Characterization and Management of Acetolactate Synthase Inhibiting Herbicide Resistant Mouse-Ear Cress (*Arabidopsis thaliana*) in Winter Wheat

Ranjeet Singh Randhawa

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Michael L. Flessner; Chair

James H. Westwood

Charles W. Cahoon

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Abstract

The first case of field evolved acetolactate synthase (ALS) inhibiting herbicide resistance in the model plant, mouse-ear cress, was reported in winter wheat fields in Westmoreland County, Virginia. A putative resistant (R) mouse-ear population was assessed for ALS resistance relative to a putative susceptible (S) and a susceptible lab population Columbia (C). Results indicated that the R population needed 23 to >2400 fold rate of thifensulfuron relative to S or C population, and it has evolved cross-resistance to sulfonylureas (SU), triazolopyrimidine sulfonanilides (TP), and sulfonylaminocarbonyltriazolinones (SCT). Further studies sequenced the whole genome for four field populations, representing two locations and two resistance levels (high and low) per location, to characterize the genetic mechanism of ALS resistance. The results revealed that all populations contained mutations in the ALS gene at the Pro_{197} site, although the Pro was substituted by Phe in one location and Thr in the other. Also, both high- and low-level resistant plants at one location had additional mutations ($Trp_{574}Leu$ or $Asp_{376}Glu$) known to confer resistance to ALS inhibiting herbicides. Patterns of herbicide cross-resistance also varied among the populations. Additionally, research was conducted to assess preemergent (PRE) and postemergent (POST) alternative herbicide options for control of ALS resistant mouse-ear cress and its interference with winter wheat. Results indicate flumioxazin, pyroxasulfone, and metribuzin can be used for effective PRE control whereas 2,4-D, dicamba, and metribuzin can be effective post control options. No mouse-ear cress interference with winter wheat was observed at density of more than 300 plants m^{-2}.

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General Audience Abstract

The first case of field evolved acetolactate synthase (ALS) inhibiting herbicide resistance in mouse-ear cress, was reported in winter wheat fields in Westmoreland County, Virginia. A putative resistant (R) mouse-ear population was assessed for ALS resistance relative to a putative susceptible (S) and a susceptible lab population Columbia (C). The ALS resistance was confirmed in greenhouse and the R population exhibited cross-resistance to three ALS herbicide chemical families. Further studies sequenced the whole genome for four field populations collected from Essex and Westmoreland Counties, Virginia to characterize the genetic mechanism of ALS resistance. The results revealed that all populations contained target site mutations. All populations had a mutation at a commonly implicated point within ALS gene; however, substitutions varied by location. Populations from one location had multiple target site mutation contrary to populations from second location which had only one mutation. Patterns of ALS cross-resistance also varied among the populations. Additionally, research was conducted to assess preemergent (PRE) and postemergent (POST) alternative herbicide options for control of ALS resistant mouse-ear cress, and its interference with winter wheat. Results indicate flumioxazin, pyroxasulfone, and metribuzin can be used for effective PRE control whereas 2,4-D, dicamba, and metribuzin can be effective post control options. No wheat yield loss was observed from mouse-ear cress interference at a density of more than 300 plants m^{-2}.

"The work and thoughts of great men are common heritage of humanity and let our society receive inspiration and guidance from it."

- Bhagat Puran Singh

Dedicated to the Beautiful Land of Five Rivers**:**

"The State of Punjab"

It starts with a dream and driven by a desire ••••••

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Chapter 1. Literature Review

Pests have been a significant threat to crop production systems as long as agriculture has existed. Various efforts have been employed to control pests like insects, diseases, and weeds. Pest management techniques include preventative, physical, chemical, and biological and cultural approaches. Chemical weed control with herbicides started after the discovery of 2,4-D during the Second World War (Duke and Powles 2008). Following which herbicide research gained pace and several herbicides with different modes of action and for use in different crops were developed (Heap 2014). Herbicides made weed control much easier than previous techniques. Herbicides are also very economical and effective (Cooper and Dobson 2007). For these reasons, herbicide use increased dramatically and is now the most widely employed weed control method. Herbicides make up 50% of total chemical use in agricultural sector worldwide (Cooper and Dobson 2007). Repeated and exclusive herbicide use has led to the evolution of herbicide resistance in weeds. This chapter will discuss weed interference in small grains and resistance to ALS inhibiting herbicides.

Winter Annual Broadleaf Weed Interference

According to Weed Science Society of America (1967), 'Weed' was defined as plants, which grow in an undesirable place. Later in 1989 this term was changed to define weed as plants, which are unacceptable or cause some intrusion with the wellbeing of humans (Humburg et al. 1989). Particularly in no- and reduced-tillage systems, winter annual weeds can directly intrude with crops (Hayden et al. 2012). Some winter annual weeds can geminate in the fall and spring and interfere with production of both winter and summer crops (Cici and Van Acker

2009).

Weed density, critical weed free period, and competitive ability of weeds are the major facets of weed interference with crops (Aldrich 1987).

Weed Competitiveness. Weed competition with crops is a significant challenge for growers (Afifi and Swanton 2012). Competitiveness studies examine the process-based competition extended by different weed species (Rajcan and Swanton 2001). Weeds compete for resources like light, nutrients, and moisture (Zimdahl 2007). In order to better exploit the potential of ecological weed management approaches, competitiveness of different weed species needs to be understood (Rajcan and Swanton 2001).

Soil Moisture. The presence of weeds may impact plant available moisture, possibly leading to drought stress in the crop (Tollenaar et al. 1997). If water stress occurs during corn pollination for example, crop yield may be adversely affected (Robins and Domingo 1953), but during vegetative growth stages water stress may limit crop height, leaf growth rate, and vegetative biomass (Denmead and Shaw 1960). Water stress leads to lower transpiration rate thus reducing the leaf water potential, stomata closure, and reduced photosynthesis, ultimately reducing grain yield (Nissanka et al. 1997). Other research indicates a reduced water absorbance by crop roots during weedy conditions (Thomas and Allison 1975).

Nutrients. Nutrient stress can reduce yield potential (Ali et al. 1997). Reduced plant leaf chlorophyll content is an outcome of weedy condition that eventually lowers crop yield (Tollenaar et al. 1997).

In corn, adequate amount of nutrients such as nitrogen are required from early in the season until five wk post silking stage, but weed presence reduces nitrogen availability and uptake (Rajcan and Swanton 2001).

Light. Weeds also compete with crops for light needed for photosynthesis, reduced light availability leads to the reduction of dry matter accumulation in plants. Crop yield is more affected by lower dry matter accumulation than reduced photosynthetic rates (Rajcan and Swanton 2001). Also, light quality received by the lower leaves is higher in far-red radiations due to the absorption of red light, thus light received in the lower canopy has a lower far-red/red ratio which alters morphological properties including reducing leaf thickness and leading to fewer branches (Ballare and Casal 2000). Competition for light mainly occurs in short crops such as soybean and is mainly affected by broadleaf weeds such as jimsonweed (*Datura stramonium* L.) and velvetleaf (*Abutilon theopbrasti* Medic.) (Stoller and Woolley 1985).

Critical Weed Free Period

The critical weed free period (CWFP) is defined as the maximum time for which a crop must remain free from weeds after planting to avoid yield loss (Weaver et al. 1992). Crop yield is reduced with an increase in weed-infested period (Welsh et al. 1999). As the critical weed free period is increases, more time and effort are required for controlling weeds to avoid yield loss (Tamado et al. 2002). The study of CWFP is very important for designing suitable weed control techniques to prevent yield loss (Burnside et al. 1998). Studies indicate that lentils (*Lens culinaris* Medik.) sown in winter produce higher yield than sown in spring, but the critical weed free period for lentils sown in winter is longer (53 days) than when sown in the spring (35 days) (Karimmojeni et al. 2015). Analysis of weed removal experiments in dry beans (*Phaseolus vulgaris* L.) established the CWFP from 21 to 42 days after planting (Burnside et al. 1998). The CWFP for corn (*Zea mays*) was found to be from 10 to 30 days after crop emergence (Vernon and Parker 1983). The CWFP for organically grown winter wheat was found to be between 506

^{0}C days after sowing and 1023 ^{0}C days with a base temperature of 0^{0}C at sowing, and crop yield was found to be reduced 46% if no weed control measures were adopted (Welsh et al. 1999).

Herbicide Resistance

Herbicide resistance (HR) is the situation in which weeds are able to survive a previously effective herbicide treatment (Heap 2014). HR has emerged as a major weed management problem. Resistance evolution is attributed to frequent and exclusive use of same chemistry of herbicides (Chahal et al. 2015). In 1957, the first incidence of HR was reported in wild carrot (*Daucus corota* L.) that was described as being insensitive to synthetic auxins (Whitehead and Switzer 1963). First triazine HR was documented in the 1970s, when winter annual broadleaf weed common groundsel (*Senecio vulagris* L.) evolved resistance to atrazine and simazine (Ryan 1970). There are multiple mechanisms through which weeds can acquire resistance, including altered target site and non-target site based resistance mechanism, the later also known as enhanced metabolism based resistance mechanism. Non- target site based resistance includes herbicides metabolism by enzymes such as cytochrome p-450 monooxygenases (P450s) and glutathione *S*-transferases (GSTs), reduced herbicide uptake or translocation within plants (Powles and Yu 2010), gene amplification, and increased gene copy number (Deyle et al. 2015).

ALS Inhibiting Herbicides. Acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS) is an important enzyme in plants involved in the synthesis of the branched-chain amino acids (valine, leucine, and isoleucine) (Powles and Yu 2010). The ALS inhibiting herbicides ultimately lead to plant death due to starvation of essential amino acids (Powles and Yu 2010). Secondary factors such as accumulation of toxic level of 2-ketobutyrate, protein synthesis

termination, and failure of photosynthate transport, are also implicated in causing plant death (Shaner 1991).

The first ALS herbicides, sulfonylureas, were discovered in late 1970s (Whitcomb 1999) and commercialized in 1982 (Saari et al. 1990). Since this introduction, four other chemistries of ALS inhibiting herbicides have been commercialized: imidazolinones (Shaner et al. 1984), triazolopyrimidine sulfonanilides (Gerwick et al. 1990), pyrimidinylthiobenzoates (Takahashi et al. 1991), and sulfonylaminocarbonyltriazolinones (Kraehmer et al. 2014). Acetolactate inhibiting herbicides have high toxicity to susceptible plants (Fletcher et al. 1993). As of today, more than 50 ALS herbicides have been commercialized and are being used for broadleaf and grass weed control in variety of crops (Heap 2016). Chlorsulfuron was the first ALS herbicide commercialized for the broadleaf weed control in cereals (Saari et al. 1994).

Resistance to ALS Inhibiting Herbicides. Once commercialized the ALS inhibiting herbicides became popular for winter annual broadleaf weed control due to high efficacy, low use rate, and few alternatives (Saari et al. 1994). The first case of resistance to ALS herbicides was documented in 1986, four years after the commercialization, in Australia, in *Lolium rigidum* (Gaud.). Herbicide metabolism was the purported cause of resistance (Heap and knight, 1986). In the United States, ALS resistance was first reported in prickly lettuce (*Lactuca serriola* L.); resistance was attributed to a target site mutation (Mallory-Smith et al. 1990). Thereafter, ALS resistance in kochia (*Kochia scoparia* L.) quickly followed. Cross-resistance was observed, where kochia was resistant to sulfonylurea and imidazolinone herbicides in winter wheat (Primiani et al. 1990). At present, 159 weed species have evolved resistance to ALS inhibiting herbicides (Heap 2016). Resistance to ALS herbicides is one of the most commonly reported resistances for several reasons. Comparatively, the ALS inhibiting herbicides are used more

frequently, as they have a total of 55 active ingredients commercialized from five families of chemistry, (Heap 2016). Also, the ALS inhibiting herbicides are highly effective on sensitive biotypes, thus resulting in high selection pressure (Tranel and Wright 2002).

Mechanism of Resistance to ALS Inhibiting Herbicides

Structure and Function of ALS enzyme. The ALS enzyme has two subunits: catalytic and regulatory (Powels and Yu 2010). The catalytic subunit has a thiamine di-phosphate (ThDP), an essential cofactor for ALS enzyme activity (Duggleby 2008). The regulatory subunit does not have an ALS activity of its own, but it simulates the activity of catalytic subunit. The regulatory subunit is required to terminate the activity of catalytic site. It works through feedback inhibition by the branched chain amino acids (Duggleby 2008).

Evident from the molecular structure, the herbicide binding site and catalytic site are separate on the ALS enzyme (Duggleby 2008; Zhou et al. 2007). The ALS herbicides do not bind directly to the catalytic site, but instead block the entry channel, thus hindering the substrate's ability to reach catalytic site (Powles and Yu 2010). The herbicide-binding domain has as many as eighteen different amino acids assisting in the binding of ALS herbicides (McCourt et al. 2006).

Target Site Resistance to ALS Inhibiting Herbicides. Though different ALS chemistries orient differently while binding to the herbicide domain, they still may have overlapping orientations. Thus switching of a particular amino acid within the herbicide-binding domain may confer resistance to one or more chemistries (Powels and Yu 2010).

This target site mutation is the primary cause of resistance to ALS herbicides in weeds (Mallory-Smith et al. 1990; Yu, Q. et al. 2008; Powels and Yu 2010). The first documented

substitution to result in a mutant ALS enzyme was proline 197 (Pro_{197}) substituted with either histidine (His) or threonine (Thr) (Guttieri et al. 1992). Later, twenty-one other amino acid substitutions, leading to possible resistance, at seven different sites on herbicide binding domain have been studied (Tranel and Wright 2002). By far, Pro_{197} mutations are the primary observed mutation. Pro_{197} can be substituted by 11 different amino acids to confer resistance to ALS inhibiting herbicides, among which serine (Ser) substitution is most common mutation. Pro_{197}Ser substitution is thought to be a relatively rapid evolving mutation because of single nucleotide involved in the mutation whereas other Pro_{197} substitutions requires two nucleotides switching for successful resistance in ALS enzyme and thus, is less often observed. Another, commonly occurring mutation conferring ALS resistance is leucine (Leu) replacement with tryptophan (Trp) at amino acid position 574 (Trp_{574}Leu). This mutation has so far lead to resistance evolution in 16 weed species.

Metabolism Based Resistance to ALS Inhibiting Herbicides. Plants can also evolve non-target site resistance to herbicides (Yuan et al. 2007). Non-target site resistance can involve metabolism, cytochrome P-450 enzyme mediation, altered glutathione *S*-transferases (GSTs), or altered translocation (Powels and Yu 2010).

