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**DÉBORA DE OLIVEIRA LATORRE**

# LEVANTAMENTO DA SUSCEPTIBILIDADE DE *Conyza canadensis* E RESISTENCIA CRUZADA EM *Amaranthus tuberculatus* EM NEBRASKA, ESTADOS UNIDOS DA AMERICA

**Botucatu**

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Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Doutor em Agronomia (Proteção de Plantas).

Orientador: Caio Antonio Carbonari
Coorientador: Greg Kruger

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TÍTULO: "LEVANTAMENTO DA SUSCETIBILIDADE DE *CONYZA CANADENSIS* E RESISTÊNCIA CRUZADA EM *AMARANTHUS TUBERCULOTUS* EM NEBRASKA, ESTADOS UNIDOS DA AMÉRICA"

**AUTORA: DÉBORA DE OLIVEIRA LATORRE**
**ORIENTADOR: CAIO ANTONIO CARBONARI**
**COORIENTADOR: GREG KRUGER**

Aprovada como parte das exigências para obtenção do Título de Doutora em AGRONOMIA (PROTEÇÃO DE PLANTAS), pela Comissão Examinadora:

Prof. Dr GREG KRUGER
Department of Agronomy and Horticulture / University of Nebraska - Lincoln

Prof. Dr. EDIVALDO DOMINGUES VELINI
Departamento de Produção e Melhoramento Vegetal / FCA / UNESP de Botucatu/SP

Prof. Dr. ULISSES ROCHA ANTUNIASSI
Depto de Engenharia Rural / Faculdade de Ciências Agrônomica - UNESP

Dr. RAMIRO FERNANDO LOPEZ OVEJERO
REGULAMENTAÇÃO MONSANTO DO BRASIL / DEPTO MONSANTO DO BRASIL

PROFESSOR ASSOCIADO PEDRO JACOB CHRISTOFFOLETI
Departamento de Produção Vegetal / ESCOLA SUPERIOR DE AGRICULTURA LUIZ DE QUEIROZ - USP

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*Aos meus amados pais e irmã,*
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## RESUMO

Os herbicidas são um dos fatores mais importantes que vem consideravelmente contribuindo no aumento na proteção das culturas, devido sua inovação no controle de plantas daninhas ao longo dos últimos 70 anos. O uso continuo de um mesmo ingrediente ativo ou modo de ação impõe uma alta pressão de seleção em uma população de plantas daninhas e a seleção de indivíduos resistentes a herbicidas pode ocorrer. A intensidade da seleção imposta pelos herbicidas e a frequência inicial de indivíduos resistentes a herbicidas dentro de uma população de plantas daninhas são fatores chave importantes no processo de evolução da resistência. Fluxo gênico via pólen, sementes e propágulos vegetativos são uma potencial fonte de distribuição de resistência a herbicidas, como previamente reportado em *Conyza canadensis* e *Amaranthus* ssp. *Conyza canadensis* e *Amaranthus* ssp são potencialmente capazes de transferir genes que conferem resistência a herbicidas via pólen e/ou sementes, por produzirem pólen que pode ser disseminado a longas distancia e grande número de sementes. Os objetivos gerais dos estudos realizados foram caracterizar o nível de resistência de duas espécies de plantas daninhas de Nebraska, Estados Unidos da América. Um primeiro estudo em casa de vegetação foi conduzido para caracterizar o nível de resistência a glyphosate de populações de buva coletadas em áreas não cultivadas foi conduzido. Experimentos de dose-resposta com 9 doses de glyphosate e 28 populações de buva foram avaliados. Um segundo estudo em casa de vegetação foi conduzido para caracterizar o nível de uma população de caruru resistente a 2,4-D a diferentes formulações de herbicidas fenóxicos. De acordo com o primeiro estudo de dose-resposta, menos de sete por cento das populações de *Conyza canadensis* em áreas de pastagem próximas a áreas de cultivo expressaram “resistência prática” a glyphosate (plantas sobreviventes a dose de glyphosate mais usual em Nebraska – 1,260 g ae $ha^{-1}$). Baseado em nossos resultados, foi detectado baixa frequência de resistência a glyphosate em populações de *Conyza canadensis* em áreas de pastagem de Nebraska, indicando que indivíduos resistentes a glyphosate dispersos das áreas de cultivo não são o biótipo predominante nessas áreas. Os resultados do segundo estudo mostraram que a população de *Amarantus tuberculatus* resistente a 2,4-D foi significativamente mais suscetíveis às formulações dos herbicidas Dicamba DGA, Dicamba DMA, Corasil, 2,4-DP, e 2,4-DP-p, enquanto sobreviveram a altas doses dos herbicidas 2,4-D 2EHE, 2,4-D EE, 2,4-DB, MCPB, MCPA, MCPA 2EHE, CMPP e CMPP-p.



**Palavras-chave**: Plantas daninhas resistentes. Herbicidas. Pressão de seleção. Glyphosate. Herbicidas auxínicos.

## ABSTRACT

SUSCEPTIBILITY OF *Conyza canadensis* AND CROSS-RESISTANCE OF *Amaranthus tuberculatus* SURVEY IN NEBRASKA, UNITED STATED OF AMERICA

Herbicides are one of the most important factors that have contributed to protect crop yields. This is due to innovative weed control over the last 70 years. The over-reliance on a single herbicide active ingredient or mode of action impose a high selection pressure on a weed population and the selection of herbicide-resistant individual plants may occur. The intensity of selection imposed by herbicides and the initial frequency of herbicide resistant in a weed population play a major role in the herbicide resistance evolution. Gene flow by pollen, seed, and vegetative propagules have the potential to move herbicide-resistant weed species, as reported previous reported in *Conyza canadensis* and *Amaranthus* genus. *Conyza canadensis* and *Amaranthus tuberculatus* are potentially able to proliferate herbicide resistance by pollen and/or seeds due to be prolific seed producer and its pollen are capable to be disseminated for long distances. The general objectives of these studies were to characterize the herbicide resistance level of two weed species in Nebraska, United States. A greenhouse study was performed to characterize the fold of glyphosate resistance in horseweed populations from non-crop areas. Dose-response experiments with 28 horseweed populations were evaluated across nine glyphosate rates. A second greenhouse study was performed to characterize the level of a 2,4-D-resistant waterhemp population resistance to various auxinic herbicides. According to the first dose-response study, less than seven percent of the rangeland Conyza canadensis populations screened expressed “practical” resistance to glyphosate (plants surviving to most common glyphosate rate used in Nebraska of 1,260g ae $ha^{-1}$). Therefore, low frequency of GR in horseweed populations was detected in Nebraska rangeland indicating that GR individuals dispersed from row crops into rangeland are not the predominant biotype in these non-row crop areas. For the second study, the results showed that 2,4-D-WR population were significantly more sensitive to Dicamba DGA, Dicamba DMA, Corasil, 2,4-DP, and 2,4-DP-p herbicides formulations, whereas survived to the higher doses of 2,4-D 2EHE, 2,4-D EE, 2,4-DB, MCPB, MCPA, MCPA 2EHE, CMPP and CMPP-p. The founds on this studied showed the 2,4-D-WR population exhibits cross-resistance to 2,4-D 2EHE, 2,4-D EE, 2,4-DB, MCPB, MCPA, MCPA 2EHE, CMPP and CMPP-p herbicides.



**Keywords**: Weed resistance. Herbicides. Selection pressure. Glyphosate. Auxinic herbicides

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# GENERAL INTRODUCTION

Herbicides are by far the most important factors that have been contributing considerably to increase crop yields due innovating weed control over the last 70 years (1946-2017), since the introduction of 2,4-dichlorophenoxyacetic acid (2,4-D) in 1946 during world war II (HEAP, 2014; KIRBY, 1980). Herbicides are chemical compounds used to manage unwanted plants, binding to and inhibiting function in a specific target site in plants pathways. Most target sites are enzymes, even though some herbicides can interact with photosynthesis or mimicking plant hormones (e.g., paraquat and 2,4-D, respectively) (BECKIE; WOLF, 1999). Nowadays herbicides are the primary weed control method in agronomic crops due provide effective, reliable and economical weed control in large weeds species worldwide. Nevertheless, the continuous herbicide application to a plant population has some drawbacks, as “weed shifts” and the arrival of herbicide-resistant weeds. The prolonged and repeated use of an herbicide is a strong selection pressure for individual plants carrying genes conferring herbicide resistance (POWLES; PRESTON, 1995).

This general introduction will summarize aspects of herbicide resistance in 5 different sections: (1) Resistance, Tolerance, Cross Resistance and Multiple Resistance definitions; (2) Resistance Origin and Dispersion, (3) Global Herbicide resistance situation, (4) New technologies and (5) Final Considerations.

## 1. RESISTANCE, TOLERANCE, CROSS RESISTANCE AND MULTIPLE RESISTANCE DEFINITIONS

To avoid confusion among terms will be used on this literature review, it is important to understand some concepts descrbed by the Weed Science Society of America (WSSA) and by the Herbicide Resistance Action Committee (HRAC). The plants can respond herbicide application in three main ways. **Susceptibility** is the inherited ability of a population to be controlled after exposure to an herbicide application. **Tolerance** is the natural inherent ability of a species to survive and reproduce after exposure to an herbicide application (WSSA,1998). **Resistance** is the evolved capacity of a weed population survive an herbicide application that usually controlled it and reproduce, and can occur naturally or induced by techniques of genetic engineering or selection in tissue culture or mutagenesis (HEAP, 2014; HRAC, 2017a; WSSA, 1998). **Cross resistance** is the expression of a genetically-endowed

mechanism conferring the ability of plants to withstand herbicides from a similar chemical group (POWLES; PRESTON 1995). **Multiple resistance** is the ability of individuals or populations to withstand herbicides with different sites of action (POWLES; PRESTON 1995; THILL, 2003).

## 2. HERBICIDE RESISTANCE ORIGIN AND DISPERSION

The evolution of herbicide resistance in weeds has become a global threat to modern agriculture, and to food security. There were no confirmed reports of weed resistance until the late 1950s, when the first reported herbicide-resistant case was with the spreading dayflower (*Commelina diffusa* Burm.) to 2,4-D in Hawaiian sugar cane fields in 1957 (HILTON, 1957). Since that time, there have been increasing reports of herbicide resistance to many herbicides classes. Resistance has now been reported to all main herbicides mode of actions and troublesome weed species, in all continents worldwide (HEAP, 2017).

Weed populations develop resistance to herbicides by natural selection of pre-existing resistant biotypes within a plant population, or by occasional natural mutation (KISSMAN, 2003). Numerous factors influence the evolution and dispersion of herbicide-resistant weeds, including herbicide mode of action, its use rate, the site of action, the weed species, the population size, the environment, genetic variation for resistance, initial frequency of resistance alleles, fitness, inheritance of resistance, and gene flow (BUSI et al., 2013; JASIENIUK; BRÛLÉ-BABEL; MORRISON, 1996; PRESTON; POWLES, 2002; SARANGI et al., 2017). For Holsinger (2000), an additional factor that can lead an additive genetic variation for resistance is the rate of herbicide resistance evolution in weed populations exposed to repeated selection of a sublethal dose. Basically, resistant weed can survive herbicide application by two types of mechanisms (BECKIE; TARDIF, 2012; DÉLYE; JASIENIUK; LE CORRE, 2013). Target-site resistance (TSR) mechanism is a result of modifications in the 3D structure of the herbicide target protein decreasing herbicide binding, or increasing activity (e.g. amplification of the target-site gene) of the target protein. Nontarget-site (NTSR) include any another mechanism (e.g. differences in metabolism, reduced leaf uptake or sequestration) (DÉLYE; JASIENIUK; LE CORRE, 2013; POWLES; YU, 2010; YUAN; TRANEL; STEWART, 2007).

Numerous weed species are prone to develop herbicide resistance due totheir innate genetic variability and also, for some herbicides, numerous plant mutations can confer target site mutations (HEAP, 2014). *Lolium perenne ssp. multiflorum* (Italian Ryegrass) is one of few weeds in the United States that rapidly evolved resistance to multiple herbicide site of action (e.g. photosystem (PS) II-, acetolactate synthase (ALS)-, 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS)-, acetyl CoA carboxylase (ACCase)-, Glutamine synthetase (GS)-, Long chain fatty acid-inhibitors, Photosystem I Electron Diverter and Carotenoid biosynthesis), including 21 multiple resistance cases in seven countries (HEAP, 2017; LIU; HULTING; MALLORY-SMITH, 2016; MAHMOOD et al., 2016).

Amaranthaceae family is a worldwide troublesome weed family prone to evolve resistance (e.g. is *Amaranthus palmeri* (Palmer amaranth) and *Amaranthus tuberculatus* (waterhemp)). From the time its first reported resistance in South Carolina in 1989 to trifluralin (GOSSETT; MURDOCK; TOLER, 1992), Palmer amaranth has now been confirmed in 59 resistant cases to five herbicide site of action (e.g. microtubule-, photosystem (PS) II-, acetolactate synthase (ALS)-, 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS)-, and hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors) (CHAHAL et al., 2015; HEAP, 2017; JHALA; SANDELL; KRUGER, 2014; NETTO et al., 2016; SOSNOSKIE et al., 2011). Waterhemp have evolved resistant to five sites of actions (ALS-, Photosystem II-, PPO-, HPPD-inhibitors, and Synthetic Auxin) in Canada and United States (EVANS, 2016; HEAP, 2017; HORAK; PETERSON, 1995; SCHRYVER et al., 2017). In Nebraska, the two widespread and troublesome Amaranthus species are A*. tuberculatus* and *A. palmeri*. Palmer amaranth and waterhemp are obligate outcrossing species characterized by high genetic variability (CHAHAL et al., 2015; NORDBY et al. 2007). Outcrossing represents only a fraction of gene flow, which is defined as the gene movement from one population to another population (SLATKIN, 1985). Gene flow majority occurs by pollen, seed, and vegetative propagule (MALLORY-SMITH et al., 2015).

