Investigation of herbicide resistance in Japanese brome (*Bromus japonicus*) and responses of grain sorghum (*Sorghum bicolor*), & corn (*Zea mays*) to multiple herbicides at high-temperature stress

by

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Abstract

Weed infestation poses a significant challenge to crop production. Post-emergence (POST) herbicides are commonly used to control weeds in crops including, wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L. Moech), and maize or corn (*Zea mays* L.). Unfortunately, an inevitable consequence of the repeated and indiscriminate use of herbicides is the evolution of herbicide resistance in weeds. In the US, recently the first case of resistance to POST-applied acetolactate synthase (ALS)-inhibitor herbicides was found in Japanese brome *(Bromus japonicus* Thumb.), a problem weed in winter wheat (focus of Chapter 2). Although POST herbicides are excellent options for weed control, some of these herbicides are also known to cause crop injury, when exposed to abiotic stresses such as high-temperature (heat) stress, which was investigated in sorghum and corn (Chapters 3 and 4). The objectives of this thesis were to 1) confirm and characterize the ALS-inhibitor resistance in Japanese brome; 2) assess the response of grain sorghum to POST herbicides under high-temperature stress; and 3) evaluate corn response to POST herbicides under high-temperature stress. Experiments were conducted either in the greenhouse or in controlled environmental growth chambers. In objective 1, the level of resistance and mechanism of resistance to ALS-inhibitors were investigated in three Japanese brome populations, R1, R2 and R3. Results indicate ~ 167-, ~ 125-, and ~ 667-fold resistance to ALS-inhibitor, propoxycarbazone-Na in R1, R2, and R3, respectively, compared to a susceptible population. Additionally, mutations in the *ALS* gene resulting in amino acid substitutions i.e., Proline-197-Threonine in R3, R1 and Proline-197-Serine in R2, R1 were identified. The resistant populations also exhibited cross-resistance to other ALS-inhibitors, such as sulfosulfuron, mesosulfuron, pyroxsulam, and imazamox. Cross resistance to imazamox possibly via

cytochrome P450 enzyme mediated metabolism was also found in R3 population. To achieve objective 2, grain sorghum genotypes, RTx430 and P84G62, were selected and grown in growth chambers maintained at two temperature regimes: optimum (OT: 32/22 °C; day/night (d/n)) and high-temperature (HT: 40/30 °C; d/n) and were treated separately with herbicides, e.g., 2,4-D, pyrasulfotole + bromoxynil, or mesotrione. Results revealed that at HT, grain sorghum is prone to more herbicide injury when treated with mesotrione or pyrasulfotole + bromoxynil but not with 2,4-D. Additionally, P84G62 had less injury compared to RTx430, irrespective of temperature or herbicide treatments. In objective 3, a similar experimental procedure was used, and corn genotype DKC59-82RIB, plants were grown at OT (30/25 °C; d/n) and HT (40/35 °C; d/n) and treated separately with 2,4-D, dicamba, mesotrione, tembotrione, or atrazine. The results indicate that at HT, corn plants exhibit more injury when treated with the above herbicides than at OT. Overall, the outcome of this research confirms alterations in the target site of ALS-inhibitors bestow resistance in Japanese brome. More importantly, the results also highlight that under HT stress, grain sorghum and corn are prone to POST-applied herbicide injury. These findings emphasize the necessity of sustainable weed management strategies amidst challenges like evolving herbicide resistance and changing climate conditions.

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Dedication

I would like to dedicate my work to my parents who I very fondly call as amma and nanna and my brother, thammu.

Chapter 1 - Introduction and Literature Review

1.1 Evolution of herbicide resistance in weeds and their impact

Before the expansion of modern agricultural practices, farmers across the globe primarily used methods like hand weeding, hoeing, primitive tools, animal- and mechanically-powered implements to control weeds. The chemical method of weed control using herbicides began much later during the time of World War- II, with the discovery of synthetic auxin-like compounds such as 2,4-D; and 2,4,5-T with herbicidal properties (Peterson et al., 2016). This discovery further led to the development and commercialization of the first modern herbicide 2,4-D in 1944, after realizing its ability to selectively control broadleaf weeds in lawns, rangelands and monocot crop fields. (Hamner & Tukey, 1944; J. W. Mitchell et al., 1944; Quastel, 1950). As a consequence of repeated use of these herbicides, the first case of 2,4- D resistance was reported in wild carrots (*Daucus carota* L.) in 1957; which was also the first case of herbicide resistance in the world (Switzer, 1957; Whitehead & Switzer, 1963). However, it was only in 1968, that researchers and weed scientists have recognized the threat of herbicide-resistant weeds when a case of common groundsel (*Senecio vulgaris* L.) resistance to atrazine and simazine was discovered (Ryan, 1970). The evolution of herbicide resistance in weeds is a major threat to present-day agriculture as weeds are the major pests in crop production and cause severe yield losses (Oerke, 2006). Currently, there are 523 unique cases of herbicide resistant weeds globally (Heap, 2023). According to Prather et al (2000), herbicide resistance is defined as -" **the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide that would normally be lethal to the wild type."**

Widespread adoption to no-till farming led to dependence on herbicides for weed control. As a result, several herbicides with different modes of action like photosystem- II (PS-II)-,

acetolactate synthase (ALS)-, acetyl-CoA carboxylase (ACCase)-, enolpyruvylshikimate-3-phosphate synthase (EPSPS)-, 4-hydroxyphenylpyruvate dioxygenase (HPPD)- and protoporphyrinogen oxidase (PPO)-inhibitors have been used extensively in agriculture. Among these groups, ACCase and ALS-inhibitors were widely used by growers for post-emergence grass weed control and for both pre- and post-emergence grass and broadleaf weed control respectively (Takano et al., 2020; Boe, 2019). From 1980-95, a 5-fold increase in herbicide resistance cases were reported due to the selection pressure from the repeated use of these herbicides groups (Shaner, 2014). A further revolution in weed management technology happened during the late 90s, with the introduction of first-ever genetically modified glyphosate-resistant (GR) crops like canola (*Brassica napus* L.), soybean (*Glycine max* L. Merril) (1996) and corn (1998) with resistance to glyphosate under the trade name Roundup Ready ® and were rapidly adopted by the farmers (James, 2012).

With increased use of these herbicides, the first case of GR weed was rigid ryegrass (*Lolium rigidum*) from Australia discovered in 1996 (Powles et al., 1998). However, within four years of its introduction in the US, the first case of glyphosate-resistant horseweed (*Conyza canadensis* L. Cronq) population was identified in a GR soybean field of Delaware in 2001 and later in many other weeds species adding further disappointment to the growers and narrowing the herbicide options for weed management (VanGessel, 2001; Heap, 2023).

As of 2022, the global market value of herbicides accounted for US$ 31.5 billion, out of which the north American countries, including the US and Canada accounts for US$ 14.4 (45.7%) billions (www.marketresearchfuture.com). Also, the widespread adoption of genetically modified herbicide resistant crops to glyphosate, 2,4-D, dicamba in the last few years has now led them to occupy more than 90 % of the planted acres of U.S. corn, upland cotton (*Gossypium*

sp.), and soybeans (USDA-ERS 2020). To date globally, a total of 522 unique cases of herbicide resistance among 269 weed species to different sites of action have been reported (Heap, 2023). Out of all the countries, US holds the first place with 131 resistant weed species reported from different states of the country (Heap, 2023).

1.1.1 Mechanism(s) of herbicide resistance

Herbicide resistance in weeds is an evolutionary process in which resistant populations adapt to the intense selection pressure imposed by herbicides (Jasieniuk et al., 1996). Weeds evolve resistance through either of following mechanisms: a) target-site (TS) or non-target site-based mechanism (NTS) (Délye et al., 2013; Powles & Yu, 2010; Yuan et al., 2007). In TS, the resistance is bestowed mainly due to alteration in the target site, mostly an enzyme, reducing its affinity to the herbicide (Murphy & Tranel, 2019). Apart from this, TS mechanism also includes increased gene expression and amplification of herbicide target gene (Gaines et al., 2020; Sammons & Gaines, 2014) . NTS, on the other hand, involves one or more combination of resistance mechanisms such as decreased herbicide penetration into the plant, decreased rates of herbicide translocation and increased rates of herbicide sequestration/metabolism resulting in a non-lethal amount of herbicide reaching to the target site (Powles & Yu, 2010).

TS resistance to PS-II-inhibitors conferred by target-site mutation in the *psb A* gene resulting in Ser-264-Gly substitution was first reported in smooth pigweed (*Amaranthus hybridus*). (Hirschberg & McIntosh, 1983). Later, several other mutations conferring resistance against herbicides mostly belonging to ALS-inhibitors and ACCase-inhibitors were identified (Heap, 2023). In another TS mechanism, gene overexpression (or up regulation) and gene duplication (more EPSPS gene copy numbers) were identified conferring resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) and Palmer amaranth (*Amaranthus palmeri* S. watson) respectively

(Baerson et al., 2002; Gaines et al., 2010). Some of the most important and common type of TS mechanism with altered site, are reported among weed species, resistant to herbicides belonging to PS II-, ALS-, ACCase- or EPSPS-inhibitors, although widespread for ALS-inhibitor resistance (Délye et al., 2015; Heap 2023; Powles & Yu, 2010).

NTS, on the other hand, includes those resistance mechanisms that do not alter target-site but reduction in the amount of herbicide reaching the target site. Reduced herbicide absorption resulting in resistance is uncommon and was found in a few cases like 2,4-D resistance in prickly lettuce (Riar, Burke, et al., 2011); atrazine resistance in annual bluegrass (Svyantek et al., 2016), glyphosate resistance in Italian ryegrass (Ghanizadeh et al., 2016) and Johnsongrass (Riar et al., 2011). In a glyphosate-resistant rigid ryegrass (*Lolium rigidum*) population from Australia, first case of resistance due to reduced glyphosate translocation was reported (Bostamam et al., 2012). However, a common NTS mechanism in weed species is metabolic resistance as a result of detoxification of herbicides into non-toxic substances (Rigon et al., 2020). The herbicide metabolism in plants is primarily mediated by biochemical reactions, including i) Phase-I, which predominantly driven by cytochrome P450 monoxygenases (P450s; EC 1.6.2.4) involves oxidation, hydroxylation, deamination, and dealkylation of the parent herbicide reducing phytotoxicity and facilitating subsequent metabolic phase; ii) Phase-II, driven mostly by glutathione- *S* -transferases (GSTs; EC 2.5.1.18) mediated conjugation of the herbicides or its metabolites with glutathione, amino acids and sugars; and iii) Phase-III, because of the activity of ATP-binding cassette (ABC) transporters on the tonoplasts, leading to compartmentalization by sequestering the herbicide metabolites into vacuole or cell wall materials (Gaines et al., 2020; Riar, Burke, et al., 2011).

Metabolism-based resistance is found in several weed species against herbicides belonging

mainly to ALS-inhibitors, PS-II, ACCase-inhibiotors and synthetic auxins including Italian ryegrass (*Lolium perenne* L.) (Kaundun, 2010); Japanese foxtail (*Alopecurus japonicus* Steud.) (Feng et al., 2016); rigid ryegrass (*Lolium rigidum* Gaud.) (Busi et al., 2011; Gaines et al., 2014; Han et al., 2016); wild oat (*Avena fatua* L.) (Ahmad-Hamdani et al., 2013); barnyard grass (*Echinochloa crus-galli* P. Beauv.) (Riar et al., 2012); common waterhemp (*Amaranthus tuberculatus*) (Evans Jr et al., 2017; Figueiredo et al., 2018; Shergill et al., 2018; Vennapusa et al., 2018); Palmer amaranth (*Amaranthus palmeri* S. Watson) (Chahal et al., 2019; Nakka, Godar, Thompson, et al., 2017); rigid brome (*Bromus rigidus* Roth.) (Owen et al., 2012); common ragweed (*Ambrosia artemisiifolia* L.) (Simard et al., 2017); annual bluegrass (*Poa annua* L.) (Svyantek et al., 2016); wild radish (*Raphanus raphanistrum* L.) (Lu et al., 2019). Metabolic resistance can lead to cross-resistance/multiple resistance to herbicides belonging to more than one mode of action and is a major threat to weed control and crop production (Rigon et al., 2020; Yu & Powles, 2014). In this thesis, the resistance mechanism to ALS-inhibitors is investigated and hence the following section discuss more details about resistance to this group of herbicides.

1.1.2 ALS-inhibitor resistance in weeds

Since its introduction, resistance to ALS-inhibitors in weeds has been a major concern. The first ever case of ALS-inhibitor resistance for chlorsulfuron, a sulfonyurea herbicide, was reported in prickly lettuce (*Lactuca serriola* L.) and kochia (*Bassia scoparia* (L.) AJ Scott) in 1987, only 5 years after the introduction into the market (Mallory-Smith et al., 1990; Saari et al., 1990) . The ALS-inhibitors are categorized further into chemical families like sulfonylurea (SUs), sulfonylaminocarbonyltriazolinones (SCTs), imidazolinones (IMIs), pyrimidinyl benzoates (PTB) sand triazolopyrimidines (TPs). Herbicides belonging to these groups hinder the growth

and development of the plants by inhibiting the acetolactate synthase (ALS; EC 2.2.1.6) enzyme, also known as acetohydroxyacid synthase (AHAS) enzyme which is crucial in the biosynthetic pathway of branched chain amino acids like valine, leucine and isoleucine (De Felice et al., 1974; Ray, 1984).

The introduction of ALS-inhibitors was considered a breakthrough in weed management as it offered several advantages to farmers like high efficacy at lower concentrations, lower toxicity in mammals, high residual activity, high selectivity and effective control of most broadleaves and some grass weeds (Mazur & Falco, 1989; Saari & Mauvais, 2018; Whitcomb, 1999). As a result, many herbicides were discovered by different manufacturers belonging to this mode of action over the years (Tranel & Wright, 2002). As of today, a total of 58 ALS inhibiting herbicide actives have been discovered and commercialized under different trade names globally for chemical weed control (Heap, 2023). Currently, ALS-inhibitors are ranked as the group with the highest number of unique cases of resistance totaling 172 cases among 105 dicots and 67 monocots globally (Heap, 2023). Several reasons such as high selection pressure exerted through repeated use, worsened by its high herbicide efficacy on susceptible populations, little or no use of alternative herbicide mode of action and higher soil residual activity of the ALS-inhibitors (Tranel & Wright, 2002).

Resistance to ALS-inhibitors is mostly conferred by single amino acid substitutions at one of the eight conserved amino acid positions (Ala122, Pro197, Ala205, Asp376, Arg377, Trp574, Ser653 and Gly654) and multiple substitutions at a single amino acid position (Heap, 2023; Tranel & Wright, 2002). These mutations though affects plant growth and development, hasn't shown much effect on plant fitness and are mostly conferred and inherited by a single, dominant nuclear-coded genes; supporting the rapid evolution of ALS-inhibitor resistant populations (Holt

& Thill, 2018; Tranel & Wright, 2002). Cross-resistance to different ALS-inhibitors is common in weed species and is dependent on the type of amino acid substitution. The amino acid substitution Ala-122-Thr generally confers high level of resistance to IMI herbicides; Pro197 substitutions mainly to SU herbicides and moderately to TP herbicides; Ala-205-Val confers resistance to IMI herbicides; Asp-376-Glu and Trp-574 substitutions to all ALS-inhibitor classes; Arg-377-His to SU herbicides only; Ser-653 substitutions to IMI and moderately to PTB herbicides; and Gly-654 substitutions to IMI herbicides (Beckie & Tardif, 2012; Tranel & Wright, 2002). In addition to TS, NTS resistance due to rapid metabolism of ALS-inhibitors by cytochrome P450 monooxygenases, has also been reported in several ALS-resistant weed biotypes (Powles & Yu, 2010).

In winter wheat production systems, for more than two decades farmers have been using ALS-inhibitors controlling weeds across the wheat growing regions in the US. As a result, several cases of ALS-inhibitor resistance have been reported in this crop. Some of the ALS-inhibitor resistant weed species include bushy wallflower (*Erysimum repandum* L.) (Peterson et al., 2006); flixweed (*Descurainia Sophia* L.) (Peterson et al., 2009); downy brome (*Bromus tectorum* L.) ((Kumar & Jha, 2017); henbit deadnettle (*Lamium amplexicaule* L.) (Varanasi et al., 2016); Italian ryegrass (*Lolium multiflorum* Lam.) (Chandi et al., 2011; Ellis et al., 2008; Kuk & Bugos, 2007; Tehranchian et al., 2019); wild buckwheat (*Polygonum convolvulus* L.) (Pandian et al., 2020) mostly conferred due to mutations in the target site. However, in some weed species including Lolium sps (Busi et al., 2020; Busi et al., 2018; Yanniccari et al., 2020); rigid brome (Owen et al., 2012); downy brome (*Bromus tectorum* L.) (Park & Mallory-smith, 2004); feral rye (*Secale cereale* L.) (Pester et al., 2001), ALS-inhibitor resistance mainly conferred due to enhanced metabolism by cytochrome P450 enzymes. One such case of ALS-inhibitor resistance

was also reported in Japanese brome US wheat fields. Japanese brome (*Bromus japonicus* Thumb.) is one of the problematic winter annual weed in crops such as wheat (Germino et al., 2016; Haferkamp et al., 2001) and is generally controlled by acetolactate synthase (ALS)-inhibitors. Repeated use of ALS-inhibitor, propoxycarbazone-Na resulted in the evolution of resistance in three Japanese brome populations, i.e., R1, R2, and R3 in Kansas (KS). Characterization and investigation of resistance to ALS-inhibitors in these three Japanese brome populations is the focus of Chapter 2.

1.2 Importance of Weed Management in Grain Sorghum and Corn

Globally, weeds are the major pests in crop production causing up to 34% yield losses (Oerke, 2006). Weeds compete with the crops for several resources like water, nutrients, light and aid in completing the life cycle of several pests and diseases. Yield losses through weeds depends on several factors like weed emergence, weed density, type of weeds and crops (Chauhan, 2020). As discussed earlier, weed management through herbicides plays a crucial role in controlling weeds in crop fields of the US. In this section, the importance of two important summer crops (grain sorghum and corn) and its weed management is discussed.

1.2.1 Grain Sorghum

Grain sorghum is the fifth largest grown cereal in the world. This crop is grown for a variety of purposes like grain for food and feed, stem for fuel and bioethanol production (Dahlberg et al., 2011; Kimber et al., 2013; Venkateswaran et al., 2019). It is mostly grown in the arid and semi-arid regions of Asia, sub-Saharan Africa, middle east, Mexico and US (Mundia et al., 2019). It belongs to the Poaceae family and is a C_4 plant with high photosynthetic efficiency. For the year 2022/2023, globally the production of sorghum was 57.16 million metric tons (MT) mostly from countries like Nigeria, Sudan, Mexico, US, Ethiopia and others. Among these

countries, the US ranks 4th accounting for 8% of the global sorghum production, a sharp downfall from its first rank with 18% of global production in previous year (2021/22) (Hgilly, 2022). The major reasons for such decrease in acreage of grain sorghum production in the US is attributed to drought, high temperature stress, supply issues and out of control input costs (Perkins 2023). Most of the production in the US is predominantly from states like Kansas. As of 2022, Kansas is the leading producer of grain sorghum in the US.

