with 1,8-naphthalic anhydride (NA) (Jablonkai 2013). Therefore, it may be that the cold winter months of November to March as observed from the climate data could have compromised the efficacy of fluxofenim in protecting the varieties from injury or perhaps this long period of cold winter have increased the time the safener is exposed to the wheat seeds thus resulting in safener injury.

There was no injury to wheat varieties with *S*-metolachlor, dimethenamid-P or twice the label rate of pyroxasulfone used in this study. Perhaps, the varieties had recovered from any initial injury at the time injury evaluations were conducted in April. Bernards et al. (2006) observed that corn seedlings usually recovered from minor injury either at lower rates of metolachlor or when metolachlor was applied with safener. Also, reports have shown that minor injury from chloroacetamide do not result in yield loss as the crop recovers from that early season injury (Martin and Burnside 1982).

A greenhouse experiment to determine the influence of a mixture of fluxofenim safener and the commonly used fungicide-insecticide premix (Vibrance Extreme and Sharda Imidacloprid) to variety UI Castle CL+ provided no evidence of chemical incompatibility. The mixture of the seed treatments did not cause injury to variety UI Castle CL+ in the greenhouse. Results of the field studies conducted in fall 2018 were therefore inconclusive and we did not see a benefit of fluxofenim safener.

Interestingly, visual injury observations from subsequent field studies conducted in fall 2019 shows benefit of fluxofenim safener in protecting three varieties UI Sparrow, UI Magic CL+ and UI Castle CL+ from *S*-metolachlor injury at 5338 g ai ha^{-1}. Slight protection was also noted for dimethenamid-P at 3305 g ai ha^{-1}, although injury from this herbicide was high relative to untreated control plots. A major difference between our field study in 2018 and 2019 is the higher herbicide rate used in 2019 field studies. Fluxofenim safener may thus be beneficial to wheat varieties in extreme stress conditions such as that imposed by the higher herbicide rates. Although, the impact of this herbicide injury on wheat yield or test weight is yet to be determined, reduction of injury early in the season shows promise for use of *S*-metolachlor and dimethenamid-P herbicides in wheat. Fluxofenim protection of winter wheat varieties under different environmental conditions, herbicide rates and soil types would need to be further evaluated. In addition, annual grass control in wheat using these herbicides will need to be further examined.

Table 3.1a: Estimated winter wheat grain yield (kg ha^{-1}) at Moscow, Idaho location for interaction of variety, safener and herbicide treatments.

Grain yield (kg ha^{-1})												
Variety	Untreated control			*S*-metolachlor			Dimethenamid-P			Pyroxasulfone		
	-S	+S	p-value	-S	+S	p-value	-S	+S	p-value	-S	+S	p-value
UI Sparrow	7906.96	7477.64	0.1971	6887.80	6980.08	0.7810	7895.52	7246.05	0.0520	7886.26	7340.91	0.1020
LWW 15-72223	7035.99	6722.31	0.3453	6011.03	5955.46	0.8670	7369.49	6858.12	0.1250	7062.50	6969.15	0.7785
Brundage 96	7107.63	6922.57	0.5772	6072.09	6324.12	0.4480	7297.64	6909.30	0.2431	6905.11	6887.61	0.9580
UI Magic CL+	6564.75	6601.89	0.9109	6238.33	6593.73	0.2852	7040.22	7091.16	0.8780	6585.20	6638.54	0.8726
UI Palouse CL+	6368.93	6100.58	0.4192	6297.51	5931.08	0.2705	6455.52	6091.30	0.2734	6934.54	6357.09	0.0835
UI Castle CL+	6877.58	6623.71	0.4447	6306.98	6027.25	0.3998	7234.32	6429.53	0.0164	7020.71	6346.75	0.0438

-S: no safener seed treatment

+S: with safener seed treatment

Table 3.1b: Estimated winter wheat grain yield (kg ha^{-1}) at Genesee, Idaho location for interaction of variety, safener and herbicide treatments.

Grain yield (kg ha^{-1})												
Variety	Untreated control			*S*-metolachlor			Dimethenamid-P			Pyroxasulfone		
	-S	+S	p-value	-S	+S	p-value	-S	+S	p-value	-S	+S	p-value
UI Sparrow	6735.44	5386.44	0.0662	4906.57	6538.06	0.0268	4576.04	4861.63	0.6956	6678.47	5861.44	0.2640
LWW 15-72223	6821.17	5663.57	0.1144	5393.35	5962.00	0.4388	4014.32	5108.42	0.1355	6951.58	6652.48	0.6820
Brundage 96	5722.88	6979.67	0.0868	5178.35	6023.35	0.2483	5634.13	4542.10	0.1362	5419.31	5871.07	0.5362
UI Magic CL+	6125.69	6060.35	0.2574	6040.28	4922.55	0.1273	5016.07	4905.36	0.8794	5369.90	5747.88	0.6047
UI Palouse CL+	4812.01	5640.48	0.2574	4613.25	6016.81	0.0561	4241.33	4575.63	0.6466	5680.95	5325.42	0.6263
UI Castle CL+	5400.66	5305.72	0.8965	6654.06	5493.34	0.1134	5597.77	5120.04	0.5130	6988.39	5396.68	0.0306

-S: no safener seed treatment

+S: with safener seed treatment

Table 3.2a: Estimated mean wheat height (cm) at Moscow, Idaho location for variety and herbicide treatment combinations. Values are presented as mean ± SEM.