Gene flow by pollen has been occurring since the subsistence of higher plants and has significantly influenced genetic diversity in a population, the frequency of multiple or polygenic herbicides resistance, and the evolutionary dynamics of a species and adaptation of populations along years (ELLSTRAND, 1992; MALLORY-SMITH; HALL; BURGOS, 2015). On the other hand, can advance the dispersion and spread

of herbicide-resistant population to another in crop areas. Gene flow by pollen have been reported in a few weed species as *Conyza canadensis* (HUANG et al., 2015), *Amaranthus palmeri* (SOSNOSKIE et al., 2012), *Amaranthus rudis* (SARANGI et al., 2017), *Lolium rigidum* (BUSI et al., 2008*)*, *Avena fatua* (MURRAY et al., 2002), *Kochia scoparia* (BECKIE et al., 2016; STALLINGS et al., 1995), *Chenopodium album* (YERKA; DE LEON; STOLTENBERG, 2012), *Plantago lanceolata* (TONSOR, 1985), *Echinochloa crus-galli* (BAGAVATHIANNAN; NORSWORTHY, 2014). Although several types of research have been led in efforts to establish the probability of hybridization between related weed species. Previously studies have confirmed that *Amaranthus* and *Conyza* and species are accomplished on interspecific hybridization (FRANSSEN et al., 2001; SOARES et al., 2015; TRUCCO et al., 2005; ZELAYA; OWEN; VANGESSEL, 2007). Wetzel et al. (1999) reported introgression of ALS resistance between Palmer amaranth and waterhemp, even though Franssen et al. (2001); Gaines et al. (2012); Steinau; Skinner; Steinau (2003); Trucco et al. (2007) found very low introgression levels and also nonviable or sterile with majority hybrid offspring from this cross. Nandula et al. (2014) suggested introgression of EPSPS resistance from Palmer amaranth to *A. spinosus*, and Jugulam; Walsh; Hall (2014) demonstrated introgression of the MCPA-resistant characteristic from *Raphanus raphanistrum* into *Raphanus sativus*. Soares et al. (2015) and Zelaya; Owen; VanGessel (2007) found gene flow between *Conyza bonariensis* and *C. canadensis* and *C. canadensis* and *C. ramosissima*, respectively.

Several herbicides groups have a great propensity to evolve resistance (e.g. ALS- and ACCase-inhibitors) in weed species. ALS inhibitors herbicides have been used over 30 years in the most part of crops, is effective to control a broad-spectrum of weed species, and is the troublesome herbicide group due to a large number of weeds species evolving resistant worldwide. The first resistance case reported was *Lolium rigidum* metabolic resistant to chlorsulfuron in 1980 in Australia (HEAP; KNIGHT, 1982), and the most intractable resistance involved multiple resistance to several herbicide sites of action in Australia (BURNET et al., 1994a, 1994b; HEAP; KNIGHT, 1982; TARDIF; HOKUM; POWLES, 1993).

For other herbicides, there are limited target site mutations that might confer resistance, appearing them quite low-risk herbicides for resistance (e.g. synthetic auxins and glyphosate). Since the discovery of the first *Daucus carota* resistant population in Canada to auxinic herbicide, there has been a slow increase of weed

species in number to auxinic herbicide resistance, as it has related to only 34 species (HEAP, 2017). The infrequency in auxinic herbicides resistance occurrence related to other herbicides classes has been endorsed due to multiple sites of action of these herbicide class (GRESSEL; SEGEL, 1982; MORRISSON; DEVINE, 1994). Even so, this hypothesis has not been directly tested, and majority studies on inherence of auxinic herbicides resistance are conferred by a single gene dominant alleles (JASIENIUK; MORRISON; BRÛLÉ-BABEL, 1995; JUGULAM; MCLEAN; HALL, 2005; MITHILA et al., 2011; PRESTON et al., 2009; PRESTON; MALONE, 2015).

Another important factor that can influence the rate of evolution in resistance is the selection pressure by herbicides overuse. The increased use of glyphosate after glyphosate-resistant crops had been marketed (e.g. soybean in 1996 and corn in 1997) was the trigger to the evolution of glyphosate-resistant weeds (BECKIE; HALL, 2014; POWLES, 2008; PRESTON et al., 2009). For Kraehmer et al. (2014), the rapid adoption of glyphosate as the single-weed control measure in major American row crops, particularly soybean and cotton, had a profound effect not only on farmers and weed resistance evolution but on the agrochemical industry. The glyphosate overuse resulted in 37 weed species resistant, including 18 species with multiple resistance on 6 continents (HEAP, 2017), even though have been considerate as a low-risk herbicide for resistance.

## 3. GLOBAL HERBICIDE RESISTANCE SITUATION

Cases of herbicide-resistant weeds worldwide are continuously increasing. To date, resistance to at least 23 classes of herbicide has been confirmed in more than 251 weed species (146 dicots and 105 monocots) in 91 crops and 69 countries. The United States is ranked as one of the top countries in the world with more herbicide-resistant weeds documented, followed by Australia, Canada, France and Brazil (HEAP, 2017). Acknowlodgement of the environmental and main factor that affects the evolution of herbicide resistance in plants is fundamental for understanding the mechanisms of resistance (BUSI et al., 2013).

Particularly, annual weed species represents barely 22% of all known weeds, though have been developing more cases of resistance, representing 64% of currently reported herbicide resistant cases. Annual weed species frequently occurs intensively in numbers, are broadly distributed, genetically variable, and efficient and prolific seed

producers (BECKIE, 2006). Globally, the troublesome economic herbicide resistant weeds up to 11 site of action are annual species, including Rigid ryegrass (*Lolium rigidum)*, Barnyardgrass (*Echinochloa crus-galli* var. *crus-galli*), Bluegrass (*Poa annua)*, Wild oat (*Avena fatua)*, Goosegrass (*Eleusine indica)*, Ryegrass (*Lolium perenne* ssp. *multiflorum*), Blackgrass (*Alopecurus myosuroides*), Junglerice (*Echinocloa colona*), Palmer amaranth (*Amaranthus palmeri*), Common waterhemp (*Amaranthus tuberculatus*), Smooth pigweed (*Amaranthus hybridus*), Redroot pigweed (*Amaranthus retroflexus)*, Giant ragweed (*Ambrosia artemisifolia*), Horseweed (*Conyza canadensis*), and Kochia (*Kochia scoparia*) (HEAP, 2017).

In general, the vast majority herbicide resistance cases reported, weed populations found a way to acquire a combination of the TSR and NTSR mechanisms, leading to an enhanced level of herbicide resistance worldwide. Currently, nuclear monogenic control of TSR has been identified to herbicides groups ACCase-, ALS-, Microtubule-, PPO-, and EPSPS-inhibitors, whereas inherence of TSR to PSII-inhibitors (e.g. triazines) is cytoplasmatic (AVILA-GARCIA et al., 2012; BECKIE; TARDIF, 2012; POWLES; YU, 2010; SHANER; LINDENMEYER; OSTLIE, 2012), conferred by dominant or semi-dominant and recessive alleles (CHANDI et al., 2012; DIGGLE; NEVE, 2001; HUFFMAN et al., 2015; JUGULAM; MCLEAN; HALL, 2005; KERN et al., 2002; OKADA; JASIENIUK, 2014; PRESTON; POWLES, 2002; VARGAS; MORAES; BERTO, 2007). NTSR is the majority mechanism conferring resistance in EPSPS-, ACCase-, ALS-inhibitors and Synthetic auxins (BECKIE; TARDIF, 2012; CHRISTOFFOLETI et al., 2015; DÉLYE; JASIENIUK; LE CORRE, 2013; MITHILA et al., 2011; POWLES; YU, 2010; SHANER; LINDENMEYER; OSTLIE, 2012).

Presently, the United States has about double the number of reported resistant weed species of Australia and 3.5 times more reported resistant weeds cases than Brazil. There are 160 weed species that have evolved resistance in the United States in the past 60 years. Of the resistant weed biotypes, 52 are acetolactate synthase (ALS) inhibitors resistant, 26 are resistant to photosystem II inhibitors, 17 to 5-enolpyruvylshikimate acid-3-phosphate synthase (EPSP synthase) inhibitors, 15 to acetyl CoA carboxylase (ACCase) inhibitors, 11 to photosystem II inhibitors (ureas and amides), 8 to synthetic auxins, 6 to photosystem I electron diverter and microtubule inhibitors and 19 to others herbicides family groups (HEAP, 2017).

## 4. NEW HERBICIDE RESISTANCE TECHNOLOGIES

Roundup Ready® (RR) crops were introduced by Monsanto in 1996, innovating weed management and no-till methods in cropping systems. Before RR crops, growers need to identify weeds and planning strategies to control them with selective herbicides. RR soybeans were the first resistant crop released in the United States, followed by RR corn and RR cotton. RR canola, RR sugar beet, and RR alfalfa have also been released. RR crops were glyphosate-resistant, which meant that farmers could spray postemergence glyphosate for all crop season and achieve excellent and broad spectrum weed control. The success factors of this technology are due glyphosate provide excellent and economical weed control, higher yield, with an easy management, reducing the environmental impact due reduction of soil tillage (GREEN, 2012, 2014). The lack of rotation to other herbicide-resistant crops and limited use of herbicides other than glyphosate contributed to the development of glyphosate resistance. In 2000, was related the first weed species resistant to glyphosate in a row crop (soybean) (VANGESSEL, 2001). At the present, there are 37 weeds worldwide reported to be resistant to glyphosate.

In response to the evolution of glyphosate-resistant weeds, seed and agrochemical companies have been looking for new methods of weed control (e.g. new herbicides, crops genetically modified). Discovering new potentials herbicides is tough, recent estimates are that scientists need a screen over than 200,000 chemicals have been screened to discover a new commercial herbicide, that sometimes have not economical return, and demand a decade until its commercialization at a cost of over US$ 250 million (GREEN, 2014). Another alternative to control the issue of glyphosate-resistant weeds is the development of multiple herbicide-resistant crops. Genetically modified crops allow crops to survive to different herbicide groups, increasing herbicide utility (DEVINE, 2005). Up to now, BASF, Bayer, DowAgrosciences, Monsanto, and Syngenta companies are developing new herbicide-resistant crops in combination with glyphosate resistance (SERVICE, 2013).

Glyphosate and glufosinate-resistant crops are already available to help farmers to control glyphosate-resistant weed in corn, soybean, and cotton (GREEN, 2012). A new platform that stacks glyphosate resistance with 2,4-D and glyphosate resistance and glyphosate resistance with dicamba, have been tested by farmers and are waiting for the regulatory approvals for commercialization in the United States. New auxinic

herbicides formulations with less volatile salts and drift adjuvants were developed to reduce off-target movement (LI et al., 2013). Soybean glyphosate plus isoxaflutole stack have been expeteded to be commercialized in the next months. In addition, crops with a combination of resistance to glyphosate with resistance to the sulfonylurea (ALS inhibitors), a resistance to glyphosate plus glufosinate and HPPD inhibitors, glyphosate plus glufosinate, 2,4-D and ACCase inhibitors, glyphosate plus glufosinate and dicamba will be commercialized not long from now (GREEN, 2012; KRAEHMER et al., 2014).

## 5. FINAL CONSIDERATIONS

Agriculture is a major industry in the United States and in Brazil, represented basically by soybean, corn, and cotton, those have been responsible for the majority herbicide resistant cases of weed species globally. Predominantly areas in the United States and Brazil are cultivated with RR crops, leading to an overuse of the herbicide glyphosate and causing weeds to evolve resistane to that herbicide. New herbicide-resistant crops are already available or will be available in the next decade in these countries.

Benefits related to genetically modified crops are excellency of weed control, the increase in yield, the ability to use less tillage, and using safer herbicides (CERDEIRA; DUKE, 2006; NATIONAL RESEARCH COUNCIL, 2010; TERRY M. HURLEY, 2010). However, the overuse of these technologies and the recurrent use of the same herbicide or/and tank mixtures without an adequate weed management approach could increase the likelihood of selection pressure for resistance result in weed issues, as multiple-resistant weeds. No weed management technology used alone is sustainable. These new technologies are expected to enhance a new tool to the weed management toolbox for control of difficulty and resistant weeds.

To avoid evolvement of resistant-weed species, certain weed management strategies should be assumed: (1) use herbicide when necessary, (2) rotate herbicides modes of action, avoinding sequential applications of herbicides with the same active ingredient or site of action, (3) apply tank mixtures containing herbicides with dfferent mode of actions, (4) rotate crops with different life cycles and not use repeated herbicides, (5) use weed-free seed to plant in your area, (6) planting new herbicide-resistant crops, (7) combine different methods to manage weeds, as cultural,

mechanical, biological and chemical, (8) seed bank management, (9) scout fields habitually to identify weed species present, as well how weeds are responding to the currently management, (10) clean tillage and harvest equipments to avoid dispersion to areas not affected with some resistant weed biotype (HRAC, 2017b; OWEN, 2016; WSSA 2017).