Herbicide resistance due to metabolism occurs when plants make biochemical modification to the herbicide turning them into less or non-toxic metabolites. This is followed by within cell compartmentalization of the herbicide metabolites (Yuan et al. 2007). The first documented case of metabolic resistance to ALS inhibiting herbicides was in *L. rigidum,* which developed resistance to sulfosulfuron (Heap and Knight 1986).

Cytochrome P-450 mediated resistance to ALS herbicides has been more commonly observed in grass weed species compared to broadleaf weeds and includes: *Alopecurus*

myosuroides (Huds)*, L. rigidum, Echinochloa phyllopogon* (Stapf) Koss., *and Digitaria sanguinalis* (L.) *Scop.* (Powels and Yu 2010). Among broadleaf weeds, *Amaranthus hybridus* (L.) was reported resistant to chlorimuron due to enhanced rate of P-450 mediated herbicide metabolism (Manley et al. 1999).

Cross-Resistance to ALS Inhibiting Herbicides. Cross-resistance is resistance of weed biotypes to two or more herbicides by a single mechanism (Beckie and Tardif 2012). Single mechanism resistance is usually conferred by a single gene, although the possibility of multiple genes conferring the same resistance is also plausible (Preston and Mallory-Smith 2001). Cross-resistance via target site mutation is most often observed (Preston 2014). A target site mutation may confer resistance to many or all of the herbicides with same mode of action (Heap 2014). Metabolism based cross-resistance has also been observed in weeds (Yu and Powels 2014). Metabolism based cross-resistance has been mostly documented in grass weeds, *L. rigidum* and *A. myosuroides* (Yu and Powles 2014). P-450 mediated enhanced herbicide metabolism conferred cross-resistance to ALS inhibiting herbicides in *L. rigidum* (Yu and Powles 2014).

Target site cross-resistance has been observed in ALS resistant weeds (Saari et al. 1994). Sulfonylurea resistant weed biotypes exhibit different levels of cross-resistance to structurally different ALS Inhibiting herbicides such as imidazolinones and triazolopyrimidines (Christopher et al. 1991; Hall and Devine 1990). The $Asp_{376}Glu$ substitution conferred sulfonylureas, imidazolinones, triazolopyrimidines, and pyrimidinylthiobenzoates resistance in smooth pigweed (*Amaranthus hybridus* L.) (Trucco et al. 2006; Whaley et al. 2006). $Ala_{122}Thr$ mutation has conferred ALS cross-resistance to SU and IMI in redroot pigweed (*Amaranthus retroflexus*) (McNaughton et al. 2005).

The $Pro_{197}Ala$ mutation has conferred sulfonylurea, imidazolinone, triazolopyrimidine,

and pyrimidinylthiobenzoate resistance in *Brassica tournefortii* (Gouan) (Boutsalis et al. 1999). Pro_{197}Ala substitution has also been observed to confer ALS cross-resistance to SU, TP, and PTB in horseweed (*Conyza canadensis* L.) (Zheng et al. 2011). Pro_{197}Ser and Asp_{376}Glu substitutions were also found to confer ALS cross-resistance in horseweed (Zheng et al. 2011). Four different target site mutations including Pro_{197}His, Pro_{197}Leu, Pro_{197}Ser, and Pro_{197}Thr substitution were found capable of conferring ALS cross-resistance in *Lactua serriola* (Guttieri et al. 1995; Lu et al. 2007; Preston et al. 2006). Target site cross-resistance to ALS inhibitors has also been documented in other broadleaf weeds such as *Raphanus raphanistrum* (L.)*, Amaranthus tuberculatus* (Moq), and *Ambrosa trifida* (Patzoldt and Tranel 2002; Tan and Medd 2002). In 2001, ALS resistant chickweed (*Stellaria media* L.) was found to be cross-resistant to other sulfonylurea herbicides (thifensulfuron and tribenuron) (Seefeldt et al. 2001).

Multiple-Resistance in Weeds. Multiple-resistance is resistance to several herbicide chemistries and contrary to cross-resistance, it is conferred by multiple mechanisms (Heap and Lebarron 2001). Multiple-resistance usually evolves due to sequential selection by herbicides with different modes of action (Heap 2014). Interspecific gene flow has also been implicated as a possible cause of multiple-resistance in certain plant biotypes (Knispel et al. 2008). In Canola (*Brassica napus* L.), it was found that gene flow among HR varieties resulted in multiple herbicide resistance (Knispel et al. 2008). Multiple herbicide resistance results in reduction of chemical control options for weeds (Patzoldt et al. 2005).

Numerous weed biotypes have evolved multiple resistance. In grass weeds, a biotype of *L. rigidum* evolved resistance to three different modes of actions, 5-enolpyruvylshikimate-3 phosphate synthase (EPSPS), acetyl-coenzyme A carboxylase (ACCase), and ALS inhibiting herbicides, by three distinct non-target site based resistance mechanisms. It evolved resistance to

EPSPS inhibiting herbicide by reduced translocation whereas two different P450 enzymes were involved in in endowing resistance to ACCase and ALS inhibiting herbicides, respectively (Yu et al. 2009). Polos and others (1988) reported that a biotype of *Conyza canadensis* (L.) has evolved resistance to both photosystem I and photosystem II inhibiting herbicides. Similarly, *Amaranthus retroflexus* L. sequentially developed resistance to two separate photosystems II inhibitors (diuron and triazine), each targeting a separate site (Lehoczki et al 1991). Also, *Phalaris paradoxa* (L.) developed resistance to photosystem II and ACCase inhibiting herbicides (Yaacoby et al. 1986). *A. myosuroides* has accumulated resistance to a wide range of herbicides including photosystem II inhibitors, ACCase herbicides, and others (Hall et al. 1994). In Illinois, waterhemp (*Amaranthus tuberculatus*) was found to have varying levels of multiple-resistance. It was found that waterhemp had 17000-fold, 38-fold and 23-fold resistance to ALS, photosystem II, and PPO inhibitors, respectively (Patzoldt et al. 2005). Palmer amaranth (*Amaranthus palmeri* S. Wats) was found to have evolved resistance against EPSPS and ALS inhibiting herbicides (Sosnoskie et al. 2011).

Effect of Herbicide Resistance on Plant Fitness. Genetic mutations conferring herbicide resistance may affect plant functionality and reduce plant fitness. Not all resistant mutations in weeds have been properly explored for fitness cost (Powles and Yu 2010). Of the 22 known mutations for ALS inhibiting herbicide resistance, the fitness cost of only a few have been studied. Several studies have shown that mutation in ALS enzyme can lead to enhanced, reduced or no effect on ALS activity. A study conducted on two ALS resistant biotypes of *Sisymbrium orientale* (Torn.) found that the ALS enzyme had increased activity (Boutsalis et al. 1999). Another study conducted on eight ALS resistant populations of *Raphanus raphanistrum* (L.) with four different mutations (His, Ser, Ala and Thr) at Pro_{197} were found to have three to five

fold more ALS enzyme activity (Yu et al. 2003).

Resistant biotypes of *Hordeum leporinum* (Link) were found to have two to three-fold higher ALS enzyme activities as compared to sensitive biotypes (Yu et al. 2007). Certain Pro_{197} mutations have been found to increase the ALS enzyme sensitivity to feedback inhibition (Yu et al. 2010). This enhanced ALS activity adds to total resistance to ALS inhibiting herbicides (Yu et al. 2007).

Conversely, ALS resistance mutations can result in reduced ALS enzyme activity. Ashigh and Tardif (2007) found that relative to susceptible plants, imazethapyr resistant *Solanum ptychanthum* (Dunal) had 56% less ALS activity as well as reduced feedback inhibition activity (Ashigh and Tardif 2007). In *Lactua sativa* Pro_{197}His mutation in plants can result in reduced ALS enzyme sensitivity to feedback inhibition and as a consequences 1 to 1.5 times higher branched chain amino acids can be present in per gram of resistant seeds relative to susceptible seeds (Eberlein et al. 1999).

It has been found that common ALS resistance mutations such as Pro_{197}Ser have no effect on ALS enzyme activity (Powles and Yu 2010), which helps us understand the abundance of plants resistant to ALS inhibiting herbicides, particularly due to Pro_{197}Ser mutation.

Impact of ALS Resistance. The ALS herbicides have provided success in broadleaf weed control for more than three decades. Herbicide resistant evolution in weeds has also been on the rise since the advent of these herbicides. Previously, producers were able to tackle HR weeds by switching herbicide chemistry. But over the last two decades no new herbicide chemistries have been released (Duke 2011). Losing herbicide chemistry to resistance means fewer weed control options for farmers. Resistance to ALS inhibiting herbicides and other herbicides underscores the need for integrated weed management. Discovery, development, and registration of new

herbicide compounds are also demanding. Long term planning and effective execution of herbicide resistant strategies can help. Industry and university scientists have tried to combat this problem, but not much success has been achieved (Tranel and Wright 2002).

Arabidopsis thaliana: Mouse-Ear Cress

Mouse-ear cress is a member of Brassicaceae family (Meinke et al. 1998). It is considered as model plant for studying and understanding the molecular biology and genetics of many plant traits. It has a comparatively small genome size of 135 mega base pairs relative to most multicellular eukaryotes, and was the first plant to have its genome sequenced (Meyerowitz 1989).

Mouse-ear cress is a winter annual that completes its entire life cycle in only six wk. Growth is initiated by seed germination followed by formation of a rosette, bolting of main stem, and lastly seed setting (Meinke et al. 1998). It is a small plant, usually 20 to 25 cm tall and is predisposed to drought stress. Seed survive the dry summer period by remaining dormant at temperatures is above 10^0C. Temperature is the key germination que; germination occurs in early winter, when soil are typically moist allowing the plant to complete its life cycle between winter and early spring (Baskin and Baskin 1972). Mouse-ear cress is a long day plant, rapidly flowering under >12 h photoperiods and optimum light intensity of 120 to 150 μmol m^{-2} sec^{-1} (ABRC 2013). It is self-pollinated and flowers are 2 mm in length (Meinke et al. 1998). Its fruits are siliques (Hays 2002; Meinke et al. 1998)

Distribution. Mouse-ear cress is believed to have originated from western Eurasia, and more than 750 ecotypes of the weed have been collected all over the world. These ecotypes differ from each other in terms of phenotypic trait (leaf shape, hairiness) and physiological traits (flowering

time, disease resistance) (TAIR 2015). Mouse-ear cress is distributed in parts of Asia (Pakistan, India {Kashmir, Punjab} (Robins and Domingo 1953), China, Japan and Afghanistan), Europe {France, Spain and Britain}, and the mountains of east Africa (Ratcliffe 1965). *Arabidopsis lyrata*, a close wild relative of mouse-ear cress, is found in Canada and the Midwestern and East Coast regions of the United States (Mitchell-Olds 2001). In North America, mouse-ear cress grows in Midwest (MI & IN), Southeast (NC & GA), Northeast (NY & VA) and Pacific Northwest (BC & WA) (Jørgensen and Mauricio 2004).

Economic Importance. Mouse-ear cress is considered a useful plant. In the past two decades it has been adopted as a model plant for research purposes due to its very small mature plant size, tiny seed size and a very short life cycle. Additionally, it grows well in fluorescent light and different soil media making it convenient and economical to maintain plant populations under laboratory conditions. Lastly, dioecious flowers and homozygous lines make it a good choice for classical genetics studies (Meyerowitz 1989). It has small genome; therefore it is easy to induce mutations and screen for desired traits (Meyerowitz 1989). It is amenable to gene transfer though agrobacterium. Thus mouse-ear cress has several properties that make it ideal for plant genetics research and directly or indirectly economically important.

Weedy Traits. Mouse-ear cress is a diploid species with 2n=10 (Fras and Maluszynska 2003). It has very minute sized seeds, around 0.5 mm in length at maturity, which can easily get dispersed over a long distance. Seed are dispersed by what is known as “pod-shatter”, in which the silique splits apart due to mechanical pressure and seeds are subsequently spread by wind or rain (Dinneny and Yanofsky 2005). Mouse-ear cress possesses secondary dormancy and can remain viable for more than a decade, if stored dry at 4^0C to 20^0C. A room temperature seed can lose viability within two years (ABRC 2013). High propagule pressure (one plant produces

approximately 5000 seeds) and adaptation for seed dispersal to wide areas adds to its weediness (Hays 2002; Meinke et al. 1998; Stewart Jr 2009). Additionally, mouse-ear cress has the ability to grow in wide range of geographic regions, as stated above (Jørgensen and Mauricio 2004; Ratcliffe 1965).

Control Methods. There are a number of techniques used for managing weeds and preventing yield loss such as crop rotation, varietal selection, crop density, and various mechanical methods used during crop growth (Welsh et al. 1999). Different control measures can be adopted to reduce the crop weed competition caused by mouse-ear cress. Different classes of ALS herbicides such as thifensulfuron (DuPont 2010), imazamox (BASF 2015), and others are known to control the weed. However, there are reports of ALS resistance and cross-resistance in mouse-ear cress (Roux et al. 2005). The auxin herbicide, 2,4-D, has also been evaluated for control of mouse-ear cress, and it was observed that this herbicide could reduce plant growth, cause epinasty symptoms or even cause cell death (Rodríguez-Serrano et al. 2014).

Biological control may also be an option. Previous research reported that mouse-ear cress could be effectively controlled using arbuscular mycelium of *G. intraradices*; this fungi infects plant roots and has a detrimental effect on plant growth (Veiga 2012).

Objectives

The objectives of this research include verification and quantification of ALS resistant mouse-ear cress in the greenhouse. A dose response methodology will be used to quantify resistance to thifensulfuron (Harmony; DuPont, Wilmington, DE). Mechanism of resistance will also be evaluated using whole genome sequencing approach. A green-house study will be conducted to assess cross-resistance to other ALS chemistries. Alternative herbicides for control

will be assessed in both the field and greenhouse as well. Another study will evaluate density at which mouse-ear cress can cause significant yield loss in winter wheat.

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Chapter 2. Mouse-Ear Cress (*Arabidopsis thaliana*) Resistance Quantification and Cross-Resistance to ALS Inhibiting Herbicides

Abstract

During 2015, thifensulfuron (an acetolactate synthase (ALS) inhibiting herbicide) failed to control mouse-ear cress in Virginia winter wheat fields. A field putative resistant population (R) was assessed for ALS resistance relative to putative susceptible (S) and a susceptible lab population Columbia (C). Greenhouse studies quantified ALS resistance with dose response studies and assessed cross-resistance to other ALS chemistries in mouse-ear cress. Visible control and above ground biomass data were collected in both studies. Cross-resistance studies evaluated four ALS chemistries: sulfonylureas (SU), imidazolinones (IMI), triazolopyrimidine sulfonanilides (TP), and sulfonylaminocarbonyltriazolinones (SCT). The IMIs (imazethapyr and imazamox) resulted in 76 to 95% control 6 wk after treatment (WAT). No herbicides other than prosulfuron and IMIs resulted in more than 30% control 6 WAT. Prosulfuron resulted in 42 and 100% control 6 WAT in experimental runs 1 and 2, respectively. The IMIs resulted in less than 30% biomass of the non-treated check 6 WAT. These results indicate mouse-ear cress has evolved cross-resistance to SU, TP, and SCT chemistries while being susceptible to prosulfuron and IMIs.