Weeds are considered to be the major pests in grain sorghum fields with most crop-weed competition occurring during the early slow growing stages of sorghum (Burnside & Wicks, 1967; Dille et al., 2020a; Wiese et al., 1964). Both broadleaf and grass weeds are known to infest sorghum fields. Some of the most important weeds include grass weed species like large crabgrass (*Digitaria sanguinalis* (L.) Scop.) foxtail (*Setaria* spp.), shattercane (*Sorghum bicolor* (L.) Moench subsp. drummondii), longspine sandbur (*Cenchrus longispinus* (Hack.) Fernald), prairie cupgrass (*Eriochloa contracta* Hitchc.), fall panicum (*Panicum dichotomiflorum* Michx.), and witchgrass (*Panicum capillare* L) and annual broadleaf weeds like kochia (*Bassia scoparia* (L.) Schrad.), russian thistle (*Salsola kali* L.), palmer amaranth (*Amaranthus palmeri* (L.) Wats.), waterhemp (*Amaranthus rudis* L.), redroot pigweed (*Amaranthus retroflexus* L.), tumble pigweed (*Amaranthus albus* L.), venice mallow (*Hibiscus trionum* L.), velvetleaf (*Abutilon theophrasti* Medik.), common cocklebur (*Xanthium strumarium* L.), morningglory (*Ipomoea* spp.), devil's claw (*Proboscidea louisianica* (Mill.) Thell.), and common sunflower (*Helianthus annuus* L.) (Dille et al., 2020b; Thompson et al., 2019). If left uncontrolled, these weeds can potentially cause 47% yield loss in grain sorghum in the US (Dille et al., 2020). Therefore, weed management in grain sorghum is crucial.

Use of pre-(PRE) and post-(POST) emergence herbicides is commonly adopted for weed control in grain sorghum (Dille et al., 2020a; Pandian et al., 2022; Thompson et al., 2019). Most of the PRE herbicides include atrazine used by itself or tank-mix in combination with alachlor, dimethenamide, or metolachlor. However, these herbicides in sorghum growing areas are less effective especially on grasses because of dry and low soil moisture conditions (Hennigh et al., 2010). Very few or no broad-spectrum POST herbicide options are available for use in sorghum as, this crop is susceptible to several POST herbicides (Abit et al., 2011; Pandian, 2020; Regehr et al., 2008; Smith et al., 2010; Thompson et al., 2019). Commonly used POST herbicides include bromoxynil, 2,4-D, dicamba, prosulfuron, fluroxypyr, carfentrazone, or halosulfuron. Nonetheless, grain sorghum is often found to be injured from the application of these herbicides under unfavorable environmental conditions (Smith et al., 2010).

1.2.2 Corn

Corn is the most widely grown cereal in the world for both feed and biofuel production. It is a C_4 crop with wider adaptability. Global production of corn is estimated at 1,161.86 million MT for the year 2022/23 (USDA-FAS, 2023). High versatility and productivity accompanied by several breeding achievements and huge markets in the last few decades made corn more attractive to farmers worldwide. The US is the largest producer of corn in the world with total production of about 348.75 million MT in the year 2022/23 (Statista, 2023). Most of the corn in the US is produced in states of Iowa, Illinois, Nebraska, Indiana, Minnesota, Kansas, South Dakota, Ohio, Missouri and Wisconsin (USDA-NASS, 2023).

Weeds are the major pests effecting corn production worldwide (Oerke, 2006). Weeds compete with corn plants for resources like light, nutrients, space, and moisture, which affects the crop's morphology and phenology, lowers yield, makes harvesting challenging, and degrades grain

quality (Jhala et al., 2014). Therefore, it is important to control weeds in these regions. In the US, farmers mostly rely on herbicides for weed control as its effective and economical (Jhala et al., 2014). The most common weeds in corn are common lambsquarters (*Chenopodium album* L.), yellow and green foxtail (*Setaria* spp.), waterhemp, redroot pigweed (*Amaranthus retroflexus*) smooth pigweed (*Amaranthus hybridus*), palmer amaranth, ivyleaf (*Ipomoea hederacea*) and pitted morningglory (*Ipomoea lacunosa*) etc. When weed control is delayed, these weed species that compete with corn plants have been seen to reduce yield by as much as 65% (Page et al., 2012).

Herbicide-resistant corn hybrids (e.g., glyphosate-, glufosinate-, or imidazolinone-resistant corn) are heavily used in corn-based cropping systems in the US (Jhala et al., 2014). PRE herbicides like atrazine, acetochlor, isoxaflutole, saflufenacil etc are labelled for use in corn. Most of the PRE herbicide applications require rainfall within 7days of application for activation and to provide effective weed control (Hoeft et al., 2000). Several POST herbicides including 2,4-D, dicamba, tembotrione, mesotrione, isoxaflutole, atrazine and ALS-inhibitors like nicosulfuron, primisulfuron, and thifensulfuron are known to provide effective control of weeds in corn.

1.3 Post-Emergence Herbicide Options for Weed Control in Grain Sorghum and Corn

POST weed control is extremely important in any crop production systems including corn. The effective POST herbicide options available for weed management in both sorghum and corn belong to herbicides families PS-II inhibitors and synthetic auxins; while HPPD-inhibitors can also safely used in corn as POST, but not in sorghum due to crop injury.

1.3.1 PS-II inhibitors

PS II- inhibitors, including those in triazine family block the electron transport in PS-II by binding to the Q_B – site displacing plastoquinone, thereby affecting photosynthesis (Moreland et al., 1959; Trebst, 2008). Atrazine and simazine are the two most commonly used selective triazine herbicides and are registered for use in both grain sorghum and corn, to control both broadleaf and grass weeds. Corn is tolerant to atrazine by converting it into non-toxic substances through glutathione conjugation whereas grain sorghum metabolizes atrazine via the N-dealkylation pathway (Shimabukuro et al., 1968, 1970) . Apart from this corn also detoxifies atrazine through cytochrome P450 enzymes (Ohkawa et al., 1999).

Huskie®, a premixed commercial formulation of pyrasulfotole and bromoxynil, is known to provide broadleaf weed control (Watteyne et al., 2008). Pyrasulfotole when supplemented with bromoxynil has a synergistic effect (Abendroth et al., 2006; Hugie et al., 2008). When applied together, pyrasulfotole a HPPD-inhibitor (discussed later in this section) inhibits the synthesis of plastoquinone, bromoxynil a PS-II-inhibitor can be effective at lower rates; thereby can provide a better control of weeds (Sutton et al., 2002). Several studies conducted in grain sorghum, showed minimum crop injury to this combination and proved to be valuable tool for weed control including ALS-, triazine- and synthetic auxin-resistant weeds (Fromme et al., 2012; Lally, 2011; Reddy et al., 2013)

1.3.2 Synthetic Auxins

Synthetic auxin herbicides (SAH), mimic the functioning of the natural plant hormone auxins, indole aceticacid (IAA) (Grossmann, 2000, 2003). Due to the higher stability of SAH over IAA in plants, these herbicides at higher concentration can affect plant growth and development, leading to death of the plant (Grossmann, 2007). Application of the SAH results in ethylene

biosynthesis causing symptoms like epinasty, tissue swelling and initiation of stem curling or subsequently accumulation of abscisic acid followed by the release of reactive oxygen species (ROS); ultimately causing necrosis and plant death (Grossmann, 2010). Reduced uptake and translocation along with enhanced metabolism are responsible for the selectivity of SAH in monocots. 2,4-D and dicamba are commonly used POST herbicides in corn and sorghum. The selectivity of these herbicides are achieved mainly due to the detoxification of herbicide via ring hydroxylation by cytochrome P450 monooxygenase in corn and grain sorghum (Montgomery et al., 1971; Peterson et al., 2016).

1.3.3 HPPD-inhibitors

HPPD-inhibitors, are one of the more recent herbicide options accessible to farmers for the management of both broadleaf and some grass weeds (Van Almsick, 2009). These herbicides inhibit the HPPD enzyme involved in the conversion of 4-hydroxyphenyl pyruvate to homogenitase, an intermediate in tocopherol and plastoquinone biosynthesis and subsequently carotenoid biosynthesis (Beaudegnies et al., 2009; Lee et al., 1998). The carotenoids are the light harvesting molecules by quenching the triplet state chlorophyll molecules, thereby preventing the generation of highly reactive singlet oxygen molecules and plays a major role in protecting the chlorophyll from photodestruction (Anderson & Robertson, 1960; Ritz et al., 2000; Young, 1991). Plants treated with these inhibitors generally show bleaching symptoms because of chlorophyll pigments destruction. Most of the HPPD-inhibitors are broadly classified into three chemical families namely isoxazoles, pyrazolones, and triketones (van Almsick, 2009) Mesotrione and tembotrione, belonging to the triketone family are the most commonly used HPPD-inhibitors as both pre- and post-emergence applications for weed control. Mesotrione is a selective herbicide commercially used as both pre and post emergence in corn and only as pre-

emergence in grain sorghum for broadleaf weed and grass weed control (Mitchell et al., 2001; Takano et al., 2016). Mesotrione is selective to corn due to slower uptake and mainly through rapid metabolism by cytochrome P450 enzymes (Hawkes et al., 2001; Mitchell et al., 2001). Various researchers found several grain sorghum varieties that are also tolerant to the foliar-applied mesotrione (Abit et al., 2009; Pandian et al., 2023). In almost all of these varieties, the tolerance towards mesotrione is also due to the rapid metabolism through cytochrome P450 enzyme (Abit & Al-Khatib, 2009; Pandian et al., 2023). Since, no significant yield reduction is observed among these varieties even with injury symptoms at 1 and 2 weeks, mesotrione can also be a potential post-emergence herbicide option for grain sorghum in the future (Abit et al., 2009).

Tembotrione, another important selective triketone herbicide is registered only in corn for post emergence weed control. Rapid metabolism of tembotrione into nontoxic substances provides selectivity in corn which can be further enhanced from the safeners like isoxadifen-ethyl included in the commercial formulations (Santel, 2009; Schulte & Köcher, 2009). Many researchers found that grain sorghum is mostly sensitive to tembotrione applications and cannot be considered as viable herbicide option to farmers (Cunha et al., 2016; Dan et al., 2010; Krishnamurthy et al., 2021). Hence, both tembotrione and mesotrione can be effectively used in corn, whereas in grain sorghum it is phytotoxic and is not labelled for use.

1.4 Effect of High-Temperature Stress on Herbicide Efficacy

Climatic conditions like light, temperature, carbon dioxide, relative humidity, precipitation, soil moisture and wind are known to influence the herbicide efficacy (Varanasi et al., 2016). The influence of temperature on herbicide is both direct and indirect (Varanasi et al., 2016). Temperature stress can affect functioning of several physiological processes including the

cuticular development, translocation, photosynthetic and metabolic pathways important for plant growth and development (Hull et al., 1975; Van Der Zanden, 2008; Varanasi et al., 2016). Temperature stress can alter the uptake, translocation, and/or metabolism of the herbicides which can directly influence the foliar-applied herbicide efficacy in plants (Jursík et al., 2023). Elevated temperature can either improve the herbicide efficacy by increasing the absorption and translocation or can reduce the efficacy by enhanced herbicide metabolism (Johnson & Young, 2002). Herbicides, mostly used for the control of summer annual weeds in crops like corn and grain sorghum are often exposed to high temperature stress.

The effect of temperature on herbicide efficacy was reported in several weeds; nonetheless the information in this area is still limited. For example, in common and giant ragweed, high temperatures (29/17 °C day/night temperatures (d/n)) improved the efficacy of 2,4-D and glyphosate due to increased translocation (Ganie et al., 2017). However, in waterhemp, rapid metabolism of 2,4-D at high temperature (34/20 °C, d/n) resulted in reduction in the herbicide efficacy and increased the level of resistance in a 2,4-D-resistant population (Shyam et al., 2019). In kochia, reduced absorption and translocation of dicamba and glyphosate under elevated temperatures (32.5/22.5 °C, d/n) resulted in decreased herbicide efficacy (Ou et al., 2018). Enhanced metabolism at high temperatures (40/30 °C, d/n) decreased the efficacy of mesotrione in Palmer amaranth (Godar et al., 2015). High temperatures reduced the efficacy of 2,4-D due to enhanced metabolism, indicating that temperature can influence the metabolism of the herbicide in Palmer amaranth (Rudell et al., 2023). It is also evident that from several of the temperature studies that the effect of high temperature stress on herbicide efficacy varies from species to species (Johnson & Young, 2002).

1.5 Summary and Objectives of the Research

Herbicide resistance in weeds and high temperature stress affecting herbicide efficacy thereby crop injury are challenges for sustainable weed management. In 2007, lack of control of a population of Japanese brome with ALS-inhibitors was reported from the wheat fields in Kansas (Heap, 2023). Later, two additional populations were also reported with poor control of this weed with ALS-inhibitors in other locations in Kansas. All these fields have history of extensive use of propoxycarbazone-Na, an ALS-inhibitor. These populations of the Japanese brome with lack of control with ALS-inhibitors are the first cases in the US (Heap 2023). Similar incidence of ALS-inhibitor-resistant Japanese brome populations were recently reported in China (Lan et al., 2022; Li et al., 2022). The Chinese populations exhibit TS mutations conferring a high level of resistance to ALS-inhibitors. However, the level of resistance or mechanism conferring resistance to ALS-inhibitors in Japanese brome from Kansas is unknown, which is investigated the Chapter-2 of this thesis.

Crops like corn and sorghum are tolerant to POST applied herbicides (e.g., 2,4-D, dicamba, atrazine or triketones) as they can detoxify these herbicides to non-toxic substances. When grown under stress free environments these crops are less prone to herbicide injury. However, under adverse climatic conditions including high temperature stress, crops can become more sensitive to herbicide injury (Bagavathiannan et al., 2017). In most cases, high temperatures reduce herbicide efficacy in weeds which may also help in reduce herbicide injury to crops (Matzrafi et al., 2016). However, in some cases, high temperature stress can also increase herbicide efficacy leading to more injury to crops (Masiunas & Weller, 1988). This suggests that the effect of high temperature stress on herbicide efficacy is species and/or herbicide-specific. Almost no literature is available on the effect of high temperature stress on herbicide efficacy in

crops. Therefore, studying the influence of high temperature stress on herbicide efficacy in corn and grain sorghum is important.

The overall goal of this thesis was a) to confirm and characterize the mechanism of ALS-inhibitor resistance in Japanese brome populations from Kansas and b) to study the effect of high temperature stress on POST herbicide efficacy in corn and sorghum. The specific objectives include:

Chapter-2:

1) Evaluate the level of resistance to ALS-inhibitors in three, i.e., R1, R2, R3 populations of Japanese brome in comparison with a known susceptible population (S1).
2) Investigate the mechanism of resistance (TS or NTS) conferring ALS-inhibitor resistance.
3) Evaluate cross-resistance to other ALS-inhibitors in these populations.

Chapter-3:

1) Evaluate the effect of temperature (optimum or high) stress on efficacy of mesotrione, 2,4-D and pyrasulotole + bromoxynil (Huskie®) in grain sorghum.

Chapter-4:

1) Evaluate the effect of temperature (optimum or high) stress on efficacy of 2,4-D, dicamba, mesotrione, tembotrione and atrazine in corn.

Chapter 2 - Confirmation and Characterization of the First case of Acetolactate Synthase (ALS)- Inhibitor Resistance in Japanese Brome (*Bromus japonicus*) in the US

Abstract

Japanese brome (*Bromus japonicus* Thumb.) is one of the problematic winter annual weeds in crops such as wheat (*Triticum aestivum* L.) and is generally controlled by acetolactate synthase (ALS)-inhibitors. Repeated use of ALS-inhibitor, propoxycarbazone-Na resulted in the evolution of resistance in three Japanese brome populations, i.e., R1, R2, and R3 in Kansas (KS). However, the level of resistance and mechanism conferring resistance in these populations is unknown. The objectives of this research were to: 1) evaluate the level of resistance to propoxycarbazone-Na in R1, R2, and R3 in comparison with a known susceptible population (S1) of *Japanese brome*, 2) investigate the mechanism of resistance involved in conferring ALS-inhibitor resistance in these populations and 3) investigate the cross-resistance to other ALS-inhibitors. Results from the dose-response (0 to 16x; x = 44 g ai ha^{-1} of propoxycarbazone-Na) assay indicated ~167, ~125, and ~667- fold resistance in R1, R2 and R3 populations, respectively, to this herbicide compared to S1 population. *ALS* gene sequencing confirmed the mutations resulting in amino acid substitutions, i.e., Pro-197-Thr (R3, R1)/Ser (R2, R1) bestow resistance. Such amino acid substitutions also showed differential cross-resistance to sulfosulfuron, mesosulfuron, pyroxsulam and imazamox. Although pretreatment with malathion (cytochrome P 450 enzyme inhibitor), followed by propoxycarbazone-Na indicated no metabolic resistance to these herbicides in any of the resistant populations, however, cross-resistance to imazamox in R3 population potentially via P450 mediated metabolism was found Overall, these

results confirm the first case of target-site based resistance to ALS-inhibitors in Japanese brome in the US, highlighting the need for exploring alternative modes of action to enhance weed control in winter wheat.

2.1 Introduction

Japanese brome (*Bromus japonicus* Thunb.) is a non-native annual grass species introduced to North America from Eurasia. It is a close relative of other brome grasses like downy brome (*Bromus tectorum* L.) and is primarily found in the western great plains of the United States (US) (Germino et al., 2016; Haferkamp et al., 2001). It is a self-pollinating, diploid species (2n=2x=14) and competes with the native flora for nutrients and water, limiting the growth of the native plants, generally infesting rangelands and crop fields (Ogle et al., 2003; Tymo, 2022; Watson & Dallwitz, 1992). Japanese brome is a problematic weed in winter wheat fields as it can survive harsher winter climates equally or sometimes better than the hardiest winter wheat (*Triticum aestivum* L.) cultivars. (O'Connor et al., 1991). A yield loss of 2.11% to 2.24% for weed density of 4 plants/ m^{-2} of Japanese brome was reported in the winter wheat fields in China (Li et al., 2016). No such data of wheat yield loss by Japanese brome alone is available for the US. However, a moderate infestation of the brome grasses including Japanese brome caused 36% wheat yield loss in the United States (Stahlman, 1986). Therefore, management of the Japanese brome in winter wheat is warranted.