Variety	Wheat height (cm)			
	Untreated control	M	D	P
UI Sparrow	32.80 ± 0.81	27.81 ± 0.81	29.84 ± 0.81	29.80 ± 0.81
LWW 15-72223	34.06 ± 0.81	32.86 ± 0.81	31.91 ± 0.81	32.56 ± 0.81
Brundage 96	34.14 ± 0.81	31.56 ± 0.81	30.44 ± 0.81	29.08 ± 0.81
UI Magic CL+	36.13 ± 0.81	32.06 ± 0.81	33.34 ± 0.81	34.52 ± 0.81
UI Palouse CL+	28.03 ± 0.81	26.45 ± 0.81	25.98 ± 0.81	25.56 ± 0.81
UI Castle CL+	32.05 ± 0.81	29.67 ± 0.81	31.69 ± 0.81	31.48 ± 0.81

M: *S*-metolachlor; D: Dimethenamid-P; P: Pyroxasulfone

Table 3.2b: Estimated mean wheat height (cm) at Moscow, Idaho location for herbicide and safener treatment combinations. Values are presented as mean ± SEM.

Herbicide	Wheat height (cm)		
	-S	+S	p-value
Untreated control	34.67 ± 0.57	31.06 ± 0.57	<.0001
S-metolachlor	30.67 ± 0.57	29.47 ± 0.57	0.0418
Dimethenamid-P	31.43 ± 0.57	29.64 ± 0.57	0.0025
Pyroxasulfone	31.30 ± 0.57	29.70 ± 0.57	0.0069

-S: no safener seed treatment

+S: with safener seed treatment

Table 3.3a: Estimated mean wheat density (plants m^{-1} row) at Moscow, Idaho location for variety and safener treatment combinations. Values are presented as mean ± SEM.

Variety	Wheat density (plants m^{-1} row)		
	-S	+S	p-value
UI Sparrow	29.80 ± 1.54	35.52 ± 1.69	0.0137
LWW 15-72223	34.91 ± 1.68	32.72 ± 1.61	0.3474
Brundage 96	35.43 ± 1.69	34.03 ± 1.65	0.5547
UI Magic CL+	34.75 ± 1.68	33.81 ± 1.65	0.6900
UI Palouse CL+	38.22 ± 1.77	27.97 ± 1.47	<.0001
UI Castle CL+	40.40 ± 1.83	32.65 ± 1.61	0.0019

-S: no safener seed treatment
+S: with safener seed treatment

Table 3.3b: Estimated mean wheat density (plants m^{-1} row) at Moscow, Idaho location for herbicide and safener treatment combinations. Values are presented as mean ± SEM.

Herbicide	Wheat density (plants m^{-1} row)		
	-S	+S	p-value
Untreated control	42.27 ± 1.54	31.86 ± 1.30	<.0001
S-metolachlor	31.24 ± 1.29	32.44 ± 1.31	0.5145
Dimethenamid-P	33.35 ± 1.34	35.10 ± 1.38	0.3625
Pyroxasulfone	35.78 ± 1.39	31.49 ± 1.29	0.0249

-S: no safener seed treatment

+S: with safener seed treatment

Table 3.3c: Estimated mean wheat density (plants m^{-1} row) at Genesee, Idaho location for variety and safener treatment combinations. Values are presented as mean ± SEM.

Variety	Wheat density (plants m^{-1} row)		
	-S	+S	p-value
UI Sparrow	41.51 ± 2.17	42.35 ± 2.19	0.7128
LWW 15-72223	41.95 ± 2.18	44.02 ± 2.26	0.3726
Brundage 96	44.52 ± 2.28	42.23 ± 2.19	0.3265
UI Magic CL+	42.48 ± 2.20	38.38 ± 2.04	0.0693
UI Palouse CL+	41.09 ± 2.15	42.21 ± 2.19	0.6236
UI Castle CL+	45.21 ± 2.30	38.39 ± 2.04	0.0032

-S: no safener seed treatment

+S: with safener seed treatment

Table 3.4a: Estimated mean wheat head count (plants m^{-1} row) at Moscow, Idaho location for herbicide and safener treatment combinations. Values are presented as mean ± SEM.

Herbicide	Wheat head count (plants m^{-1} row)		
	-S	+S	p-value
Untreated control	94.26 ± 4.72	75.03 ± 3.84	0.0018
S-metolachlor	70.66 ± 3.65	65.61 ± 3.42	0.3140
Dimethenamid-P	65.08 ± 3.40	69.36 ± 3.59	0.3877
Pyroxasulfone	69.57 ± 3.60	62.15 ± 3.26	0.1284

-S: no safener seed treatment

+S: with safener seed treatment

Table 3.4b: Estimated mean wheat head count (plants m^{-1} row) at Genesee, Idaho location for variety, herbicide and safener treatment combinations. Values are presented as mean ± SEM.