Nomenclature: flucarbazone; imazamox; imazethapyr; mesosulfuron; prosulfuron; thifensulfuron; tribenuron; mouse-ear cress, *Arabidopsis thaliana* L.

Keywords: dose response, differential resistance, target site mutation.

Introduction

Acetolactate synthase (ALS) is required for biosynthesis of the branched-chain amino

acids valine, leucine, and isoleucine (Powles and Yu 2010). The ALS inhibiting herbicides were first commercialized in 1982, and the first case of resistance was documented four years later in *Lolium rigidum* (Gaud.) in Australia (Heap and Knight 1986). The ALS inhibiting herbicides include five distinct chemical families: sulfonylureas (SUs), imidazolinones (IMIs), triazolopyrimidine sulfonanilides (TPs), pyrimidinylthiobenzoates (PTBs) and sulfonylaminocarbonyltriazolinones (SCTs) (Kraehmer et al. 2014; Tranel and Wright 2002). At present, 159 weed species have evolved resistance to ALS inhibiting herbicides, which is the most for any herbicide mode of action (Heap 2017).

The primary mechanism of resistance to ALS inhibiting herbicides is an altered target site of the enzyme (Mallory-smith et al. 1990; Powles and Yu 2010; Tranel and Wright 2002; Yu et al. 2008). Eight different amino acid substitutions in ALS are known to confer herbicide resistance (Heap 2017). Frequently, this resistance mechanism results in very high resistance factors (>50) (Burgos et al. 2001; Patzoldt et al. 2005; Trainer et al. 2005).

Target site mutations are capable of conferring cross-resistance to multiple chemical families with the same mode of action (Tranel and Wright 2002). Similarly, target site mutations in the ALS gene have been documented to confer cross-resistance to one or all of the ALS chemistries (Saari et al. 1994). However, spectrum and magnitude of cross-resistance varies with mutation and alternate herbicide. The SU resistant weeds can have different levels of cross-resistance to structurally different ALS inhibiting herbicides such as the IMIs and TPs (Christopher et al. 1991; Hall and Devine 1990; Whaley et al. 2007). Based upon the target site mutation evolved, SU resistant weeds could have cross-resistance to zero, one, or multiple IMI herbicides (Guttieri et al. 1995; Mallorysmith et al. 1990). In certain cases, single target site alteration conferred cross-resistance across all families of ALS inhibiting herbicides (Trucco et

al. 2006; Whaley et al. 2006).

Brassicaceae weeds, including Asian mustard (*Brassica tournefortii* Gouan.) and wild mustard (*Sinapis arvensis* L.), have developed cross-resistance to the SU, IMI, TP, and SCT herbicides (Boutsalis et al. 1999; Christoffers et al. 2006). A SU and TP cross-resistant wild radish (*Raphanus raphanistrum* L.) population may or may not exhibit IMI's resistance based upon mutation evolved (Han et al. 2012; Yu et al. 2012; Yu et al. 2003).

Mouse-ear cress (*Arabidopsis thaliana* L.) is a member of the Brassicaceae that commonly occurs in winter wheat but has not been extensively studied as a weed. In 2015, growers reported thifensulfuron (Harmony SG; DuPont, Wilmington, DE), an ALS inhibiting herbicide, failed to control mouse-ear cress in Essex and Westmoreland County, Virginia. Resistance to thifensulfuron was suspected to be the reason for control failure. The objectives of this study were to evaluate mouse-ear cress populations for resistance to thifensulfuron as well as evaluate for cross-resistance to other ALS inhibiting herbicide chemistries.

Materials and Methods

Plant Material. Mouse-ear cress seed were collected from wheat fields (38°4'52" N, 76° 46'38" W) in Westmoreland County, Virginia suspected to have thifensulfuron-resistant mouse-ear cress. Initial greenhouse screening at the labeled thifensulfuron rate (26.3 g ha^{-1}) confirmed resistance (Anonymous 2012). Seeds from putative susceptible (S) mouse-ear cress populations were collected in Montgomery County, Virginia (37°8'12'' N, 80°32'33'' W). Additionally, Columbia (C), a putative susceptible population commonly used in laboratory experiments was included for evaluation. For all populations, a nursery was raised within the greenhouse using trays (42 by 32 by 7cm) containing a commercial potting mixture (Metro mix 360; Sun Gro

Horticulture, Seba beach, AB, Canada). A single seedling (2 to 3 leaf stage) of R, S, and C mouse-ear cress was transplanted into 590 cm^{-3} pots filled with Hayter loam soil (Fine-loamy, mixed, active, mesic Ultic Hapludalfs) with 5.1% organic matter and pH of 6.4 and allowed to acclimate for 2 wk prior to testing. Pots were maintained in greenhouse conditions (15 to 27 °C and average relative humidity 80%) with day length of 12 hours under supplemental light and were watered to maintain field capacity.

ALS Resistance Quantification. Greenhouse studies were conducted for dose response quantification to thifensulfuron in mouse-ear cress populations. The R, S, and C populations of mouse-ear cress were treated with thifensulfuron at 0.263, 1.31, 2.63, 13.1, 26.3, 131, 263 and 1310 g ai ha^{-1}, which corresponds to 0.01, 0.05, 0.1, 0.5, 1, 5, 10 and 50 times the maximum labeled rate (26.3 g ai ha^{-1}) (DuPont 2012). All thifensulfuron treatments included 0.25% v v^{-1} non-ionic surfactant (NIS) (Activator 90; Loveland Industries, Inc. Greeley, CO). Additionally, treated {glyphosate (Roundup Powermax; Monsanto, St. Louis, MO) at 1260 g ae ha^{-1}} and non-treated controls were included for each population. Treatments were applied at the rosette (8 cm diam) stage using a spray chamber equipped with a single TeeJet 8001 EVS nozzle (Spraying Systems Co., Wheaton, IL) calibrated to deliver 140 L ha^{-1} of total spray volume at 172 kPa. Experiments utilized a randomized complete block design with 10 replicates per treatment and were replicated in time for a total of two experimental runs. For herbicide efficacy evaluation, visible control data were estimated relative to the non-treated check on a 0 (no control) to 100 (complete plant necrosis) scale (Frans et al. 1986). Visible control was assessed 6 wk after treatment (WAT). Above ground biomass was collected 6 WAT and oven dried at 65 C for 72 h.

Cross-Resistance. The R mouse-ear cress was assessed under previously described greenhouse conditions for cross-resistance to various ALS inhibiting chemistries. Nine different herbicides

from four ALS inhibiting herbicide families were evaluated for cross-resistance (Table 1). Treatments were setup in a randomized complete block design with 6 replicates per treatment. Treatment application and data collection were conducted as previously described in the ALS resistance quantification study. Visible control was assessed at 2, 4, and 6 WAT and above ground biomass data were collected at 6 WAT. Experiment were replicated in time.

Statistical Analysis. Treated check data were not included in the analysis. For the dose response study, above ground biomass was obtained by dividing the biomass for each treatment by the biomass of the non-treated check within each block. ANOVA was performed using JMP 1.1.0 (SAS Institute Inc., Cary, NC) and effects were considered significant when $p < 0.05$. Following ANOVA, a dose-response curve model was used (Seefeldt et al. 1995) with the following equation:

$$Y = C + \{(D - C) / (1 + exp\ (b(log(x) - I_{50}))\} \qquad [1]$$

where y is response (e.g., above ground biomass) at herbicide rate x, D is upper limit for response, C is lower limit for response, I_{50} is the herbicide rate resulting in 50% response reduction (e.g. 50% biomass reduction), and b is the slope of curve at I_{50}. For the analysis, treatments were log transformed to satisfy model assumptions and back transformed for presentation purposes, and the non-treated check was artificially assigned an herbicide rate of 0.0001 kg ai ha^{-1} thifensulfuron to satisfy the model requirements (Seefeldt et al. 1995). I_{50} values were established for each population and resistance factors were calculated for R population relative to S and C populations separately.

$$Y = \text{R}\ (I_{50}) / \text{S}\ (I_{50}) \qquad [2]$$

$$Y = \text{R}\ (I_{50}) / \text{C}\ (I_{50}) \qquad [3]$$

where y is the resistance factor, R/S/C I_{50} is the herbicide rate resulting in 50% response

reduction (e.g. 50% biomass reduction) for R, S, and C population, respectively.

Non-linear regression analysis and resistance factor evaluation was performed using RStudio 3.3.0 (RStudio; Boston, MA).

Data analyses for cross-resistance study were performed using JMP 1.1.0 (SAS Institute Inc., Cary, NC). ANOVA was performed and effects were considered significant when $p < 0.05$. Subsequently, data were subjected to means separation using Fisher's protected LSD ($p < 0.05$).

Results and Discussion

ALS Resistance Quantification. A significant population by experimental run interaction was detected for visible control and above ground biomass. Therefore, data were not pooled, and separate analyses were conducted for both experimental runs. Log-logistic curves were used to explain the visible control and relative biomass patterns for field selected putative resistant and putative susceptible populations of mouse-ear cress. For both experimental runs, S and C populations resulted in drastic visible control and biomass reduction from thifensulfuron at > 131 g ha^{-1} (Figures 1 and 2). However, the R population was not significantly affected by the highest thifensulfuron rate (1310 g ai ha^{-1}); less than 40% visible control was observed for the R population at 50 times the highest labeled rate (26.3 g ai ha^{-1}) (Figure 1b). Conversely, the S and C populations were controlled greater than 90% at same rate (Figures 1b and 2b). Such poor control at 50 times the labeled thifensulfuron rate signifies a high degree of resistance in the R biotype. Other research reports poor control of ALS resistant biotypes of wild mustard (Christoffers et al. 2006; Yu et al. 2012). Similar to visible control, the R population had no or little biomass reduction from thifensulfuron at highest rate whereas same rate significantly reduced biomass of the S and C populations (Figures 1a and 2a). Fifty percent visible control and

50% reduction in above ground biomass for the R population did not occur within the rates tested (Table 4); therefore, the highest rate of thifensulfuron (1310 g ai ha^{-1}) was used as the I_{50} value to calculate resistance factor. I_{50} values from above ground biomass for the S and C populations varied with experimental run, but ranged from 0.53 to 57.8 g ai ha^{-1} for the S population and 0.53 to 15.8 g ai ha^{-1} for the C population. Similarly, I_{50} values from visible control for the S populations was observed to range from 21 to 125 g ai ha^{-1}; however, thifensulfuron at 16 g ai ha^{-1} resulted in 50% visible control for C population in both runs. Resistance factors of 23 to 2471 and 83 to 2471 were calculated for R population relative to populations S (Equation 2) and C (Equation 3), respectively. Consistent with this finding, past research has identified wild radish and wild mustard populations with resistance factors of more than 150 and 450, respectively, to ALS herbicides (Warwick et al. 2005; Yu et al. 2012; Yu et al. 2003).

Cross-Resistance. A significant treatment by experimental run interaction was observed, so data are presented separately by experimental run. Mouse-ear cress response varied by ALS inhibiting herbicide chemistries. In both experimental runs, IMIs (imazethapyr and imazamox) resulted in 76% to 95% control 6 WAT (Tables 2 and 3). None of the SU, TP, and PTB herbicides except prosulfuron (a SU herbicide) resulted in more than 30% control 6 WAT, indicating cross-resistance to these chemistries. Prosulfuron resulted in 42 and 100% control 6 WAT in experimental runs 1 and 2, respectively. Similar results have been observed in previous research where common windgrass (*Apera spica-venti* L.) expressed resistance to chlorsulfuron and mesosulfuron but not to sulfosulfuron (Krysiak et al. 2011). It has been documented that field penny cress' and wild mustards' sensitivity to ethametsulfuron and metsulfuron was unaffected while being resistant to other sulfonylurea herbicides (Beckie et al. 2007; Topuz 2007). Such differential resistance is conceivable in target site mutations because herbicides

interaction varies based on amino acid substitutions (Sada et al. 2013). The IMIs resulted in less than 30% biomass of the non-treated check 6 WAT for both experimental runs. Prosulfuron resulted in biomass similar to the IMIs for experimental run 2. However, for experimental run 1, prosulfuron caused similar biomass reduction as imazethapyr but was no different than non-treated plants (Tables 2 and 3). These results indicate broad-spectrum cross-resistance to ALS herbicides. Similar results have been documented for other weeds with ALS resistance. Zheng et al. (2011) reported horseweed (*Conyza candensis* L.) biotypes with cross-resistance to SU, TP, and PTB. Wild radish was reported to exhibit SU and TP resistance while plant sensitivity to IMIs was not effected (Yu et al. 2003;Yu et al. 2012). Current results signify that mouse-ear cress was controlled by IMI herbicides for both experimental runs while none of the other ALS inhibiting chemistries resulted in commercially acceptable control. Thus, field biotypes of mouse-ear cress have evolved cross-resistance to SU, TP, and SCT chemistries.

From past research, it is evident that broad spectrum ALS cross-resistance usually evolves due to the existence of a point mutation in the target site of the enzyme (Yu et al. 2012; Yu et al. 2003). Therefore, these results indicate the mechanism or resistance is likely a point mutation in ALS gene of mouse-ear cress. Future research is necessary to test this hypothesis.

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Table 1. Herbicides evaluated for cross-resistance to thifensulfuron resistant mouse-ear cress (*Arabidopsis thaliana*) in greenhouse in Blacksburg, VA in 2015-16.

Treatment				
ALS Chemistry	Trade name	Common name	Rate	Manufacturer
			g ai ha^{-1}	
Sulfonylurea[a]	Harmony SG	thifensulfuron	17.5	E. I. du Pont de Nemours and Company, Wilmington, DE
	Harmony Extra	thifensulfuron + tribenuron	17.5 + 8.8	E. I. du Pont de Nemours and Company
	Express	tribenuron	8.8	E. I. du Pont de Nemours and Company
	Peak	prosulfuron[c]	16	Syngenta Crop Protection LLC, Greensboro, NC
	Osprey	mesosulfuron[c]	15	Bayer CropScience LP, Research Triangle Park, NC
Triazolinone[a]	Everest	flucarbazone[d]	23	Arysta Lifescience North America, LLC, Cary, NC
Triazolopyrimidines[a]	Powerflex HL	pyroxsulam[c]	18.4	Dow AgroSciences LLC, Indianapolis, IN

Imidazolinone[b]	Pursuit	imazethapyr	52.5	BASF Corporation, Research Triangle Park, NC
	Raptor	imazamox	35	BASF Corporation

[a] Treatment included non-ionic surfactant at 0.25% v v^{-1} as per product label recommendation.