Weed management methods including prescribed burning and grazing are followed to control Japanese brome species in rangelands crops like western wheatgrass (*Pascopyrum smithii* P.A. Love) (DiTomaso et al., 2006; Mosley & Roselle, 2006; Whisenant & Bulsiewicz, 1985; Whisenant & Uresk, 1990). However, in winter wheat, the application of herbicides including acetolactate synthase (ALS)-inhibitors is commonly adopted by farmers. ALS-inhibitors control weeds by inhibiting the enzyme, acetolactate synthase (ALS; EC 4.1.3.18) also known as acetohydroxyacid synthase (AHAS; EC 2.2.1.6) involved in the biosynthesis of branched chain amino acids such as leucine, isoleucine, and valine, which are essential for plant growth and

development (De Felice et al., 1974; Ray, 1984)**.** Based on their chemistry, the ALS-inhibitor herbicides are divided into several chemical families, *viz.,* sulfonylureas (SUs; e.g., sulfosulfuron, mesosulfuron-methyl), sulfonylamino-carbonyl-triazolinones (SCTs; e.g., propoxycarbazone-Na), imidazolinones (IMIs; e.g., imazamox), pyrimidinyl benzoates (PTBs; e.g., pyrithiobac) and triazolopyrimidines (TPs; e.g., pyroxsulam). Propoxycarbazone-Na, sulfosulfuron, pyroxsulam are the common ALS-inhibitors used for controlling brome grass species and other broad-leaf weeds in wheat (Reddy et al., 2013). Recently, imazamox also became an effective option to control brome grasses with the introduction of IMIs tolerant Clearfield® wheat production systems.

Repeated use of ALS-inhibiting herbicides increased selection pressure and resulted in the evolution of weed populations resistant to these herbicides. Currently, a total of 172 weed species have been known to evolve herbicide resistance to ALS-inhibitors (Heap 2023). Two major mechanisms that confer resistance to herbicides in weeds include target-site resistance (TSR) and non-target site resistance (NTSR). TSR in weeds evolves as a result of mutation, amplification and/or increased expression of target genes of herbicide; while NTSR results from either reduced absorption, translocation or increased metabolism of herbicides (Gaines et al., 2020; Jugulam & Shyam, 2019; Powles & Yu, 2010). In most cases, the evolution of resistance to ALS-inhibitors was reported because of a single nucleotide alterations in in the *ALS* gene, resulting in amino acid substitutions in the herbicide target site (Boutsalis et al., 1999; Tranel & Wright, 2002). Multiple amino acid substitutions at eight specific sites (Ala122, Pro197, Ala205, Asp376, Arg377, Trp574, Ser653 and Gly654) in five conserved domains of ALS enzyme have been identified to confer resistance to these herbicides in weed species (Murphy & Tranel, 2019; Heap, 2023). NTSR due to enhanced metabolism via cytochrome P450 monooxygenase enzyme

activity has also been reported in several ALS-inhibitor resistant weed species (Powles & Yu, 2010b).

In 2007, lack of control of Japanese brome population with ALS-inhibitors was found in some wheat fields in KS (referred to as R1 population in this research). More recently, two more populations of Japanese brome with lack of control using these herbicides were also found in other winter wheat fields of KS (referred as R2 and R3). All the three populations survived the application of propoxycarbazone-Na, an SCT herbicide, which is generally effective in controlling these weeds in the winter wheat fields. However, the level of resistance and the mechanism conferring resistance to ALS-inhibitors in these populations were still unknown. Recently, a single nucleotide alteration in *ALS* genes was reported to confer resistance to ALS-inhibitors in a Japanese brome population in China (Lan et al., 2022; Li et al., 2022). Based on these reports, we hypothesized that the lack of control of R1, R2 and R3 of Japanese brome with ALS-inhibitors may also be due to modifications in the *ALS* genes. The objectives of this research were to: 1) evaluate the level of resistance in R1, R2, R3 Japanese brome populations in comparison with a known susceptible population (S1), 2) investigate the mechanism of resistance (TSR or NTSR) involved in conferring ALS-inhibitor resistance and 3) investigate the presence of cross-resistance to other ALS-inhibitors in these populations.

2.2 Materials and Methods

2.2.1 Plant materials and growing conditions

The three Japanese brome populations, R1, R2, R3 and a known susceptible population (S1) from KS were used in this experiment. The seeds were pretreated with gibberellic acid (1000 ppm) at 4 °C for 24 hours to improve the germination. The seeds were then transferred to a germination tray (21 by 6 by 4 cm) filled with potting mix (Pro-Mix® premium potting mix,

Premier Tech Home and Garden, Mississauga, ON, Canada) and were placed in a greenhouse maintained at a temperature of 20/15 ±2 °C (d/n; day/night) with a 16-hour photoperiod, where natural sunlight was supplemented with mercury halide lamps, providing a minimum of 500 µmol m^{-2} s^{-1} photon flux at plant canopy level. Two weeks after planting, the germinated seedlings were transplanted individually into a plastic pot (6 x 6 x 6.5 cm) filled with the potting mix and were grown in same greenhouse until they reached the appropriate stage of herbicide application.

2.2.2 Whole-plant dose-response experiment

At 4- to 5-leaf stage, the Japanese brome plants were treated with various doses of propoxycarbazone-Na (Olympus®, Bayer CropScience LP 800 North Lindbergh Blvd. St. Louis, MO, US), including 0 (NT, non-treated), 11, 22, 44 (1x), 88, 176, 352, 704 (where 1x is the recommended field dose=44 g ai ha^{-1}) for R1, R2, R3 and at 0 (NT, non-treated), 2.75, 5.5, 11, 22 doses for S1 along with non-ionic surfactant (NIS) at 0.25% v/v. The herbicide application was done using a bench-type sprayer (Research Track Sprayer, De Vries Manufacturing, 86956 State Highway 251, Hollandale, MN, US) fitted with a single flat-fan nozzle (8002 TeeJet tip, Spraying System Co., 100 W. Lake Drive, Glendale Heights, Wheaton, IL, US) which delivers a total volume of 187 L ha^{-1} at a speed of 4.85 km h^{-1} at 207 kPa pressure. After treatment, all the plants were placed back in the same greenhouse as mentioned above. At least three replicates of each population were kept for each treatment and the plants were watered regularly. At 3 weeks after treatment (WAT), the plants were assessed for visual injury on a scale of 0-100%, where 0%- no injury to 100%- plant death in comparison with their respective non-treated checks. The above-ground biomass was harvested at 3 WAT and was dried in an oven maintained at 60 °C for 72 hours, after which the dry biomass was weighed.

2.2.3 *ALS* gene amplification and sequencing

Leaf tissue (50 mg) was collected from 10 plants of each Japanese brome population and genomic DNA was isolated using the CTAB method with slight modifications (Doyle, 1990). The concentration of the DNA in each sample was quantified using a spectrophotometer (NanoDrop 1000, Thermo Fisher Scientific, 3411 Silverside Road, Bancroft Building, Suite 100, Wilmington, DE, US) and the quality was determined by 0.8% agarose gel electrophoresis. The *ALS* gene sequence encompassing the 8 recognized sites of mutations for ALS- inhibitor resistance was amplified using several primer combinations (Table 2.1). These primers were designed by retrieving the partial *ALS* gene sequence of Japanese brome (ON456299.1) and *B. tectorum* (MK492423.1) from the gene bank collection available in the National Centre of Biotechnology Information website (NCBI, www.ncbi.nlm.nih.gov) using OligoAnalyzer 3.1 (IDT SciTools, 2014; Integrated DNA Technologies, Inc., 1710 Commercial Park, Coralville, IA, US). The specific *ALS* gene regions were amplified through polymerase chain reaction (PCR) with the primers using T100™ Thermal Cycler (Bio-Rad Inc., 1000 Alfred Noble Drive, Hercules, CA, US). The PCR mixture used contained 50-80 ng of gDNA, 0.5 µM each of forward and reverse primer, and 1 × of GoTaq® G2 Green Master Mix (Promega™, Promega Corporation, 2800 Woods Hollow Road, Madison, WI, US). The two separate PCR conditions used for the amplification of two overlapping *ALS* gene sequences are provided below. For primer pairs ALSf1 and ALSr1, the PCR conditions used were initial denaturation: 94 °C for 5 min, followed by 30 cycles of denaturation: 94 °C for 30 s, annealing: 59 °C for 30 s and extension: 72 °C for 1 min and final extension: 72 °C for 7 min to amplify a total of ~ 700 bp of *ALS* gene region. A similar PCR profile was used for the primer pair ALSf2 and ALSr2, except with an annealing temperature of 60 °C for 45 s for 35 cycles to amplify a total of ~1900 bp

fragment. The PCR product was resolved on 1% agarose gel to confirm the desired amplicon size followed by gel extraction using GeneJET™ gel purification kit (Thermo scientific™, Thermo Fisher Scientific, 168 Third Avenue, Waltham, MA, US) and the concentration was determined using the Nanodrop spectrophotometer. The gel purified PCR products were then sequenced by Sanger sequencing (GENEWIZ, Agenta Life Sciences, 115 Corporate Boulevard, South Plainfield, NJ, US) and the results were analyzed by clustal omega multiple gene sequence alignment tool (EMBL's European Bioinformatics Institute (EMBL-EBI), Wellcome genome Campus, Hinxton, Cambridgeshire, United Kingdom) to identify for any mutations in the resistant (R1, R2 R3) compared to the susceptible (S1) plants.

Table 2.1 Primers used to amplify partial acetolactate synthase (ALS) gene to assess target site modification in Japanese brome.

Primers	**Sequence**	**Tm (°C)**	**Mutation sites present in the amplicon[a]**
ALSf1	5' ATGGCCACACCCACCACA 3'	60.1	Ala122, Pro197
ALSr1	5' GACCTGCTCAAGCGATTCAGT 3'	57.4	
ALSf2	5' TCGACGTGTTTGCCTACCCG 3'	59.9	Pro197, Ala205, Asp376, Arg377, Trp574, Ser653 and Gly654
ALSr2	5' ATGACAGCTTATCCCTACAAAGC 3'	54.9	

2.2.4 Evaluation of Japanese brome cross-resistance to other ALS- inhibitors

To determine cross-resistance of R1, R2 and R3 of Japanese brome to other ALS-inhibitors, i.e., SUs, TPs, SCTs and IMIs, the R1, R2, R3 as well as S1 plants were raised in the greenhouse as described earlier. At 4-5 leaf stage, at least 10-12 plants from each population were treated separately with mesosulfuron-methyl (Osprey™, Bayer CropScience LP P.O. Box 12014, 2 T.W. Alexander Drive Research Triangle Park, NC, US), sulfosulfuron (Outrider® Valent U.S.A. Corporation, P.O. Box 8025, Walnut Creek, CA, US), pyroxsulam (Powerflex®, Corteva Agriscience™, 9330 Zionsville Road, Indianapolis, IN, US) and imazamox (Beyond®, BASF corporation, 26 Davis Drive, Research Triangle Park, NC, US) at recommended field dose as given in Table 2.2. The survival percentage of plants was recorded at 3 WAT.

Table 2.2 List of herbicides and doses used to determine cross-resistance to ALS-inhibitors in Japanese brome populations.

Herbicide	**Trade name**	**Chemical family**	**Manufacturer**	**Recommended field dose (g ai ha^{-1})**
Mesosulfuron methyl	Osprey™	Sulfonylurea (SUs)	Bayer CropScience www.cropscience.bayer.us	34.75
Sulfosulfuron	Outrider®	Sulfonylurea (SUs)	Valent U.S.A. Corporation www.valent.com	14.57
Pyroxsulam	Powerflex®	Triazolopyrimidines (TPs)	Corteva Agriscience www.corteva.us	18.38
Imazamox	Beyond®	Imidazolinones (IMIs)	BASF corporation agriculture.basf.us	36
Propoxycarbazone-Na	Olympus®	sulfonylamino-carbonyl-triazolinones (SCTs)	Bayer CropScience www.cropscience.bayer.us	44

2.2.5 Treatment with malathion

Malathion is an organophosphate insecticide, known to inhibit cytochrome P450 monoxygenase (P450) mediated herbicide metabolism in plants (Christopher et al., 1994; Kreuz & Fonné-Pfister, 1992). In order to determine presence of P450-mediated metabolism of ALS-inhibitors confer resistance in R1, R2 and R3, all the populations of Japanese brome were treated with malathion (Spectracide®; United Industries Corporation, St Louis, MO, US) at 1000 g ai ha^{-1} along with NIS at 0.25% v/v, followed by treatment with propoxycarbazone-Na at 44 g ai ha^{-1} (1x) or imazamox (1x,2x, 4x where x= 36 g ai ha^{-1}). Additionally, soil drenching of 5 mM malathion, 24 hours after primary application as a booster dose was also given. The experiment was conducted using non-treated, malathion-treated, herbicide (propoxycarbazone-Na or imazamox) treated and malathion+herbicide (propoxycarbazone-Na or imazamox) treatments with at least 4 replicates of each population in each treatment and the experiment was repeated. The dry biomass (in grams) was collected at 3 WAT and the data was analyzed using Tukey's HSD among the treatments at a significance level of alpha=0.05.

2.2.6 Statistical analysis

The dry biomass data collected was expressed as relative dry biomass and was calculated using the following formula,

Equation 1

$$Relative\ dry\ biomass\ (\%\ of\ non-treated) = \frac{\text{dry biomass of individual treated plants}}{\text{average dry biomass of all the non} - \text{treated plants}} \times 100$$

The relative dry biomass data was subjected through a non-linear regression analysis

using the *drc* package in R (Knezevic et al., 2007; Ritz et al., 2015)**.** A three-parameter log-logistic model was selected through the lack of fit test, where the relationship between the herbicide dose and relative dry biomass was described as follows.

Equation 2

$$Y = d / (1 + \exp \{b[\log(x) - \log (GR50)]\})$$

where Y is the response (dry biomass) expressed as a percentage of the non-treated control, d is the asymptotic value of Y at the upper limit, b is the slope of the curve around GR_{50} (the herbicide rate giving response halfway between d and the lower asymptotic limit, which was set to 0), and x is the herbicide dose. The ratio between the GR_{50} values (resistance index) were calculated between resistance and susceptible populations to give the level of resistance of each resistant population (R1, R2, R3) in comparison to the susceptible (S1).

2.3. Results

2.3.1 Propoxycarbazone-Na dose response experiment

A high level of resistance to propoxycarbazone-Na was confirmed from the results of dose-response assay in the resistant populations (R1, R2, R3) of Japanese brome in comparison to susceptible populations (S1) (Figure 2.1A). Data from two separate runs of dose-response experiments were combined as there was no significant difference between the runs ($P>0.05$). Among the three resistant populations, all were able to survive up to 706 g ai ha^{-1} (16x of the field-recommended dose), whereas the S1 population exhibited severe visual injury and plant death at 2.64 g ai ha^{-1} (0.06x) of propoxycarbazone-Na. However, the GR_{50} estimates from the dose-response curves (Figure 2.1B) indicated that the R3 had the highest GR_{50} value of 719.34 g ai ha^{-1} followed by R1 and R2 at 180.73 g ai ha^{-1} and 134.90 g ai ha^{-1}, respectively. In contrast,

the S1 had a GR_{50} value of only 1.09 g ai ha^{-1}. The resistant index (R/S) calculated from the GR_{50} estimates suggests that the R1, R2 and R3 exhibit approximately ~167, ~125, and ~667- fold resistance to propoxycarbazone-Na relative to S1, respectively (Table 2.3).

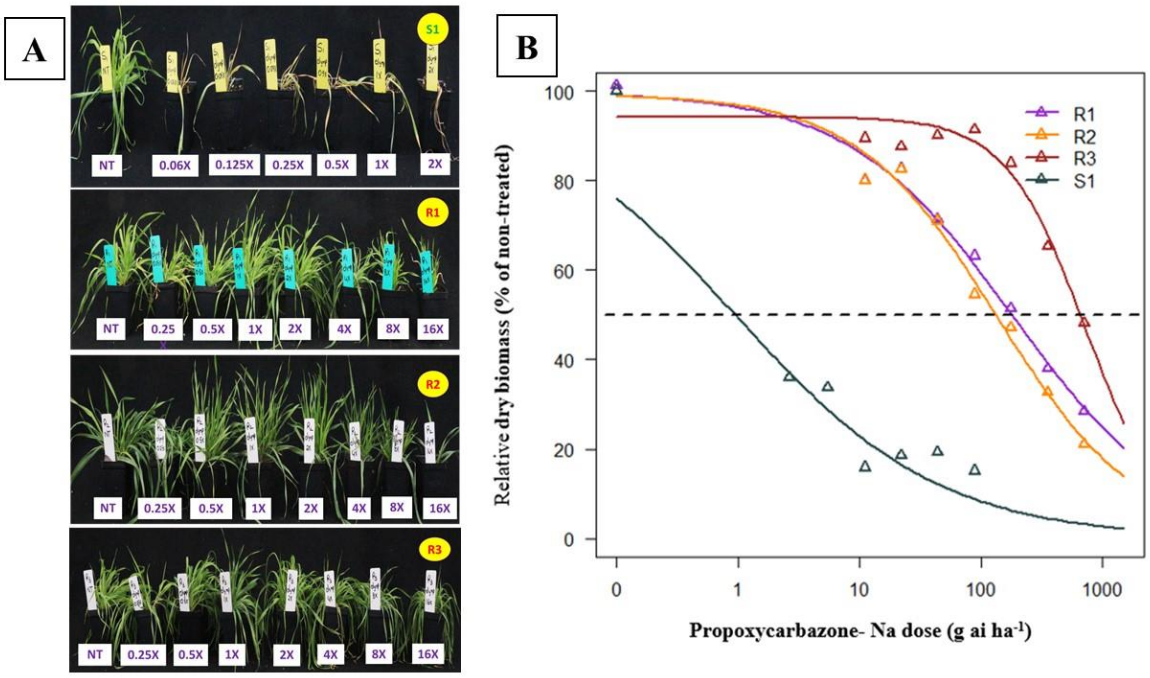

Figure 2.1 A) Propoxycarbazone-Na dose-response of susceptible (S1) and resistant (R1, R2, R3) populations of Japanese brome at 3 weeks after treatment (WAT) with NT= non-treated control and 1x = recommended field dose = 44 g ai ha^{-1}. B) Propoxycarbazone- Na dose response curves from the non-linear regression analysis of susceptible (S1) and resistant (R1, R2, R3) populations of Japanese brome aboveground dry biomass at 3 weeks after treatment (WAT). Dashed horizontal line represents 50% dry biomass reduction.

Table 2.3 GR_{50} values describing the response of the S1 (susceptible) and R1, R2, R3 (resistant) of Japanese brome aboveground biomass to different rates of propoxycarbazone-Na at 3 weeks after treatment (WAT). The values are estimated from the regression model using equation 2.