## 6. DISSERTATION OBJECTIVES

Herbicide-resistant weeds evolution is a natural-selection process and occurs basically due to herbicide-susceptible plants have been controlled, while plants with an herbicide-resistant gene can survive and reproduce, leading to increase the frequency of herbicide resistance gene. One of a couple sources of resistance genes is the gene flow, which can occur by pollen, seed, and vegetative propagule. Gene flow by pollen is significantly a potential for evolve herbicide resistance in weed species, as reported in *Conyza canadensis* and *Amaranthus* genus. Horseweed and waterhemp are potentially able to transfer herbicide resistance by pollen and/or seeds due to be prolific seed producer and its pollen are capable to be disseminated for long distances.

The general objectives of these studies were to characterize the herbicide resistance level of *Conyza canadensis* and *Amaranthus tuberculatus* species in Nebraska, United States.

A study with horseweed was performed under greenhouse conditions, where was characterize the status of herbicide resistance in horseweed populations in non-crop areas.

A second study with a 2,4-D-resistant waterhemp population was achieved in greenhouse conditions with the objective to characterize the level of resistance of a 2,4-D resistant waterhemp population to various auxinic herbicides.

# CHAPTER 1

## GLYPHOSATE-RESISTANT HORSEWEED (*Conyza canadensis*) PRESENT AT LOW FREQUENCY IN NEBRASKA RANGELAND

Débora de O. Latorre[1], Spencer Samuelson[2], Caio A. Carbonari[3], Rodrigo Werle[4] and Greg R. Kruger[5]

Glyphosate-resistant (GR) horseweed (*Erigeron canadensis*) populations have been commonly documented in no-till cropping systems. Long distance seed dispersal by wind increases the chance for GR-horseweed establishment into new areas, including rangeland. The objective of this study was to confirm and quantify the level of glyphosate resistance in 28 horseweed populations collected in Nebraska's rangeland. In the greenhouse, horseweed populations were treated with 0, 217, 434, 868, 1,736, 3,472, 6,944, 13,888, or 27,776 g ae $ha^{-1}$ of glyphosate. At 21 days after treatment, visual estimates of plant injury (I) were collected, plants were harvested,

[1] Postdoctoral Research Associate (ORCID: 0000-0002-1385-4887), University of Nebraska-Lincoln, West Central Research and Extension Center, North Platte, NE, USA; [2] Graduate Research Assistant, West Central Research and Extension Center, North Platte, NE, USA; [3] Assistant Professor, College of Agricultural Sciences, São Paulo State University, Botucatu, SP, 18610-307 Brazil; [4] Assistant Professor, University of Nebraska-Lincoln, West Central Research and Extension Center, North Platte, NE, USA; [5] Associate Professor, University of Nebraska-Lincoln, West Central Research and Extension Center, North Platte, NE, USA.

Author for correspondence: Debora de O. Latorre, Postdoctoral Research Associate, University of Nebraska-Lincoln, 402 West State Farm Road, North Platte, NE 69101 USA. (E-mail: deboraolatorre@gmail.com)

and dry weights measured and converted to biomass reduction (BR). According to the dose-response study, less than seven percent of the rangeland horseweed populations screened expressed “practical” resistance to glyphosate (plants surviving to the field label rate of 1,260g ae $ha^{-1}$). Therefore, low frequency of GR in horseweed populations was detected in Nebraska rangeland indicating that GR individuals dispersed from row crops into rangeland are not the predominant biotype in these non-row crop areas.

**Nomenclature:** Glyphosate; horseweed, *Erigeron canadensis* L.

**Key words:** Dose-response, marestail, pasture, Resistance to glyphosate.

## INTRODUCTION

Herbicides have revolutionized agriculture over the last several decades, contributing significantly to increased crop yields due to their serving as an effective and economic strategy for weed control. Because of over-reliance on herbicides as sole measure for weed control and the lack of new sites of action, herbicide resistance has become a major concern in current row crop management (Dayan and Duke 2014; Heap 2014, 2018). Glyphosate has become the most widely used and is considered the most successful herbicide in history (Duke et al. 2012; Duke and Powles 2008). During the first 22 years of glyphosate commercialization (1974–1996), no glyphosate-resistant (GR) weeds were documented (Duke 2017; Sammons and Gaines 2014). In the United States, the adoption of GR (Roundup Ready Technology, Monsanto) soybean, cotton and corn crops climbed from 10% in 1997 to more than 90% in 2014-2016, intensifying the glyphosate use from 11 ton in 1996 to more than 113 ton in 2014 (Duke 2017, USDA-NASS, 2016).

As a result of over-relying on glyphosate as the major weed control method, 41 weed species have evolved GR on six continents, including the troublesome species horseweed *(Erigeron canadensis* L.) (Heap 2018). In addition, increasing adoption of GR crop technology allowed farmers to reduce the reliance on tillage for weed control, and this switch has favored establishment of traditionally noncropland and small seeded weed species such as horseweed to move and infest cropland (Bruce and Kells 1990; Duke 2017; Nandula et al. 2006). Compounding this problem, since the first documented case of GR-horseweed in the US in 2000, horseweed populations have evolved resistance to glyphosate in 25 states, including Nebraska (VanGessel 2001; Knezevic 2006; Heap 2018). Production of GR soybean and corn monocultures in Nebraska and consequent reliance on glyphosate for weed control have favored the evolution and establishment of GR horseweed populations across the state. A greenhouse evaluation of 130 horseweed populations collected from row crop areas in 40 Nebraska counties revealed that 98% were confirmed GR (Samuelson 2017).

High seed production (up to 1,340,00 seeds per plant), long-distance seed dispersal (from 100 m to 500 km), and cross-pollination at low rates (4%), could lead to evolution and establishment of GR horseweed populations in non-row crop production areas such as rangeland (Dauer et al. 2006; Davis et al. 2010; Kruger et al. 2010; Regehr and Bazzaz 1979; Shields et al. 2006). In Nebraska, 10.5 million ha of rangeland coexist with approximately 20 million ha of cropland (USDA-NASS 2017); thus, GR horseweed seed dispersal from row crops to rangeland becomes inevitable. There is a lack of published information on occurrence of GR horseweed populations dispersed to areas where glyphosate use is uncommon (e.g., pastures and rangeland) and whether those areas could be acting as “reservoirs” for herbicide

resistance. We hypothesized that most horseweed populations in rangeland areas of Nebraska are originated from row crops and GR. Therefore, the objective of this study was to characterize the distribution and level of GR in horseweed populations from rangeland areas of Nebraska.

## MATERIALS AND METHODS

**Plant Material.** Seeds from horseweed populations were arbitrarily collected from 28 rangeland sites in 17 Nebraska counties (Banner, Chase, Cheyenne, Deuel, Dundy, Fillmore, Frontier, Grant, Hayes, Hitchcock, Kearney, Keith, Lincoln, Logan, Morrill, Perkins, and Red Willow) in October 2015 (Figure 1.1, Table 1.1). These counties were chosen based on the concurrent occurrence of row crops and rangeland next to each other. Seeds from at least 20 mature plants from each population were collected and their respective GPS coordinates documented. Seeds from each population were combined in an individual plastic bag and stored at 4 C until use.

**Glyphosate Dose-response Study.** The study was conducted under greenhouse conditions at the West Central Research and Extension Center, University of Nebraska-Lincoln, in North Platte, NE, in a completely randomized design with five replications spatially and two replications temporally. Seeds from each population and a glyphosate-susceptible (GS)-horseweed population as a control reference (collected in Lancaster County, NE) were scattered on the surface of separate aluminum pans (24.4 cm in diameter and 2.87 cm deep) filled with commercial potting mix (Berger™ BM7 Bark Mix, Saint-Modeste, QC, Canada) in August 2016. Seedlings were watered regularly to provide adequate soil moisture and minimize desiccation. After seedlings reached the first pair of true leaves, individual plants were transplanted (11 to 14 days after planting) to cone-tainers (RLC4 Cone-tainer™

cells Stuewe and Sons Inc., Corvallis, OR), supplied with water as needed and maintained in greenhouse with controlled temperature and light conditions (31/22 ± 5 C day/night with 12 h photoperiod).

The study was arranged in a factorial design with 29 horseweed populations and nine glyphosate doses (Roundup PowerMax® Monsanto Company, St. Louis, MO). Glyphosate solution was prepared by adding water, ammonium sulfate at 0.25% v/v (Bronc®, Wilbur-Ellis, Aurora, CO), and glyphosate. Glyphosate doses (0, 217, 434, 868, 1,736, 3,472, 6,944, 13,888, and 27,776 g ae $ha^{-1}$) were applied when horseweed rosettes reached 4 to 6 cm in diameter using a single-track research spray chamber (Generation III, DeVries Manufacturing, Hollandale, MN) calibrated to deliver 93.5 L $ha^{-1}$ with an AI95015EVS nozzle (Spraying Systems Co., Glendale Heights, IL) at 345 kPa. Applications were performed at 4.9 km $h^{-1}$ with the nozzle positioned 15 cm above the target.

**Data Collection.** Visual estimates of horseweed injury (I) were collected at 21 days after treatment (DAT) using a scale of 0 to 100%, where 0% indicates no herbicide injury and 100% indicates complete control or plant death. The injury was based on a typical glyphosate toxicity symptom on horseweed that included meristematic and leaf margin necrosis, leaf chlorosis especially in the area between veins, and arrested plant growth (Zelaya et al. 2004). Aboveground plant biomass was harvested 21 DAT by clipping plants near soil surface, placing in bag and oven dried at 60 C to a constant weight. Dry weight data were converted into percentage of biomass reduction (BR) by averaging the non-treated control plants for each population and comparing them to the dry weight of the glyphosate-treated plants (Equation 1):

$$BR = \left(\frac{(X - Z)}{X}\right) * 100 \quad [1]$$

where *x* represents the average dry weight of the non-treated plants, and *z* represents the dry weight of an individual treated experimental unit.

**Statistical Analysis.** Percentage of biomass reduction and visual estimation of injury data were combined across experimental runs. Data from all treatments were analyzed with a nonlinear, three-parameter log-logistic regression model using the *drc* package (Ritz et al. 2015) in R 3.4.3 (R Foundation for Statistical Computing, Wien, Austria) as proposed by Knezevic et al. (2007):

$$y = \frac{d}{1 + exp[b(logx - loge)]} \quad [2]$$

where *y* is the response variable (BR or I), x is herbicide dose, *b* is the slope at the inflection point, *d* is the upper limit (fixed to 100%), and *e* is the inflection point corresponding the 50% effective dose ($BR_{50}$ or $I_{50}$).

Resistance indices (RI) were calculated for the response variables by dividing the respective $BR_{90}$ and $I_{90}$ values estimated from the statistical analysis by the most common rate of glyphosate use in Nebraska soybean and cron crops (1,260 g ae $ha^{-1}$). For each population, the confidence interval for $BR_{90}$ or $I_{90}$ was estimated, and populations with the upper limit of the 95% confidence interval (CI) greater than 1,260 g $ha^{-1}$ of glyphosate were considered as having “practical” resistance. Practical resistance occurs when a pesticide applied at its label rate no longer provides effective control of a pest species (Tabashnik et al. 2014). Biomass reduction for specific glyphosate doses within and among horseweed populations were compared by box plots, including the outliers, constructed in R.

## RESULTS AND DISCUSSION

**Visual Estimation of Injury.** According to our results, the RI from horseweed populations in rangelands from Nebraska varied from 0.02-fold (susceptible) to 1.25-fold (Table 1.2, Figure 1.2). Across horseweed populations, the Lin1 population was the most susceptible, requiring 26.8 g $ha^{-1}$ of glyphosate to injury plants by 90% ($I_{90}$). The Kea1 and Fil1 horseweed populations in this study were least susceptible to glyphosate, requiring 1,520 g $ha^{-1}$ and 1,574 g $ha^{-1}$, respectively, representing 1.20- and 1.25-fold difference in relation to the most common rate of glyphosate (1,260 g $ha^{-1}$) (Table 1.2, Figure 1.2). According to our dose-response model, the most common rate of glyphosate caused 88.9% and 89.0% injury to Kea1 and Fil1 populations, whereas the same dose resulted in 100% of injury (complete plant death) to the remaining 27 populations (Table 1.2, Figure 1.2, Figure 1.3).

**Biomass Reduction.** Biomass reduction in response to glyphosate was different among horseweed populations (Table 1.3, Figure 1.3). The dose-response model determined that the Hay2 horseweed population was the most susceptible with $BR_{90}$ dose of 97.8 g $ha^{-1}$. Corroborating the I results, Kea1 and Fil1 horseweed populations expressed 'practical' resistance and the glyphosate dose required to reduce biomass by 90% were estimated to be 1,520 g $ha^{-1}$ and 3,076 g $ha^{-1}$, respectively, representing RI of 1.2- and 2.6-fold more in relation to the most common rate of glyphosate (1,260 g $ha^{-1}$). Based on the dose-response model, the most common rate of glyphosate caused 88.9% and 84.0% BR of the Kea1 and Fil1 populations, whereas the same dose resulted in 100% of injury (complete plant death) to the remaining 27 populations (Table 1.2).