[b] Treatment included methylated seed oil at 1% v v^{-1} as per product label recommendation.

[c] Treatment included ammonium sulfate at 3360 g ha^{-1} as per product label recommendation.

[d] Treatment included ammonium sulfate at 1660 g ha^{-1} as per product label recommendation.

Table 2. Mouse-ear cress (*Arabidopsis thaliana*) visible control and above ground biomass (experimental run 1) from various ALS inhibiting herbicides in Blacksburg, VA in 2015-16.

ALS Chemical Family	Herbicide	Control			Relative[1] Biomass
		2 WAT[2]	4 WAT	6 WAT	6 WAT
		%			
Imidazolinone	imazethapyr	45 B[3]	47 B	90 A	28 EF
	imazamox	42 B	37 B	76 B	23 F
Sulfonylurea	prosulfuron	23 C	17 C	42 C	63 DE
	thifensulfuron	17 CD	13 C	17 D	83 CD
	tribenuron	12 CDE	2 D	0 E	163 A
	mesosulfuron	10 DE	3 D	3 E	135 AB
	thifensulfuron + tribenuron	8 DE	3 D	0 E	104 BC
Triazolinone	flucarbazone	5 E	3 D	3 E	98 BCD
Triazolopyri-midines	pyroxsulam	3 E	0 D	3 E	128 AB
	non-treated	-[4]	-	-	100 BCD

[1] Relative above ground biomass was calculated relative to the non-treated check within each block.

[2] Abbreviation: WAT, wk after treatment.

[3] Treatments without a same letter are significantly different by Fisher's protected LSD ($p < 0.05$) within column.

[4] Non-treated check data for visible control was not included in data analysis.

Table 3. Mouse-ear cress (*Arabidopsis thaliana*) visible control and above ground biomass (experimental run 2) from various ALS herbicides in Blacksburg, VA in 2015-16.

ALS Chemical Family	Herbicide	Control			Relative Biomass[1]
		2 WAT[2]	4 WAT	6 WAT	6 WAT
		%			
Imidazolinone	imazethapyr	27 AB[3]	85 A	95 A	9 D
	imazamox	43 A	78 A	90 A	12 D
Sulfonylurea	prosulfuron	27 AB	85 A	100 A	12 D
	thifensulfuron	0 C	10 C	0 C	126 B
	tribenuron	7 C	0 C	0 C	172 A
	mesosulfuron	5 C	3 C	0 C	201 A
	thifensulfuron + tribenuron	5 C	8 C	17 BC	131 B
Triazolinone	flucarbazone	13 BC	38 B	23 BC	81 C
Triazolopyri-midines	pyroxsulam	29 AB	40 B	28 B	73 C
	non-treated	-[4]	-	-	100 BC

[1] Relative above ground biomass was calculated relative to the non-treated check within each block.

[2] Abbreviation: WAT, wk after treatment.

[3] Treatments without a same letter are significantly different by Fisher's protected LSD ($p < 0.05$) within column.

[4] Non-treated check data for visible control was not included in data analysis.

Table 4. Mouse-ear cress visible control best-fit regression parameters (± standard errors) for the log-logistic regression model used for thifensulfuron resistance quantification in Blacksburg, VA in 2015-16.

	Data type	Population	C	D	b	I_{50}	R*f*
			%			g ai ha^{-1}	
Experimental		Resistant	15.75 (2.3)	4262 (908)	-0.5 ($-^{3}$)	-	-
Run 1	Visible Control	Susceptible	0.005	214	-0.33	125	10.5[4]
		Columbia	3.9 (13)	106 (15.3)	-0.78 (0.6)	16 (1.53)	82
Experimental	Visible	Resistant	*	*	*	*	-
Run 2	Control						
		Susceptible	1.18 (3.2)	97.5 (3.7)	-3.8 (0.9)	21 (1.08)	62
		Columbia	4.5 (6.14)	73.7 (6.8)	-1.8 (1.55)	16.5 (1.36)	79
Experimental	Biomass	Resistant	-0.001 (3.2)	1.1 (0.06)	1.89 (2.42)	1430 (18.2)	-
Run 1							
		Susceptible	-0.001 (0.2)	1.1 (0.24)	0.38 (0.24)	0.53 (2.69)	2471
		Columbia	-0.001 (0.07)	1.07 (0.14)	0.48 (0.17)	0.53 (2.13)	2471
Experimental	Biomass	Resistant	-0.001 (-)	1.5 (0.3)	-0.25 (0.05)	-	-
Run 2							
		Susceptible	-0.001 (-)	1.24 (.225)	0.79 (-)	57.8 (-)	23
		Columbia	0.23 (0.97)	2.5 (0.45)	1.6 (4.6)	15.8(4.34)	83

[1] Regression parameters are determined by the log-logistic regression model explained by equation $Y = C + \{(D - C) / (1 + \exp (b(\log(x) - I_{50}))$.

[2] Abbreviations: C, lower limit; D, upper limit; b slope at I_{50}; I_{50}, thifensulfuron rate resulting a 50% visible control relative to non-treated check; Rf, resistance factor.

[3] Confidence interval could not be calculated as the model calculated the respective values to be insignificant.

[4] Resistance factors are calculated by equations $Y = R (I_{50}) / S (I_{50})$ and $Y = R (I_{50}) / C (I_{50})$.

[*] No visible control was observed at any treatment level so regression was not possible.

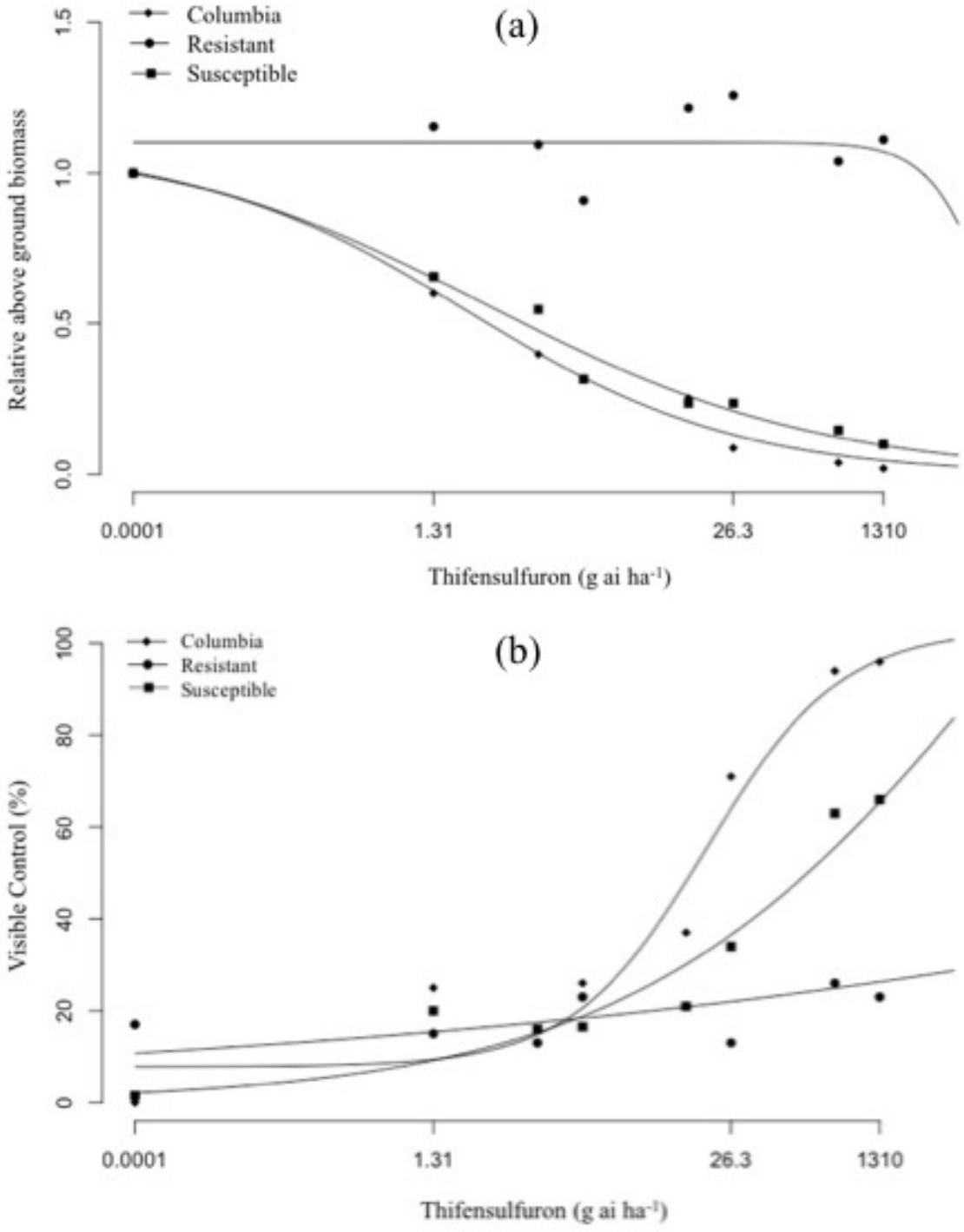

Figure 1. Experimental run 1 log-logistic (4 parameter) curves for relative dry biomass (a) and visible control (b) for dose response of three mouse-ear cress populations in, Blacksburg, VA in

2015-16. Non-treated check was artificially assigned an herbicide rate of 0.0001 g ai ha^{-1} thifensulfuron to satisfy the model requirements. Points on the plot represent treatment means for each respective population.

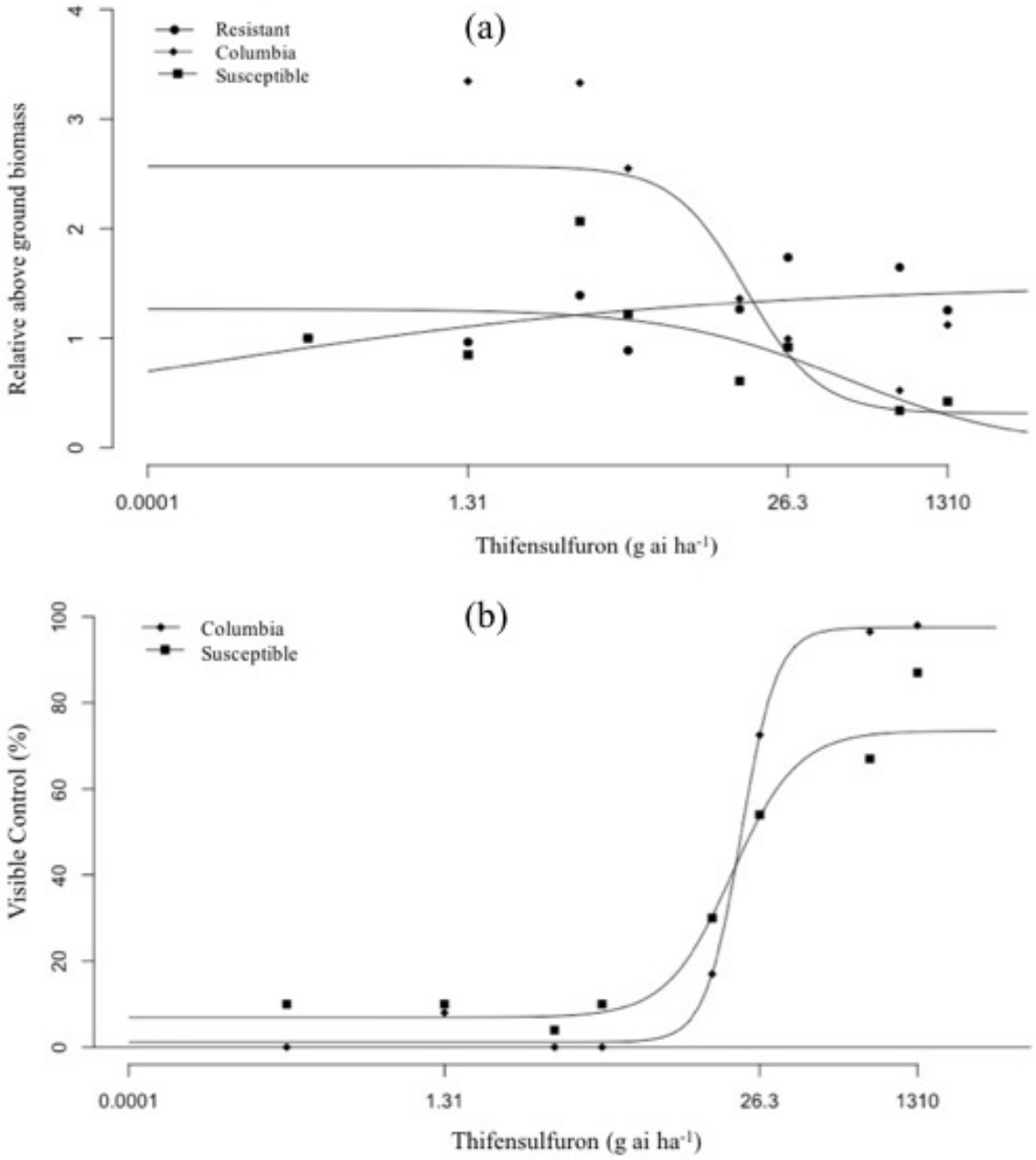

Figure 2. Experimental run 2 log-logistic (4 parameter) curves for relative above ground biomass (a) and visible control (b) for dose response of three mouse-ear cress populations in Blacksburg, VA in 2015-16. No visible control curve for resistant population is shown as no control was observed with any treatment. Non-treated check was artificially assigned an herbicide rate of

0.0001 g ai ha^{-1} thifensulfuron to satisfy the model requirements. Points on the plot represent treatment means for each respective population.