Wheat head count (plants m^{-1} row)												
Variety	Control			*S*-metolachlor			Dimethenamid-P			Pyroxasulfone		
	-S	+S	p-value	-S	+S	p-value	-S	+S	p-value	-S	+S	p-value
UI Sparrow	104.26 ± 7.56	82.32 ± 6.32	0.0175	73.69 ± 5.83	101.48 ± 7.40	0.0018	75.63 ± 5.94	66.69 ± 5.42	0.2380	88.34 ± 6.66	69.83 ± 5.60	0.0241
LWW 15-72223	126.64 ± 8.80	118.29 ± 8.34	0.4551	91.52 ± 6.84	122.50 ± 8.57	0.0026	79.26 ± 6.14	104.97 ± 7.60	0.0051	102.30 ± 7.45	115.17 ± 8.16	0.2096
Brundage 96	94.67 ± 7.02	106.51 ± 7.68	0.2216	89.93 ± 6.75	86.44 ± 6.55	0.6913	86.70 ± 6.57	82.44 ± 6.33	0.6183	82.42 ± 6.33	83.09 ± 6.36	0.9367
UI Magic CL+	104.50 ± 7.57	100.06 ± 7.33	0.6500	98.12 ± 7.21	93.77 ± 6.97	0.6412	85.65 ± 6.51	90.79 ± 6.80	0.5593	98.60 ± 7.24	92.54 ± 6.90	0.5147
UI Palouse CL+	102.47 ± 7.46	113.23 ± 8.06	0.2908	94.65 ± 7.02	105.78 ± 7.76	0.2530	67.89 ± 5.49	84.72 ± 6.46	0.0353	97.64 ± 7.19	87.75 ± 6.63	0.2784
UI Castle CL+	97.23 ± 7.16	100.33 ± 7.34	0.7449	106.88 ± 7.70	103.75 ± 7.53	0.7540	88.35 ± 6.66	80.09 ± 6.19	0.3321	106.81 ± 7.70	96.05 ± 7.10	0.2695

-S: no safener seed treatment

+S: with safener seed treatment

Table 3.5: Mean response of variety UI Castle CL+ to fluxofenim and fungicide-insecticide mix in the presence of *S*-metolachlor herbicide. Values are presented as mean ± SEM.[a,b,c,d]

	Biomass (g plant^{-1})
Untreated control	0.0585 ± 0.0019 A
Fluxofenim + *S*-metolachlor	0.0498 ± 0.0019 B
Fluxofenim + FIP + *S*-metolachlor	0.0546 ± 0.0019 AB

[a] Fluxofenim seed treatment at 0.4 g ai kg^{-1} seed

[b] *S*-metolachlor applied at 1782 g ai ha^{-1}

[c] FIP, Fungicide-Insecticide Premix seed treatment at 9.32 ml kg^{-1} seed

[d] Means presented within each column with same letters are not significantly different ($\alpha = 0.05$).

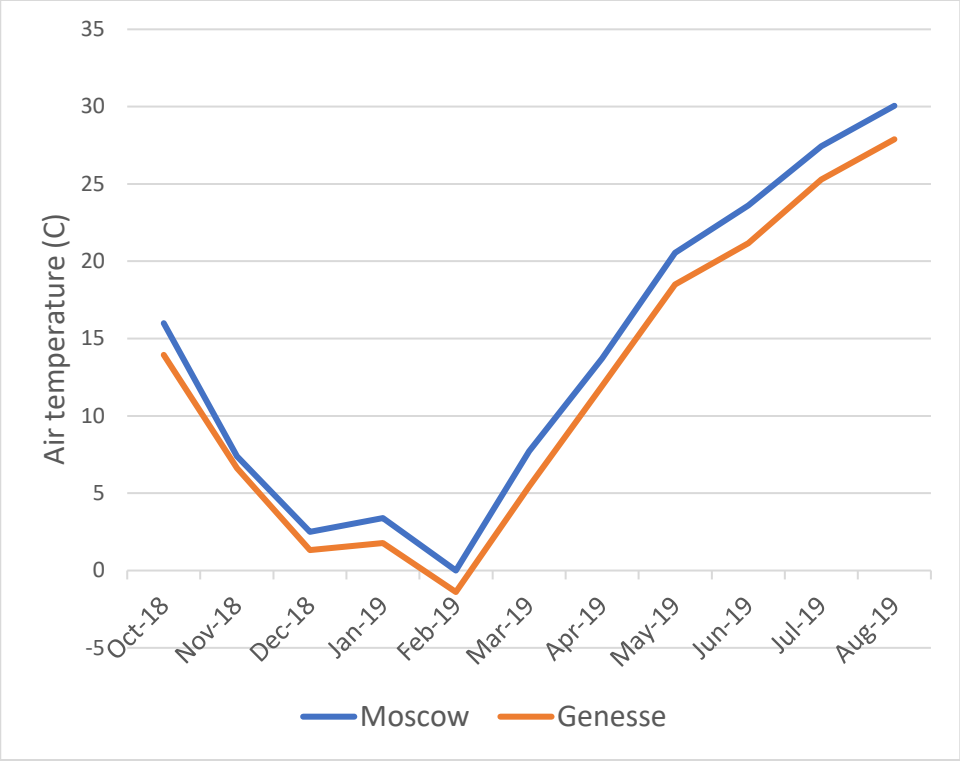

Figure 3.1a: Maximum air temperature at Moscow and Genesee, Idaho climate stations.