Population	**GR_{50} [a]**	**S.E. [b]**	**R.I. [c]**
R1	180.73	42.1	167
R2	134.9	26.77	125
R3	719.34	127.12	667
S1	1.07	0.89	

[a] GR_{50} is the dose of herbicides (g ai ha^{-1}) required for 50% reduction in the dry biomass

[b] S.E is the standard error

[c] R.I. (Resistance Index) is the ratio between the GR_{50} values of resistant (R1, R2, R3) and susceptible (S1) population depicting the level of resistance

2.3.2 Target-site resistance to ALS-inhibitor in Japanese brome

The high level of resistance exhibited by R1, R2 and R3 of Japanese brome was due to a single nucleotide alteration in the *ALS* gene. Upon sequencing of the *ALS* gene, our results indicated a single amino acid substitution from proline (Pro) to either serine (Ser) or threonine (Thr) at the 197th position in resistant populations (R1, R2, R3). Among the resistant populations, all ten samples of R3 that were sequenced predominantly had Pro (CCC)-197- Thr (ACC)

substitution (Figure 3), which confers the highest level of resistance to propoxycarbazone-Na (Table 3); whereas, in R2 with a lower level of resistance, all the samples that were sequence had Pro (CCC)-197-Ser (TCC) substitution (Figure 3; Table 3). Interestingly, in the case of the R1 population, out of the 10 samples sequenced, 6 (60%) had Pro-197-Thr substitution while the remaining 4 (40%) showed Pro-197-Ser substitution (Figure 2.2).

Figure 2.2 Nucleotide sequence alignment of *ALS*- gene of susceptible (S1) and resistant (R1, R2, R3) populations of Japanese brome. Red text in the nucleotide sequence codes for proline, blue text codes for serine and the green text codes for threonine. The nucleotide/amino acid numbering is in reference to the position in *Arabidopsis thaliana ALS* gene sequence.

2.3.3 Cross-resistance Japanese brome to other ALS-inhibitors

To assess cross-resistance of Japanese brome to other ALS-inhibitors, including SUs, TPs, SCTs and IMIs, both the resistant and susceptible populations were treated with these herbicides and the survival percentage in comparison to non-treated was recorded (Table 2.4). The resistant populations (R1, R2, R3) exhibited 100% survival in response to treatments with field-recommended doses of sulfosulfuron, mesosulfuron-methyl, pyroxsulam in addition to propoxycarbazone-Na, indicating that the R1, R2 and R3 populations have evolved cross-resistance to SUs, SCTs, and TPs. However, upon treatment with imazamox, an IMI herbicide, a differential response was observed among the resistant populations. Specifically, R2 plants did not survive the application of imazamox, suggesting that the Pro-197-Ser substitution provided resistance to SUs, SCTs, TPs but not IMIs. In contrast, the R3 population with Pro-197-Thr substitution exhibited cross-resistance to IMIs. However, in case of the R1 population with a combination of both Pro-197 to Ser or Thr substitutions, only 60 % of plants survived imazamox treatment (Table 2.4).

Table 2.4 Percent survival of R1, R2, R3, and S1 of Japanese brome populations treated with field recommended dose of ALS-inhibitors belonging to different chemical families at 3 weeks after treatment.

Herbicide	Recommended field rate (g ai ha^{-1})	Survival Percentage [a]			
		R1	R2	R3	S1
Mesosulfuron methyl	34.75	100	100	100	0
Sulfosulfuron	14.57	100	100	100	0
Pyraoxsulam	18.38	100	100	100	0
Propoxycarbazone-Na	44	100	100	100	0
Imazamox	36	60	0	100	0

[a] Survival percentage (%) is calculated as the number of plants that survived out of the total 20-24 B. japonicus plants in each R1, R2, R3 and S1 populations.

2.3.4 Japanese brome response to malathion treatment

When Japanese brome plants were treated with malathion alone, there was no difference in plants response in any of the populations (Figure 2.3A and Figure 2.3B). The S1 plants treated with propoxycarbazone-Na or imazamox alone or malathion + propoxycarbazone-Na or imazamox did not survive (Figure 2.3A and Figure 2.3B). However, the resistant populations

treated with propoxycarbazone-Na alone and malathion + propoxycarbazone-Na, showed similar responses without any injury and biomass reduction (Figure 2.3A). These results suggest that the pretreatment with malathion failed to reverse the resistance to propoxycarbazone-Na at the field-recommended rate. However, the resistant populations, particularly the R3 population treated with imazamox alone, showed ~50% visual injury at the field-recommended dose implying a moderate level of resistance compared to S1 (Figure 2.3B). However, when treated with malathion + imazamox (1x), an increase in sensitivity (75% injury) was found in only R3 plants (Figure 2.3B). The R1 plants exhibited 70% visual damage when treated with imazamox (1x) alone, while all R2 plants were dead (Figure 2.3B). Among all the resistant populations, only R3 showed a significant difference with biomass accumulation in response to either imazamox alone or malathion + imazamox treatment (Table 2.5). These results indicated that in addition to target site mutations, a possible P450-based metabolism may confer cross-resistance to imazamox in R3 population.

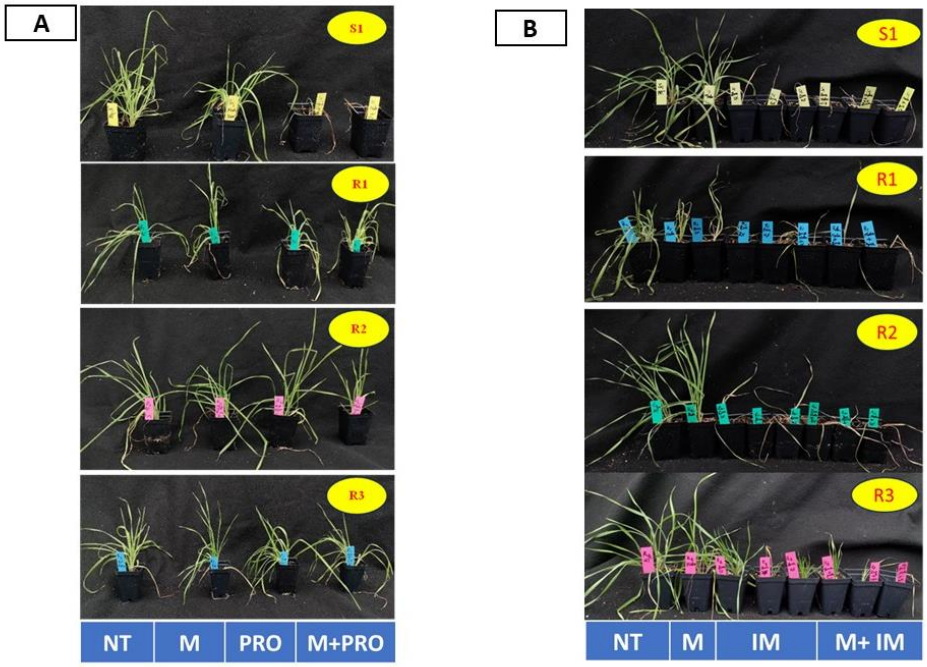

Figure 2.3 A) Visual injury of S1, R1, R2 and R3 populations of Japanese brome treated with malathion alone (1000 g ai ha^{-1}), propoxycarbazone-Na (44 g ai ha^{-1}), combination of malathion (1000 g ai ha^{-1}) and propoxycarbazone-Na (44 g ai ha^{-1}) at 3 weeks after treatment (WAT). B) Visual injury of S1, R1, R2 and R3 populations of Japanese brome treated with malathion alone (1000 g ai ha^{-1}), imazamox (1x, 2x, 4x where x=36 g ai ha^{-1}), combination of malathion (1000 g ai ha^{-1}) and imazamox (1x, 2x, 4x where x=36 g ai ha^{-1}) at 3 weeks after treatment (WAT).

Abbrev. NT-Non-treated, M-Malathion treatment alone, PRO- Propoxycarbazone-Na (1x-44 g ai ha^{-1}), M+PRO –Malathion+ propoxycarbazone-Na; IM- imazamox, M+IMI- Malathion+imazamox.

Table 2.5 Biomass reduction (% to the non-treated) in S1, R1, R2 and R3 populations of Japanese brome treated with imazamox (1x,2x,4x where x=36 g ai ha^{-1}), or combination of malathion (1000 g ai ha^{-1}) and imazamox (1x,2x,4x where x=36 g ai ha^{-1}) at 3 weeks after treatment (WAT). Significance level, alpha=0.05

Treatments	**Imazamox doses**	**Biomass reduction (% to respective non-treated)**			
		R1	R2	R3	S1
IM	1x	33.39	15.33	**54.09***	13
	2x	11.15	7	11.59	10.3
	4x	8.74	3.94	12.29	9.8
M+IM	1x	22.07	12.22	**18.72***	5.93
	2x	9.86	8.25	14.26	5.76
	4x	9.59	3.87	11.01	5.06

* $P<0.001$

2.4 Discussion

The evolution of ALS-inhibitor resistance in R1, R2 and R3 Japanese brome, like most of the weed species is likely due to extensive use of the ALS-inhibiting herbicides, with strong selection pressure and higher activity on sensitive biotypes below the recommended dose (Tranel & Wright, 2002). Recently two Japanese brome populations from different regions of China were found have 66.7-fold and 120-fold resistance to flucarbazone sodium, a SCT herbicide in

comparison to their respective susceptible populations (Lan et al., 2022; Li et al., 2022). In another brome grass species, *B. tectorum*, a 425-fold resistance to propoxycarbazone- Na along with 317-fold resistance to primisulfuron, and 263-fold resistance to sulfosulfuron, was reported in a population collected from research plots of Kentucky bluegrass *(Poa pratensis* L.*)* intensively sprayed with these herbicides (Park & Mallory-Smith, 2004).

In most of the weed populations, the presence of high-level of resistance and/or cross-resistance to ALS-inhibitors is predominantly dependent on specific mutations found in the *ALS* gene (Gaines et al., 2020). Among all the eight known amino acid substitutions in ALS enzyme, the most common substitution was found at Pro-197 position, which was reported in 40 weed species (Heap 2023). In this research, amino acid substitutions at Pro-197 position resulted in conferring resistance to propoxycarbazone-Na in the Japanese brome populations. Among the three populations, the R3 with Pro-197-Thr substitution had a high level of resistance compared to R1 or R2 with Pro-197-Ser substitution (Figure 2.2 and Table 2.3). The chromatograms of sanger sequencing of *ALS* gene did not indicate heterozygosity either. Such a high level of resistance in R3 population with only Pro-197-Thr with no possible indication of metabolic resistance to propoxycarbazone- Na is intriguing and needs further investigation. This is similar to ALS-inhibitor-resistant Japanese brome from China and *B. tectorum* from the US (Lan et al., 2022; Park & Mallory-Smith, 2004) had the amino acid substitution of Pro-197-Ser, conferring a high level of resistance. Nonetheless, the substitution of Asp-376-Glu was also found to endow resistance in ALS-inhibitors in another Japanese brome population from China (Li et al., 2022). Apart from these, several cases of multiple amino acid substitutions at Pro-197 position have also been reported to confer ALS- inhibitor resistance in weed species like rigid ryegrass *(Lolium rigidum* Gaud.) (Kaundun et al., 2012; Yu et al., 2008); silky bent grass (*Apera spica-venti* (L.)

P.B.) (Hamouzová et al., 2014; Krysiak et al., 2011); mayweed chamomile (*Anthemis cotula* L.) (Intanon et al., 2011). Our results also confirm Pro-197 to Ser or Thr substitution bestow a high-level of resistance in Japanese brome (Figure 2.2). Another downy brome populations from imidazolinone-tolerant (IMI-tolerant or Clearfield™) winter wheat fields, showed a Ser-653-Asn substitution with high level of resistance to imazamox (Kumar and Jha, 2017).

A differential cross-resistance response to other ALS-inhibitors was found among resistant populations particularly in case of R3 Japanese brome in this research (Table 2.4) Cross-resistance to ALS-inhibitors in the weed species is highly dependent on the type of amino acid substitutions in the target enzyme. The substitutions at Pro-197 are generally known to confer resistance to ALS-inhibitors belonging to SU and SCT families but not to IMIs; on the other hand, substitutions at Ala-122 or Ser-653 provide resistance only to IMIs but not to SUs (Tranel & Wright, 2002). So far, at Pro-197 position the highest number of 11 substitutions conferring resistance mainly to SUs and SCTs were found (Heap 2023). However, a few exceptions were also reported. For example, Pro-197-Thr substitution in resistant populations of corn poppy (*Papaver rhoeas* L.) (Délye et al., 2011); prickly lettuce (*Lactuca serriola* L.) (Preston et al., 2006); mayweed chamomile (*Anthemis cotula* L.) (Intanon et al., 2011); shortawn foxtail (*Alopecurus aequalis* Sobol.) (Xia et al., 2015); Hare Barley (*Hordeum murinum* ssp. Leporinum) (Owen et al., 2012), exhibited a high to moderate level of resistance to SUs, SCTs with an intermediate level of resistance to IMIs. Our results suggest that the Pro-197-Thr substitution showed cross-resistance to SUs, SCTs, TPs and IMIs whereas, Pro-197-Ser did not exhibit resistance to IMI herbicide.

Pretreatment with malathion did not reverse the resistance to propoxycarbazone-Na at recommended field rates, suggesting a possible lack of metabolic resistance to this herbicide

(Figure 2.3A). However, to completely rule out this possibility it is important to measure the metabolites of propoxycarbazone-Na using radiolabelled herbicide. On the other hand, treatment with malathion along with imazamox increased the sensitivity of R3 plants suggesting a potential of metabolic resistance to imazamox in this population (Figure 2.3B and Table 2.5). Involvement of P450 metabolism was reported to confer resistance to multiple ALS-inhibitors in several weed species including other brome grasses. For example, in one of the resistant biotype of downy brome, metabolic resistance to propoxycarbazone-Na due to enhanced metabolism involving cytochrome P450 activity was reported (Park et al., 2004). Likewise, in another biotype of rigid brome grass (*Bromus rigidus* Roth.), upon treatment with malathion, a reversal of resistance to sulfosulfuron was reported (Owen et al., 2012). In feral rye (*Secale cereale*), malathion treatment in population A indicated the involvement of metabolism-based resistance to imazamox along with target site resistance (Soni et al., 2022). The occurrence of resistance to both bensulfuron-methyl and imazethapyr in Chinese arrowhead (*Sagittaria trifolia*) due to the presence of both Pro-197 substitution and P450 metabolism mechanisms was confirmed in a study by Zou et al. (2023). These findings underscore the involvement of distinct mechanisms in conferring cross-resistance to various ALS-inhibitors in the same population. Also, transcriptome analysis of P450 enzymes suggested overexpression of different P450 genes within the *CYP81A* subfamily bestow cross-resistance to all chemical groups of ALS-inhibitors in barnyardgrass (*Echinochloa phyllopogon*) (Dimaano et al., 2020).

2.5 Conclusions

In conclusion, this is the first case of ALS-inhibitor resistance in Japanese brome in the

US. The evolution of ALS- inhibitor resistance was confirmed in three Japanese brome populations found in winter wheat fields in KS and therefore, can have huge impact on the management of this weed using ALS-inhibitors. The presence of TSR with amino acid substitutions at Pro-197-Ser and Pro-197-Thr conferred a high level of resistance to ALS-inhibitors. Furthermore, the evolution of cross resistance to several chemical families of ALS-inhibitors had been observed, further limiting the available herbicide options for managing this weed. The evolution of resistance to multiple herbicides highlights the necessity for the development of new modes of action or crop varieties that are tolerant to different herbicides, in order to expand the range of choices available to farmers.

Chapter 3 - Response of Grain Sorghum to Multiple Herbicides at High Temperature stress

Abstract

Post-emergence (POST) weed control is critical in grain sorghum production. High-temperature stress can have a negative impact on crop growth in response to POST herbicide application. The effect of temperature stress on the growth and development of grain sorghum when POST herbicides, with different modes of action groups, e.g., 2,4-D, Huskie® and mesotrione is unknown. In this research, the response of grain sorghum genotypes, RTx430 and P84G62 grown under temperature stress to POST application of the above herbicides was evaluated. Grain sorghum plants were grown in two separate growth chambers maintained at either optimum temperature (OT) of 32/22 °C or high temperature (HT) of 40/30 °C conditions. When plants reached 4-5 leaf stage, they were treated with multiple doses (0 to 8x; x = field recommended dose) of the above herbicides. The above-ground plant biomass was harvested three weeks after treatment (WAT), and dry biomass was measured. Using a non-linear regression model, GR_{50} (dose required for 50% biomass reduction) was determined. The results indicate that both genotypes of sorghum showed less injury with higher GR_{50} when treated with Huskie® or mesotrione at OT than HT. However, these genotypes exhibited less injury to 2,4-D at HT than OT. Among the two genotypes, the P84G62 had less injury than RTx430 in response to herbicide treatments, regardless of temperature stress. Overall, these results suggest that HT stress can cause more injury to grain sorghum when treated with mesotrione or Huskie, but not with 2,4-D, possibly because of differences in the mode of action of these herbicides.

3.1 Introduction

Grain sorghum (*Sorghum bicolor* L. Moench) is the fifth-largest cereal crop grown in the world for a variety of purposes including food, feed, and fuel (Dahlberg et al., 2011; Kimber et al., 2013; Venkateswaran et al., 2019). In recent years, one of the major consequences of global warming and climate change is the increasing temperatures. By the end of this century, a temperature increases from 2.6 - 4.8°C is expected with frequent episodes of heat waves (IPCC, 2022). Grain sorghum, predominantly grown in arid and semi-arid tropics regions is often exposed to high-temperature (HT) stress with temperatures reaching as high as 40 °C (Peacock, 1982; Tack et al., 2017). In grain sorghum, HT stress can reduce the chlorophyll concentration and photosynthetic rate and increase membrane damage (Djanaguiraman et al., 2010). HT stress also affects several phenological, growth, physiological, and yield traits including plant height, leaf area, leaf growth, pollen germination, seed set, and seed size leading to significant yield losses in grain sorghum (Nguyen et al., 2013; Prasad et al., 2008; Prasad et al., 2021; Singh et al., 2016).

Weeds are the most important pests in grain sorghum and can cause severe yield losses if uncontrolled (Oerke, 2006). In grain sorghum, weed infestation at the early stages i.e., within 4 weeks after planting can cause severe yield losses (Burnside & Wicks, 1969). If left uncontrolled, weeds can cause up to 47% yield losses in grain sorghum in the United States (Dille et al., 2020). The use of herbicides for weed management is the most common practice adopted by farmers. Post-emergence (POST) herbicide options are limited in grain sorghum because of potential crop injury. Some of the POST herbicides including 2,4-D, dicamba, prosulfuron, bromoxynil are routinely used for weed control in grain sorghum. Previous research

indicate that POST application of pyrasulfotole+bromoxynil or mesotrione did not cause injury to grain sorghum while providing broad spectrum grass weed control (Abit et al., 2009; Besançon et al., 2016; Fromme, Dotray, James Grichar, et al., 2012; Pandian et al., 2023). Huskie®, a commercial premix product with pyrasulfotole and bromoxynil is labelled for use in grain sorghum, although mesotrione is registered for preemergence application only.