Chapter 3. A Pooled Whole-Genome Sequencing Approach to Characterizing ALS Inhibitor Resistance in Mouse-Ear Cress (*Arabidopsis thaliana*)

Abstract

Following the confirmation of field evolved ALS resistance in mouse-ear cress, studies were conducted to characterize the mechanism and associated cross-resistance patterns of field evolved ALS inhibitor resistance. Two field populations, O and B, were collected from Essex and Westmoreland Counties, Virginia, respectively. Each population consisted of putative resistant lines collected from within a field with thifensulfuron control failures and corresponding putative susceptible lines, collected from outside each field. Mouse-ear cress DNA were pooled from multiple lines of each population based on their location and high (HR) or low (LR) resistance to thifensulfuron (no true susceptible plants were identified). Thus, a total of four different genomic libraries were developed (O-HR, O-LR, B-HR, and B-LR) and these were sequenced. The populations were evaluated for cross-resistance to four ALS inhibiting herbicide families: sulfonylureas (SU), imidazolinones (IMI), triazolopyrimidine sulfonanilides (TP), and sulfonylaminocarbonyltriazolinones (SCT). The results revealed that O populations contain the $Pro_{197}Thr$ mutation that has been previously reported to confer resistance to ALS inhibiting herbicides. Additionally, the B populations contained a novel $Pro_{197}Phe$ mutation. Furthermore, O-HR has additional mutations, one previously reported ($Trp_{574}Leu$) and two ($Asn_{554}Gln$ & $Val_{559}Ile$) novel mutations. The O-LR has additional mutations as well, including a previously documented $Asp_{376}Glu$. Few other mutations, possibly related to population divergence, were present across the mouse-ear cress genome. The O-HR and O-LR populations exhibited broad range cross-resistance to all four ALS inhibitor chemical families evaluated. On

the contrary, B-HR and B-LR were partially sensitive to IMI's which resulted in 59 to 60% control 6 weeks after treatment (WAT). Uniquely, O-LR and B populations exhibited differential resistance to SUs and were found susceptible to prosulfuron which controlled the biotypes 48 and 92% 6 WAT, respectively.

Nomenclature: flucarbazone; imazamox; imazethapyr; mesosulfuron; prosulfuron; thifensulfuron; tribenuron; mouse-ear cress, *Arabidopsis thaliana* L.

Keywords: differential resistance, novel mutations, point mutation, target site mutation.

Introduction

The acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS) enzyme, encoded by the ALS gene, is the primary enzyme in biosynthesis of essential branched chain amino acids (valine, leucine and isoleucine) (Powles and Yu 2010; Warwick et al. 2005). The ALS inhibiting herbicides can cause plant death due to starvation of essential amino acids (Powles and Yu 2010) or due to toxic level of 2-ketobutyrate accumulations, protein synthesis termination, and photosynthate transport failure (Tranel and Wright 2002). Currently, more than 50 herbicides within five distinct chemical classes targeting the ALS enzyme have been commercialized for broadleaf and grass weed management in a variety of crops (Heap 2014). These classes include sulfonylureas (SU), imidazolinones (IMI), triazolopyrimidine sulfonanilides (TP), pyrimidinylthiobenzoates (PTB), and sulfonylaminocarbonyltriazolinones (SCT) (Gerwick et al. 1990; Kraehmer et al. 2014; Tranel and Wright 2002; Whitcomb 1999).

The ALS inhibiting herbicides are popular because of their high efficacy, low use rate, and broad spectrum weed control (Saari et al. 1994; Warwick et al. 2008). However, these factors also render high selection pressure on weeds (Tranel and Wright 2002), which has led to the evolution of ALS herbicide resistance in 158 different cases, more than any other herbicide

mode of action (Heap 2017). Altered target site has been documented as the primary cause of resistance in most cases (Duggleby et al. 2008; Mallorysmith et al. 1990; Powles and Yu 2010; Tranel and Wright 2002; Yu et al. 2008). Currently, eight different alterations in the ALS amino acid sequence are known to confer ALS resistance (Heap 2017). Target site resistance is generally monogenic (Chahal et al. 2015) and spreads very quickly (Jugulam and Godar 2013).

Certain ALS resistant Brassicaceae weed biotypes have been documented to possess multiple target-site mutations associated with resistance evolution. The ALS resistant wild radish (*Raphanus raphanistrum* L.) biotypes were reported to have $Ala_{122}Tyr$, $Pro_{197}Ala$, $Pro_{197}Thr$, $Pro_{197}His$, $Pro_{197}Ser$, $Asp_{376}Glu$, or $Trp_{574}Leu$ mutations (Han et al. 2012; Yu et al. 2012; Yu et al. 2003) whereas ALS resistant hedge mustard (*Sisymbrium officinale* L.) biotypes had $Pro_{197}Ile$ or $Trp_{574}Leu$ mutations (Boutsalis et al. 1999). Different populations of other Brassicaceae weeds, such as wild mustard (*Sinapsis arvensis* L.) have been documented with $Trp_{574}Leu$, $Pro_{197}Ser$, or $Asp_{376}Glu$ mutations (Bahmani et al. 2015; Warwick et al. 2005).

Generally, target site mutations confer cross-resistance to more than one or all chemistries having the same mode of action (Tranel and Wright 2002). Target site mutations in the ALS gene also generally confer broad spectrum cross-resistance, which varies by affected amino acid loci and specific substitution (Whaley et al. 2007). The $Pro_{197}Leu$ substitution has been observed to confer ALS cross-resistance to SU, IMI, PTB, and TP in redroot pigweed (*Amaranthus retroflexus* L.) (Sibony et al. 2001); however, $Ala_{122}Thr$ substitutions conferred high IMI resistance along with no or low resistance to the other ALS chemical families (Whaley et al. 2006). The $Asp_{376}Glu$ substitution has been documented to confer cross-resistance to all five ALS chemistries in two distinct *Amaranthus* species (Ashigh et al. 2009; Whaley et al. 2007).

In Brassicaceae weeds, $Pro_{197}Ala$ has conferred SU, IMI, TP, and PTB resistance in African mustard (*Brassica tournefortii* (Gouan)) (Boutsalis et al. 1999). In wild radish biotypes, Ala_{122} substitution conferred cross-resistance to SU, TP, and IMI, (Han et al. 2012) however, four different Pro_{197} substitutions resulted in cross-resistance to SU and TP without affecting IMI herbicide sensitivity (Yu et al. 2012; Yu et al. 2003)

Mouse-ear cress is a winter annual member of the Brassicaceae (Meinke et al. 1998). It is considered as model plant for studying and understanding the molecular biology and genetics of many plant traits (Meyerowitz 1989). It has not been studied extensively as a crop weed under field conditions however mouse-ear cress biotypes present on railway lines are known to have evolved resistance to atrazine, a photosynthetic inhibitor herbicide (Mohamed et al. 2005). During 2015, in Essex and Westmoreland County, Virginia mouse-ear cress control failures were reported with thifensulfuron (a SU) in winter wheat. Preliminary green-house studies indicated possible resistance in field biotypes of mouse-ear cress. Resistance quantification using dose response methodology resulted in a very high (> 2400 fold) resistance factor relative to susceptible population which indicated possible target site mutations in field biotypes (Randhawa et al. 2017). Most studies across different ALS resistant weed species to date have focused on studying the mutation evolved in the ALS region of genome however there is the possibility of mutations outside the ALS gene reducing herbicide sensitivity. No studies have been conducted to study genome of herbicide resistant field biotypes of mouse-ear cress. The objectives of this study were to characterize the whole genome of mouse-ear cress populations collected from Virginia for understanding the molecular mechanism of ALS inhibiting herbicide resistance and to evaluate the relation between target site mutations and cross-resistance patterns in the same populations.

Materials and Methods

Whole Genome Characterization: A lab study was designed to characterize the genetic basis of ALS inhibiting herbicide resistance in mouse-ear cress. Two field populations, Oakland Road (O) and Balderson's farm (B) were collected from Essex (37°53'56"N, 76°57'14"W) and Westmoreland (38°4'52" N, 76° 46'38"W) Counties, Virginia. Each population consisted of multiple putative resistant lines, collected from within a field with known history of control failures following thifensulfuron application and putative susceptible lines, were collected from outside the field within 10 m of the field margins. Plants were collected from the field and transplanted into 590 cm^{-3} pots filled with Hayter loam soil (fine-loamy, mixed, active, mesic Ultic Hapludalfs) with 5.1% organic matter and pH of 6.4. Pots were maintained in greenhouse conditions with a day length of 12 hours under supplemental light and were watered as required to prevent drought stress. At flowering, each plant was isolated with transparent sheets to prevent cross-pollination. Seeds were harvested from each plant separately and sown in square trays (12 by 12 by 7cm) filled with potting mixture (Metro Mix 360; Sun Gro Horticulture, Seba beach, AB, Canada). Seeds were subjected to vernalisation treatment at 4^0 C for four days to synchronize germination, and then were maintained in growth chamber conditions: day temperature: 24^0C, night temperature: 19^0C, daylight: 10 hours. Seedlings were thinned to produce five uniformly dispersed plants per tray. After three weeks, DNA was extracted from 1-2 leaves of each plant using the cetyl trimethylammonium bromide (C-TAB) method (Murray and Thompson 1980). The plants were then treated with thifensulfuron (Harmony; DuPont, Wilmington, DE) at 394 g ai ha^{-1}, which is 15x of the labeled rate, and 0.25% v v^{-1} non-ionic surfactant (Activator 90, Loveland Industries, Inc. Greeley, CO). Herbicide was applied using a spray chamber equipped with a TeeJet 8001 EVS nozzle (Spraying Systems Co., Wheaton, IL)

calibrated to deliver a spray volume of 140 L ha^{-1} at 172 kPa. Plants were returned to the growth chamber after herbicide application. Three weeks after treatment (WAT), plants were visibly evaluated and scored for level of resistance on a scale from 0 (no injury) to 5 (all plants completely necrotic). There were no truly susceptible lines, but resistance levels varied, so all lines were categorized as high resistance (HR) or low resistance (LR) for each location. All populations exhibited phenotypic differences following herbicide applications (Figures 3, 4, and 5). The O-HR consisted of 14 parental lines come from within the field however, O-LR consisted of two parental lines from within the field and one from margins. On the contrary, B-HR had total six parental lines including two from the field margins and B-LR had three parental lines, all coming from margins. The previously isolated DNA was then pooled separately for HR and LR lines for each location. Whole genome sequencing was completed using Illumina HiSeq 2 x 125bp paired-end sequencing by Genewiz (South Palinfield, NJ 07080) to produce four different libraries: O-HR, O-LR, B-HR and B-LR.

Cross-Resistance Evaluation: All four mouse-ear cress populations were evaluated under greenhouse conditions for cross-resistance to ALS inhibiting herbicides. Experiments including 10 different ALS herbicides along with treated and non-treated checks were set up in a randomized complete block design with six replications per treatment. Herbicide treatments (Table 5) were made using a spray chamber equipped with TeeJet 8001 EVS nozzle calibrated to deliver a total spray volume of 140 L ha^{-1} at 172 kPa. The experiment was replicated in time for a total of two experimental repetitions for each set of populations. Visible weed control was evaluated relative to the non-treated check on a 0 (no control) to 100 (complete plant necrosis) scale (Frans et al. 1986). Visible control was assessed at 2, 4, and 6 WAT and above ground dry biomass was measured 6 WAT.

Data Analysis: Whole genome sequencing data were first entered into Trimmomatic 0.35 (USADEL; Aachen, Germany), which was used to trim raw sequences and remove adapters and low quality (<Q30) reads. Following trimming, ~138X genome coverage was observed. Genome wide alignment was completed using genomes with BWA v0.7 (MSI; Minneapolis, MN) followed by visualization of mutations with IGV 2.3_67 (Broad Institute; Cambridge, MA). Mutations were observed within the ALS gene however, no mutations possibly associated with resistance were found to be present across the rest of the genome. Therefore, a custom Python script was used to capture the ALS gene region and translate to protein sequence while MAFFT (Multiple Alignment using Fast Fourier Transform) (EMBL-EBI; London, UK) was used for protein alignment to assess possible mutations causing the differential resistance in mouse-ear cress.

Cross-resistance data analyses were performed using JMP 1.1.0 (SAS Institute Inc., Cary, NC). Data analysis was performed using ANOVA and effects were considered significant when $p < 0.05$. Subsequently, data were subjected to means separation using Tukey's HSD ($p < 0.05$).

Results and Discussion

To determine the molecular basis of ALS resistance in mouse-ear cress the pooled whole genome sequences of each of the four populations were compared with a previously available mouse-ear cress genome (TAIR 2016). Different target site mutations were found to be present in all populations. Target site mutations are predominant in conferring ALS inhibitor resistance in different weed species (Saari et al. 1994). The results revealed a Pro_{197} mutation across all four population pools. In SU based herbicide selection, Pro_{197} is the most commonly observed mutation (Yu et al. 2003). However, substituted amino acids differ by population pools: both O

population pools had $Pro_{197}Thr$ while both, B populations had $Pro_{197}Phe$ mutation (Table 6). Previous research has shown that different populations of ALS resistant weeds, like flixweed (*Descurainia sophia* L.) and wild radish have different substitutions such as serine, alanine, and leucine at Pro_{197} (Cui et al. 2012; Yu et al. 2012). The $Pro_{197}Thr$ mutation has been documented in 13 different ALS resistance cases (Heap 2017), however $Pro_{197}Phe$ has not been reported before. Three Brassicaceae weeds: wild radish, flixweed, and shepherd's-purse (*Capsella bursa-pastoris* L.), have been documented with $Pro_{197}Thr$ mutation (Heap 2017). Furthermore, $Pro_{197}Thr$ has been previously known to confer a high level (>10 fold) of SU and TP resistance (Yu et al. 2012).

Additionally, the O-HR population has one previously reported ($Trp_{574}Leu$) and two ($Asn_{554}Gln$ and $Val_{559}Ile$) novel mutations (Table 6). Similar, cases of multiple ALS mutations have been reported in wild radish where two different populations simultaneously exhibited three different ALS mutations at Pro_{197}, Asp_{376}, and Trp_{574} (Yu et al. 2012). The presence of multiple ALS conferring mutations within same population has been attributed to cross pollination in wild radish (Yu et al. 2012). Mouse-ear cress is mostly self-pollinated, but it is possible that out-crossing has led to stacking of multiple mutations that originally could have evolved into separate plants.

The $Trp_{574}Leu$ has been documented to a cause ALS resistance in 36 different weed species including six Brassicaceae weeds (wild radish, wild mustard, flixweed, hedge mustard, smallseed false flax (*Camelina microcarpa* DC.), and radish (*Raphanus sativus* L.) (Heap 2017). The $Trp_{574}Leu$ mutation is known to confer high levels of ALS resistance across all five ALS chemistries (Deng et al. 2017; Pandolfo et al. 2016). On the contrary, O-LR population has $Asp_{376}Glu$ mutation, besides $Pro_{197}Thr$. The $Asp_{376}Glu$ is known to confer low to high resistance

to different ALS chemistries (Heap 2017). $Asp_{376}Glu$ has also been reported to confer cross-resistance to the entire range of ALS herbicides in Powell amaranth (*Amaranthus powelli* S.), smooth pigweed (*Amaranthus hybridus* L.), and wild radish (Ashigh et al. 2009; Whaley et al. 2007; Yu et al. 2012).