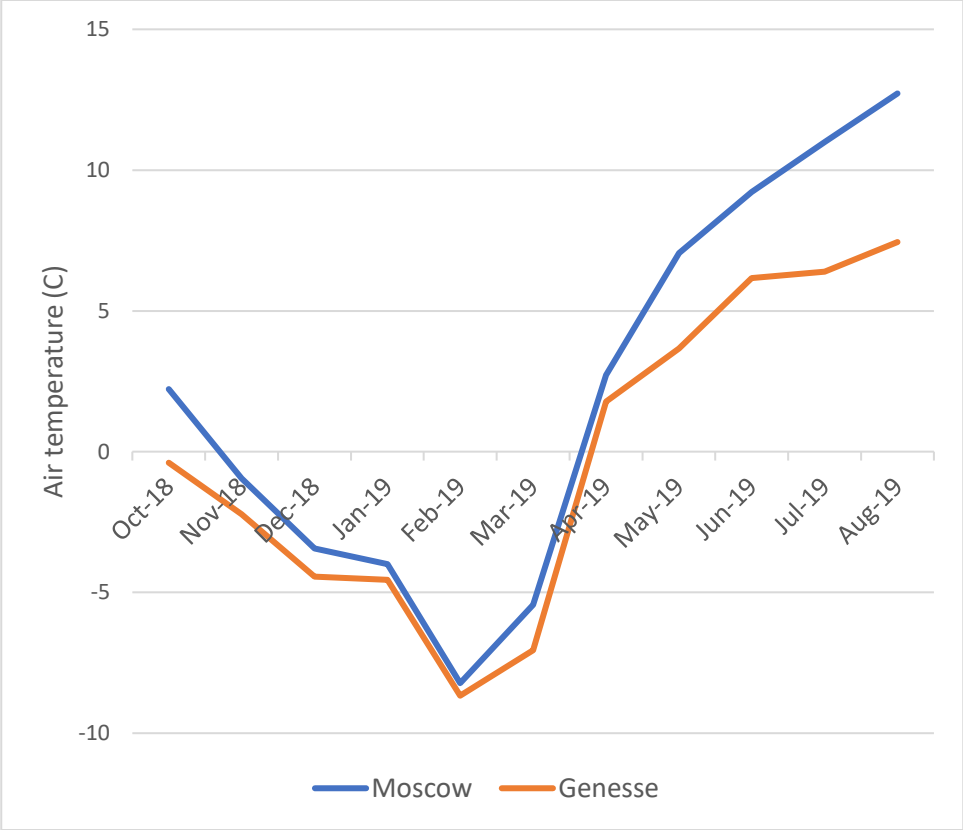

Figure 3.1b: Minimum air temperature at Moscow and Genesee, Idaho climate stations.

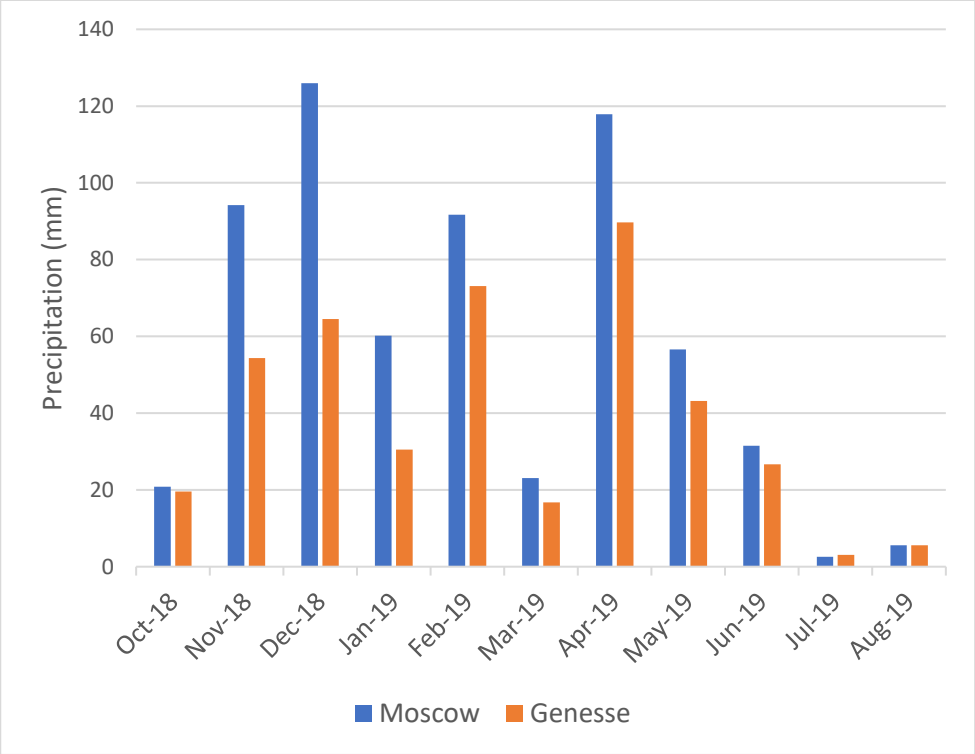

Figure 3.2: Precipitation at Moscow and Genesee, Idaho climate stations. Values are for total rainfall and melted snow in millimeters.