There was variability evident in BR within and among horseweed populations across the selected glyphosate doses (Figure 1.3). The glyphosate treatment of 217

g $ha^{-1}$ provided a median BR greater than 60% within horseweed populations and there were only two and three surviving plants by 21 DAT within Hay2 and Hay3 populations, respectively (Figure 1.3-A). Widest spread among populations was observed when plants were treated with 434 g $ha^{-1}$ of glyphosate. Two horseweed populations, Fil1 and Kea1, had less BR than other populations (Figure 1.3-B). The 868 g $ha^{-1}$ of glyphosate had a large BR in a majority of the populations, which was similar to the visual estimations of injury (Figure 1.3-C). One, one, and three plants were alive in the 1,736 g $ha^{-1}$ of glyphosate treatment for GS populations Cha1, Che1, and Lin3, respectively, whereas three plants were alive for each of the Fil1 and Kea1 populations, representing 84% and 88% of BR, respectively (Figure 1.3-D). There were three surviving plants harvested in the population Fil1 in the 3,472 g $ha^{-1}$ of glyphosate treatment (Figure 1.3-E). One plant from population Fil1 survived exposure to 6,944 g $ha^{-1}$ of glyphosate, whereas no survived plants within the other populations (Figure 1.3-F). There were no survivors when treated with 13,888 and 27,776 g $ha^{-1}$ of glyphosate.

Glyphosate-resistant horseweed in Nebraska row crops was first documented in 2006 (Knezevic 2006). In a recent survey, Samuelson (2017) reported a high frequency of glyphosate resistance within horseweed populations collected in Nebraska's row crops, with $ED_{50}$ values (on the basis of the dose necessary to reduce dry weight in 50%) ranging from 4 to 18,000 g $ha^{-1}$ of glyphosate among the populations. Horseweed seed dispersion by wind, coupled with high seed production and possibly pollen-mediated gene flow through outcrossing suggests that the spread of herbicide-resistant horseweed plants across agricultural and nonagricultural settings could be very fast. Davis et al. (2007) proposed that horseweed establishment in a habitat was very dependent on seed production and

dispersion. Models can provide information about the spread of horseweed pollen and seeds. Dauer et al. (2007) developed predictive models that showed the spatial pattern of horseweed seed deposition up to more than 1.5 km, and Shields et al. (2006) found that horseweed seed dispersal can easily exceed 500 km in a single dispersal event if seeds entered the planetary boundary layer. Therefore, a neighbor's field that contains GR horseweed could act as a source for resistance to surrounding fields and rangelands. In addition, field margins and surrounding nonagricultural landscapes may provide a refuge where GR horseweed could potentially outcross with GS horseweed (Gage et al. 2015).

Herbicides influence the evolution of plant species in managed landscapes. Gage et al (2015) suggested that crop rotation management reduced horseweed GR levels. Davis et al. (2009) found that management increased the shift of GS compared to the GR horseweed biotype after 4 years of crop rotation and residual non-glyphosate herbicides. Glyphosate resistance may be associated with a fitness cost, defined as the reduction of plant fitness in an herbicide-free environment and could be estimated by the difference in fitness between an herbicide-resistant and -susceptible biotype (Vila-Aiub et al. 2005). If there is a fitness cost associated with resistance, removal of the selection pressure of herbicide may allow the regression of resistance (Gage et al. 2015).

This research showed that 93% of horseweed populations from Nebraska's rangelands were susceptible to glyphosate, rejecting our initial hypothesis. The lack of significant differences in GR horseweed in rangeland areas is somewhat surprising considering a survey of soybean fields in Nebraska in 2017 showed that 98% were GR horseweed in the 130 populations evaluated in 40 counties (Samuelson 2017). The rangeland sites used on this study were chosen based upon neighboring

soybean and corn row crops that had presence of horseweed and surrounding areas of the areas that had demonstrated horseweed GR by Samuelson (2017). It is likely that GR horseweed did not establish in rangeland, except for the two populations from Filmore (Fil1) and Kearney (Kea1) counties. On the basis of the most common rate of glyphosate (1,260 g $ha^{-1}$), only 30% of the plants of both GR populations were alive and showed a BR average of 60% to the Kea1 and 45% to the Fil1 populations, demonstrating low frequency of resistance. Dauer et al. (2009) proposed predictive model could show the influence that reducing the selection pressure for resistant genes to persist may slow the spread of GR horseweed. The lack of establishment of GR horseweed in rangeland may be correlated with a combination of factors, such as genetic, biologic and physiologic of the individuals within the populations, gene flow and fitness cost (Davis et al. 2009, Gage et al. 2015, Maxwell et al. 1990, Nandula et al. 2006, Vila-Aiub et al. 2005).

Glyphosate-resistant horseweed was not the predominant biotype in rangeland and these areas were not acting as reservoirs for resistant horseweed populations. We conclude from these results that removal of the selection pressure of glyphosate allowed the regression of resistance, an observation that might be associated with fitness cost. One limitation of our research was that the horseweed dose-response experiments were conducted under greenhouse conditions and may have impacted ecological fitness performance. The cumulative effects to understand the adaptation of GR horseweed from row crops into rangeland when compared to native biotypes infesting these areas and the possibility of any fitness cost impacting on GR adaptability in rangeland needs to be further evaluated.

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## TABLES AND FIGURES

Table 1.2 - Geographic location of horseweed populations collected in October 2015 in Nebraska, US.

| Population[a] | County | N Latitude[b] | W Longitude[b] |
|---|---|---|---|
| Ban 1 | Banner | 41.48 | 103.45 |
| Cha 1 | Chase | 40.68 | 101.66 |
| Che 1 | Cheyenne | 41.37 | 103.19 |
| Che 2 | Cheyenne | 41.35 | 102.75 |
| Che 3 | Cheyenne | 41.37 | 102.64 |
| Deu 1 | Deul | 41.11 | 102.25 |
| Dun 1 | Dundy | 40.13 | 101.40 |
| Fil 1 | Fillmore | 40.69 | 97.60 |
| Fro 1 | Frontier | 40.55 | 100.62 |
| Gra 1 | Grant | 42.02 | 101.63 |
| Hay 1 | Haynes | 40.68 | 101.15 |
| Hay 2 | Haynes | 40.63 | 101.06 |
| Hay 3 | Haynes | 40.57 | 100.89 |
| Hit 1 | Hitchcock | 40.11 | 100.85 |
| Hit 2 | Hitchcock | 40.07 | 100.90 |
| Hit 3 | Hitchcock | 40.13 | 101.22 |
| Kea 1 | Kearney | 40.63 | 98.80 |
| Kei 1 | Keith | 41.34 | 101.82 |
| Kei 2 | Keith | 41.16 | 101.96 |
| Kei 3 | Keith | 41.16 | 101.84 |
| Lan 10[c] | Lancaster | 40.51 | 96.30 |
| Lin 1 | Lincoln | 41.03 | 100.52 |
| Lin 2 | Lincoln | 40.91 | 100.37 |
| Lin 3 | Lincoln | 40.71 | 100.33 |
| Log 1 | Logan | 41.46 | 100.50 |
| Mor 1 | Morril | 41.64 | 102.98 |
| Per 1 | Perkins | 40.85 | 101.61 |
| Per 2 | Perkins | 40.73 | 101.63 |
| Red 1 | Red Willow | 40.28 | 100.60 |
| Red 2 | Red Willow | 40.16 | 100.69 |

[a] Horseweed populations were designated by the three first letters of the Nebraska county where the population was collected, followed by the number of populations collected per county.
[b] Geographical coordinates in decimal degree unit.
[c] Reference susceptible population (Lancaster county).

Table 1.3 - Growth reduction estimate of regression parameters and glyphosate dose required for 50% ($GR_{50}$) and 90% ($GR_{90}$) growth reduction of 27 horseweed populations at 21 DAT.

| Horseweed population[a] | Regression parameter[b] *b* | $GR_{50}$[c] | | $GR_{90}$[d] | | GR: GS ratio[e] |
|---|---|---|---|---|---|---|
| | | ---------kg ae ha$^{-1}$ (SE)[f] ---------- | | | | |
| Mor 1 | -0.376 | (0.113) | < 0.001 | (0.001) | 0.137 | (0.118) | - |
| Ban 1 | -1.611 | (0.513) | 0.093 | (0.031) | 0.363 | (0.057) | 2.645 |
| Cha 1 | -0.907 | (0.312) | 0.030 | (0.025) | 0.333 | (0.076) | 2.424 |
| Che 1 | -0.785 | (0.234) | 0.012 | (0.012) | 0.191 | (0.064) | 1.394 |
| Che 2 | -1.771 | (0.379) | 0.123 | (0.021) | 0.427 | (0.057) | 3.109 |
| Che 3 | -1.866 | (0.469) | 0.119 | (0.023) | 0.385 | (0.051) | 2.803 |
| Deu 1 | -0.791 | (0.214) | 0.010 | (0.010) | 0.165 | (0.058) | 1.202 |
| Dun 1 | -2.385 | (0.828) | 0.127 | (0.028) | 0.320 | (0.047) | 2.328 |
| Fil 1 | -1.224 | (0.469) | 0.072 | (0.039) | 0.433 | (0.102) | 3.154 |
| Fro 1 | -1.088 | (0.194) | 0.104 | (0.025) | 0.780 | (0.150) | 5.683 |
| Gra 1 | -2.711 | (0.856) | 0.142 | (0.024) | 0.319 | (0.037) | 2.322 |
| Hay 1 | -1.540 | (0.544) | 0.080 | (0.034) | 0.334 | (0.051) | 2.433 |
| Hit 1 | -1.504 | (0.406) | 0.124 | (0.030) | 0.533 | (0.113) | 3.880 |
| Hit 2 | -1.404 | (0.463) | 0.076 | (0.033) | 0.361 | (0.059) | 2.627 |
| Hit 3 | -1.690 | (0.403) | 0.133 | (0.025) | 0.489 | (0.084) | 3.560 |
| Kea 1 | -0.533 | (0.247) | 0.043 | (0.053) | 2.664 | (2.348) | 19.401 |
| Kei 1 | -1.508 | (0.526) | 0.127 | (0.038) | 0.546 | (0.151) | 3.976 |
| Kei 2 | -3.514 | (1.359) | 0.162 | (0.021) | 0.303 | (0.037) | 2.206 |
| Kei 3 | -1.256 | (0.514) | 0.100 | (0.047) | 0.574 | (0.194) | 4.184 |
| Lan 10 | -1.188 | (0.482) | 0.067 | (0.043) | 0.426 | (0.116) | 3.103 |
| Lin 2 | -1.492 | (0.426) | 0.108 | (0.031) | 0.471 | (0.094) | 3.431 |
| Lin 3 | -1.664 | (0.544) | 0.129 | (0.033) | 0.482 | (0.109) | 3.510 |
| Log 1 | -2.356 | (0.850) | 0.170 | (0.028) | 0.432 | (0.099) | 3.146 |
| Per 1 | -1.114 | (0.348) | 0.033 | (0.023) | 0.235 | (0.044) | 1.708 |
| Per 2 | -1.619 | (0.512) | 0.075 | (0.029) | 0.292 | (0.035) | 2.129 |
| Red 1 | -1.357 | (0.245) | 0.164 | (0.027) | 0.826 | (0.166) | 6.015 |
| Red 2 | -0.572 | (0.171) | 0.003 | (0.004) | 0.141 | (0.082) | 1.026 |

[a] Horseweed populations were designated by the three first letters of the Nebraska's county where the population was collected, followed by the number of populations collected per county. The populations Hay 2, Hay 3, and Lin 1 model did not converge.

[b] Regression parameter: a four-parameter log logistic model $y = c + \frac{d-c}{1+exp[b(logx-loge)]}$ was used, where *b* is the relative slope around *e*, *c* is the lower limit fixed = 0, *d* is the upper limit fixed = 100, and *e* is the $GR_{50}$.

[c] Estimated herbicide dose necessary to cause 50% of biomass reduction ($GR_{50}$).

[d] Estimated herbicide dose necessary to cause 90% of biomass reduction ($GR_{90}$).

[e] GR:GS ratios were calculated by dividing the $GR_{90}$ of each population by the relative $GR_{90}$ value of the most susceptible population from this dose-response.

[f] kilograms of glyphosate acid equivalent per hectare (kg ae ha$^{-1}$); Standard Error (SE)

Table 1.4 - Independent t tests of $I_{90}$ (on the basis of visual injury estimated) values from the dose-response of horseweed plant populations from Nebraska.

| | Based on visual estimation of injury | |
|---|---|---|
| Populations[a] | $I_{90}$[b] | *P* -value |
| Lin1 vs Kei 2 | 0.0265 vs 0.111 | 0.8188 |
| Lin1 vs Ban 1 | 0.0265 vs 0.149 | 0.8267 |
| Lin 1 vs Per 2 | 0.0265 vs 0.152 | 0.8282 |
| Lin 1 vs Per 1 | 0.0265 vs 0.178 | 0.8130 |
| Lin 1 vs Hit 1 | 0.0265 vs 0.212 | < 0.0001 |
| Lin 1 vs Lan 10 | 0.0265 vs 0.213 | 0.8140 |
| Lin 1 vs Dun 1 | 0.0265 vs 0.216 | < 0.0001 |
| Lin 1 vs Lin 2 | 0.0265 vs 0.234 | < 0.0001 |
| Lin 1 vs Deu 1 | 0.0265 vs 0.251 | 0.8057 |
| Lin 1 vs Hit 2 | 0.0265 vs 0.252 | < 0.0001 |
| Lin 1 vs Che 2 | 0.0265 vs 0.259 | 0.8053 |
| Lin 1 vs Kei 1 | 0.0265 vs 0.264 | 0.8052 |
| Lin 1 vs Hay 1 | 0.0265 vs 0.268 | 0.8065 |
| Lin 1 vs Gra 1 | 0.0265 vs 0.281 | < 0.0001 |
| Lin 1 vs Cha 1 | 0.0265 vs 0.286 | 0.8135 |
| Lin 1 vs Lin 3 | 0.0265 vs 0.286 | < 0.0001 |
| Lin 1 vs Che 3 | 0.0265 vs 0.294 | 0.8068 |
| Lin 1 vs Hit 3 | 0.0265 vs 0.307 | < 0.0001 |
| Lin 1 vs Kei 3 | 0.0265 vs 0.311 | 0.8037 |
| Lin 1 vs Log 1 | 0.0265 vs 0.326 | < 0.0001 |
| Lin 1 vs Fro 1 | 0.0265 vs 0.353 | < 0.0001 |
| Lin 1 vs Red 1 | 0.0265 vs 0.475 | < 0.0001 |
| Lin 1 vs Kea 1 | 0.0265 vs 1.88 | 0.8079 |

[a] Horseweed populations were designated by the first three letters of the Nebraska county where the population was collected, followed by the number of populations collected per county. The populations Hay 2, Hay 3, Mor 1, and Red 2 model did not converge.