The $Pro_{197}Phe$ substitution in the B population has been previously implicated as the cause of ALS resistance in different weeds (Heap 2017; Yu et al. 2003) and is presently attributed as the cause for the ALS resistance. Similarly, the O-HR population has two mutations, $Pro_{197}Thr$ and $Trp_{574}Leu$, which have been previously implicated as cause of ALS resistance in multiple weeds (Heap 2017). However, the roles of $Asn_{554}Gln$ and $Val_{559}Ile$ in conferring ALS resistance are unknown and require further research to establish potential impact on response to ALS herbicides. The mutation patterns are correlated for all populations collected within the field and outside, as they have Pro_{197} mutations.

All mutations mentioned were present at 100% frequency within respective DNA pool. There were other mutations presents across the genome which had lower frequency. This research included a limited number of individuals for DNA pool of each sub-population. Further research with more-individuals would be needed to categories the exact frequency of other mutations.

Cross-Resistance Evaluation. There was a significant population by treatment interaction for visible control data, so data for O and B populations were analyzed separately. Sub-populations within the O population had a significant population by treatment interaction however, no such interaction was observed for sub-populations within population B, therefore data were analyzed separately for O-HR and O-LR while data were pooled for B-HR and B-LR.

O-HR Herbicide Response: No significant WAT effect was observed so data were pooled across rating dates. No ALS herbicide resulted in more than 36% visible control over the period of 6 WAT (Table 7). All herbicides except prosulfuron resulted in less than 20% control while prosulfuron resulted in 36% control (Table 7). Above ground biomass analysis for 6 WAT resulted in none of the ALS herbicides reducing biomass relative to the non-treated (Table 7). From these results, it is evident that O-HR population has evolved resistance to all four ALS chemistries (SUs, SCTs, TPs, and IMIs) evaluated. Broad range cross-resistance was expected due to the occurrence of similar cross-resistance patterns associated with $Pro_{197}Thr$ and $Trp_{574}Leu$ (Heap 2017). The $Pro_{197}Thr$ is responsible for SU and TP cross-resistance while $Trp_{574}Leu$ confers IMI resistance (Yu et al. 2012). All three Brassicaceae weeds: wild radish, shepherd's-purse, and flixweed, with the $Pro_{197}Thr$ mutation have been reported as resistant to SUs while wild radish was cross-resistant to TPs (Heap 2017). Additionally, wild radish biotypes with $Trp_{574}Leu$ exhibited SU, TP, and IMI cross resistance relative to SU and TP cross-resistance in biotypes with only $Pro_{197}Thr$ (Yu et al. 2012).

O-LR Herbicide Response: A significant WAT effect was observed so data are presented separately across rating dates. Prosulfuron was the only the SU herbicide to control mouse-ear cress approximately 50% at 2, 4, and 6 WAT while all other SU, SCT, and TP herbicides controlled the weed 21% or less over the same period (Table 8). The IMI herbicides, imazethapyr and imazamox, provided 25 and 12% control 2 WAT, respectively, however control increased to 35% at 6 WAT, which was similar to prosulfuron. No herbicide resulted in less above ground biomass than the non-treated 6 WAT (Table 8). Poor control from SU and TP was expected due to the occurrence of $Pro_{197}Thr$ (previously described) whereas $Asp_{376}Glu$ is possibly responsible for poor control by SCT and IMI herbicides. Past research has characterized

the $Asp_{376}Glu$ mutation as responsible for cross-resistance to all ALS chemistries in smooth pigweed (Whaley et al. 2007) and Powell amaranth (Ashigh et al. 2009). However, other research in horseweed (*Conyza canadensis* L.) (Zheng et al. 2011) and wild radish (Yu et al. 2012) observed high resistance (>10 fold) to SU and TP and low resistance (<10 fold) to IMI herbicides. Similar results were observed in the current study where IMIs resulted in greater (but not absolute) control than other chemistries. Current results indicate broad range ALS cross-resistance in the O-LR mouse-ear cress population. Data indicate that the O-LR population has differential resistance to SUs which is possible because different amino acid substitutions at Pro_{197} are known to cause differential resistance among SU herbicides (Sada et al. 2013). It was found that ALS (SU) resistant rock bulrush (*Schoenoplectus juncoides* Toxb.) exhibited three different Pro_{197} substitutions {Serine (Ser), Analine (Ala) and Histidine (His)}, which resulted in the highest resistance to three different SU herbicides (Sada et al. 2013).

B-HR and B-LR Herbicide Response: No significant difference between visible control of B-HR and B-LR populations was observed so data were pooled. Prosulfuron resulted in the highest weed control at all rating dates, providing 43% control 2 WAT and 92% control 6 WAT (Table 9). However, no other SU herbicide resulted in more than 30% control at any point during the study. Such differential resistance among SU herbicides is possible because different herbicides interact differently with different amino acid substitutions (described previously for Pro_{197} based substitutions). Similar cross-resistance trends were observed in silky windgrass (*Apera spica-venti* L.) where $Ala_{122}Val$ conferred cross-resistance to chlorsulfuron and mesosulfuron but not to sulfosulfuron (Krysiak et al. 2011). The greatest control observed from pyroxsulam and flucarbazone was below 45% at any rating date. Both IMI herbicides resulted in >50% control 4 and 6 WAT (Table 9). Corresponding to visible control, prosulfuron treated plants had the least

above ground biomass for B-HR and B-LR populations, but less above ground biomass compared to the non-treated was observed only in the B-HR population. For both populations, plants treated with mesosulfuron, pyroxsulam, flucarbazone, imazethapyr, and imazamox had biomass similar to plants treated with prosulfuron but biomass was not different than the non-treated. It is clearly evident that the ALS resistant mouse-ear cress B population is being controlled by prosulfuron however other herbicides like imazethpyr and imazamox result in moderate control and biomass reduction.

The mouse-ear cress populations evaluated possibly have evolved differential resistance due to presence of different target site mutations. Mouse-ear cress O-HR population has two previously reported ($Pro_{197}Thr$, $Trp_{574}Leu$) and two novel ($Asn_{554}Gln$ & $Val_{559}Ile$) mutations and this population was cross-resistant to all four ALS (SU, SCT, TP and IMI) chemistries evaluated whereas O-LR has two ($Pro_{197}Thr$ and $Asp_{376}Glu$) known mutations and has similar cross-resistance patterns. However, only one novel substitution ($Pro_{197}Phe$) was detected for B-HR and B-LR and both populations were resistant to SUs whereas partial IMI resistance was observed. These results support previous findings that weeds with only $Pro_{197}Thr$ have no or low resistance to IMI herbicides (Tranel and Wright 2002). Therefore, it is concluded that target site mutations are present in mouse-ear cress which confer ALS resistance.

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Table 5. Treatments used for cross-resistance evaluations of mouse-ear cress (*Arabidopsis thaliana*) in greenhouse in Blacksburg, Virginia in 2017.

ALS Chemical Family	Herbicides		Rate (g ai ha^{-1})	Manufacturer
	Product Name	Common Name		
Sulfonylurea[1]	Harmony	thifensulfuron	17.5	E. I. du Pont de Nemours and Company, Wilmington, DE
	Harmony Extra	thifensulfuron + tribenuron	17.5 + 8.8	E. I. du Pont de Nemours and Company
	Express	tribenuron	8.8	E. I. du Pont de Nemours and Company
	Peak	prosulfuron[3]	16	Syngenta Crop Protection LLC, Greensboro, NC
	Osprey	mesosulfuron[3]	15	Bayer CropScience LP, Research Triangle Park, NC
Triazolinone[1]	Everest	flucarbazone[4]	23	Arysta Lifescience North America, LLC, Cary, NC
Triazolopyrimidines[1]	Powerflex HL	pyroxsulam[3]	18.4	Dow AgroSciences LLC, Indianapolis, IN
Imidazolinone[2]	Pursuit	imazethapyr	52.5	BASF Corporation, Research Triangle Park, NC
	Raptor	imazamox	35	BASF Corporation

[1] Treatments with non-ionic surfactant 0.25% v v^{-1} as per product label recommendation.

[2] Treatments with methylated seed oil 1% v v^{-1} as per product label recommendation.

[3] Treatments with ammonium sulfate at 3360 g ha^{-1} as per product label recommendation.

[4] Treatment with ammonium sulfate at 1660 g ha^{-1} as per product label recommendation.

Table 6. Mutations detected in the ALS gene of different mouse-ear cress (*Arabidopsis thaliana*) populations collected from Essex County, Virginia and their resistance status to ALS inhibiting herbicide chemistries.

Mouse-Ear Cress Population	Mutations	ALS inhibitor chemistry			
		SU[1]	TP[1]	SCT[1]	IMI[1]
O-HR	$Pro_{197}Thr$	R[2]	R	R	R
	$Asn_{554}Gln$				
	$Val_{559}Ile$				
	$Trp_{574}Leu$				
O-LR	$Pro_{197}Thr$	R*	R	R	R
	$Asp_{376}Glu$				
B-HR	$Pro_{197}Phe$	R*	R	R	r[3]
B-LR	$Pro_{197}Phe$	R*	R	R	r

[1] Abbreviations: SU, sulfonylureas; TP, triazolopyrimidine sulfonanilides; SCT, sulfonylaminocarbonyltriazolinones; IMI, imidazolinones.

[2] R: high resistance.

[3] r: partial resistance.

* Indicates populations with differential resistance to sulfonylureas.

Table 7. Visible control and above ground biomass of mouse-ear cress (*Arabidopsis thaliana*) O-HR population under greenhouse conditions in Blacksburg, Virginia in 2017.

Herbicide	Rate	Mouse-Ear Cress Control[1]	Biomass (6 WAT[2])
	g ai ha^{-1}	%	g
thifensulfuron	17.5	0 C[3]	0.76 A
thifensulfuron + tribenuron	17.5 + 8.8	0 C	0.53 AB
tribenuron	8.8	6 BC	0.56 AB
mesosulfuron[4]	15	1 C	0.62 AB
prosulfuron[4]	16	36 A	0.29 B
pyroxsulam[5]	18.4	3 B	0.57 AB
flucarbazone[4]	23	4 CB	0.58 AB
imazethapyr	52.5	17 B	0.55 AB
imazamox	35	8 BC	0.67 A
non-treated	-[6]	-	0.58 AB

[1] Visible control data were pooled across rating dates due to lack of significance.

[2] Abbreviation: WAT, weeks after treatment.

[3] Treatment means within columns with no common letters are significantly different according to Tukey's HSD pairwise comparison ($p < 0.05$).

[4] Treatment included ammonium sulfate at 3360 g ha^{-1} as per product label recommendation.

[5] Treatment included ammonium sulfate at 1660 g ha^{-1} as per product label recommendation.

[6] Visible mouse-ear cress control data for non-treated were not included in the analysis.

Table 8. Visible control and above ground biomass of mouse-ear cress (*Arabidopsis thaliana*) O-LR population under greenhouse conditions in Blacksburg, Virginia in 2017.

Herbicide	Rate	Mouse-Ear Cress Control			Biomass
		2 WAT[1]	4 WAT	6 WAT	6 WAT
	g ai ha^{-1}		%		g
thifensulfuron	17.5	0 C[2]	0 C	0 B	0.91 A
thifensulfuron + tribenuron	17.5 + 8.8	0 C	0 C	0 B	0.92 A
tribenuron	8.8	0 C	0 C	0 B	0.83 A
mesosulfuron[3]	15	4 BC	19 BC	19 AB	0.69 A
prosulfuron[3]	16	53 A	54 A	48 A	0.35 A
pyroxsulam[4]	18.4	3 BC	11 BC	12 B	0.74 A
flucarbazone[3]	23	5 BC	21 BC	10 B	0.70 A
imazethapyr	52.5	25 B	36 AB	35 AB	0.54 A
imazamox	35	12 BC	40 AB	35 AB	0.67 A
non-treated		-[5]	-	-	0.83 A

[1] Abbreviation: WAT, weeks after treatment.

[2] Treatment means within columns with no common letters are significantly different according to Tukey's HSD pairwise comparison ($p < 0.05$).

[3] Treatment included ammonium sulfate at 3360 g ha^{-1} as per product label recommendation.

[4] Treatment included ammonium sulfate at 1160 g ha^{-1} as per product label recommendation.

[5] Visible mouse-ear cress control data for non-treated were not included in the analysis.

Table 9. Visible control and above ground biomass of mouse-ear cress (*Arabidopsis thaliana*) B-HR and B-LR populations under greenhouse conditions at Blacksburg, Virginia in 2017.

		Mouse-Ear Cress Control			Biomass	
Herbicide	Rate	2 WAT[1]	4 WAT	6 WAT	B-HR	B-LR
	g ai ha^{-1}	%			g	
thifensulfuron	17.5	0 C[2]	2 E	4 EF	0.45 A	0.50 A
thifensulfuron + tribenuron	17.5 + 8.8	1 C	0 E	1 F	0.30 ABC	0.52 A
tribenuron	8.8	0 C	0 E	1 F	0.36 AB	0.44 AB
mesosulfuron[3]	15	16 BC	28 D	27 DE	0.14 BCD	0.29 ABC
prosulfuron[3]	16	43 A	79 A	92 A	0.02 D	0.073 C
pyroxsulam[4]	18.4	23 AB	37 BCD	34 CD	0.14 BCD	0.27 ABC
flucarbazone[4]	23	23 AB	41 BCD	43 BCD	0.13 BCD	0.19 BC
imazethapyr	52.5	38 AB	55 B	59 BC	0.08 CD	0.25 ABC
imazamox	35	41 AB	52 BCD	60 B	0.09 CD	0.13 C
non-treated		-[5]	-	-	0.32 ABC	0.36 ABC

[1]Abbreviation: WAT, weeks after treatment.

[2] Treatment means within columns with no common letters are significantly different according to Tukey's HSD pairwise comparison ($p < 0.05$).

[3] Treatment included ammonium sulfate at 3360 g ha^{-1} as per product label recommendation.

[4] Treatment included ammonium sulfate at 1160 g ha^{-1} as per product label recommendation.

[5] Visible mouse-ear cress control data for non-treated were not included in the analysis.

Figure 3. Mouse-ear cress (*Arabidopsis thaliana*) O-HR population herbicide response to thifensulfuron at 394 g ai ha^{-1} (15x of labeled rate) 3 wk after treatment in Blacksburg, Virginia in 2016-17.

Figure 4. Mouse-ear cress (*Arabidopsis thaliana*) O-LR population herbicide response to thifensulfuron at 394 g ai ha^{-1} (15x of labeled rate) 3 wk after treatment in Blacksburg, VA in 2016-17.