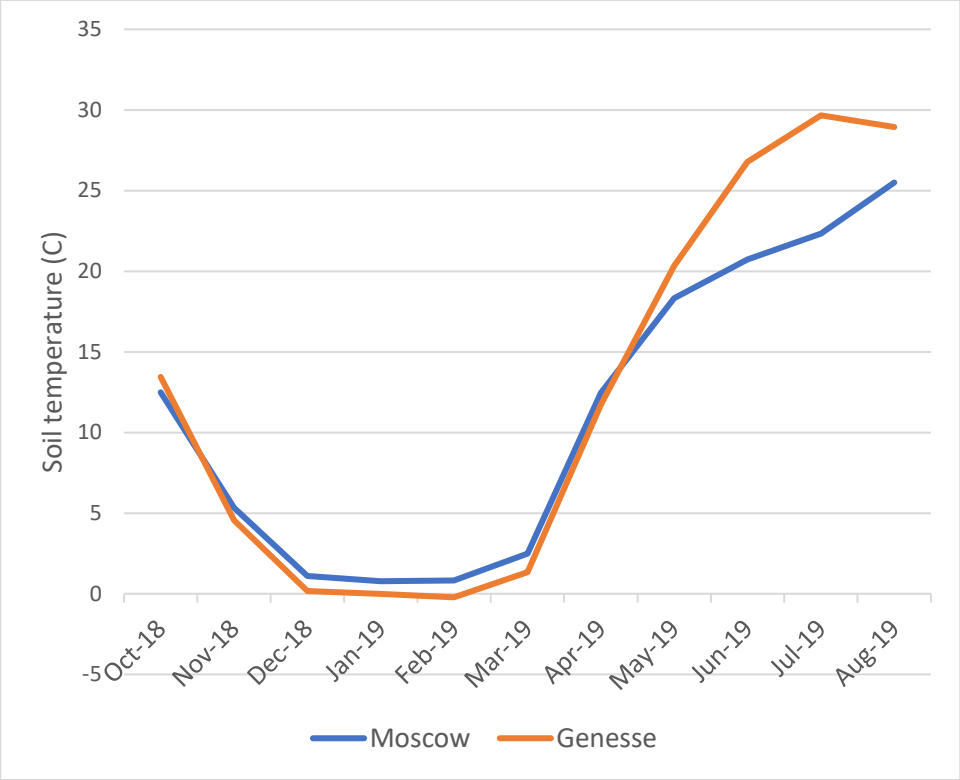

Figure 3.3a: Maximum soil temperature at 10 cm depth, Idaho climate stations.

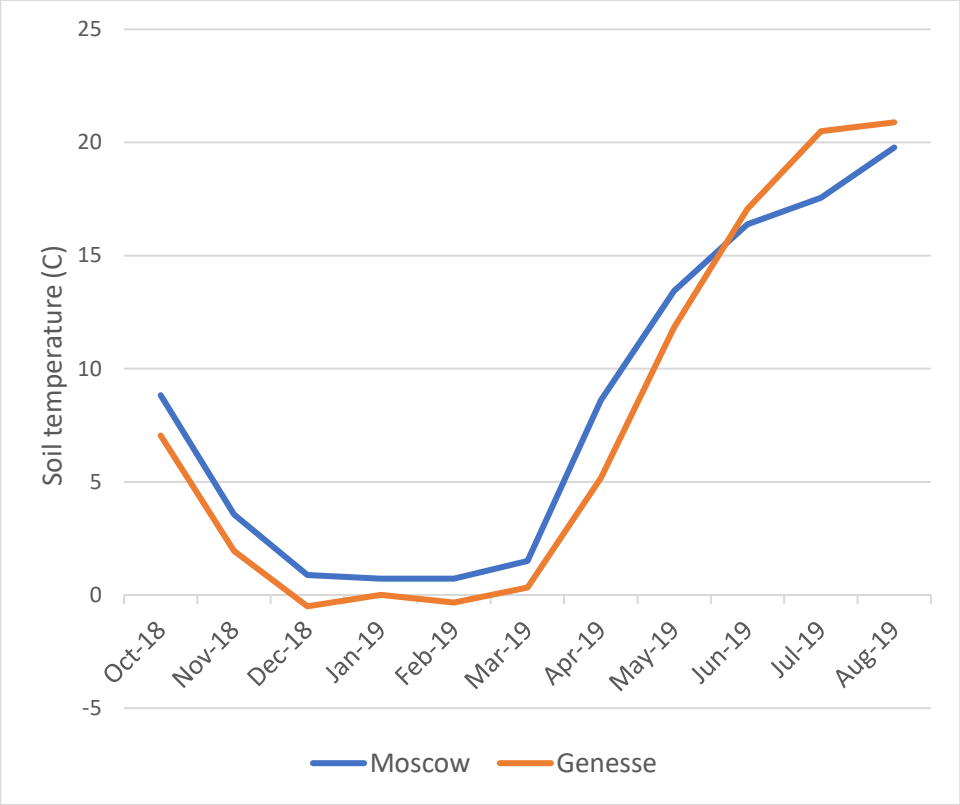

Figure 3.3b: Minimum soil temperature at 10 cm depth, Idaho climate stations.

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APPENDIX A: DOSE-RESPONSE ANALYSIS OF SIX VARIETIES TO FLUXOFENIM TREATMENTS

Table A1: Parameter estimates from dose-response analysis of six varieties to incremental doses of fluxofenim-alone treatment. Biomass data collected at 21 DAT were fitted to a two-parameter log-logistic equation, $y = K*100/(K + \exp(b(\log(x) - \log(ED_Q))))$, where $ED_Q = ED_{10}$ when K = 9.

Parameter	Estimate	Standard Error	Pr > |t|	95 % Confidence Limit	
UI Sparrow, ED_{10}	0.8510	0.1985	<.0001	0.4604	1.2415
LWW 15-72223, ED_{10}	1.1478	0.1875	<.0001	0.7788	1.5168
Brundage 96, ED_{10}	0.6735	0.2356	0.0045	0.2100	1.1370
UI Magic CL+, ED_{10}	0.5468	0.1762	0.0021	0.2000	0.8936
UI Palouse CL+, ED_{10}	1.2335	0.2259	<.0001	0.7891	1.6780
UI Castle CL+, ED_{10}	0.8684	0.1469	<.0001	0.5794	1.1574
UI Sparrow, *b*	0.9074	0.2056	<.0001	0.5028	1.3120
LWW 15-72223, *b*	1.4421	0.2638	<.0001	0.9230	1.9613
Brundage 96, *b*	0.7731	0.2249	0.0007	0.3307	1.2155
UI Magic CL+, *b*	0.6126	0.1546	<.0001	0.3084	0.9168
UI Palouse CL+, *b*	1.2444	0.2713	<.0001	0.7106	1.7781
UI Castle CL+, *b*	1.3060	0.2061	<.0001	0.9004	1.7116

Table A2: Parameter estimates from dose-response analysis of six varieties to incremental doses of fluxofenim-alone treatment. Height data collected at 14 DAT were fitted to a two-parameter log-logistic equation, $y = K*100/(K + \exp(b(\log(x) - \log(ED_Q))))$, where $ED_Q = ED_{10}$ when K = 9.