Mesotrione is a triketone herbicide belonging to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors group of herbicides. The HPPD is an important enzyme in the production of tocopherol and plastoquinone of the carotenoid biosynthetic pathway (Beaudegnies et al., 2009). Carotenoids are essential for light harvesting and plant membrane protection (Siefermann-Harms, 1987). Depletion of carotenoids due to HPPD inhibition results in chlorophyll breakdown, bleaching, cell membrane damage, and eventually plant death (Prisbylla et al., 1993). Huskie® is a premix herbicide of pyrasulfotole and bromoxynil, each of which has a unique mode of action. Pyrasulfotole, like mesotrione, is a selective herbicide that inhibits the HPPD enzyme (Freigang et al., 2008). Bromoxynil is a photosystem II inhibitor that disrupts the photosynthetic process in sensitive plants by blocking electron transport (Reddy et al., 2012). This inhibition leads to the production of singlet, triplet chlorophyll molecules and singlet oxygen molecules, leading to lipid peroxidation and eventual plan death (Hess, 2000). This synergistic effect of HPPD and PS-II inhibition offers better weed control in grain sorghum (Reddy et al., 2012). 2,4-D is a synthetic auxin herbicide and mimics the action of natural plant hormone, auxin. Upon treatment with synthetic auxins, including 2,4-D, they disturb normal growth processes, causing uncontrolled growth, twisting, curling, and eventually death in susceptible broadleaf plants (Grossmann, 2000, 2003). However, the effectiveness of these herbicides is dependent on several environmental factors.

Environmental factors such as low/high temperature stress, high humidity, high precipitation etc. influence both herbicide efficacy and the response of the targeted species (Varanasi et al., 2016). Under these stress conditions, crops though can withstand herbicides application, may often show injury (Gunsolus & Curran, 1991). HT stress may improve the herbicide efficacy, resulting in damage to crops (Matzenbacher et al., 2014). For example, in glyphosate-resistant soybean, increase in temperature from 15 to 35 °C increased glyphosate efficacy causing crop injury (Pline et al., 1999). Additionally, grain sorghum was found to show more phytotoxicity to MCPA, 2, 4-D, bromoxynil and dicamba application at HT stress (Pannacci & Bartolini, 2018), possibly due to alterated herbicide absorption, translocation and metabolism (Hatzios & Penner, 1982).

In crops like grain sorghum, tolerance to herbicide (e.g., synthetic auxins or HPPD-inhibitors) injury is bestowed likely because of detoxification of herbicides, mediated by cytochrome P450 (P450) enzyme activity (Abit & Al-Khatib, 2009; Pandian et al., 2023; Peterson et al., 2016). The activity of P450 is known to be influenced by temperature, thereby herbicide efficacy (Mahan et al., 2004). The response of problem weeds of grain sorghum (e.g., Palmer amaranth, kochia) to herbicide such as 2,4-D, glyphosate, dicamba and mesotrione at HT stress was evaluated (Ou et al., 2018; Ganie et al., 2017; Godar et al., 2015; McCurdy et al., 2008; Rudell et al., 2023; Shyam et al., 2019a). For example, in Palmer amaranth, high temperature stress decreased herbicide efficacy to 2,4-D and mesotrione (Godar et al., 2015; Rudell et al., 2023). However, no information is available regarding grain sorghum response to POST herbicides, e.g., 2,4-D, Huskie® and mesotrione at HT stress.

Based on the previous reports, we hypothesize that grain sorghum may exhibit herbicide injury at HT stress. The objective of this study was to evaluate grain sorghum response to 2,4-D, mesotrione and Huskie® application at HT stress by performing dose-response assays.

3.2 Materials and Methods

3.2.1 Materials

Two grain sorghum genotypes; Pioneer 84G62 (P84G62) and RTx430, were selected for this experiment. RTx430 genotype is the most commonly used inbred line for several breeding programs in grain sorghum (Miller, 1984). P84G62 is a popular high-yielding commercial hybrid cultivated in the US (Maulana & Tesso, 2013).

3.2.2 Plant growth and conditions

The seeds of grain sorghum, P84G62 and RTx430 were placed individually in square pots (6×6×6 cm) filled with potting mixture (ProMix Ultimate, Premier Tech Horticulture, Mississauga, Ontario, Canada). Initially, for both mesotrione and Huskie dose-response experiments, the seed was germinated and raised in a greenhouse maintained at a 32/22°C, 15/9 h day/night photoperiod with a photosynthetic photon flux density of 750 µ mol m^{-2} s^{-1} at a relative humidity of 60±10%. At 3-4 leaf stage, i.e., two days prior to the herbicide application the plants were moved to two separate growth chambers maintained either at optimum temperature (OT; 32/22°C, day/night) or HT (40/30°C, day/night) regimes to acclimatize. The other conditions, *viz*., relative humidity of 60 ± 5% delivering 330 µ mol m^{-2} S^{1} photon flux at the canopy level supplemented by incandescent, fluorescent bulbs and LED lamps for a 12-hour photoperiod were maintained the same in both growth chambers. For 2,4-D dose-response, experiments, the grain sorghum seeds of both the genotypes were germinated and raised in growth chambers maintained at OT. At 3-4 leaf stage (2 days before spraying), a set of plants

were transferred from OT to the HT growth chambers, prior to herbicide treatment. All the plants were watered regularly as needed.

3.2.3 Whole plant dose-response assay

At 4-5 leaf stage, the plants (RTx430 and P84G62) from both growth chambers (OT and HT) were treated separately with either 2,4-D, mesotrione or Huskie® along with the recommended adjuvants at different doses (Table 1). Herbicide applications were done using a bench-top track spray chamber (Generation III; De Vries Manufacturing, Hollandale, MN, US) equipped with a single flat-fan nozzle (80015LP TeeJet tip; Spraying Systems, Wheaton, IL, US) moving with a speed of 2.96 mph delivering 187 L ha^{-1} at a pressure of 36.5 psi. Following treatment with herbicides, grain sorghum plants were returned to their corresponding growth chambers (within 30 min after treatment). At 1, 2, and 3 weeks after treatment (WAT), the plants were scored for herbicide injury relative to the non-treated plants, using a scale ranging from no injury (0%) and complete death of the plant (100%). The above ground biomass was harvested at 3 WAT, dried in a hot air oven at 60°C for 72 hours, and weighed. In each experiment for each herbicide at least, 4 replicates were maintained for each treatment and each experiment was repeated. The data of the leaf area of the fully open young leaf was collected at 3 WAT in plants treated with only with 2,4-D using LI-3100 leaf area meter (Li-COR inc. Lincoln, Nebraska, US).

Table 3.1 List of the herbicides applied in this study at different doses

Trade name	Mode of action[a]	Adjuvants used[b]	Doses sprayed
Callisto	HPPD-inhibitors	1% COC	NT, 0.5x, 1x (105 g ai ha^{-1}), 2x, 4x, 6x, 8x
Huskie	HPPD-inhibitors+PS-II inhibitors	0.25% NIS + 1.5% AMS	NT, 1x (288 g ai ha^{-1}), 2x, 4x, 8x
2,4-D amine solutions	Synthetic auxins	no adjuvant	NT, 0.5x, 1x (560 g ai ha^{-1}), 2x, 4x, 8x

[a] **HPPD- inhibitors:** 4-Hydroxyphenylpyruvate dioxygenase inhibitors, **PS-II inhibitors:** Photosystem-II inhibitors**, SAH: S**ynthetic auxinic herbicides [b] **COC:** crop oil concentrate, **NIS**: Non-ionic surfactant, **AMS**: Ammonium sulfate

3.2.4 Statistical analysis

The data from both runs of experiments for each herbicide were pooled as there was no significant difference between the runs from Lavene's test ($P>0.05$). All the experiments were conducted in a completely randomized design and were repeated in time. The dry biomass data collected from each experiment was converted to relative dry biomass by using the following Equation 1

Equation-1:

Relative dry biomass (% of non-treated) =

dry biomass of individual treated plants/ average dry biomass of all the non-treated plants × 100

The relative dry biomass calculated was subjected to non-linear regression analysis using a three-parameter log-logistic model using a 'drc' package (Knezevic et al., 2007; Ritz et al., 2015) available in R software (Development Core Team 2013).

Equation-2:

$$Y=\{d/1+\exp[b(\log-\log e)]\}$$

In the equation above Y is the response, and b is the dose, where d is the upper limit, b is the slope of the curve, and e is GR_{50} which is the herbicide dose required for 50% reduction in plant biomass. To assess the fit of data to the regression model, a Lack-of-fit test was performed using the model fit function of 'drc.'

3.3. Results

3.3.1 Response of RTx430 and P84G62 sorghum to mesotrione application at HT and OT

In response to 1x dose (105 g ai ha^{-1}) of mesotrione, at 1 WAT, the grain sorghum cultivar, P84G62 showed 10-15 % injury at OT and 20% at HT; while the RTx430 plants had 25% injury at OT than 30-35 % at HT. Both these genotypes recovered better from herbicide injury at OT than HT. A substantial increase in visual injury was found at HT when RTx430 and P84G62 were treated with mesotrione at 2x and 4x, respectively. At 3 WAT, RTx430 plants showed increased sensitivity to mesotrione at HT than OT (Figure 3.1 A). This increased sensitivity was particularly evident both for visual injury and biomass accumulation at 3 WAT (Figure 3.1A). At HT, the GR_{50} of mesotrione in RTx430 was 254.929 g ai ha^{-1} (> 2x), compared to OT where value was 891.721 g ai ha^{-1} (~8x) (Table 3.2 and Figure 3.1 B); suggesting 4 times increase in mesotrione efficacy at HT on this genotype. In contrast, P84G62 plants did not

exhibit injury from mesotrione application at HT (Figure 3.2A); with GR_{50} of 1215 and 1001 g ai ha^{-1}, of mesotrione at HT and OT, respectively (Table 3.2 and Figure 3.2B). Notably, both of these values were greater than eight times the field dose (>8x) of mesotrione.

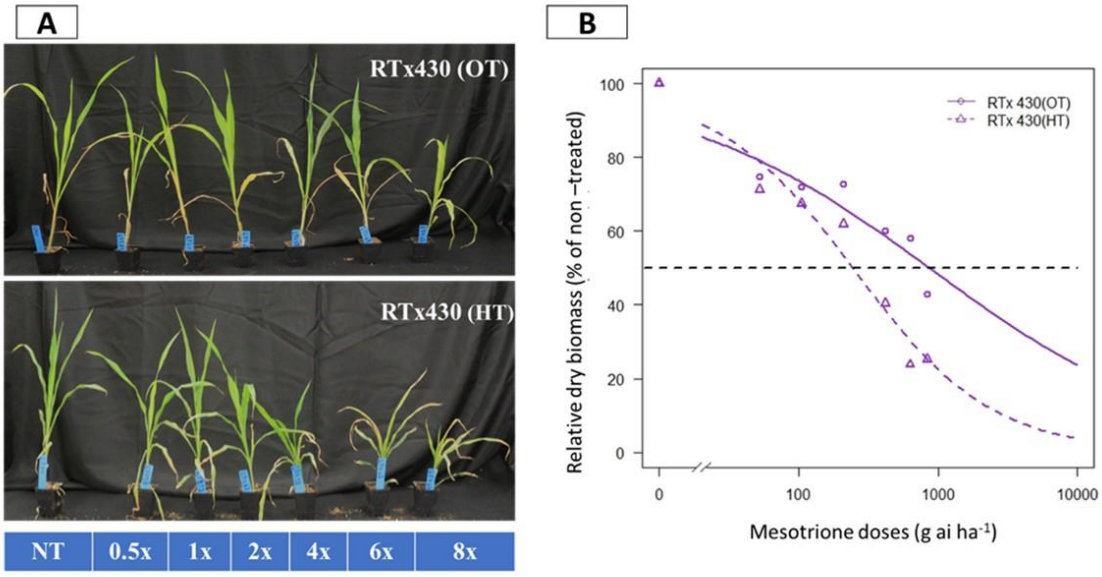

Figure 3.1 A) Response of RTx430 sorghum to different doses of mesotrione application at OT (32/22 °C) and HT (40/30 °C). NT is non-treated check and 1x is the field recommended dose of mesotrione, 105 g ai ha^{-1}. B) Mesotrione dose-response curves representing the relative dry biomass of RTx430 at both OT (solid line) and HT (dashed line) generated using the three-parameter log-logistic model. The dashed horizontal line represents the GR_{50} values.

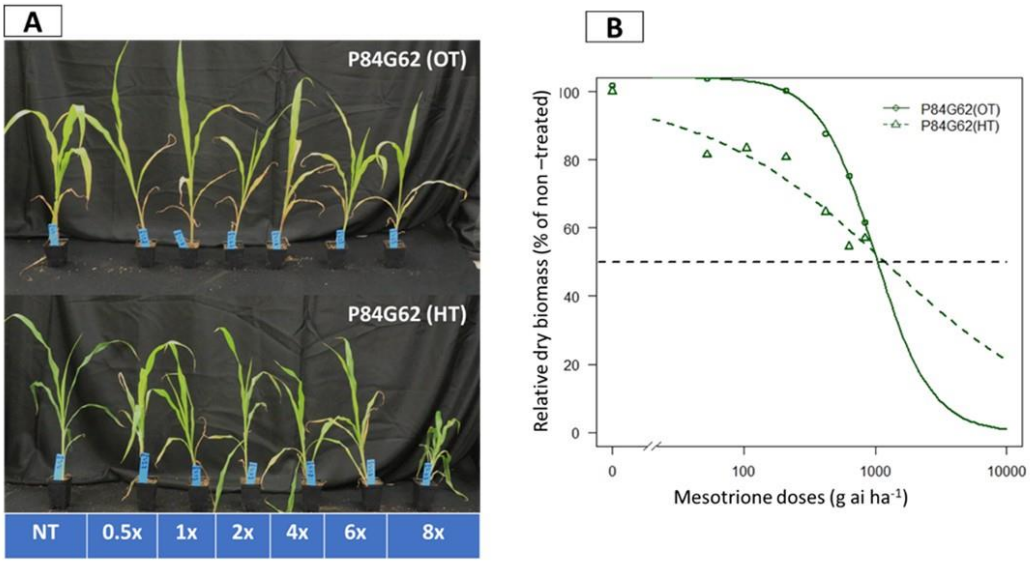

Figure 3.2 A) Response of P84G62 sorghum to different doses of mesotrione application at OT (32/22 °C) and HT (40/30 °C). NT is non-treated check and 1x is the field recommended dose of mesotrione, 105 g ai ha $^{-1}$. B) Mesotrione dose-response curves representing the relative dry biomass of P84G62 at both OT (solid line) and HT (dashed line) generated using the three-parameter log-logistic model. The dashed horizontal line represents the GR_{50} values.

3.3.2 Response of RTx430 and P84G62 sorghum to pyrasulfotole+bromoxynil (Huskie®) application at OT and HT

In response to 1x dose (288 g ai ha^{-1}) of Huskie® at 1 WAT, the grain sorghum cultivar, P84G62 showed 10-15 % injury at OT and 20% at HT; while the RTx430 plants had 10-15% injury at OT compared to 30-35 % at HT. Both these genotypes recovered better from herbicide injury at OT than HT. A substantial increase in visual injury was found at HT when RTx430 and P84G62 were treated with Huskie® at 4x and 8x, respectively. At 3 WAT, RTx430 plants showed increased sensitivity to Huskie® at HT than OT (Figure 3.3 A). This increased

sensitivity was particularly evident both for visual injury and biomass accumulation at 3 WAT (Figure 3.3A). At HT, the GR_{50} of Huskie® in RTx430 was 929 g ai ha^{-1} (> 4x), compared to OT where value was 1675 g ai ha^{-1} (~8x) (Table 3.2 and Figure 3.3B); suggesting 2 times increase in Huskie® efficacy at HT. In contrast, P84G62 plants did not exhibit significant injury from Huskie® application at HT (Figure 3.4A); with GR_{50} of 2011.25 and 2824.06 g ai ha^{-1}, of Huskie® at HT and OT, respectively (Table 3.2 and Figure 3.4B). Also, both of these values were times the field dose (10x) of Huskie®.

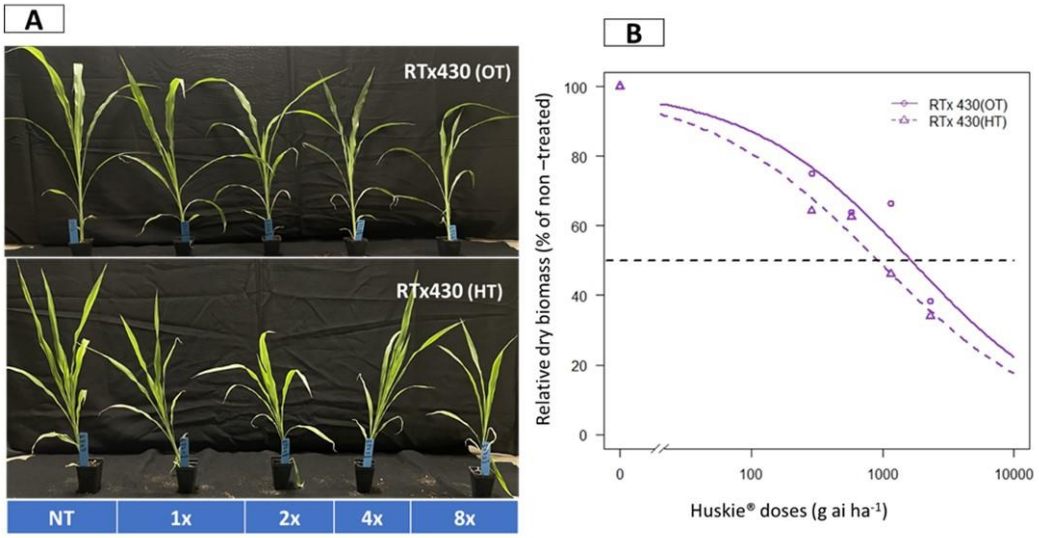

Figure 3.3 A) Response of RTx430 sorghum to different doses of Huskie® (pyrasulfotole + bromoxynil) application at OT (32/22 °C) and HT (40/30 °C). NT is non-treated check and 1x is the field recommended dose of Huskie® (pyrasulfotole + bromoxynil), 288 g ai ha^{-1}. B) Huskie® (pyrasulfotole + bromoxynil) dose-response curves representing the relative dry biomass of RTx430 at both OT (solid line) and HT (dashed line) generated using the three-parameter log-logistic model. The dashed horizontal line represents the GR_{50} values.