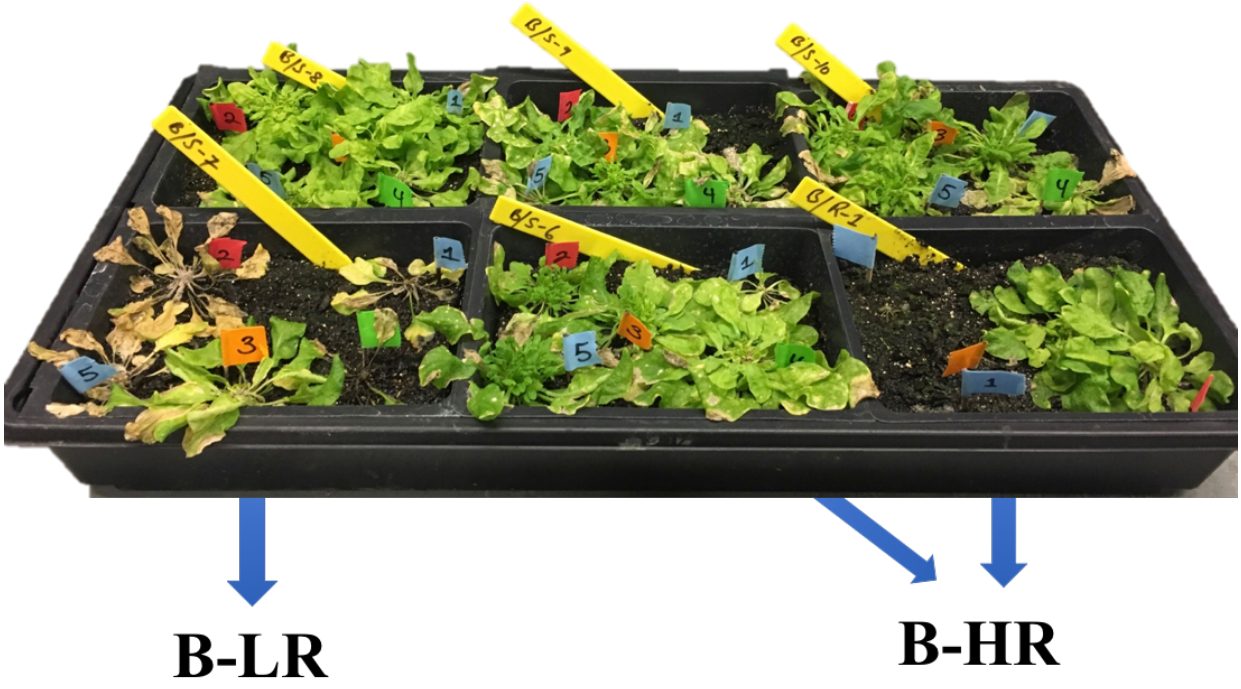

Figure 5. Mouse-ear cress (*Arabidopsis thaliana*) B-HR and B-LR population herbicide response to thifensulfuron at 394 g ai ha^{-1} (15x of labeled rate) 3 wk after treatment in Blacksburg, Virginia in 2016-17.

Chapter 4. ALS Resistant Mouse-Ear Cress (*Arabidopsis thaliana*) Alternative Herbicide Control Options and Interference in Winter Wheat

Abstract

Following the confirmation of field evolved ALS resistance in mouse-ear cress, research was conducted to evaluate alternative PRE and POST herbicide options as well as mouse-ear cress interference in winter wheat. The PRE and POST herbicide studies consisted of 7 and 11 treatments, respectively; visible control, visible crop injury, and yield data were collected. The interference study consisted of two treatments (whole plots), thifensulfuron and non-treated. Mouse-ear cress density counts were taken from nine random 1 m^2 subplots at the flowering growth stage and subsequent wheat yield was collected. One subplot was made weed-free via hand weeding. In the PRE study, flumioxazin, pyroxasulfone, and metribuzin resulted in >80% control 15 wk after treatment (WAT) across all sites. No crop injury was observed in PRE study, and only metribuzin resulted in greater yield than non-treated check. In the POST study, 2,4-D, dicamba, and metribuzin resulted in >75% control in the field and greenhouse. Dicamba and pyroxsulam resulted in 20% crop injury 3 WAT at all sites. None of the treatments resulted in yield differences relative to the non-treated check. No yield loss was observed by mouse-ear cress density greater than 300 plants m^{-2}, indicating that mouse-ear cress is not competitive with winter wheat.

Nomenclature: bromoxynil; dicamba; flumioxazin; fluroxypyr; metribuzin; nitrogen; pendimethalin; pyroxasulfone; saflufenacil; thifensulfuron; tribenuron; 2,4-D; mouse-ear cress, *Arabidopsis thaliana* L.; wheat, *Triticum aestivum* L.

Keywords: PRE control, POST control, density, yield loss.

Introduction

Mouse-ear cress (*Arabidopsis thaliana* L.) is a Brassicaceae winter annual weed in small grains. It originated from western Eurasia (TAIR 2015), however it can now be found all over the globe including the United States (Mitchell-Olds 2001). Albeit it is considered a model organism for plant genomics, it has been mostly ignored as a crop weed due to its small size and short life cycle of six wk (Meyerowitz 1989). Mouse-ear cress is a long day plant, showing rapid flowering under >12 h photoperiods and optimum light intensity of 120 to 150 µmol m^{-2} sec^{-1} (ABRC 2013). It is self-pollinated and flowers are 2 mm in length (Meinke et al. 1998). Its fruits are sliques (Hays 2002; Meinke et al. 1998). It has minute sized seeds, around 0.5 mm in length at maturity, which can easily be dispersed over long distances. Seed is dispersed via what is known as "pod-shatter", in which the silique splits apart due to mechanical pressure and is subsequently spread by wind or rain (Dinneny and Yanofsky 2005). Mouse-ear cress seeds possess secondary dormancy and can remain viable for more than a decade, if stored dry at 4^0C to 20^0C. At room temperature seed can lose viability within two years (ABRC 2013). High propagule pressure (one plant produces approximately 5000 seeds) and adaptation for seed dispersal to wide geographical areas adds to its weediness (Hays 2002; Meinke et al. 1998; Stewart Jr 2009).

In 2015, growers in Essex and Westmoreland Counties, Virginia reported failures of mouse-ear cress control in winter wheat from the ALS inhibiting herbicide thifensulfuron in Essex County, Virginia. ALS-resistance was confirmed in 2016 as well as cross-resistance to other sulfonylurea herbicides, triazolopyrimidine sulfonanilides, and sulfonylaminocarbonyltriazolinones (Randhawa et al. 2017). With almost all ALS-inhibiting herbicides registered for use in winter wheat being ineffective, alternative herbicides are needed,

but little is known regarding their efficacy.

Furthermore, little is known about the impact of mouse-ear cress on winter wheat yield. Weeds can cause significant yield loss due to crop-weed interference. Weed density is one of the primary factors affecting crop-weed interference (Faria et al. 2014). Winter annual weeds such as littleseed canarygrass (*Phalaris minor* Retz.) and wild oat (*Avena fatua* L.) can cause 35 to 40% yield loss in wheat when present at 150 plants m^{-2} (Oad et al. 2007). Other research has documented 64% yield loss in spring wheat from wild oat at 300 plants m^{-2} (Carlson and Hill 1985). Downy brome (*Bromus tectorum* L.) reduced winter yield up to 33% at density of 40 plants m^{-2} (Refsell 2013). Brassicaceae weeds, the same family as mouse-ear cress, can also interfere and reduce wheat yield. Wild radish (*Raphanus raphanistrum* L.) can cause yield loss of more than 50% in wheat, when present at densities of 75 to 200 plants m^{-2} (Hashem and Wilkins 2002).

Therefore, objectives of this study were to 1) assess alternative PRE and POST herbicide options for ALS inhibiting herbicide resistant mouse-ear cress in winter wheat, 2) assess mouse-ear cress interference with winter wheat by determining the density at which mouse-ear cress will cause yield loss in winter wheat.

Material and Methods

Herbicidal Control. Mouse-ear cress was assessed for alternative herbicidal control options under field conditions. Field locations were established in Essex and Westmoreland Counties, Virginia based on farmers' report on ineffectiveness of thifensulfuron-based herbicides for mouse-ear cress control. Two field trails were setup to evaluate alternative PRE and POST herbicide control options. Site years 1, 2, and 3 for PRE and POST field studies were conducted

at these locations (37°53'56"N, 76°57'14"W; 38°4'52" N, 76° 46'38"W and 37°53'54"N, 76°55'31"W) and (37°53'56"N, 76°57'14"W; 37°54'32"N, 76°57'44"W and 37°53'54"N, 76°55'31"W), respectively. Winter wheat was grown using standard practices for the region (Table 10).

PRE Herbicide Control. The PRE herbicide control of mouse-ear cress was assessed relative to weed germination. Study consisted of seven treatments including a non-treated check (Table 11) and was conducted in three site-years previously mentioned. Plots were 3 by 7.6 m and arranged in a randomized complete block design with four replicates per treatment. Herbicide treatments were made using a hand-held spray boom equipped with four TeeJet 11002 AIXR nozzles (Spraying Systems Co., Wheaton, IL) with 46 cm spacing calibrated to deliver total spray volume of 140 L ha^{-1} at 207 kPa. Visible weed control and crop injury were evaluated relative to the non-treated check on a 0 (no control or injury) to 100 (complete plant necrosis) scale (Frans et al. 1986). Visible control data were collected 15 wk after initial treatment (WAIT). Visible control evaluation timing was based on usual scouting for POST application by growers. Wheat yield data were also collected. Data analyses were performed using JMP 1.1.0 (SAS Institute Inc., Cary, NC). ANOVA was performed and effects were considered significant when $p < 0.05$. Subsequently, data were also subjected to means separation using Tukey's HSD pairwise comparison ($p < 0.05$). For yield data, multiple comparison using Fisher's protected LSD ($p < 0.05$) was conducted to examine treatment effect.

POST Herbicide Control. Mouse-ear cress was assessed for alternative POST herbicidal control options under field conditions. The study consisted of 10 treatments including a non-treated check (Table 12). The study was conducted across three site-years previously mentioned. Experimental design, treatment application, data collection, and data analyses were conducted as

previously described in the PRE study. Treatments were applied in first wk of March and visible control was assessed at 3, 5, and 7 wk after treatment (WAT). Wheat yield data were also collected.

Mouse-ear cress was also assessed for alternative POST herbicidal control options under greenhouse conditions in 2015 and 2016. Thifensulfuron resistant mouse-ear cress seeds were collected from fields in Essex County, Virginia. Initial screening at the labeled herbicide rate (26.3 g ha^{-1}) confirmed resistance and plants were thereafter labeled thifensulfuron resistant (R) (Randhawa et al. 2017). Mouse-ear cress seeds were germinated in flat trays (42 by 32 by 7cm) containing a potting mixture (Metro Mix 360; Sun Gro Horticulture, Seba beach, AB, Canada). Single seedlings were transplanted into 590 cm^3 plastic pots with Hayter loam soil (Fine-loamy, mixed, active, mesic Ultic Hapludalfs) with 5.1% organic matter and pH of 6.4 and allowed to acclimate for 2 wk prior to any further testing. Pots were maintained in greenhouse conditions (15 to 27 °C and average relative humidity of 80%) with day length of 12 hours under supplemental light, and were watered to maintain field capacity. Treatments evaluated were the same as the field study with one exception, nitrogen was replaced with bromoxynil + pyrasulfotole (Huskie; Bayer CropScience LP, Research Triangle Park, NC**)** + ammonium sulfate at 207 + 37 + 1120 g ai ha^{-1}. The study utilized a randomized complete block design with six replicates for each treatment. The study was duplicated in time under similar greenhouse conditions for a total of two experimental replications.

Herbicide treatments were made using a spray chamber equipped with a single 8001 EVS nozzle calibrated to deliver total spray volume of 140 L ha^{-1} at 172 kPa. For herbicide efficacy evaluation, visible weed control data were collected as previously described 2, 4, and 6 WAT. Above ground biomass was collected 6 WAT and oven dried at 65 C for 72 h. Data analyses

were performed using JMP 1.1.0 (SAS Institute Inc., Cary, NC). ANOVA was performed and effects were considered significant when $p < 0.05$. Subsequently, data were subjected to means separation using Tukey's HSD pairwise comparison ($p < 0.05$).

Mouse-Ear Cress Interference in Wheat. A field study was conducted to assess the interaction of mouse-ear cress density with winter wheat yield. Field locations (same as the POST study) with history of thifensulfuron resistant mouse-ear cress were chosen in 2015 and 2016 in Essex County, Virginia. The experiment was replicated across three site-years previously described. Winter wheat was grown using standard practices for the region (Table 10). A two-treatment study was setup in a randomized complete block design with six replicates for each treatment. Each plot measured 6.1 by 10.7 m. Treatments included thifensulfuron at 17.5 g ai ha^{-1} and a non-treated check. Treatment application was similar to the POST study; however, application was made in last wk of February in each year. Nine randomly assigned 1 m^2 subplots were marked within each main plot (treatment) and mouse-ear cress density counts were taken at the full flowering growth stage. One of the nine subplots in each replicate was selected randomly and made weed free by hand weeding at the time of the census (2 wk after herbicide application). Any weeds other than mouse-ear cress were removed by hand from all subplots at the time of the census. Winter wheat was hand harvested within subplots and fed into a combined harvester for threshing and yield data were collected. Data analyses were performed using JMP 1.1.0. ANOVA and effects were considered significant when $p < 0.05$. Subsequently, yield was regressed against mouse-ear cress density.

Results and Discussion

PRE Herbicide Control. A significant interaction between herbicide treatment and location was observed, therefore data are presented separately for each location. Mouse-ear cress control varied among herbicide treatments in general (Table 13). Metribuzin, flumioxazin, and pyroxasulfone resulted in >80% visible control across all three sites. Metribuzin is known to provide >85% weed control in wheat, which agrees with the current research (Pandey et al. 2006). Saflufenacil and pendimethalin resulted in 67 and 71% control, respectively, at site 1. Saflufenacil resulted in >85% mouse-ear cress control, at sites 2 and 3, 15 WAIT whereas pendimethalin resulted in 45 and 83% mouse-ear cress control, at sites 2 and 3, respectively. Lesser activity by saflufenacil and pendimethalin at site 1 is possibly due to lack of activating rainfall (rainfall data not available). Previous research has reported similar (78%) blue mustard (*Chorispora tenella* Pallas) control by saflufenacil (Geier et al. 2009). Thifensulfuron resulted in no more than 40% weed control except at site two where it resulted in 61% control 15 WAIT, which corroborates the field history of ALS resistant mouse-ear cress. No crop-injury was observed with any of the herbicide treatments (data not shown).