[b] $I_{90}$ is the effective dose (kg ae $ha^{-1}$) of glyphosate required for 90% control.

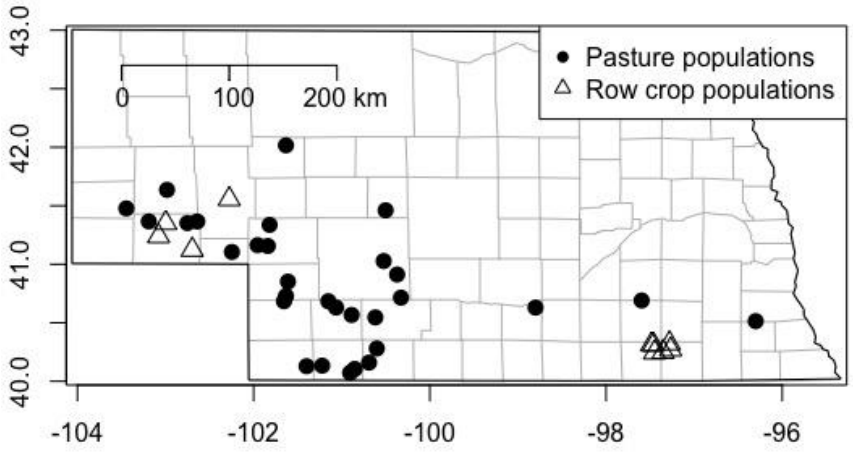



Figure 1.1 - Nebraska geographical area of horseweed populations collection.

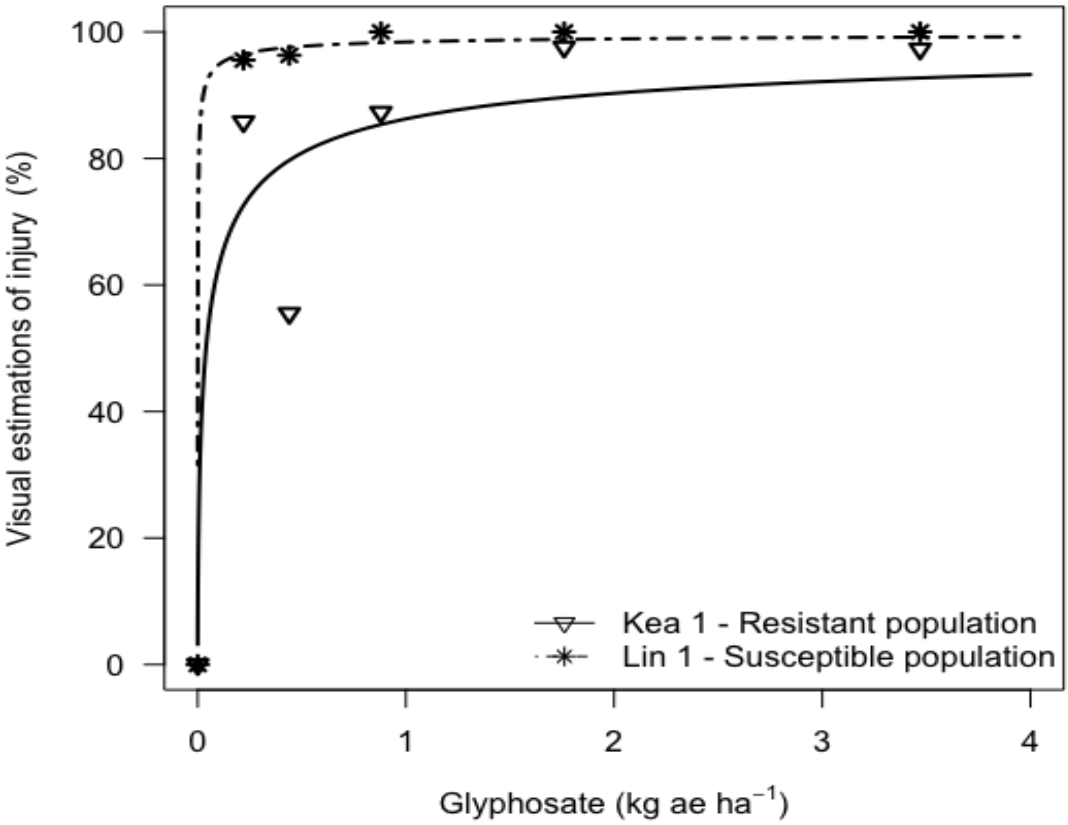



Figure 1.2 - Visual estimations of injury (percentage) of Lin 1 and Kea 1 horseweed populations at 21 DAT.

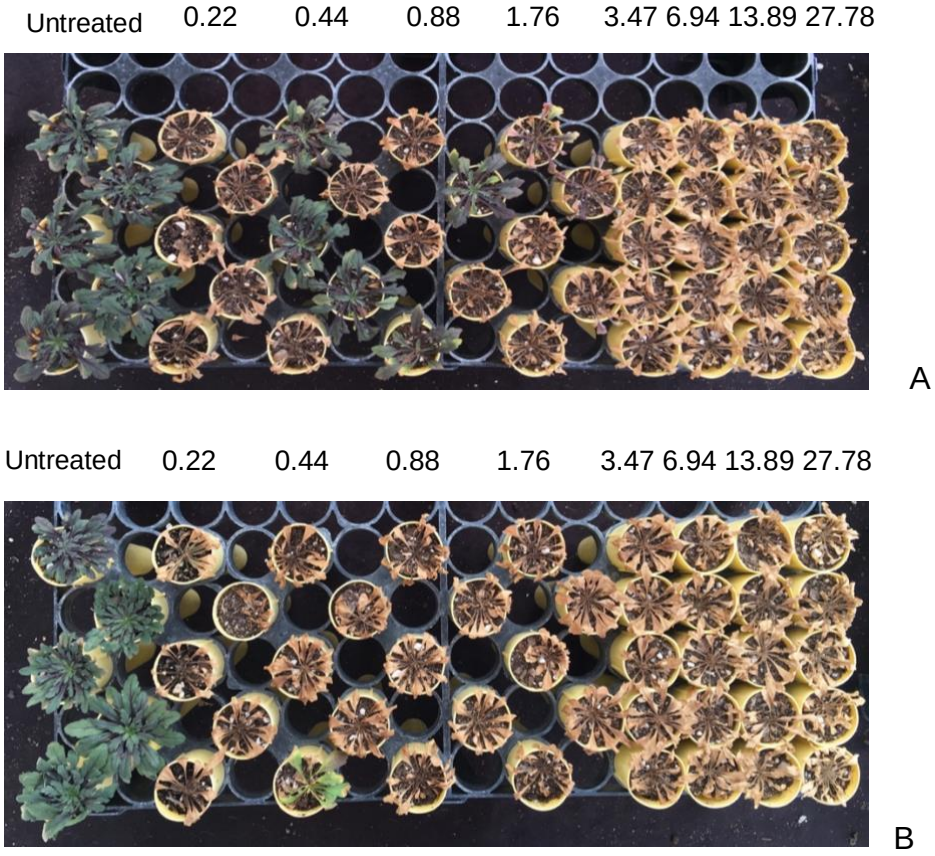
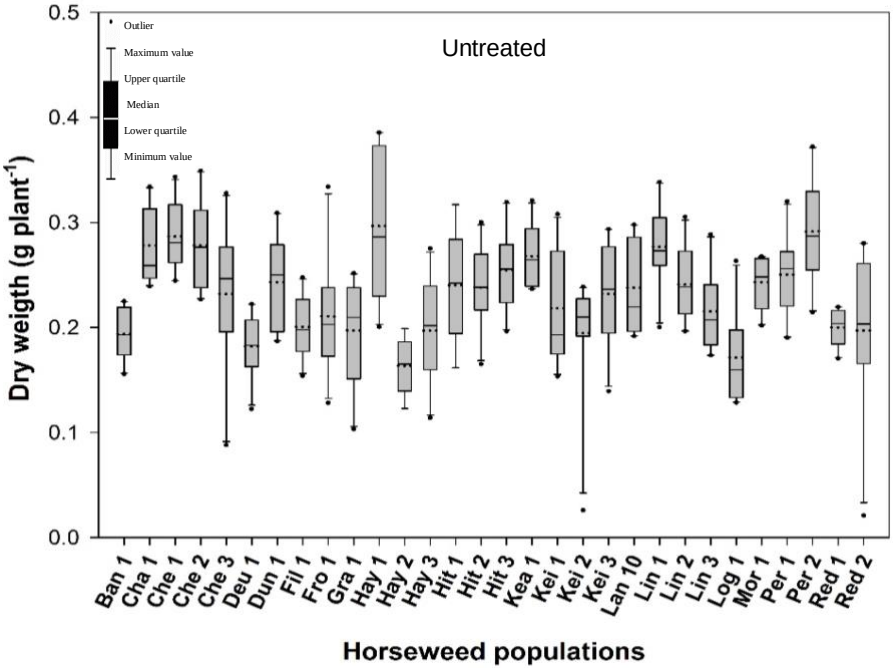
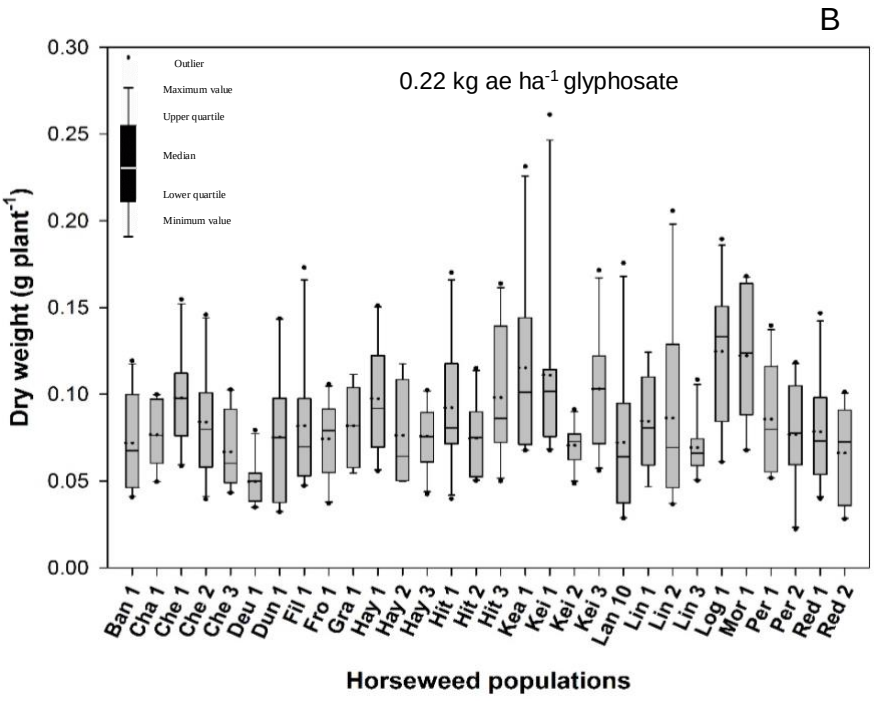



Figure 1.3 - Glyphosate (kg ae $ha^{-1}$) dose-response of (A): Kea 1 (glyphosate-resistant) and (B): Lin 1 (glyphosate susceptible) horseweed populations.