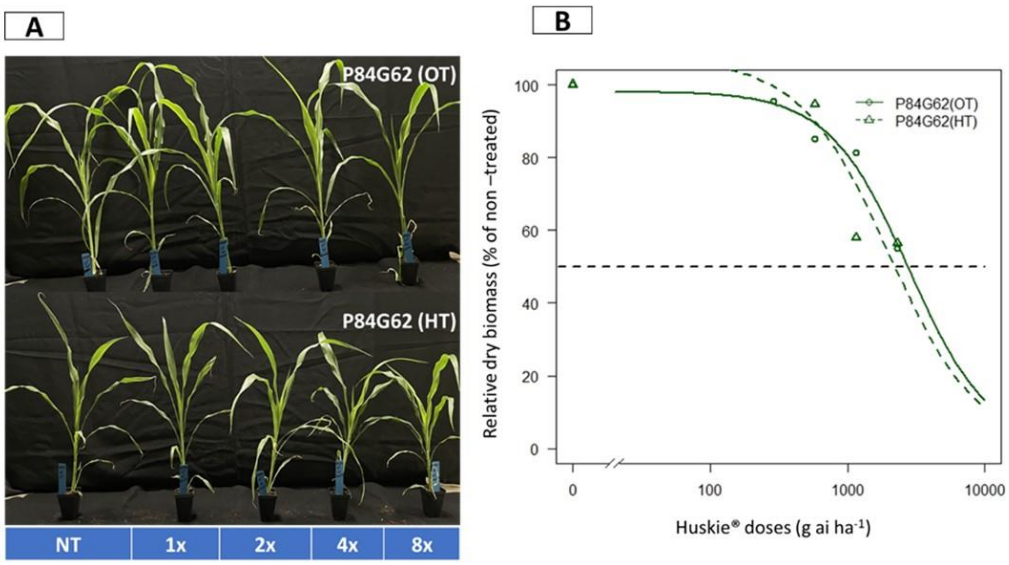

Figure 3.4 A) Response of P84G62 sorghum to different doses of Huskie® (pyrasulfotole + bromoxynil) application at OT (32/22 °C) and HT (40/30 °C). NT is non-treated check and 1x is the field recommended dose of Huskie® (pyrasulfotole + bromoxynil), 288 g ai ha $^{-1}$. B) of Huskie® (pyrasulfotole + bromoxynil) dose-response curves representing the relative dry biomass of P84G62 at both OT and HT generated using the three-parameter log-logistic model where the dashed horizontal line represents the GR_{50} values.

3.3.3 Response of RTx430 and P84G62 sorghum to 2,4-D application at OT and HT

In response to 1x dose (560 g ae ha^{-1}) of 2,4-D, at 1 WAT, the grain sorghum cultivar, P84G62 showed 35-40 % injury at OT and 30% at HT, while the RTx430 plants had 40% injury at OT than 30% at HT. Both these genotypes have lesser herbicide injury at HT than OT. A substantial increase in visual injury was found at OT when RTx430 and P84G62 treated with 2,4-D at 1x and 2x, respectively. At 3 WAT, RTx430 plants showed increased sensitivity to 2,4-

D at OT than HT (Figure 3.5A). This increased sensitivity was particularly evident both for visual injury and biomass accumulation at 3 WAT (Figure 3.5A). At HT, the GR_{50} of 2,4-D in RTx430 was 1214.30 g ae ha^{-1} (~2x), compared to OT where value was 394.11 g ae ha^{-1} (<1x) (Table 3.2 and Figure 3.5B); suggesting 2 times decrease in 2,4-D efficacy at HT. Similarly, P84G62 plants exhibited significant injury from 2,4-D application at OT (Figure 3.6A); with GR_{50} of 1114.09 and 797.21 g ae ha^{-1}, of 2,4-D at HT and OT, respectively (Table 3.2 and Figure 3.6B).

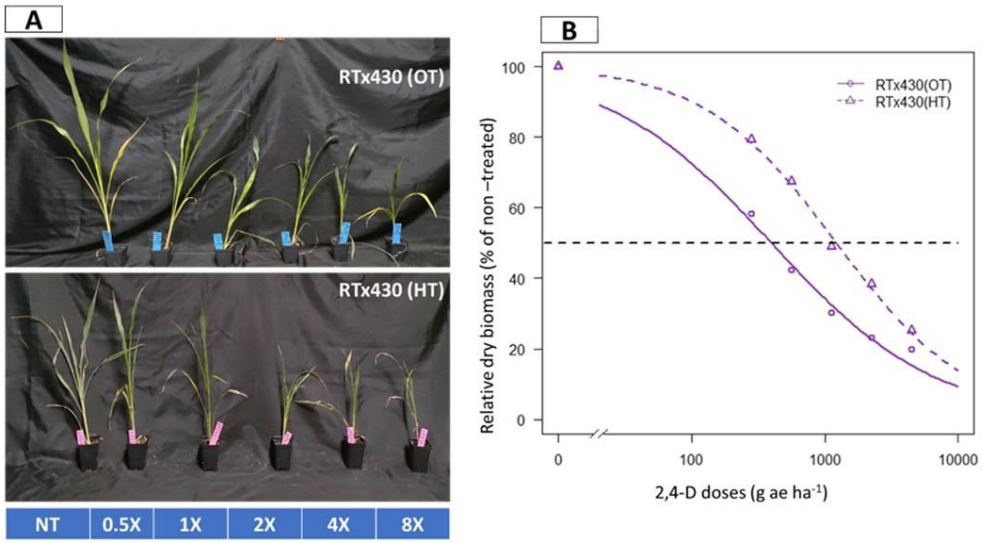

Figure 3.5 A) Response of RTx430 sorghum to different doses of 2,4-D application at OT (32/22 °C) and HT (40/30 °C). NT is non-treated check and 1x is the field recommended dose of 2,4-D, 560 g ae ha^{-1}. B) 2,4-D dose-response curves representing the relative dry biomass of RTx430 at both OT (solid line) and HT (dashed line) generated using the three-parameter log-logistic model. The dashed horizontal line represents the GR_{50} values.

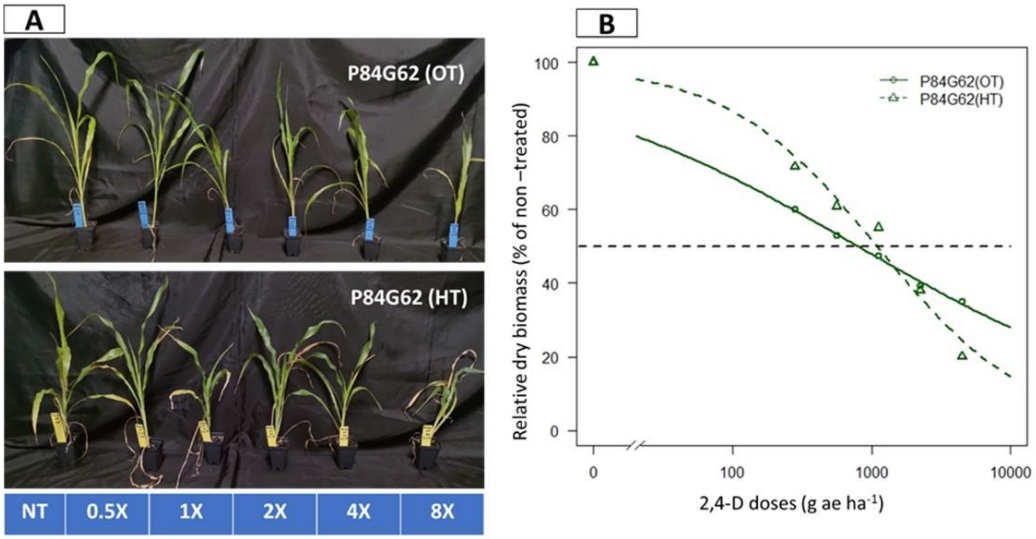

Figure 3.6 A) Response of P84G62 sorghum to different doses of 2,4-D application at OT (32/22 °C) and HT (40/30 °C). NT is non-treated check and 1x is the field recommended dose of 2,4-D, 560 g ae ha $^{-1}$. B) 2,4-D dose-response curves representing the relative dry biomass of P84G62 at both OT and HT generated using the three-parameter log-logistic mode where the dashed horizontal line represents the GR_{50} values.

Table 3.2 Regression parameters describing the response of sorghum (RTx430 and P84G62) to herbicide treatments of 0x to 8x doses at OT (32/22 °C) and HT (40/30 °C; HT), where GR_{50} is the dose of herbicides required for 50% dry biomass reduction and S.E. (numerical within parenthesis) is the standard error.

Herbicide	GR_{50} values (± S.E.) in g ai/ae ha^{-1}			
	RTx430		P84G62	
	OT	HT	OT	HT
Mesotrione	891.7 (313.4)	254.9 (47.5)	1215 (461.9)	1001.6 (138.6)
Pyrasulfotole+bromoxynil (Huskie®)	1675.6 (532.2)	929.5 (280.7)	2824 (540.9)	2011.2 (304.6)
2,4-D	394.1 (106.5)	1214.3 (259.9)	797.2 (329.2)	1114.0 (244.2)

Overall, there was a significant biomass reduction of up to 35-55% in the non-treated grain sorghum plants (RTx430 and P84G62) at HT compared to OT. Such an effect suggests that the temperature alone had an influence on the plant growth. However, the chlorophyll index and PS-II efficiency (data not shown) were not different in the non-treated plants grown either at OT or HT. Nonetheless, the non-treated grain sorghum plants showed a significant reduction in leaf area at HT than OT (data not shown).

3.4 Discussion

Crop injury with visual symptoms is a primary indicator for herbicide effect on plant growth. In most cases, an inverse relationship is found between crop injury and yield, implying that an increase in visible injury can decrease crop yield. However, in some cases, the crop may recover from the initial herbicide injury and avoid substantial yield losses (Carvalho et al., 2009).

In our results, sorghum plants (RTx430 and P84G62) at both OT or HT, initially showed higher visual injury (more bleaching or chlorosis) upon application with field recommended doses of mesotrione and Huskie® at 1 WAT (data not shown), but plants subsequently revered from this injury (Figure 3.1, 3.2, 3.3 and 3.4). Previously, the response of P84G62 grain sorghum to mesotrione at field recommended dose suggested more injury initially after treatment, however there was no yield penalty as the plants recovered from the herbicide injury (Abit et al., 2009). Treatment with pyrasulfotole + bromoxynil mixture although showed initial chlorosis and stunting in sorghum plants, bu did not show any yield losses (Fromme et al., 2012). Similarly, treatment with Huskie® had up to 5-10% injury (necrosis) on sorghum at 1 WAT, nonetheless fully recovered at 3 WAT (Currie & Geier, 2016).

HT stress is known to affect plant growth and may result in increased herbicide injury (Bagavathiannan et al., 2017). In our experiments, it is evident that both genotypes of grain sorghum (RTx430 and P84G62) showed more injury to mesotrione or Huskie® at HT than OT (Figure 3.1, 3.2, 3.3 and 3.4). Among these genotypes, P84G62 performed better at both the temperature regimes upon treatment with these herbicides (Figure 3.2, 3.4). However, previous reports suggest at HT, mesotrione treatment may result in increased or decreased efficacy on weeds depending on the species. For example, Palmer amaranth (*Amaranthus palmeri*), a C_4 summer annual dicot weed in grain sorghum, showed less injury or decreased mesotrione efficacy at HT (40 °C) (Godar et al., 2015). On the other hand, perennial ryegrass (*Lolium perenne*) and large crabgrass (*Digitaria sanguinalis*), grown at varying temperatures (18, 26, or 34 °C) showed no difference in mesotrione efficacy or injury on these weeds (McCurdy et al., 2008, 2009). Further, the impact of temperature stress on mesotrione foliar activity was enhanced at HT (32 °C) in common cocklebur (*Xanthium strumarium*) and velvetleaf (*Abutilon*

theophrasti) with up to threefold increase in susceptibility while common waterhemp (*Amaranthus tuberculatus*), and large crabgrass showed six- and sevenfold greater susceptibility to mesotrione at 18 °C compared to 32 °C, respectively (Johnson & Young, 2002). Therefore, it appears that the plant response to mesotrione application at HT stress varies from species to species.

Similar to mesotrione effect, the treatment with Huskie® had more injury on grain sorghum grown at HT (Figure 3.3 and 3.4). Although, to our knowledge there are no reports investigated the temperature effect on efficacy of Huskie® in sorghum or weeds, a study on the influence of the times of day (TOD), on the efficacy of premix, tolpyralate + atrazine (HPPD + PS-II inhibitors) when treated at temperatures ranging across 14 °C (6:00 h) to 30 °C (15:00 h) showed no difference in weed control or corn yield (Langdon et al., 2021). Additionally, Stewart et al. (2009) showed maximum air temperatures recorded between 12:00 and 18:00 h (24-31 °C) increased the efficacy of POST applied atrazine, bromoxynil, and glufosinate with increased weed control in corn.

2,4-D is widely used in grain sorghum and previous studies on treatment with this herbicide had a varied activity across hybrids causing injury (abnormal growth and lodging) and yield reduction (Burnside & Wicks, 1972). In this study, both the genotypes of sorghum showed higher injury at lower dose of 2,4-D at both OT (<1x) and HT (<2x), with more injury at OT than HT (Figure 3.5 and 3.6). In Palmer amaranth, the increased efficacy of 2,4-D and dicamba was found at low temperatures (LT; 31/20 °C) than HT (41/30 °C) (Johnston et al., 2019). Also, recently, reduced 2,4-D efficacy at HT than LT was reported in both Palmer amaranth and common waterhemp (Rudell et al., 2023; Shyam et al., 2019). In contrast, increased efficacy of 2,4-D and glyphosate was found in common (*Ambrosia artemisiifolia*) and giant (*Ambrosia*

trifida) ragweed grown at HT (29/17 °C) compared to OT (20/11 °C) (Ganie et al., 2017). Therefore, the effect of temperature on herbicide efficacy appears to be species specific and also depend on the mode of action of herbicides (Johnson & Young, 2002; Varanasi et al., 2016a).

Temperatures stress can improve herbicide performance by increasing the absorption and translocation of herbicides (Devine, 1989; Kells et al., 1984). This may be due to the increasing permeability of cuticular wax with increase in temperature (Schönherr et al., 1979). However, in some cases HT stress can reduce herbicide uptake due to rapid drying of spray droplets to solid deposits (Price, 1983). In the case of synthetic auxins like 2,4-D, dicamba an increase in temperature from 30 to 40 °C had increased volatility (Behrens & Lueschen, 1979; Ouse et al., 2018). Importantly, the HT stress is known to reduce herbicide activity by rapid metabolism (Johnson & Young, 2002; Hatzios & Penner, 1982), primarily via P450 activity (Frear et al., 1969; Frear & Swanson, 1996). The efficacy of herbicides such as mesotrione and 2,4-D at HT stress can be influenced by altering either herbicide absorption, translocation or metabolism (Ganie et al., 2017; Godar et al., 2015; Ou et al., 2018; Rudell et al., 2023; Shyam et al., 2019). Future studies are warranted to assess the differences in absorption, translocation and metabolism of mesotrione, Huskie® or 2,4-D in grain sorghum grown at HT or OT in order to gain better understanding of the physiological basis of herbicide induced injury at temperature stress.

Difference in response of grain sorghum to herbicides at temperature stress can also attributed to the different mode of action of the herbicides. Both HPPD- and PS-II inhibitors though differ in their specific site of action, have significant influence on plant photosynthetic pathway. HPPD inhibitors inhibits the function of HPPD enzyme causing reduction in plastoquinone which is essential components of electron transport and carotenoid biosynthesis

(Amesz, 1973; Lee et al., 1997). PS- II inhibitors, on the other hand competes with plastoquinone for binding to the Q_b binding site of the D1 protein, thereby disrupting electron transport (Jansen et al., 1993; Trebst et al., 1993). Synthetic auxin herbicides, on the other hand, interact with the auxin receptor complex by binding to Aux/IAA proteins, which function as transcriptional repressors of auxin-responsive genes, such as those in charge of producing ethylene and ABA (Mithila et al., 2011; Peterson et al., 2016). Also, herbicides like 2,4-D do not specifically target a single element of photosynthesis, like HPPD or PSII inhibitors, but causes a comprehensive transcriptional down-regulation of numerous components involved in the photosynthetic process (Gaines, 2020). This suggests that understanding the influence of temperature injury due to herbicides can be rather complex as each mode of action affects different plant processes and metabolic pathways. Similar to our results a previous study showed reduction in sorghum biomass at HT (40/30 °C) compared to OT (32/22 °C), as a result of decreased photosynthetic rate, antioxidant enzyme activity and increase in reactive oxygen species (ROS) leading to damage to the thylakoid membranes (Djanaguiraman et al., 2010)

3.5 Conclusions

In conclusion, the results of this research suggest that HT can influence sorghum growth and development it's response to POST herbicide application. With increasing temperatures and prevailing herbicide resistance, weeds acquire a competitive advantage over crops, emphasizing the need for better weed management strategies. However, with limited options for POST herbicides and possible crop damage under the high temperatures stress, it can be particularly challenging to control weeds in grain sorghum. Apart from temperature, other plant and environmental factors like relative humidity, light and precipitation can also influence herbicide performance and crop injury. Therefore, further research focusing on the effects of abiotic and

plant-related variables on herbicide efficacy and sorghum injury will be critical for the sensible use of herbicides in weed management.

Chapter 4 - Response of Corn to Multiple Herbcides at High-Temperature Stress

Abstract

Post-emergence (POST) weed control is critical in corn production. High-temperature stress can have a negative impact on crop growth in response to POST herbicide application. The effect of temperature stress on the growth and development of corn when POST herbicides, with different modes of action, e.g., 2,4-D, dicamba, atrazine, tembotrione, and mesotrione is unknown. This research investigated the response of corn (DKC59-82RIB) grown under temperature stress to the POST application of the above herbicides. Corn plants were grown in two separate growth chambers maintained at 30/25 ℃ (OT) and 40/35 ℃ (HT) conditions and were treated with multiple doses (0 to 8x; x = filed recommended dose) of the above herbicide at the 4-5 leaf stage. The above-ground plant tissue was harvested three weeks after treatment (WAT), and dry biomass was measured. Using a non-linear regression model, GR_{50} (dose required for 50% biomass reduction) was determined. The results indicated that the corn plants showed high injury at HT stress with herbicides of 2,4-D, dicamba, atrazine, tembotrione, and mesotrione with lower GR_{50} values at HT than OT. It is concluded that HT stress can affect the response of corn to POST herbicide application and increase herbicide injury.

4.1 Introduction

Corn (*Zea mays*), also known as "maize," is one of the important cereal crops cultivated in the world. Globally, a total of 1,161.86 million MT was estimated to be produced for the year 2022/23 (USDA, 2023), with the United States (US) being the leading producer of about 348.75 million MT in the year 2022/23 (Statista 2023). The optimum temperature for corn production is 30-34 °C, generally recorded in tropical regions of corn-growing regions (Lizaso et al., 2018; Wang et al., 2018). However, due to climate change and global warming, temperature can increase from 2-5 °C by the end of the century (IPCC, 2022). The projected increase in growing season temperatures can lead to significant yield losses in corn (Zhu et al., 2019). High-temperature stress has a negative impact on various physiological processes in corn, such as photosynthesis, cellular homeostasis, and antioxidant metabolism (Mathur et al., 2021). This can result in a decrease in photosynthetic parameters, disruption of cellular activities, and an increase in the production of reactive oxygen species and ultimately can affect the growth and yield of corn (Li & Zhang, 2022; Tiwari & Yadav, 2020).