Parameter	Estimate	Standard Error	Pr > |t|	95 % Confidence Limit	
UI Sparrow, ED_{10}	0.2787	0.0596	<.0001	0.1615	0.3960
LWW 15-72223, ED_{10}	0.5062	0.0887	<.0001	0.3316	0.6807
Brundage 96, ED_{10}	0.7704	0.1398	<.0001	0.4955	1.0453
UI Magic CL+, ED_{10}	0.3274	0.0748	<.0001	0.1804	0.4745
UI Palouse CL+, ED_{10}	0.3623	0.0750	<.0001	0.2148	0.5099
UI Castle CL+, ED_{10}	0.4017	0.0692	<.0001	0.2655	0.5378
UI Sparrow, *b*	0.6753	0.0810	<.0001	0.5160	0.8346
LWW 15-72223, *b*	0.8213	0.1045	<.0001	0.6157	1.0269
Brundage 96, *b*	0.7516	0.1259	<.0001	0.5039	0.9993
UI Magic CL+, *b*	0.6352	0.0875	<.0001	0.4630	0.8073
UI Palouse CL+, *b*	0.6648	0.0861	<.0001	0.4954	0.8342
UI Castle CL+, *b*	0.8254	0.0922	<.0001	0.6439	1.0069

APPENDIX B: DOSE-RESPONSE ANALYSIS OF ITALIAN RYEGRASS TO S-METOLACHLOR AND DIMETHENAMID-P HERBICIDES

Table B1: Parameter estimates from dose-response analysis of an Italian ryegrass population to incremental doses of *S*-metolachlor and dimethenamid-P herbicides. Biomass data collected at 21 DAT were fitted to a two-parameter log-logistic equation, $y = K*100/(K + \exp(b(\log(x) - \log(ED_Q))))$, where $ED_Q = ED_{50}$ or ED_{90} when K = 1 or 0.1111 respectively.

Parameter	Estimate	Standard Error	Pr > |t|	95 % Confidence Limit	
Dimethenamid-P					
ED_{50}	22.9648	6.7002	0.0008	9.7325	36.1970
Slope, *b*	0.9780	0.2152	<.0001	0.5529	1.4031
ED_{90}	213.04	67.2960	0.0019	80.1389	345.94
Slope, *b*	0.9887	0.2151	<.0001	0.5639	1.4135
S-metolachlor					
ED_{50}	71.4062	12.9773	<.0001	45.7774	97.0351
Slope, *b*	1.1261	0.2151	<.0001	0.7013	1.5509
ED_{90}	558.12	187.62	0.0034	187.60	928.64
Slope, *b*	1.0621	0.2192	<.0001	0.6292	1.4950

APPENDIX C: DOSE-RESPONSE ANALYSIS OF THREE VARIETIES TO FLUXOFENIM TREATMENTS AT TWO HERBICIDE RATES

Table C1: Parameter estimates from dose-response analysis of varieties UI Sparrow, Brundage 96 and UI Castle CL+ to incremental doses of fluxofenim safener at 1010 g ai ha^{-1} rate of *S*-metolachlor herbicide. Biomass data collected at 21 DAT were fitted to an exponential function, $y = C + A*(1 – \exp(-b*\text{rate}))$. $ED_{90} = \log (0.1*(A+C)/A)/-b$, where Max = A + C.

Variety	Parameter	Estimate	Standard Error	Pr > |t|	95 % Confidence Limit	
UI Sparrow	Max	153.76	8.9256	<.0001	136.11	171.40
	A	54.5583	15.7101	0.0007	23.5061	85.6105
	B	4.9401	3.2045	0.1254	-1.3938	11.2739
	C	99.1980	13.1800	<.0001	73.1467	125.25
	ED_{90}	0.2564	0.1750	0.1452	-0.0896	0.6024
Brundage 96	Max	162.99	25.0042	<.0001	113.57	212.41
	A	58.4874	21.1650	0.0065	16.6531	100.32
	B	2.3358	3.1513	0.4598	-3.8929	8.5645
	C	104.50	15.5175	<.0001	73.8291	135.17
	ED_{90}	0.5470	0.7778	0.4830	-0.9905	2.0845
UI Castle CL+	Max	158.56	7.3226	<.0001	144.09	173.03
	A	58.5175	15.2595	0.0002	28.3559	88.6791
	B	19.0363	77.2111	0.8056	-133.58	171.65
	C	100.04	13.4156	<.0001	73.5256	126.56
	ED_{90}	0.0686	0.2806	0.8072	-0.4860	0.6232

Table C2: Parameter estimates from dose-response analysis of varieties UI Sparrow, Brundage 96 and UI Castle CL+ to incremental doses of fluxofenim safener at 1782 g ai ha^{-1} rate of *S*-metolachlor herbicide. Biomass data collected at 21 DAT were fitted to an exponential function, $y = C + A*(1 – \exp(-b*\text{rate}))$. $ED_{90} = \log (0.1*(A+C)/A)/-b$, where Max = A + C.