A

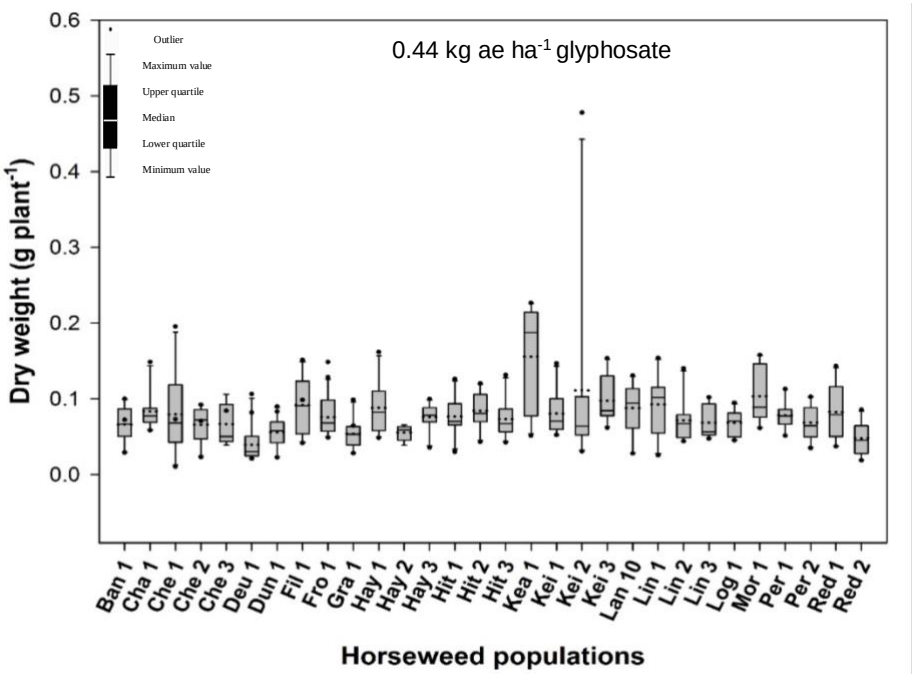

Untreated
Outlier
Maximum value
Upper quartile
Median
Lower quartile
Minimum value
Dry weigth (g plant-1)
0.0
0.1
0.2
0.3
0.4
0.5
Ban 1
Cha 1
Che 1
Che 2
Che 3
Deu 1
Dun 1
Fil 1
Fro 1
Gra 1
Hay 1
Hay 2
Hay 3
Hit 1
Hit 2
Hit 3
Kea 1
Kei 1
Kei 2
Kei 3
Lan 10
Lin 1
Lin 2
Lin 3
Log 1
Mor 1
Per 1
Per 2
Red 1
Red 2
Horseweed populations

B

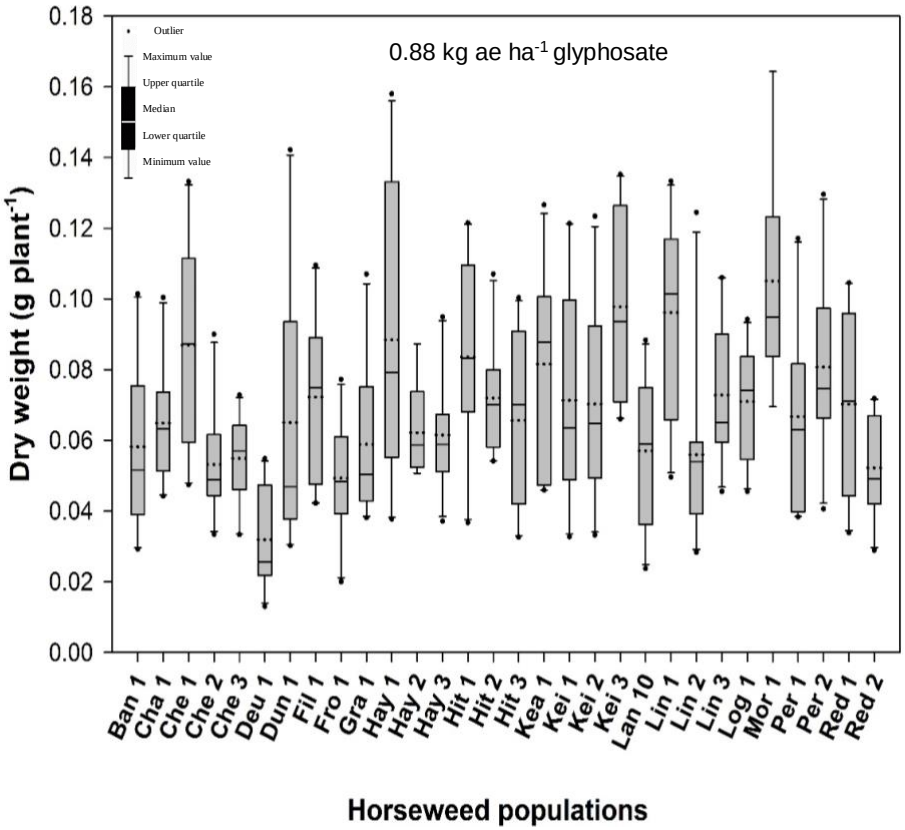

0.22 kg ae ha-1 glyphosate
Outlier
Maximum value
Upper quartile
Median
Lower quartile
Minimum value
Dry weight (g plant-1)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Ban 1
Cha 1
Che 1
Che 2
Che 3
Deu 1
Dun 1
Fil 1
Fro 1
Gra 1
Hay 1
Hay 2
Hay 3
Hit 1
Hit 2
Hit 3
Kea 1
Kei 1
Kei 2
Kei 3
Lan 10
Lin 1
Lin 2
Lin 3
Log 1
Mor 1
Per 1
Per 2
Red 1
Red 2
Horseweed populations

Continue

C


0.44 kg ae ha-1 glyphosate
Outlier
Maximum value
Upper quartile
Median
Lower quartile
Minimum value
Dry weight (g plant-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Ban 1
Cha 1
Che 1
Che 2
Che 3
Deu 1
Dun 1
Fil 1
Fro 1
Gra 1
Hay 1
Hay 2
Hay 3
Hit 1
Hit 2
Hit 3
Kea 1
Kei 1
Kei 2
Kei 3
Lan 10
Lin 1
Lin 2
Lin 3
Log 1
Mor 1
Per 1
Per 2
Red 1
Red 2
Horseweed populations

D


0.88 kg ae ha-1 glyphosate
Outlier
Maximum value
Upper quartile
Median
Lower quartile
Minimum value
Dry weight (g plant-1)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
Ban 1
Cha 1
Che 1
Che 2
Che 3
Deu 1
Dun 1
Fil 1
Fro 1
Gra 1
Hay 1
Hay 2
Hay 3
Hit 1
Hit 2
Hit 3
Kea 1
Kei 1
Kei 2
Kei 3
Lan 10
Lin 1
Lin 2
Lin 3
Log 1
Mor 1
Per 1
Per 2
Red 1
Red 2
Horseweed populations

Continue

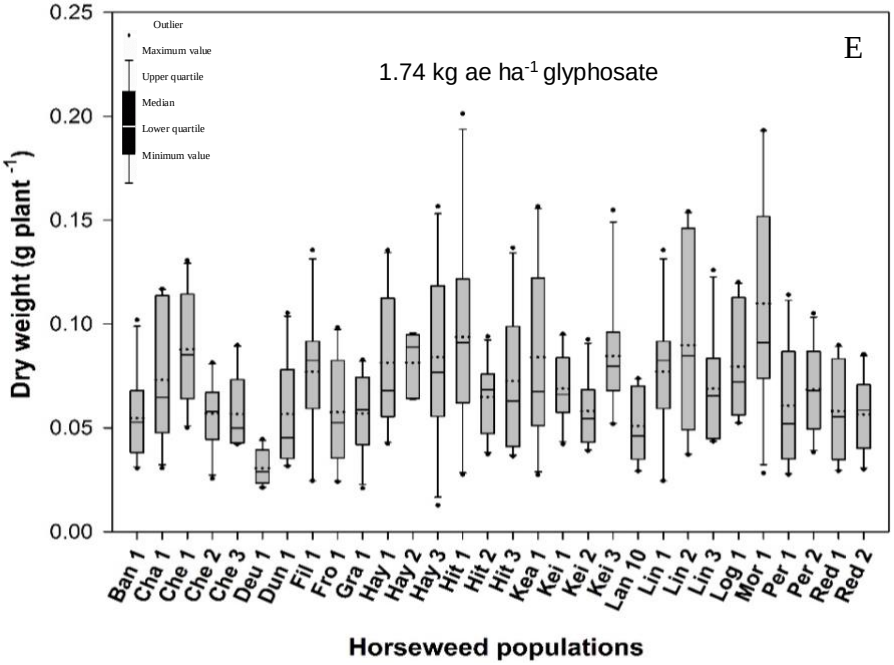



Figure 1.4 - Box-and-whisker plots for horseweed plant dry weights at 21 DAT of 30 horseweed populations. …  represent the mean per population.

# CHAPTER 2

## PHENOXY HERBICIDES EVALUATION ON 2,4-D RESISTANT WATERHEMP

RESUMO

Herbicidas fenóxicos são uma classe de herbicidas auxínicos que apresentam uma estrutura similar ao hormonio natural auxina. Herbicidas deste grupo podem atuar em multiplos locais dentro da planta, interferindo no balanço de hormônios e síntese de proteínas, levando as plantas de um crescimento descontrolado a morte. Resistencia aos herbicidas fenóxicos foi originalmente relatado em 1957 a *Commelina diffusa* Burm. f. no Hawai e a *Daucus carota* L. em Ontário. Até o momento, foram registradas oito espécies de plantas daninhas resistentes a este grupo de herbicida nos Estados Unidos da América, incluindo *Amaranthus tuberculatus*. O objetivo deste estudo foi caracterizar o nível de resistência de uma população de *Amaranthus tuberculatus* resistente a 2,4-D a diferentes formulações de herbicidas fenóxicos. Duas populações de *Amaranthus tuberculatus* de Nebraksa suscetível e resistente ao herbicida 2,4-D foram foram comparadas com os herbicidas: 2,4-D, 2,4-D 2EHE, 2,4-D EE, 2,4-D DB, MCPA, MCPA 2EHE, MCPB, CMPP, CMPP-p, 2,4-DP, 2,4-DP-p, Corasil, Dicamba DMA e, Dicamba DGA em experimento em casa-de-vegetacao. Os resultados deste experimento mostraram que indivíduos da população resistente ao herbicida 2,4-D foram significativamente mais suscetíveis aos herbicidas Dicamba DGA, Dicamba DMA, Corasil, 2,4-DP e 2,4-DP-p, enquanto sobreviveram às altas doses de 2,4-D 2EHE, 2,4-D EE, 2,4-DB, MCPB, MCPA, MCPA 2EHE, CMPP e, CMPP-p. A partir de nossos resultados podemos sugerir que a populacao de *Amaranthus tuberculatus de* Nebraska apresenta resistência cruzada aos herbicidas 2EHE, 2,4-D EE, 2,4-DB, MCPB, MCPA, MCPA 2EHE, CMPP e CMPP-p.

**Palavras-chave:** Amaranthaceae, 2,4-D, Dicamba, Herbicidas auxínicos, Reguladores de Crescimento, Dose-resposta.

ABSTRACT

Phenoxy herbicides are a class of auxinic herbicides structurally similar to the natural phytohormone auxin. This herbicide group can act in multiple sites of the plant, disrupting hormonal balance and protein synthesis, leading to uncontrolled growth, causing the plant to grow itself to death. Resistance to phenoxy herbicides was originally related in 1957 to *Commelina diffusa* Burm. f. in Hawaii and *Daucus carota* L. in Ontario. Up to now, in the United States eight weed species evolved resistance to auxin herbicide group, including *Amaranthus tuberculatus*. The objective of this research is to characterize the level of resistance of a 2,4-D resistant waterhemp population to various phenoxy herbicides. The responses of a susceptible and 2,4-D-resistant waterhemp populations from Nebraska were compared with 2,4-D, 2,4-D 2EHE, 2,4-D EE, 2,4-D DB, MCPA, MCPA 2EHE, MCPB, CMPP, CMPP-p, 2,4-DP, 2,4-DP-p and Corasil phenoxy herbicides, and Dicamba DMA and Dicamba DGA benzoic acid herbicides in an experiment. The results of this study showed that 2,4-D-WR population were significantly more sensitive to Dicamba DGA, Dicamba DMA, Corasil, 2,4-DP, and 2,4-DP-p herbicides formulations, whereas survived to the higher doses of 2,4-D 2EHE, 2,4-D EE, 2,4-DB, MCPB, MCPA, MCPA 2EHE, CMPP and CMPP-p and this suggests that 2,4-D-WR population exhibits cross-resistance to these herbicides.

**Keywords:** Pigweed, 2,4-D, Dicamba, Auxinic herbicides, Growth regulators, Dose-response.

## 3.1 INTRODUCTION

Phenoxy herbicides are a class of auxinic herbicides structurally similar to the natural phytohormone auxin, as indole-3-acetic acid (IAA). Nevertheless, they endure more in the plants metabolism due to be more stable and effective than IAA (GROSSMANN, 2007). Auxins play a major role in cell division, elongation, differentiation, rooting formation (brace roots), apical dominance, inhibition of lateral bud growth and phototropism (TAIZ; ZEIGER, 2010; COFFMAN 2004). Uptake of phenoxies occurs predominantly by the leaves, occasionally by plant stem, buds, and roots, and are rapidly translocated to the meristems of the plant mainly by phloem via ion trapping (PETERSON et al. 2016; RIEDERER, 2005).

The mode of action of this herbicide group apparently include bind to some or all of the same sites as IAA, although remains not completely clear (BECKIE; WOLF, 1999; MITHILA et al., 2011; WEINTRAUB, 1953). This herbicide group can act in multiple sites of the plant, disrupting hormonal balance and protein synthesis, leading to uncontrolled growth, twisting, curving and swelling shoot, causing the plant to grow itself to death (PETERS; METZGER, 1994; KLINGMAN et al., 1983). The phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) transport is commanded by auxin influx and efflux (ENDERS; STRADER, 2015; GRONES; FRIML, 2015), and its concentration, as well IAA concentration could be controlled by transporting IAA toward the vacuole by tonoplast-bound protein (GRONES; FRIML, 2015). Though, fewer 2,4-D efflux occurs, allowing high 2,4-D concentration inside the cells providing an improvement in herbicide activity (DELBARRE et al., 1996; PETERSON et al., 2016). Once 2,4-D absorption occurs via IAA carrier protein (e.g., TIR1), a sequence of events might follow, as: 1. cell wall losing its structural function, leading to epinastic effects, 2. activation of gene expressions responsible by reactive oxygen species (ROS) synthesis resulting in ROS overproduction, 3.ROS may easily penetrate cell wall and plasmatic membrane, and interact with plasmatic membrane phospholipids, resulting in cell death (FIGUEIREDO et al., 2016; MITHILA et al., 2011; TAN et al., 2007).