Treatment effect on yield was significant ($p = 0.04$); however, treatment by location interaction was not significant. Therefore, data were pooled for treatments across locations (Table 13). Herbicides that resulted in the best mouse-ear cress control (metribuzin, flumioxazin, and pyroxasulfone) resulted in similar yield but only metribuzin and pendimethalin (4617 and 4589 kg ha^{-1}) had greater yield than the non-treated (3983 kg ha^{-1}). Wheat yield in plots treated with thifensulfuron were not different than non-treated plots.

POST Herbicide Control. For field visible weed control, data were pooled across locations and all rating timings as no significant treatment by location interaction and WAT effects were observed, respectively. Additionally, for yield data, no significant treatment by location

interaction was observed, however location effect (Table 14) was significant. Data were pooled across locations for each treatment.

2,4-D resulted in 95% mouse-ear cress control while dicamba and metribuzin resulted in 84% control each (Table 14). All three herbicides resulted in greater control than all other treatments. No other herbicide treatments including thifensulfuron based herbicides controlled mouse-ear cress more than 50%.

For greenhouse studies, a significant treatment by experimental run interaction was observed so data were analyzed and presented separately for each experimental run. Weed control was similar between field and greenhouse conditions for 2,4-D, dicamba, metribuzin, pyroxsulam, and thifensulfuron treatments. 2,4-D and dicamba resulted in 100% mouse-ear cress control 6 WAT in both experimental runs (Table 15) whereas metribuzin resulted in 100% and 91% control 6 WAT in experimental run 1 and 2, respectively. Past research has also documented similar results where ALS resistant wild mustard was controlled by 2,4-D (Heap 2017). None of the thifensulfuron based herbicides resulted in commercially acceptable (>90%) mouse-ear cress control. Wild radish biotypes were previously documented with multiple resistance to thifensulfuron, tribenuron, and dicamba (Heap 2017), but the mouse-ear cress biotype under evaluation in this study does not have multiple resistance to dicamba as this herbicide had high efficacy.

Bromoxynil + pyrasulfotole resulted in 100% and 83% control 6 WAT in experimental run 1 and 2, respectively (Table 15). Bromoxynil + pyrasulfotole treatment had greater efficacy in the greenhouse compared to bromoxynil alone in field and greenhouse conditions. Fluroxypyr had a greater efficacy (100% control 6 WAT in both experimental runs) than field conditions (46% control).

Dicamba resulted in 20% crop injury 3 WAT across all three field sites which dissipated at the 5 and 7 WAT ratings (Table 14). Dicamba injury results corroborates past research where 20% wheat injury was observed 2 WAT and plants eventually recovered (Orr et al. 1996; Robinson et al. 2015). Pyroxsulam caused 20% and 7 % crop injury at site 1 and 2, 3 WAT however no injury was observed at site 3. Metribuzin resulted in 35% crop injury 3 WAT at site 3; however, recovery was observed 5 WAT of which 10% injury was observed. No crop injury was observed 7 WAT from any of the treatments (data not shown) indicating that crop injury was transient.

Yield data collected from the field experiment indicated that while mouse-ear cress control differences were observed, these did not result in a yield loss. No differences were observed between the non-treated check and any herbicide treatment. No significant yield difference even with complete weed control signals towards lower crop-weed competition, which could be possibly attributed to its very small mature plant size and a very short life cycle of six wk (Meyerowitz 1989).

Mouse-Ear Cress Interference in Wheat. Data were analyzed using a nested model where mouse-ear cress density was nested into treatment as a random effect. Treatment by location interaction was not significant so data were pooled across locations. Treatment and density main effects were not found to be significant; herbicide treatment and mouse-ear cress density did not affect yield. Simple linear regression and non-linear regression of yield data against mouse-ear cress density was insignificant and indicating that there was no relation between mouse-ear cress density and winter wheat yield (Figure 6). Mouse-ear cress at density greater than 300 plants m^{-2} did not result in yield loss. The current results concur with the previous research where henbit (*Lamium amplexicaule* L.) density up to 156 plants m^{-2} did not result in yield loss in winter

wheat (Refsell 2013). These results also correspond to yield trends observed in PRE and POST study where treatments with >90% control did not result in greater yield than non-treated.

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Table 10. Winter wheat production information of PRE and POST field sites for control of thifensulfuron resistant mouse-ear cress (*Arabidopsis thaliana*) in Essex County, Virginia in 2015-16.

Location	Year	Variety	Planting Date	Fertilizer (kg ha^{-1})	
				Fall Applied	Spring Applied
PRE/POST Site 1	Fall 2015	USG 3404	10/20/15	37-67-135-13[1]	118-0-0-15
PRE Site 2	Fall 2015	Dyna-Gro 9042	10/17/15	34-101-101-0	-
POST Site 2	Fall 2015	Pioneer[2]	10/03/15	34-0-67-4.5	112-0-0-15
PRE/POST Site 3	Fall 2016	Hilliard	10/15/17	45-0-112-13	53-0-0-7

[1] Fertilizer are listed in order of N-P_2O_5-K_2O-S.

[2] Variety unknown.

Table 11. Herbicide treatments evaluated for PRE control of thifensulfuron resistant mouse-ear cress (*Arabidopsis thaliana*) in winter wheat in Essex County, Virginia in 2015-16.[1]

Trade name	Common name	Rate	Application timing	Manufacturer
		g ai ha^{-1}		
Valor	flumioxazin	71.5	7 DBP[2]	Valent U.S.A Corporation, Walnut Creek, CA
Sharpen	saflufenacil	50	7 DBP	BASF Corporation, Research Triangle park, NC
Zidua	pyroxasulfone	119	7 DAP[2]	BASF Corporation
Prowl H_2O	pendimethalin	1600	7 DAP	BASF Corporation
Tricor	metribuzin	105	21 DAP	United Suppliers, Inc., Eldora, IA
Harmony	thifensulfuron	17.5	21 DAP	E. I. du Pont de Nemours and Company, Wilmington, DE

[1] All treatments included 0.25% v v^{-1} of non-ionic surfactant (Activator 90; Loveland Industries, Inc. Greeley, CO).

[2] Abbreviations: DBP, days before planting; DAP, days after planting.

Table 12. Herbicide treatments evaluated for POST control of thifensulfuron resistant mouse-ear cress (*Arabidopsis thaliana*) in wheat winter wheat in Essex County, Virginia in 2015-16.[1]

Trade name	Common name	Rate	Manufacturer
		g ai/ae ha^{-1}	
Harmony	thifensulfuron	17.5	E. I. du Pont de Nemours and Company, Wilmington, DE
Harmony Extra	thifensulfuron + tribenuron	17.5 + 8.8	E. I. du Pont de Nemours and
2,4-D LVE	2,4-D	1060	United Suppliers, Inc., Eldora, IA
Banvel	dicamba	560	Arysta Lifescience North America, LLC, Cary, NC
Tricor	metribuzin	105	United Suppliers, Inc.
Buctril	bromoxynil	420	Bayer CropScience LP, Research Triangle Park, NC
Starane Ultra	fluroxypyr	157	Dow AgroSciences LLC, Indianapolis, IN
Poweflex HL	pyroxsulam[2]	18.4	Dow AgroSciences LLC,
Urea	nitrogen	25700	Southern States Cooperation Inc., Richmond, VA

[1] All treatments included 0.25% v v^{-1} of non-ionic surfactant (Activator 90; Loveland Industries, Inc. Greeley, CO).

[2] Treatment included ammonium sulfate at 3360 g ha^{-1} as per product label recommendation.

Table 13. Pre-emergence herbicidal control of thifensulfuron resistant mouse-ear cress (*Arabidopsis thaliana*) and winter wheat yield in Essex County, Virginia in 2015-16.

Herbicide	Rate		Site 1	Site 2	Site 3	Yield
			15 WAIT[1]	15 WAIT	15 WAIT	
	g ai ha^{-1}		———	%	———	kg ha^{-1}
flumioxazin	71.5	7 DBP[1]	84 AB[2]	81 A	89 A	4312 ABC
saflufenacil	50	7 DBP	67 AB	85 A	90 A	4063 C
pyroxasulfone	119	7 DAP[1]	90 A	88 A	90 A	4340 ABC
pendimethalin	1600	7 DAP	71 AB	45 B	83 A	4589 AB
metribuzin	17.5	21 DAP	85 A	91 A	81 A	4617 A
thifensulfuron	105	21 DAP	5 C	61 A	0 B	4153 BC
nontreated[3]	-	-	-[3]	-	-	3983 C

[1] Abbreviations: WAIT, wk after initial treatment; DBP, days before planting; DAP, days after planting.

[2] Treatment means within columns with no common letters are significantly different according to Tukey's HSD pairwise comparison and Fisher's protected LSD for visible control and yield, respectively ($p < 0.05$).

[3] Visible mouse-ear cress control data for non-treated were not included in analysis.

Table 14. Post-emergence herbicidal control of thifensulfuron resistant mouse-ear cress (*Arabidopsis thaliana*) and winter wheat injury and yield in Essex County, Virginia in 2015-16.

Herbicide	Control	Yield	Crop Injury					
			Site 1		Site 2		Site 3	
			3 WAT[1]	5 WAT	3 WAT	5 WAT	3 WAT	5 WAT
	%	kg ha^{-1}	%					
thifensulfuron	0 C[2]	3906 A	0 B	0 C	0 C	0 B	0 D	0 B
thifensulfuron + tribenuron	0 C	3904 A	0 B	0 C	0 C	0 B	0 D	0 B
2,4 D	95 A	4000 A	0 B	0 C	0 C	0 B	6 C	1 AB
dicamba	84 A	4110 A	21 A	20 A	21 A	20 A	21 B	6 AB
metribuzin	84 A	4085 A	0 B	0 C	0 C	0 B	35 A	10 A
bromoxynil	33 B	3731 A	0 B	0 C	0 C	0 B	0 D	0 B
fluroxypyr	46 B	3930 A	0 B	0 C	0 C	0 B	0 D	0 B
pyroxsulam	0 C	3732 A	20 A	12 B	7 B	0B	0 D	0B
nitrogen	0 C	3912 A	0 B	0 C	0 C	0 B	0 D	0 B
nontreated	-[3]	3784 A	-[3]	-	-	-	-	-

[1] Abbreviation: WAT, wk after treatment.

[2] Treatment means within columns with no common letters are significantly different according to Tukey's HSD pairwise comparison ($p < 0.05$).

[3] Visible mouse-ear cress control and injury data for non-treated were not included in analysis.

Table 15. Post-emergence herbicidal control of thifensulfuron resistant mouse-ear cress (*Arabidopsis thaliana*) under green-house conditions in Blacksburg, Virginia in 2015-16.[1]

Herbicide	Run 1			Run 2		
	2 WAT[2]	4 WAT	6 WAT	2 WAT	4 WAT	6 WAT
	%					
thifensulfuron	49 BC[3]	54 B	45 B	11 C	0 B	0 B
thifensulfuron + tribenuron	38 C	20 C	6 C	2 D	10 B	13B
2,4-D	84 AB	100 A	100 A	65 AB	95 A	100 A
dicamba	77 B	100 A	100 A	60 AB	86 A	100 A
metribuzin	100 A	100 A	100 A	67 A	80 A	91 A
bromoxynil	71 AB	21 C	20 BC	33 C	0 B	0 B
fluroxypyr	65 BC	100 A	100 A	60 AB	98 A	100 A
pyroxsulam	36 C	32 BC	23 BC	20 C	26 B	4 B
bromoxynil + pyrasulfotole	100 A	100 A	100 A	35 BC	75 A	83 A

[1] Visible mouse-ear cress control data for non-treated were not included in analysis.

[2] Abbreviation: WAT, wk after treatment.

[3] Treatment means within columns with no common letters are significantly different according to Tukey's HSD pairwise comparison ($p < 0.05$).

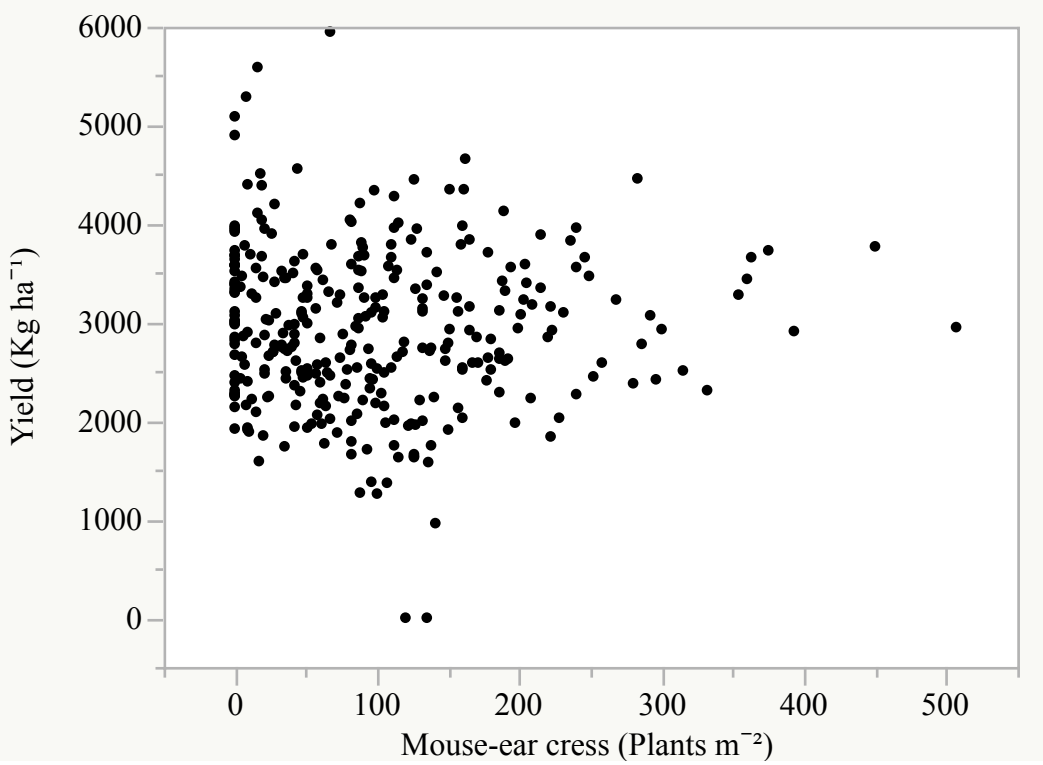

Figure 6. Relationship between mouse-ear cress density (x-axis) and winter wheat yield (y-axis) in Essex County, Virginia in 2015-16. Linear and non-linear regression were not significant.