Variety	Parameter	Estimate	Standard Error	Pr > |t|	95 % Confidence Limit	
UI Sparrow	Max	191.52	7.7068	<.0001	176.29	206.76
	A	91.1506	14.2140	<.0001	63.0553	119.25
	B	7.9592	4.6792	0.0911	-1.2896	17.2079
	C	100.37	12.4710	<.0001	75.7217	125.02
	ED_{90}	0.1960	0.1165	0.0948	-0.0344	0.4264
Brundage 96	Max	155.42	13.2838	<.0001	129.16	181.67
	A	53.5861	16.0151	0.0010	21.9310	85.2411
	B	2.8363	2.2417	0.2078	-1.5946	7.2671
	C	101.83	12.2215	<.0001	77.6744	125.99
	ED_{90}	0.4364	0.3659	0.2349	-0.2868	1.1596
UI Castle CL+	Max	163.70	7.1155	<.0001	149.63	177.76
	A	63.7438	14.1424	<.0001	35.7903	91.6973
	B	8.0346	5.5380	0.1490	-2.9118	18.9809
	C	99.4512	12.3763	<.0001	75.4886	124.41
	ED_{90}	0.1692	0.1200	0.1607	-0.0681	0.4064

Table C3: Parameter estimates from dose-response analysis of varieties UI Sparrow, Brundage 96 and UI Castle CL+ to incremental doses of fluxofenim safener at 647 g ai ha^{-1} rate of dimethenamid-P herbicide. Biomass data collected at 21 DAT were fitted to an exponential function, $y = C + A*(1 – \exp(-b*\text{rate}))$. $ED_{90} = \log (0.1*(A+C)/A)/-b$, where Max = A + C.

Variety	Parameter	Estimate	Standard Error	Pr > |t|	95 % Confidence Limit	
UI Sparrow	Max	134.14	6.2523	<.0001	121.78	146.50
	A	35.3747	13.2123	0.0083	9.2597	61.4898
	B	6.6264	4.5084	0.1438	-2.2848	15.5377
	C	98.7677	11.3370	<.0001	76.3593	121.18
	ED_{90}	0.1463	0.1144	0.2029	-0.0798	0.3724
Brundage 96	Max	147.03	25.1817	<.0001	97.2607	196.81
	A	46.3330	23.9853	0.0554	-1.0758	93.7417
	B	1.3402	1.5770	0.3968	-1.7768	4.4572
	C	100.70	10.5090	<.0001	79.9296	121.47
	ED_{90}	0.8564	1.1923	0.4737	-1.5002	3.2130
UI Castle CL+	Max	136.17	9.4338	<.0001	117.52	154.81
	A	36.2871	14.1086	0.0111	8.4004	64.1738
	B	3.3591	2.7571	0.2251	-2.0906	8.8087
	C	99.8803	11.0464	<.0001	78.0462	121.71
	ED_{90}	0.2918	0.2699	0.2815	-0.2418	0.8253

Table C4: Parameter estimates from dose-response analysis of varieties UI Sparrow, Brundage 96 and UI Castle CL+ to incremental doses of fluxofenim safener at 1005 g ai ha^{-1} rate of dimethenamid-P herbicide. Biomass data collected at 21 DAT were fitted with an exponential function, $y = C + A*(1 – \exp(-b*\text{rate}))$. $ED_{90} = \log (0.1*(A+C)/A)/-b$, where Max = A + C.

Variety	Parameter	Estimate	Standard Error	Pr > |t|	95 % Confidence Limit	
UI Sparrow	Max	180.52	6.6941	<.0001	167.28	193.75
	A	80.4309	16.8142	<.0001	47.1925	113.67
	B	16.5072	23.4375	0.4824	-29.8242	62.8387
	C	100.09	15.3860	<.0001	69.6703	130.50
	ED_{90}	0.0905	0.1300	0.4874	-0.1665	0.3475
Brundage 96	Max	154.12	18.0469	<.0001	118.44	189.79
	A	57.1639	20.0569	0.0050	17.5152	96.8127
	B	1.7990	1.3548	0.1863	-0.8792	4.4771
	C	96.9520	12.0797	<.0001	73.0726	120.83
	ED_{90}	0.7286	0.6053	0.2307	-0.4679	1.9252
UI Castle CL+	Max	136.42	7.0873	<.0001	122.41	150.43
	A	35.7496	15.0731	0.0190	5.9529	65.5463
	B	10.1109	14.9593	0.5002	-19.4608	39.6825
	C	100.67	13.3219	<.0001	74.3351	127.00
	ED_{90}	0.0953	0.1493	0.5244	-0.1999	0.3905

Table C5: Parameter estimates from dose-response analysis of varieties UI Sparrow, Brundage 96 and UI Castle CL+ to incremental doses of fluxofenim safener at 246 g ai ha^{-1} rate of pyroxasulfone herbicide. Biomass data collected at 21 DAT were fitted with an exponential function, $y = C + A*(1 – \exp(-b*\text{rate}))$. $ED_{90} = \log (0.1*(A+C)/A)/-b$, where Max = A + C.