Weeds are a significant pest in crop production, including corn (Oerke, 2006). Results from research conducted across the corn-producing states in the US and Canada from year 2007 to 2013 showed around 50% yield losses in corn due to weeds (Soltani et al., 2016). Chemical control methods like herbicides are widely adapted weed management strategies among farmers. Post-emergence herbicides are generally used in corn for control of several grasses and broadleaf weeds. Some of the post-emergence herbicides used in corn are 2,4-D, dicamba, fluroxypyr, atrazine, bromoxynil, nicosulfuron, prosulfuron, mesotrione, and tembotrione etc. Corn plants can naturally tolerate most herbicides by detoxifying them through N-alkylation, hydroxylation,

or peptide conjugation. This is done primarily through cytochrome P450 monooxygenase (P450) or Glutathione-S-transferases (GST), with the latter being more effective at detoxifying atrazine (Barrett, 1995; Jensen et al., 1977). However, corn sometimes shows herbicide injury symptoms including ear pinching, ear bottlenecking, internode stacking, onion leafing, rat tailing, brace root malformation, and green snap (Legleiter, 2012).

Synthetic auxinic herbicide (SAH) like 2,4-D and dicamba are commonly used in corn for broadleaf weed control. Corn hybrids have differential sensitivity to auxinic herbicides like 2,4-D and can sometimes lead to injury even under recommended doses (Peterson et al., 2016; Rodgers, 1952). SAH herbicides influence several physiological processes involved in plant growth and development that can lead to the development of several symptoms like brittleness, epinasty, rolled or buggy whipped shoots and stunted growth in corn (www.btny.purdue.edu). Photosystem-II (PS- II)inhibitors like atrazine are widely used to control broadleaf weeds in crops including corn, either alone or combined with other herbicides (Hamill & Zhang, 1997; Richburg et al., 2019). PS- II inhibitor that disrupts the photosynthetic process in sensitive plants by blocking electron transport (Reddy et al., 2012). The use of atrazine can affect both corn and weeds, resulting in chlorosis, necrosis, stunted growth, and even plant death ((Bibi et al., 2019). Tembotrione and mesotrione are triketone herbicides belonging to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors family and is mostly used in corn production for both pre- and post-emergence for wide range of broadleaf and grasses weed control (James et al., 2006; Santel, 2009; Schulte & Köcher, 2009). These herbicides inhibit HPPD enzyme and influences the production of tocopherol and plastoquinone of the carotenoid biosynthetic pathway. The most common symptom of HPPD injury is bleached-white foliage in sensitive plants, which may recover, but in severe cases, tissue death can occur.

Environmental conditions including high/low temperatures, humidity etc during postemergence herbicide application can significantly affect herbicide effectiveness in corn as it affects plants natural ability to deactivate and metabolize the herbicide (Fawcett et al., 1987). For example, a study conducted in 13 corn hybrids showed under controlled conditions showed that more herbicide injury to trifluralin at 15 °C decreased as temperature changes from 15 to 25 °C (Roggenbuck & Penner, 1987). Another example in glyphosate-resistant soybean, showed that increase in temperature from 15 to 35 °C increased glyphosate efficacy causing more crop injury (Pline et al., 1999). High temperature (HT) stress conditions can also lead to increase in herbicide injury to post-emergence herbicides like 2,4-D, dicamba, atrazine, tembotrione and mesotrione in corn.

Effect of HT stress on plant response to herbicide injury is mainly due to differences in absorption, translocation and enzyme activity of P450 and GST on herbicide detoxification (Jensen et al., 1977; Matzrafi, 2019; Varanasi et al., 2016a). Some previous studies have been conducted to study the effect of HT stress on herbicide efficacy in some problems weeds of corn. For example, in Palmer amaranth *(Amaranthus palmeri*), a decrease in herbicide efficacy of 2,4-D, mesotrione was observed at HT. However, the effect of HT stress on herbicide injury varies with the temperature regimes selected, herbicide sprayed and target plant species (Varanasi et al., 2016b).

Based on the previous reports, we hypothesized that corn may exhibit herbicide injury at HT stress. The objective of this study was to evaluate corn response to 2,4-D, dicamba, mesotrione, tembotrione and atrazine application at HT stress by performing dose-response assays.

4.2 Materials and Methods

4.2.1 Plant growth and conditions

Corn seeds (DKC59-82RIB) were placed individually in square pots (6×6×6 cm) filled with potting mixture (ProMix Ultimate, Premier Tech Horticulture, Mississauga, Ontario, Canada). Initially, for both 2,4-D and dicamba dose-response experiments, the seed was germinated and raised in a growth chamber maintained at a 25/23 °C, 15/9 h day/night photoperiod with a photosynthetic photon flux density of 450 µ mol m^{-2} s^{-1} at a relative humidity of 60±10%. When the plant reaches 3-4 leaf stage viz., two days prior to the spraying, the plants were shifted to growth chambers maintained either at optimum temperature (OT; 30/25 °C, day/night) or high temperatures (HT; 40/35 °C, day/night) with a relative humidity of 60 ± 5% delivering 330 µ mol m^{-2} s^{1} photon flux at the canopy level supplemented by incandescent, fluorescent bulbs for a 12-hour photoperiod for acclimatization. In the remaining experiments with tembotrione, mesotrione and atrazine, the corn seeds were germinated and raised in growth chambers maintained at OT. Later, at 3-4 leaf stage, a few plants were transferred from the OT growth chambers to the HT growth chambers. Plants were watered regularly.

4.2.2 Whole plant dose-response essay with HPPD-, PS-II inhibitors and synthetic auxins (SAH)

At the 4-5 leaf stage, corn plants from both growth chambers (OT and HT) were treated with one of the three different modes of action (HPPD, SAH, PS-II) herbicides along with the recommended adjuvants at different rates (Table 1). Application of herbicide was done using a bench-top track spray chamber (Generation III; De Vries Manufacturing, Hollandale, MN, US) equipped with a single flat-fan nozzle (80015LP TeeJet tip; Spraying Systems, Wheaton, IL, US)

moving with a speed of 2.96 mph delivering 187 L ha^{-1} at a pressure of 36.5 psi. Following the treatment with different herbicides, the plants were returned to their corresponding growth chambers (within 30 min after treatment). At 1, 2, and 3 weeks after treatment (WAT), the plants were rated relative to the non-treated plants, with no injury (Visual injury=0%) and plants died (Visual injury = 100%). The above-ground biomass was harvested at 3 WAT, dried in a hot air oven at 60°C for 72 hours, and weighed. In each experiment for all the herbicides listed, 4 replicates were kept for each treatment and each experiment was repeated.

Table 4.1 List of the herbicides applied in this study at different doses

Herbicide	**Trade name**	**Mode of action [a]**	**Adjuvants used [b]**	**Doses sprayed**
2,4-D	2,4-D amine	SAH	No adjuvant	NT, 0.5x, 1x (560 g ae ha^{-1}), 2x, 4x
Dicamba	Clarity	SAH	No adjuvant	NT, 0.5x, 1x (560 g ae ha^{-1}), 2x, 4x, 8x
Tembotrione	Soberan	HPPD-inhibitors	1% MSO +1% AMS	NT, 0.5x, 1x (92 g ai ha^{-1}), 2x, 4x, 8x
Mesotrione	Callisto	HPPD-inhibitors	1% COC +2.5% AMS	NT, 0.5x, 1x (105 g ai ha^{-1}), 2x, 4x, 8x
Atrazine	Aatrex 4L	PS-II inhibitors	1% COC	NT, 0.5x, 1x (2250 g ai ha^{-1}), 2x, 4x, 8x

[a] **HPPD- inhibitors:** 4-Hydroxyphenylpyruvate dioxygenase inhibitors, **PS-II inhibitors:** Photosystem-II inhibitors**, SAH: S**ynthetic auxinic herbicides**.** [b] **COC:** Crop oil concentrate, **MSO**: Methylated seed oil, **AMS**: Ammonium sulfate

4.2.3 Statistical analysis

The data from both runs of experiments for each herbicide were pooled as there was no significant difference between the runs from Lavene's test ($P>0.05$). All the experiments were conducted in a completely randomized design and were repeated in time. The dry biomass data collected from each experiment was converted to relative dry biomass by using the following Equation 1**.**

Equation-1:

Relative Dry biomass (% of non-treated) =

dry biomass of individual treated plants/ average dry biomass of all the non-treated plants × 100

The relative dry biomass calculated was subjected to non-linear regression analysis using a three-parameter log-logistic model using a 'drc' package (Ritz et al. 2005) available in R software (Development Core Team 2013). To assess the fit of data to various regression models, a Lack-of-fit test was performed using the model fit function of 'drc.'

Equation-2:

$$Y=\{d/1+\exp[b(\log-\log e)]\}$$

In the equation above Y is the response, and b is the dose, where d is the upper limit, b is the slope of the curve, and e is GR_{50} which is the herbicide dose required for 50% reduction in plant biomass.

4.3 Results

4.3.1 Response of corn plants to synthetic auxinic herbicides like 2,4-D and dicamba application at OT and HT

At 3 WAT, corn plants showed increased sensitivity to both 2,4-D and dicamba at HT than OT (Figure 4.1 A and 4.2 A). This increased sensitivity was particularly evident for visual injury and biomass accumulation at 3 WAT (Figure 4.1A). At HT, the GR_{50} of 2,4-D in corn was 802.52 g ae ha^{-1} (~2x), compared to OT where the value was 2180.69 g ae ha^{-1} (~4x) (Table 4.2 and Figure 4.1 B); suggesting 4 times increase in 2,4-D efficacy at HT. Similarly, crop plants exhibited significant injury from dicamba application at HT (Figure 4.2A); with GR_{50} of 2615.22 g ae ha^{-1} (>4x) and 1579.16 g ae ha^{-1} (>2x), of dicamba at OT and HT, respectively (Table 4.2 and Figure 4.2B).

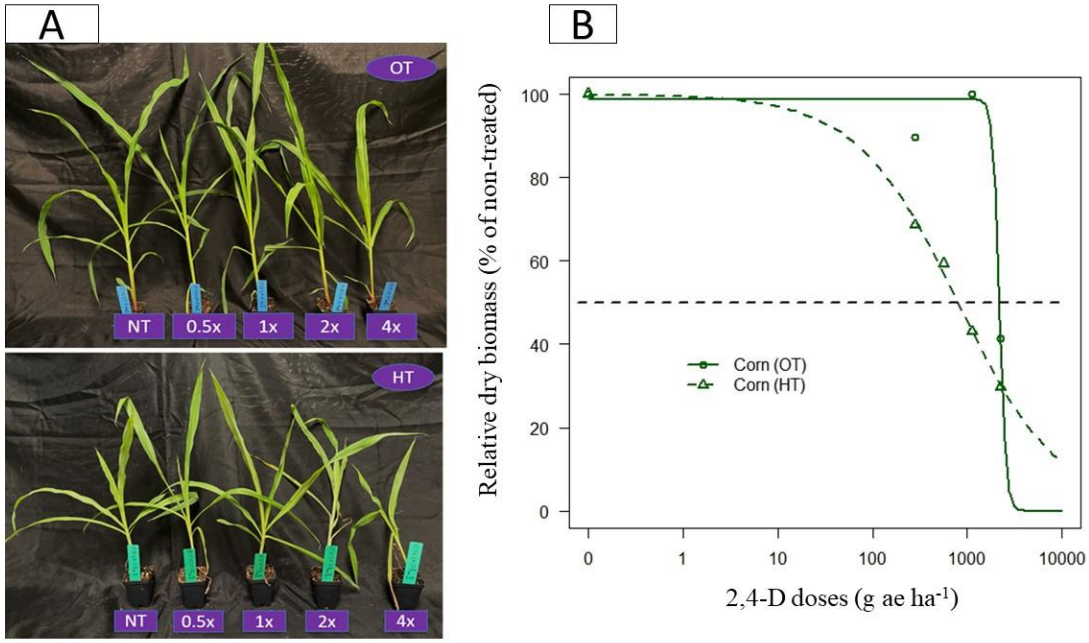

Figure 4.1 A) Response of corn to different doses of 2,4-D application at OT (30/25 °C) and HT (40/35 °C). NT is non-treated check and 1x is the field recommended dose of 2,4-D, 560 g ae ha $^{-1}$. B) 2,4-D dose-response curves representing the relative dry biomass of corn at both OT (solid line) and HT (dashed line) generated using the three-parameter log-logistic model. The dashed horizontal line represents the GR_{50} values.

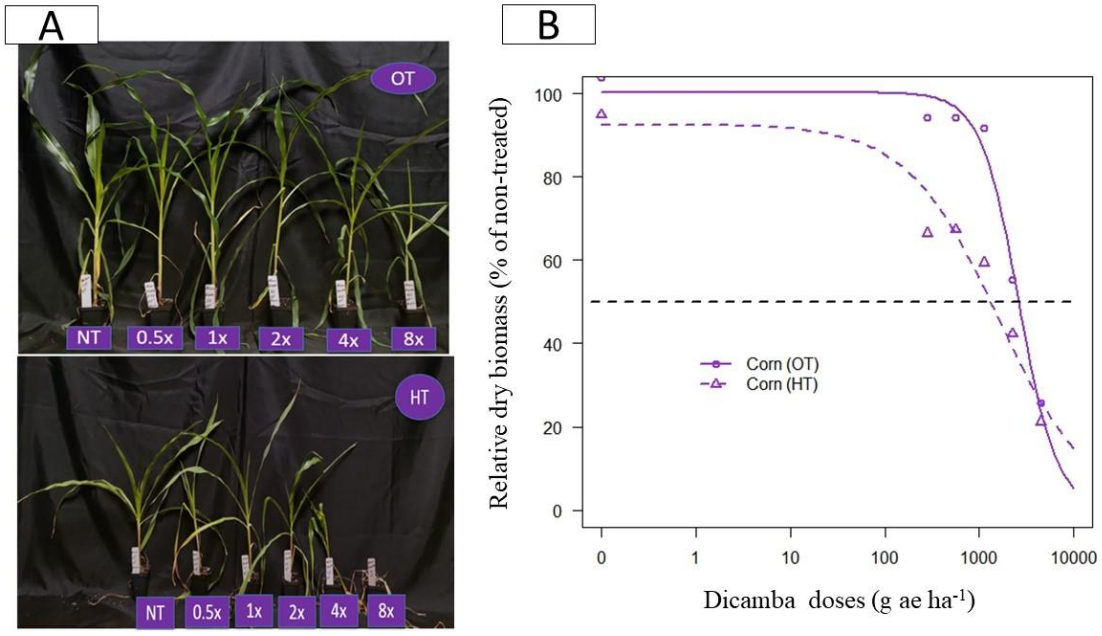

Figure 4.2 A) Response of corn to different doses of dicamba application at OT (30/25 °C) and HT (40/35 °C). NT is non-treated check and 1x is the field recommended dose of dicamba, 560 g ae ha $^{-1}$. B) Dicamba dose-response curves representing the relative dry biomass of corn at both OT (solid line) and HT (dashed line) generated using the three-parameter log-logistic model. The dashed horizontal line represents the GR_{50} values**.**

4.3.2 Response of corn plants to PS- II herbicide like atrazine application at OT and HT

At 3 WAT, corn plants showed increased sensitivity to atrazine at HT than OT (Figure 4.3A). This increased sensitivity was particularly evident for visual injury and biomass accumulation at 3 WAT (Figure 4.3A). At HT, the GR_{50} of atrazine in corn was 10109 g ai ha^{-1} (>4x), compared to OT where the value was 21355.1 g ai ha^{-1} (~10x) (Table 4.2 and Figure 4.3B); suggesting 2.5 times increase in atrazine efficacy at HT.

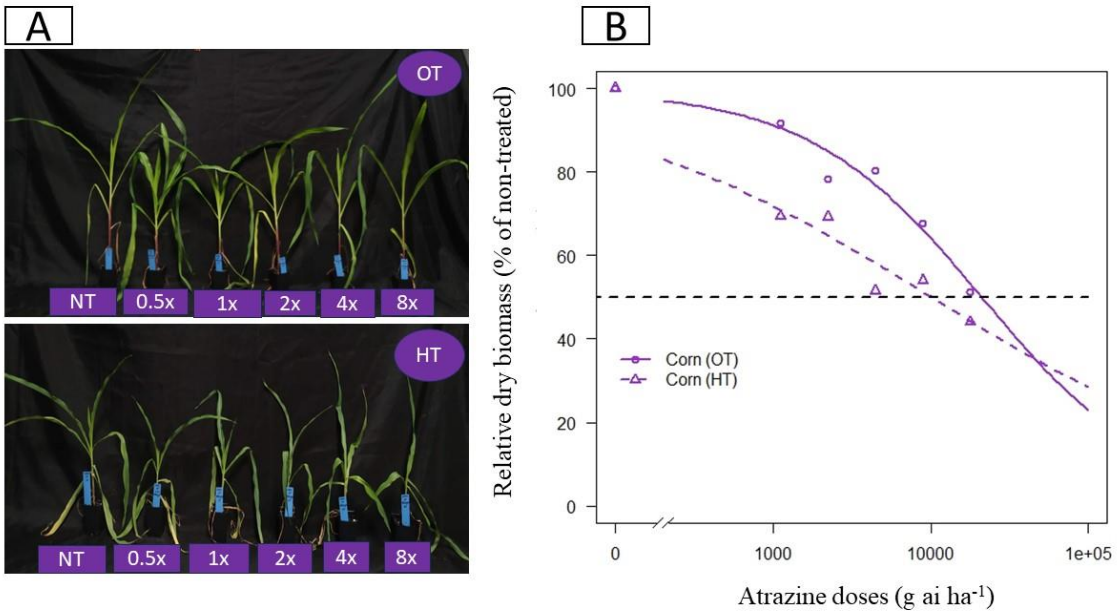

Figure 4.3 A) Response of corn to different doses of atrazine application at OT (30/25 °C) and HT (40/35 °C). NT is non-treated check and 1x is the field recommended dose of atrazine, 2240 g ai ha^{-1}. B) Atrazine dose-response curves representing the relative dry biomass of corn at both OT (solid line) and HT (dashed line) generated using the three-parameter log-logistic model. The dashed horizontal line represents the GR_{50} values.