The phenoxy herbicides perform as an important efficacious and economical method to control a wide spectrum of broadleaf weeds and woody plants in various crop and non-crop areas due to its selectivity and low application costs (BOVEY, 2001; MITHILA et al., 2011; PETERSON et al., 2016). 2,4-D and 2-methyl-4-chlorophenoxyacetic acid (MCPA) were the first group of phenoxy herbicides

developed in 1940's when 2,4-D was used as one of the ingredients in Agent Orange mixture utilized during the World War II (KIRBY 1980; TURNER, 1977). The 2,4-D formulation as an amine salt is the most used phenoxy herbicide to control broadleaf weed species in grass; in addition, the phenoxy butanoic herbicides (e.g., 2,4-DB / MCPB) are also selective in many clovers and legumes (PHENOXY, 2017). All phenoxy herbicides are organic acids having an aromatic ring, an oxygen atom substituted for one hydrogen bonded to the ring with a carboxylic side chain (JAYAKODY; HARRIS; COGGON, 2015).

The members of this herbicide group can be distinguished by the length of the carboxylic side chain and/or substituents and their location on the ring (Jayakody et al. 2015) (Figure 2.1). Phenoxies are usually formulate as esters and salts of their corresponding parent acid, including the compounds 2,4-D, 2,4-D-2-ethylhexyl ester (2,4-D 2EHE), 2,4-D ethyl ester, 4-(2,4-dichlorophenoxy) butanoic acid (2,4-DB), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), MCPA, 2-methyl-4-chlorophenoxyacetic acid 2-ethylhexyl ester (MCPA 2EHE), 4-(4-chloro-2-methylphenoxy)butyric acid (MCPB), 2-(-4-chloro-2-methylphenoxy)propanoic acid (CMPP), 2-(4-chloro-2-methylphenoxy) propanoic acid (CMPP-p), 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), 2-(2,4-dichlorophenoxy) propionic acid (2,4-DP-p) and Dichlorprop-P 2-ethylhexyl ester (Corasil). Dicamba is a benzoic acid in auxinic herbicide group, selective to control broadleaf weed species as phenoxy group (SENSEMAN, 2007).

With the introduction of new technologies that implement transgenic resistance to auxin herbicides, as corn and soybean crops with resistance to 2,4-D and cotton and soybean crops with resistance to dicamba, it is crucial to understand the status of cross-resistance of weeds, mostly pigweeds that are troublesome weeds in the US. Though nowadays glyphosate-resistant weeds are major problems in cropping systems, 2,4-D and dicamba herbicides will be an effective weed management tool in a short-term available. Some concerns have been discussed about the phenoxy herbicides use in tolerant crops, as the possibility of volatility and drift of herbicide to non-target susceptible crops and weeds, increasing herbicide selection pressure in resistant weeds biotype, and eventually result in new herbicide resistant populations evolving (CHRISTOFFOLETI et al., 2015; EGAN et al., 2011).

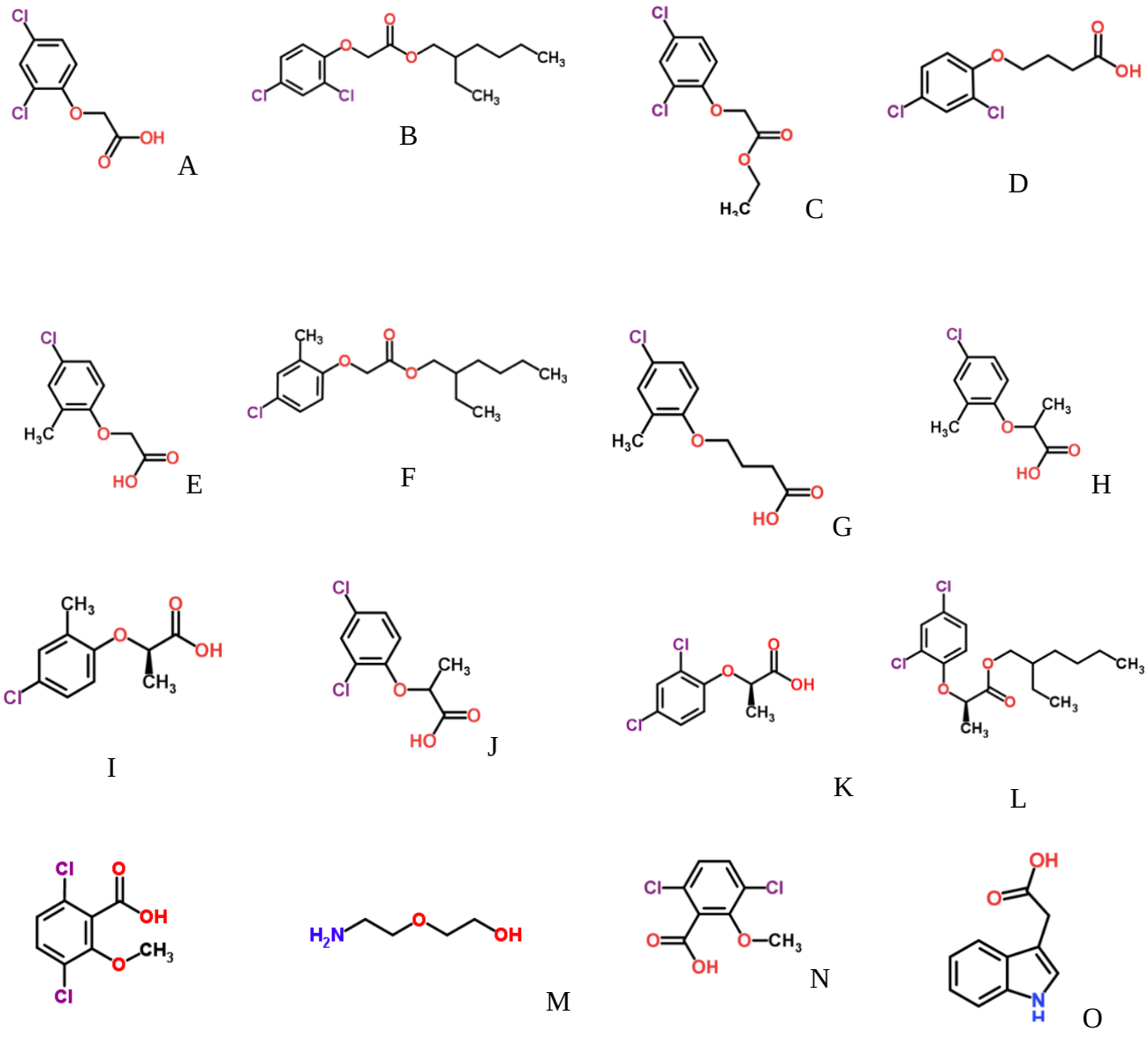



**Figure 2.1 -** Herbicides chemical structure. [A]2,4-D, [B]2,4-D 2EHE, [C]2,4-D ethyl ester, [D] 2,4-DB, [E] MCPA, [F] MCPA 2 EHE, [G]MCPB, [H]CMPP, [I]CMPP-p, [J]2,4-DP, [K]2,4-DP-p, [L] Dichlorprop-p 2 EHE, [M]Dicamba DGA, [N]Dicamba DMA, and [O] IAA.

Resistance to phenoxy herbicides was originally related in 1957 to Spreading dayflower (*Commelina diffusa* Burm. f.) in Hawaii and Wild carrot (*Daucus carota* L.) in Ontario. Currently, 34 weed species evolved resistance to this group, and have been reported in 20 countries globally. Up to now, in the United States eight weed species evolved resistance to auxin herbicide group (e.g., waterhemp, kochia) (HEAP, 2017),

and in 2009 Bernards et al., 2012 reported waterhemp (*Amaranthus tuberculatus*) resistant to synthetic auxin group in a grass seed production field situated in Nebraska. In addition to this case, in 2016 waterhemp has evolved multiple resistance to five herbicide sites of action (ALS inhibitors, Photosystem II inhibitors, PPO inhibitors, HPPD inhibitors, and Synthetic Auxins), in corn and soybean fields in Illinois (HEAP, 2017; SCHULTZ et al., 2015).

Waterhemp is part of the Amaranthaceae family, native to the Great Plains region of the United States and commonly find in agricultural production systems (SAUER, 1957; STECKEL; SPRAGUE, 2004; WASELKOV; OLSEN, 2014). It is the most prominent and troublesome summer annual broadleaf, acknowledge for its prolific growth characteristics (e.g., seed production, growth rate) and highly competitive ability with agronomic crops (GOWER; LEE, 2001; HORAK; PETERSON, 1995; NORDBY et al. 2007, SAUER, 1957; SELLERS et al., 2003).

Waterhemp interference can reduce soybean yield up to 43% if remained for the whole season (HAGER et al., 2002; WAGGONER; BRADLEY, 2011) reported an average of 182 kg $ha^{-1}$ soybean yield loss in Missouri. Corn can suffer 74% yield loss due to waterhemp density of 60 to 300 $m^{-2}$ (STECKEL; SPRAGUE, 2004). Nevertheless, the differential fitness among waterhemp populations and also environmental conditions (e.g., humidity, temperature, light, soil fertility) plays an important factor related to penalty, predominance, and competitiveness, as well, unlike herbicides responses.

Amaranthaceae Family is one of the five weed families that are very prone to evolve herbicide resistance (HEAP, 2017). However, some researchers had been hypothesized that the current low number of auxinic-resistant cases if compared with other herbicides groups (e.g., acetolactase synthase [ALS] inhibitors) is due to phenoxy herbicides performance at multiple sites of action inside plants. Therefore, members of the phenoxy herbicides family will act in several routes sites and can be an alternative way to control resistant weeds to another phenoxy groups, minimizing herbicide selection pressure. The objective of this research is to characterize the level of resistance of a 2,4-D resistant waterhemp population to various phenoxy herbicides.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Plant Growth and Phenoxies Herbicides Application

Experiments were conducted in the greenhouse located at West Central Research and Extension Center, University of Nebraska-Lincoln, in North Platte, Nebraska. Seeds from a putative susceptible (2,4-D-WS) and 2,4-D-resistant population (2,4-D-WR) (i.e., 2,4-D-resistant; collected in a field of southwest Nebraska; reported by Bernards et al. (2012)) were planted in August and September of 2016 in aluminum pans (71 cm length x 32 cm width x 9 cm depth) filled with a commercial potting mix (Berger™ BM7 Bark Mix, Saint-Modeste, Québec G0L 3W0, Canada) limed to 5.5 to 6.5 pH. Seedlings were fertilized with UNL 5-1-4 0.2% $v.v^{-1}$ (Wilbur-Ellis Agribusiness, Suite 500, Aurora, CO 80014) blending with water regularly to provide adequate soil moisture and minimize dehydration. After seedlings had reached the second pair of true leaves, individual plants were transplanted (8 to 11 days after planted) into RLC4 Cone-tainer™ cells (Stuewe and Sons Inc., Corvallis, OR 97389). After transplanting, plants were watered and fertilized as needed until harvest with a three-misting-jet garden nozzle. LED growth lights provided supplemental lighting to ensure a 15-h photoperiod. Daytime temperatures were 31 $\pm$ 5 C, and nighttime temperatures were 22 $\pm$ 5 C.

The experiment had a completely randomized design (CRD) with five replications and repeated twice. The treatments were composed of 14 herbicides formulations and two tank mixtures (Nufarm Limited, 103-105 Pipe Road, Laverton North Victoria 3026, Australia) (Table 2.1), arranged in a factorial treatment design with 2 waterhemp populations and 12 rates. Populations dose-response was measured with herbicides rates at 0, 0.125x, 0.250x, 0.5x, 0.75x, 1x, 1.5x, 2x, 3x, 4x, 6x and 9x each herbicide recommended rate, as showed on Table 2.1.

Waterhemp plants were treated when achieved 4 to 6 cm tall (10 to 15 days after transplanting). Herbicide treatments were applied with a single-tip spray chamber (DeVries Manufacturing, Hollandale, MN 56045), fitted with a TP 9504 EVS nozzle (Spraying Systems Co., North Avenue, Wheaton, IL, 60139), calibrated to deliver 187 L $ha^{-1}$ carrier volume at 276 kPa, at a speed of 6.44 km $h^{-1}$, with nozzle positioned approximately 35 cm above the target. For the Treatment 12, the speed was reduced

to 4.25 km $h^{-1}$ and 2.83 km $h^{-1}$ for the rates 6x and 9x, respectively, to provide the correct treatment flow rate.