Variety	Parameter	Estimate	Standard Error	Pr > |t|	95 % Confidence Limit	
UI Sparrow	Max	236.72	13.3825	<.0001	210.27	263.17
	A	113.25	20.9580	<.0001	71.8243	154.67
	B	3.2211	1.0919	0.0037	1.0628	5.3794
	C	123.47	16.0789	<.0001	87.8046	148.75
	ED_{90}	0.4859	0.1710	0.0051	0.1479	0.8240
Brundage 96	Max	188.57	39.1033	<.0001	111.28	265.86
	A	70.2896	37.2366	0.0611	-3.3113	143.89
	B	1.2721	1.4911	0.3950	-1.6753	4.2194
	C	118.28	15.4165	<.0001	87.8046	148.75
	ED_{90}	1.0343	1.3907	0.4583	-1.7146	3.7833
UI Castle CL+	Max	143.43	14.2935	<.0001	115.18	171.69
	A	37.5719	21.0623	0.0766	-4.0593	79.2031
	B	3.2645	3.8611	0.3992	-4.3673	10.8963
	C	105.86	16.3415	<.0001	73.5623	138.16
	ED_{90}	0.2950	0.3962	0.4577	-0.4881	1.0780

APPENDIX D: MEAN OF HERBICIDE TREATED CONTROLS FROM DOSE-RESPONSE ANALYSIS OF THREE VARIETIES TO FLUXOFENIM TREATMENTS AT TWO HERBICIDE RATES

Table D1: Mean of herbicide treated controls for the three herbicides used in dose-response experiments.

Variety	*S*-metolachlor		Dimethenamid-P		Pyroxasulfone
	1010	1782	647	1005	247
			g ai ha^{-1}		
			Mean values of herbicide treated control		
			g $plant^{-1}$		
UI Sparrow	0.0281	0.0228	0.0314	0.0188	0.0320
Brundage 96	0.0342	0.0354	0.0344	0.0289	0.0542
UI Castle CL+	0.0425	0.0378	0.0411	0.0341	0.0603

APPENDIX E: HERBICIDE SAFENERS

Table E1: List of safeners used in crop protection.[1]

Year reported	Crop	Safener[2]	Chemical class	Herbicide[2]	Application timing	Company
1971	Corn	NA (Protect)		EPTC, butylate, vernolate	Seed treatment	Gulf Oil Company
1972	Corn	Dichlormid	Dichloroacetamide	Acetochlor (TopNotch, Surpass), metolachlor (Stalwart C)	Preemergence; premixed with herbicide	Stauffer now Syngenta
1978	Sorghum	Cyometrinil (Concep I)	Oxime ethers	Metolachlor	Seed treatment	Ciba-Geigy now Syngenta
1982		Oxabetrinil (Concep II)				
1986		Fluxofenim (Concep III)				
1983	Sorghum	Flurazole (Screen)	Thiazole carboxylic acid	Alachlor	Seed treatment	Monsanto now Bayer CropScience AG
1983	Rice	Fenclorim	Phenyl-pyrimidine	Pretilachlor	Preemergence; premixed with herbicide	Ciba-Geigy now Syngenta
1987	Corn	MG-191	Dichloromethyl-ketal (Dichloromethyl-1,3-dioxolane)	Thiocarbamate herbicides	Preemergence; premixed with herbicide	Central Research Institute for Chemistry, Hungarian Academy of Sciences
1988	Corn	Benoxacor	Dichloroacetamide	*S*-metolachlor or racemic metolachlor (Dual II Magnum, Bicep II Magnum, Camix)	Preemergence; premixed with herbicide	Ciba-Geigy now Syngenta

1989	Cereals	Cloquinocet-mexyl	8-quinolinoxy-carboxylic esters	Clodinafop-propargyl (Discover), pinoxaden (Axial), pyroxsulam (Powerflex)	Postemergence; premixed with herbicide	Ciba-Geigy now Syngenta
1989	Cereals e.g. wheat, barley	Fenchlorazole-ethyl	1,2,4-triazolcarboxylates	ACCase inhibitors e.g. Fenoxaprop-ethyl	Postemergence; premixed with herbicide	Hoechst AG now Bayer CropScience AG
1991	Corn and sorghum	Furilazole	Dichloroacetamide	Acetochlor (Degree, Harness), sulfonylurea e.g. Halosulfuron-methyl (Permit)	Preemergence; premixed with herbicide	Monsanto now Bayer CropScience AG
1999	Cereals e.g. wheat, barley, rye	Mefenpyr-diethyl	Dihydropyrazole-dicarboxylate	Mesosulfuron-methyl and iodosulfuron (Atlantis WG-Europe), pyrosulfatole (Huskie)	Postemergence; premixed with herbicide	AgrEvo now Bayer CropScience AG
2001	Corn and rice	Isoxadifen-ethyl	Dihydroisoxazole-carboxylate	Tembotrione (Laudis OD), foramsulfuron (Option), fenoxaprop-ethyl (Ricestar), nicosulfuron (Accent Q)	Postemergence; premixed with herbicide	Bayer CropScience AG
2008	Corn and sorghum	Cyprosulfamide	Arysulfonyl-benzamide	Pre- and postemergence herbicides e.g. isoxaflutole alone (Balance Flexx) or in combination with thiencarbazone (Corvus)	Pre- and postemergence	Bayer CropScience AG

1986 (Registered for grass and broadleaf weed control)	Rice and cotton	Clomazone	O, O-diethyl-O-phenyl phosphorothioate	Dietholate	Seed treatment	FMC
	Corn	AD-67	Dichloroacetamide	Acetochlor	Preemergence; premixed with herbicide	Monsanto now Bayer CropScience AG
	Rice	Daimuron or Dymron	Urea	Pretilachlor, pyributicarb	Postemergence/water surface application	
	Rice	Cumyluron	Urea	Sulfonyureas	Postemergence/water surface application	
	Rice	Dimepiperate	Piperidine–1–carbothioate	Sulfonylurea e.g. Bensulfuron, Azimsulfuron	Postemergence/water surface application; premixed with herbicide	

[1] References (Jablonkai 2013, Rosinger 2014)

[2] Names in parenthesis are trade names of the commercial products.