4.3.3 Response of corn plants to HPPD-inhibitor herbicide like tembotrione and mesotrione application at OT and HT

At 3 WAT, corn plants showed increased sensitivity to both mesotrione and tembotrione at HT than OT (Figure 4.4A and 4.5A). This increased sensitivity was particularly evident for visual injury and biomass accumulation at 3 WAT (Figure 4.4A and 4.5A). At HT, the GR_{50} of tembotrione in corn was 325.7 g ai ha^{-1} (>2x), compared to OT where the value was 602.4 g ai ha^{-1} (~6x) (Table 4.2 and Figure 4.4B); suggesting 3 times increase in tembotrione efficacy at HT. Similarly, crop plants exhibited significant injury from mesotrione application at HT (Figure 4.5A); with GR_{50} of 1403.2 g ai ha^{-1} (>10x) and 583.1 g ai ha^{-1} (>4x), of mesotrione at OT and HT, respectively (Table 4.2 and Figure 4.5B).

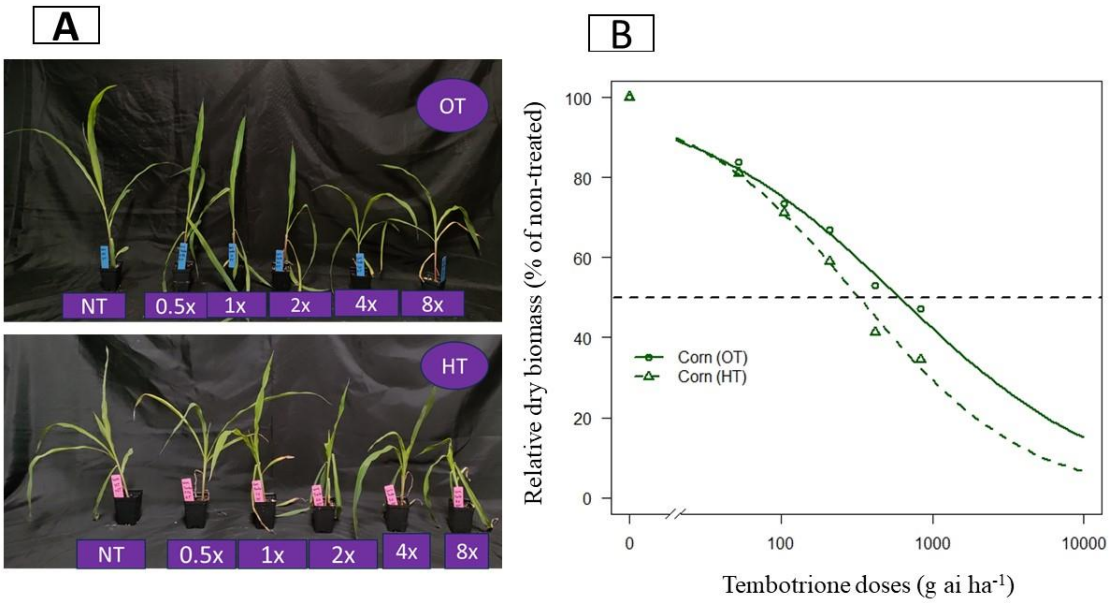

Figure 4.4 A) Response of corn to different doses of tembotrione application at OT (30/25 °C) and HT (40/35 °C). NT is non-treated check and 1x is the field recommended dose of

tembotrione, 92 g ai ha $^{-1}$. B) Tembotrione dose-response curves representing the relative dry biomass of corn at both OT (solid line) and HT (dashed line) generated using the three-parameter log-logistic model. The dashed horizontal line represents the GR_{50} values.

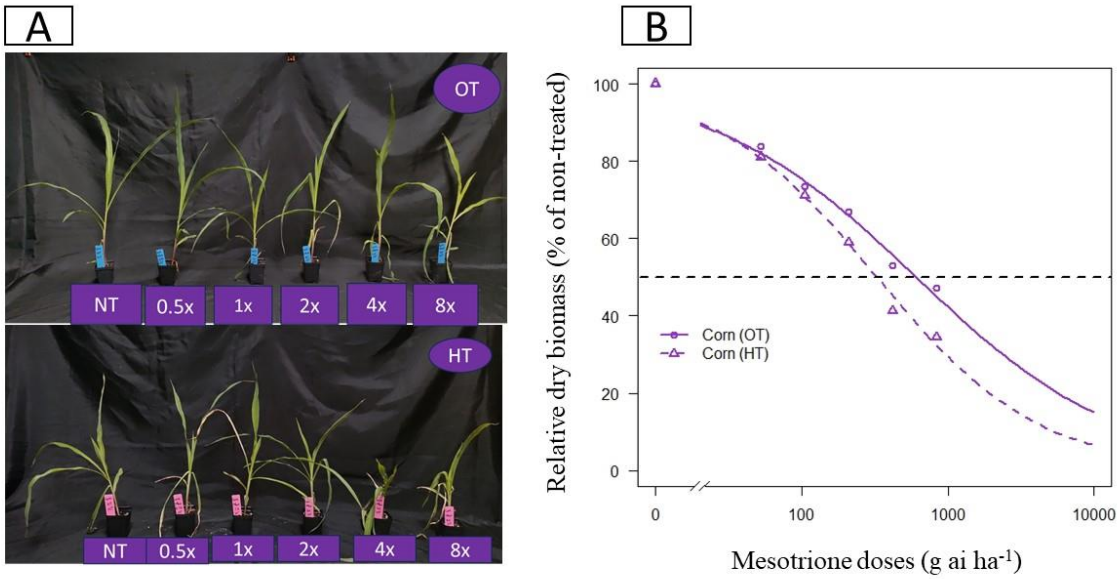

Figure 4.5 A) Response of corn to different doses of mesotrione application at OT (30/25 °C) and HT (40/35 °C). NT is non-treated check and 1x is the field recommended dose of mesotrione, 105 g ai ha $^{-1}$. B) Mesotrione dose-response curves representing the relative dry biomass of corn at both OT (solid line) and HT (dashed line) generated using the three-parameter log-logistic model. The dashed horizontal line represents the GR_{50} values.

Table 4.2 Regression parameters describing the response corn plants to herbicide treatments of 0x to 8x doses at OT (30/25 °C) and HT (40/35 °C), where GR_{50} is the dose of herbicides required for 50% dry biomass reduction and S.E. (numerical within parenthesis) is the standard error.

Herbicide	**GR_{50} (± S.E.) Values (g ae/ai ha^{-1})**	
	OT	HT
2,4-D	2180.7 (439.1)	802.5 (146.7)
Dicamba	2615.2 (285.9)	1579.2 (391.4)
Atrazine	21355.1 (4746.2)	10109.9 (2568.2)
Mesotrione	1403.2 (417.4)	583.07 (168.7)
Tembotrione	602.4 (75.8)	325.7 (42.9)

4.4 Discussion

The study revealed an increased injury of corn plants to both 2,4-D and dicamba under HT conditions as compared to OT (figure 4.1 and 4.2). This increased injury was evident from the data of visual injury and biomass reduction. These findings suggested that prolonged exposure to HT intensifies the stress imposed on corn plants by herbicide application. Improved efficacy of 2,4-D and glyphosate in controlling common ragweed (*Ambrosia artemisiifolia* L.) and giant ragweed (*Ambrosia trifida* L.) at high temperatures (Ganie et al., 2017). Increasing temperatures had little to no influence on the dicamba, picloram and 2,4-D ester injury in buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) (Friesen & Dew, 1966). In contrast, HT (34/24

°C) stress decreased efficacy to 2,4-D with less injury in KCTR populations at OT (24/14 °C) in Palmer amaranth (Rudell et al., 2023). Temperature also influenced the effectiveness of 2,4-D and dicamba with plants showing higher phytotoxicity at LT (31/20 °C; low temperature) than HT (41/30 °C) conditions (Johnston et al., 2019). In common waterhemp (*Amaranthus tuberculatus*), HT (34/20 °C) decreased the efficacy of 2,4-D and increased the level of resistance in resistant WHR (Shyam et al., 2019). Elevated temperatures reduced the efficacy of dicamba and glyphosate in controlling kochia (*Bassia scoparia*), attributing this to decreased absorption and translocation of the herbicides (Ou et al., 2018). In synthetic auxins like 2,4-D, dicamba an increase in temperature from 30 to 40 °C causes increased volatility and increases drift (Behrens & Lueschen, 1979; Ouse et al., 2018). In soybeans (*Glycine max*), dicamba drift resulted in increased injury when temperature increased from 15 to 30 °C, however did not increase further at higher temperature up to 40 °C (Behrens & Lueschen, 1979). Therefore, it is evident that the effect of temperature on herbicide efficacy is species specific and varies among herbicides (Johnston et al., 2019; Varanasi et al., 2016b).

PS-II inhibitor like atrazine injury observed in corn is attributed to the buildup of atrazine absorbed through the foliage and the weakened condition of the plants cultivated under stressful environmental conditions (Thompson et al., 1970).The results from this study emphasize the crucial role of temperature in influencing the response of corn plants to atrazine, with higher temperatures enhancing the herbicide impact on herbicide efficacy and biomass accumulation. Elevated temperatures increased atrazine absorption, metabolism, and reduced photosystem-II activity in common bean (*Phaseolus vulgaris*) and redroot pigweed (*Amaranthus retroflexus*), thus increasing foliar-applied atrazine phytotoxicity. High temperatures and wider temperatures

fluctuations increased the injury to corn following post-emergence herbicide applications of tolpyralate + atrazine (Metzger et al., 2019).

Corn plants exhibited increased sensitivity to both mesotrione and tembotrione at HT compared to OT (Figure 4.4 and 4.5). HT stress showed increased foliar activity of mesotrione in plants (Johnson & Young, 2002). In Palmer amaranth, the effectiveness of mesotrione declined significantly as the temperature rose from 25 °C to 40 °C, suggesting a potential enhancement in mesotrione metabolism at elevated temperatures (Godar et al., 2015). In another case, no significant differences were observed that different temperatures (18, 26, or 34 °C) on mesotrione efficacy and injury in perennial ryegrass (*Lolium perenne*) and large crabgrass (*Digitaria sanguinalis*) (McCurdy et al., 2008, 2009). In the study conducted by Johnson and Young (2002), they observed that common water hemp and large crabgrass (*Digitaria sanguinalis* L.), both C4 plants, were more susceptible to mesotrione at 18 °C compared to 32 °C. Conversely, cocklebur (*Xanthium strumarium* L.) and velvetleaf (*Abutilon theophrasti* (L.) Medic.), C3 plants, displayed higher susceptibility to mesotrione efficacy at 32 °C as opposed to 18 °C.

The response of crops or plants to herbicide application can be attributed to temperature's influence on various morphological and physiological processes. In general, higher temperatures can enhance herbicide performance by increasing absorption and translocation, potentially due to the greater permeability of the cuticular wax layer at elevated temperatures (Schönherr et al., 1979). However, it's important to note that in some cases, high temperatures can reduce herbicide uptake, primarily due to the rapid drying of spray droplets into solid deposits (Price, 1983). Additionally, high-temperature stress may decrease herbicide activity in plants by accelerating metabolic processes (Johnson & Young, 2002). Temperature variations can also impact the rate

of herbicide degradation, influencing their toxicity on plants (Hatzios & Penner, 1982). Cytochrome P450 monooxygenase (P450) enzymes play a role in herbicide detoxification through xenobiotic metabolism (Frear et al., 1969; Frear & Swanson, 1996) . In most weed species, the efficacy of herbicides like mesotrione and 2,4-D in the above examples is affected by changes in absorption, translocation, and metabolism under high-temperature stress conditions (Ganie et al., 2017; Godar et al., 2015; Ou et al., 2018; Rudell et al., 2023; Shyam et al., 2019)

4.5 Conclusions

In conclusion, the findings of this study indicate that HT can affect the growth and development of corn and its response to herbicide application after planting. With increasing temperatures and herbicide resistance becoming more prevalent, weeds have a competitive advantage over crops, highlighting the need for better weed management practices. However, controlling weeds in corn can be particularly challenging due to the limited options for herbicides and the potential for crop damage under high temperature stress. In addition to temperature, other factors such as relative humidity, light, and precipitation can also affect herbicide performance and crop damage. Therefore, further research is necessary to investigate the impact of environmental and plant-related variables on herbicide efficacy and corn damage, which is critical for the judicious use of herbicides in weed management.

Chapter 5 - General Discussion, Conclusions, Implications and Future directions

Problems addressed:

Weeds are one of the important pests that threaten sustainable crop production (Oerke, 2006). Although several management strategies are available, growers primarily depend on chemical control methods using herbicides to control weeds. Over-reliance on herbicides as a sole method of weed control resulted in the evolution of resistance to herbicides in numerous weed species in major crops across the globe. Importantly, resistance to most commonly used in cropping systems such as photosystem (PS-II)-, acetolactate synthase (ALS)-, acetyl-CoA carboxylase (ACCase)-inhibitors, and glyphosate poses a serious challenge for weed management. ALS-inhibitor resistance is a serious challenge within herbicide resistance management (Heap, 2023) and prevalent worldwide (Yu and Powles 2014). These herbicides are valuable for broad-spectrum weed management in wheat. However, recently, the first case of ALS-inhibitor resistance in Japanese brome was found in wheat fields in Kansas. Characterization of resistance to ALS-inhibitors is the focus of Chapter 2 of this thesis.

In addition to management of herbicide resistant weeds, another major crop production challenge is constant and steady change in global climate. Global warming caused by climate change is predicted to have an increase in temperature of up to 2-5 °C by the end of this century (IPCC, 2022). Environmental conditions like high/low temperature, precipitation, relative humidity etc, can influence the herbicide's performance or efficacy (Varanasi et al., 2016). High or low temperatures can influence the rate of herbicide, absorption, translocation and/or degradation, potentially impacting its effectiveness (Hatzios & Penner, 1982; Johnson & Young, 2002). Temperature influence on herbicide efficacy was studied in several weeds; however, little to no

literature was found in crops such as grain sorghum and corn. Weed species and crops may respond differently to herbicides under high-temperature stress. Some may become less susceptible to herbicides, while others exhibit increased sensitivity. This variability can complicate weed management strategies adopted by the farmers and, therefore, is essential to investigate crop response to herbicides under temperature stress. Chapters 3 and 4 of this thesis, studied the response of grain sorghum and corn to post-emergence (POST) herbicides such as synthetic auxins, PSII and HPPD-inhibitors.

Results

***Chapter 2*:** Japanese brome is a winter annual weed belonging to the brome family and is one of the important weeds in winter wheat. ALS inhibitors like propoxycarbazone-Na are known to control this weed effectively and are widely used. In Kansas, three resistant populations (R1, R2, R3) of Japanese brome from winter wheat fields were failed to be controlled with propoxycarbazone-Na, an important ALS-inhibitor. This Chapter investigated the level of resistance as well as mechanism of resistance to this herbicide in Japanese brome. Dose-response results suggested a high level of resistance in the three populations R1, R2, and R3 (167, 125, and 667-fold, respectively) compared to a known susceptible population (S1). Importantly, Pro-197-Thr (R3, R1)/Ser (R2, R1) amino-acid substitutions in *ALS* gene were found to confer resistance. Different cross-resistance patterns were also found among the resistant populations to other ALS-inhibitors, such as sulfosulfuron, mesosulfuron, pyroxsulam, and imazamox, depending on the type of amino-acid substitution. Similarly, ALS-inhibitor-resistant populations due to target site mutation were identified in two resistant populations of Japanese brome in China., with 66.7- 120-fold resistance to flucarbazone- Na, due to Pro-197-Ser and Asp-376-Glu amino acid substitutions (Lan et al., 2022; Li et al., 2022).

Chapter 3: Grain sorghum genotypes, RTx430 and P84G62, were grown in growth chambers maintained at two temperature regimes: optimum (32/22 °C; OT; day/night (d/n)) and high-temperature (40/30 °C; HT; d/n) conditions and were treated separately with POST herbicides, including 2,4-D, pyrasulfotole + bromoxynil, or mesotrione. The results revealed that upon treatment with mesotrione or pyrasulfotole + bromoxynil sorghum plants exhibit more herbicide injury at HT than OT but not with 2,4-D. D, possibly because of differences in the mode of action of these herbicides. Further, the sorghum hybrid, P84G62 had less injury compared to RTx430, irrespective of temperature stress or herbicide treatment, suggesting that such response is likely genotype specific in sorghum.

Chapter 4: In this Chapter, corn (DKC59-82RIB) plants were grown at OT (30/25 °C; d/n) and HT (40/35 °C; d/n) were treated separately with 2,4-D, dicamba, atrazine, mesotrione, or tembotrione to evaluate its response to these herbicides at temperature stress. The data indicate that corn plants exhibit more injury when treated with the above herbicides at HT than OT.

Overall, the outcome of Chapter 3 and 4 suggest that HT can negatively impact grain sorghum and corn growth in response to POST herbicide application. The results from this research provide insights about judicious use of herbicides in in these crops.

Future prospects:

Investigation of inheritance of ALS-inhibitor resistance in Japanese brome will help understand the recombination and expression of mutations in *ALS* gene in the progeny. Crossing between resistant (R1, R2, R3) and S1 plants of Japanese brome to generate F1 progeny and subsequently, F2 progeny (i.e., self-pollination of F1 plants) and/or backcrosses (BC) will help understand the segregation of the two mutations found in resistant parents, thereby the cross-resistance pattern as well. It was suggested that depending on the resistance gene/mechanism,

varied patterns of inheritance were found in weed species (Ghanizadeh et al., 2019). One of the possible reasons for high frequency of occurrence of ALS- inhibitor resistance compared to other herbicides in weed species is because of inheritance of this herbicide resistance primarily via a single, dominant, nuclear-encoded gene (Tranel & Wright, 2002). To fully understand the impact of the Pro-197 mutations on resistance levels, further research should involve conducting an ALS enzyme assays of R3, R2, and R1. This assay, using propoxycarbazone and imazamox, would allow for a precise measurement of the resistance conferred by the two Pro 197 mutations. Such a detailed enzymatic analysis would provide valuable insights into the functional consequences of these mutations and contribute to a more nuanced understanding of herbicide resistance mechanisms in these biotypes.

Global warming with constant increase in atmospheric temperature is a threat to crop production. Temperature stress can impact crop performance in response to herbicide-based weed control. Importantly, as mentioned above, when plants are exposed to temperature stress, herbicide absorption, translocation, and/or degradation are known to be altered, ultimately affecting their performance. Although results of Chapter 3 and 4 suggests at HT grain sorghum and corn exhibit more injury in response to POST herbicide treatments than at OT, the physiological basis for such response is not known. Future research using radiolabeled (^{14}C) herbicides to assess the absorption, translocation, and metabolism of 2,4-D, mesotrione, or atrazine in sorghum and corn exposed to temperature stress are needed.

Future research focusing on effect of temperature stress on post-herbicide injury comparing different widely grown hybrids/varieties of grain sorghum/corn, rather than parental line vs hybrid is needed for understanding the practical implications of temperature stress on crop injury to herbicides and also to understand temperature-dependent dynamics of post-

herbicide effects. Such comparative approach is also valuable for optimizing herbicide application strategies tailored to different genetic backgrounds.

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