**Table 2.1** - Herbicides chemical names according to International Union of Pure and Applied Chemistry (IUPAC) nomenclature and recommend dose.

| Herbicide | Rate |
|---|---|
| | g ae $ha^{-1}$ |
| 1. Non-treated control | ___ |
| 2. 2,4-D-dichlorophenoxy acetic acid (2,4-D) | 455 |
| 3. 2-ethylhexyl 2-(2,4-dichlorophenoxy)acetate (2,4-D 2EHE) | 659 |
| 4. ethyl 2-(2,4-dichlorophenoxy)acetate (2,4-D ethyl ester) | 800 |
| 5. 4-(2,4-dichlorophenoxy) butanoic acid (2,4-D DB) | 500 |
| 6. 2-(4-chloro-2-methylphenoxy)acetic acid (MCPA) | 443 |
| 7. 2-ethylhexyl 2-(4-chloro-2-methylphenoxy)acetate (MCPA 2EHE) | 443 |
| 8. 4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB) | 400 |
| 9. 2-(4-chloro-2-methylphenoxy)propanoic acid (CMPP) | 600 |
| 10. (2R)-2-(4-chloro-2-methylphenoxy)propanoic acid (CMPP-p) | 600 |
| 11. 2-(2,4-dichlorophenoxy)propanoic acid (2,4-DP) | 600 |
| 12. (2R)-2-(2,4-dichlorophenoxy)propanoic acid (2,4-DP-p) | 600 |
| 13. (R)-2-(2,4-dichlorophenoxy) propanoic acid, 2-ethylhexyl ester (Corasil) | 25 |
| 14. 2-(2-aminoethoxy)ethanol;3,6-dichloro-2-methoxybenzoic acid (Dicamba DGA) | 480 |
| 15. 3,6-dichloro-2-methoxybenzoic acid (Dicamba DMA) | 500 |
| 16. 2,4-D + 2,4-DP | 455 + 600 |
| 17. 2,4-D + CMPP | 455 + 600 |

### 2.2.2 Data Collection and Statistical Analysis

Visual estimations of plant injury (I) were performed at 3, 7, 14, and 21 days after treatment (DAT) based on growth suppression and epinastic effects compared with untreated control plants, rating each plant on a scale of 0 (no injury) to 100% (dead plants). At 21 DAT, plants were severed at the base and dried at 62 C ± 5 C to a constant moisture prior to recording dry weight (growth reduction). The dry weight of individual plants was recorded.

The effective dose to control the population by 50% ($ED_{50}$) and 90% ($ED_{90}$) for waterhemp-resistant and waterhemp-susceptible was determined using the four-parameter log-logistic curve of the *drc* package (drc 1.2, Christian Ritz and Jens Strebig, R 2.5, Kurt Hornik, online) in R 3.1.1 (R statistical software, R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org) (KNEZEVIC et al. 2007) (Equation 1):

$$y = c + \frac{d-c}{1+exp[b(logx-loge)]} \quad [1]$$

In this model, *y* is the visual estimation of plant injury (%) or the Dry weight (g plant$^{-1}$), *d* is the upper limit, *c* is the lower limit of the model, *b* is the relative slope around the parameter, *e* is the $I_{50}$ or $ED_{50}$, and *x* is the herbicide rate in g ae ha$^{-1}$.

The resistance level (R:S) was calculated by dividing the $ED_{50}$ and $ED_{90}$ of the 2,4-D-WR by the $ED_{50}$ and $ED_{90}$, respectively, of the 2,4-D-WS. The R:S indices for the respective effective dose between the 2,4-D-WR and 2,4-D-WS were compared using the EDcomp function of package *drc* in R. The EDcomp function compares the ratio of effective doses using t-statistics, where *P*-value <0.05 indicates that herbicide $ED_{90}$ values are different between the 2,4-D-WR and 2,4-D-WS (OLIVEIRA et al. 2017).

## 2.3 RESULTS AND DISCUSSION

Estimated repregression parameters, $ED_{50}$, $ED_{90,}$ and resistance level values based on biomass and visual estimation of injury are representes on Table 2.2 and Table 2.3. Biomass and Visual estimation of injury dose-responses of 2,4-D waterhemp-resistant and susceptible are represented on Figure 2.2 to 2.9, and Figures 2.10 to 2.17, respectively.

Populations-by-treatment on biomass data interaction was not significant for all treatments; on visual estimations of injury data, only 2,4-DP-p treatment data population interaction was significant; therefore, treatment data were combined; Dose-response curves based on biomass (g $plant^{-1}$) suggested 2,4-D-WR population was most resistant to 2,4-D amine formulation when compared with all phenoxy herbicides treatments presented (Table 2.2; Figure 2A). Although dose-response curves based on $I_{90}$ showed 2,4-D-WR population higher resistant level when treated with 2,4-D 2EHE herbicide (Table 2.3; Figure 2.10A; Figure 2.16A).

On the basis of $ED_{90}$ and $I_{90}$ values, the analysis showed a 9-fold and 20-fold, respectively, resistance to 2,4-D comparative to the susceptible population. The label rate of 2,4-D (455 g ae $ha^{-1}$) provided about 50% control of the 2,4-D-WR, while the effective dose to control the waterhemp-susceptible population by 90% ($ED_{90}$) was 349 g ae $ha^{-1}$ and 178 g ae $ha^{-1}$ to achieve 90% plant injury (Table 2.2; Figure 2.2A; Table 2.3; Figure 2.10A; Figure 2.16A).

A 2,4-D 2 EHE rate of 1756 g ae $ha^{-1}$ and 723 g ae $ha^{-1}$ was necessary to achieve 90% the $ED_{90}$ and $I_{90}$, respectively, in 2,4-D-WR (Table 2.2; Figure 2.2B; Table 3 2.; Figure 2.10B), considering label rate as 659 g ae $ha^{-1}$. In contrast, the dose necessary to control 90% of the susceptible population was 548 g ae $ha^{-1}$ and 30 g ae $ha^{-1}$ for $I_{90}$. Therefore, was necessary 25-fold increase in the 2,4-D 2 EHE rate required do achieve the $I_{90}$ in the 2,4-D-WR population (Table 2.3; Figure 2.10B; Figure 2.16B).

The 2,4-D EE recommended dose (800 g ae $ha^{-1}$), provided 80% control of 2,4-D-WR, with $ED_{90}$ value for biomass (g $plant^{-1}$) 1255 g ae $ha^{-1}$, 5 times more than the $ED_{90}$ value necessary to control 2,4-D-WS (261 g ae $ha^{-1}$) (Figure 2.3A; Table 2.2; Figure 2.16C).

The resistance level in $ED_{90}$ between 2,4-D-WR and 2,4-D-WS populations treated with 2,4-DB was less than $I_{90}$, 3-fold and 6-fold, respectively. For the resistant population, an estimated dose of 631 g ae $ha^{-1}$ would be predicted to result in 90%

**Table 2.2 -** Estimated $ED_{50}$ and $ED_{90}$ values based on biomass (g ae $ha^{-1}$) in 2,4-D-resistant (2,4-D-WR) and susceptible (2,4-D-WS) waterhemp populations 21 days after treatment in a dose-response study with 16 phenoxy herbicides treatments, conducted under greenhouse conditions at the West Central Research and Extension Center, University of Nebraska-Lincoln in North Platte, Nebraska.

| Population [a] | Herbicide [b] | Regression parameters [c] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *b* | *c* | *d* | $ED_{50}$ | *P*-value [d] | R:S [e] | $ED_{90}$ | *P*-value [f] | R:S [g] |
| | | | | | g ae $ha^{-1}$ | | | g ae $ha^{-1}$ | | |
| 2,4-D-WS | 2,4-D | 1.06 (0.86) | 0.09 (0.05) | 0.61 (0.07) | 44 (28) | 0.22 | 10 | 349 (483) | 0.59 | 9 |
| 2,4-D-WR | | 1.14 (0.39) | 0.05 (0.10) | 0.75 (0.06) | 450(167) | | | 3098 (2738) | | |
| 2,4-D-WS | 2,4-D 2EHE | 0.42 (0.71) | 0.08 (0.11) | 0.61 (0.03) | 3 (12) | 0.81 | 76 | 548 (2924) | 0.89 | 3 |
| 2,4-D-WR | | 1.07 (0.35) | 0.08 (0.06) | 0.73 (0.06) | 228 (70) | | | 1756 (1316) | | |
| 2,4-D-WS | 2,4-D EE | 0.71 (0.55) | 0.04 (0.03) | 0.64 (0.03) | 12 (19) | 0.55 | 31 | 261 (269) | 0.46 | 5 |
| 2,4-D-WR | | 1.78 (0.36) | 0.06 (0.02) | 0.56 (0.03) | 364 (51) | | | 1255 (332) | | |
| 2,4-D-WS | 2,4-DB | 0.99 (0.55) | 0.8 (0.02) | 0.64 (0.03) | 23 (15) | 0.18 | 9 | 212(150) | 0.43 | 3 |
| 2,4-D-WR | | 1.97 (0.68) | 0.16 (0.02) | 0.58 (0.03) | 207 (31) | | | 631 (279) | | |
| 2,4-D-WS | MCPA | 1.66 (0.45) | 0.04 (0.04) | 0.64 (0.04) | 274 (51) | 0.71 | 1.5 | 1029 (411) | 0.64 | 7 |
| 2,4-D-WR | | 0.75 (0.30) | -0.05 (0.14) | 0.57 (0.05) | 383 (290) | | | 7122 (12743) | | |
| 2,4-D-WS | MCPA 2EHE | 1.02 (0.37) | 0.06 (0.02) | 0.63 (0.04) | 38 (13) | 0.01 | 11.5 | 330 (205) | 0.29 | 4.5 |
| 2,4-D-WR | | 1.86 (0.57) | 0.06 (0.03) | 0.53 (0.03) | 442 (65) | | | 1440 (541) | | |
| 2,4-D-WS | MCPB | 0.40 (0.26) | -0.07 (0.24) | 0.64 (0.04) | 108 (185) | 0.70 | 39 | 26843 (136863) | 0.88 | 6 |
| 2,4-D-WR | | 0.60 (0.18) | -0.26 (0.52) | 0.58 (0.04) | 4204 (7899) | | | 167474 (465336) | | |
| 2,4-D-WS | CMPP | 1.09 (0.27) | 0.04 (0.02) | 0.63 (0.04) | 86 (18) | <0.01 | 4.5 | 646 (314) | 0.33 | 3 |
| 2,4-D-WR | | 1.32 (0.34) | 0.05 (0.03) | 0.55 (0.03) | 369 (71) | | | 1957 (956) | | |
| 2,4-D-WS | CMPP-p | 1.03 (0.26) | 0.03 (0.02) | 0.55 (0.03) | 92 (22) | 0.02 | 4.5 | 771 (404) | 0.39 | 2.5 |
| 2,4-D-WR | | 1.39 (0.35) | 0.03 (0.03) | 0.45 (0.03) | 422 (91) | | | 2056 (1017) | | |
| 2,4-D-WS | 2,4-DP | 1.89 (0.45) | 0.04 (0.01) | 0.41 (0.02) | 126 (18) | <0.01 | 2.5 | 404 (121) | 0.45 | 1.5 |

Continue

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 2,4-D-WR | | 3.87 (0.94) | 0.06 (0.01) | 0.41 (0.02) | 309 (23) | | | 545 (85) | | |
| 2,4-D-WS | 2,4-DP-p | 2.36 (0.67) | 0.07 (0.01) | 0.46 (0.03) | 163 (24) | 0.52 | 1 | 413 (106) | 0.47 | 1.5 |
| 2,4-D-WR | | 2.01 (0.65) | 0.05 (0.01) | 0.49 (0.04) | 190 (32) | | | 569 (160) | | |
| 2,4-D-WS | Corasil | 2.50 (0.82) | 0.07 (0.01) | 0.47 (0.03) | 3 (0.4) | 0.15 | 0.5 | 8 (2) | 0.43 | 2 |
| 2,4-D-WR | | 1.22 (0.41) | 0.05 (0.02) | 0.51 (0.03) | 2 (0.6) | | | 14 (7) | | |
| 2,4-D-WS | Dicamba DGA | 1.15 (0.41) | 0.07 (0.02) | 0.62 (0.03) | 38 (11) | 0.48 | 0.5 | 257 (133) | 0.09 | 0.5 |
| 2,4-D-WR | | 1.36 (1.19) | 0.08 (0.02) | 0.62 (0.03) | 22 (21) | | | 112 (63) | | |
| 2,4-D-WS | Dicamb DMA | 2.61 (0.72) | 0.08 (0.01) | 0.62 (0.03) | 68 (6) | 0.68 | 1 | 158 (36) | 0.85 | 1 |
| 2,4-D-WR | | 2.59 (0.61) | 0.06 (0.01) | 0.62 (0.03) | 72 (6) | | | 168 (35) | | |
| 2,4-D-WS | 2,4-D + 2,4-DP[h] | 1.98 (0.94) | 0.06 (0.01) | 0.56 (0.03) | 0.08 (0.02) | 0.03 | 2.5 | 0.25 (0.08) | 0.21 | 2 |
| 2,4-D-WR | | 2.64 (0.70) | 0.04 (0.01) | 0.44 (0.03) | 0.23 (0.03) | | | 0.52 (0.12) | | |
| 2,4-D-WS | 2,4-D + CMPP[h] | 2.01 (1.20) | 0.05 (0.01) | 0.56 (0.03) | 0.07 (0.03) | 0.05 | 6 | 0.22 (0.08) | 0.07 | 8.5 |
| 2,4-D-WR | | 1.53 (0.35) | 0.03 (0.02) | 0.45 (0.08) | 0.45 (0.08) | | | 1.89 (0.64) | | |

[a] Waterhemp populations were designated as 2,4-D-WR, 2,4-D-dichlorophenoxy acetic acid-resistant collected from a grass seed production field situated in Nebraska in 2011; 2,4-D-WS, 4-D-dichlorophenoxy acetic acid-susceptible collected from a field in Lancaster county, NE in 2012.
[b]: Phenoxy herbicides formulations (g ae $ha^{-1}$).
[c]: Regression parameters: four-parameter log logistic model $y = c + \frac{d-c}{1+exp[b(logx-loge)]}$ was used, where: *b* is the relative slope around *e*, *c* is the lower limit, *d* is the upper limit, and *e* is the $ED_{50}$, $\pm$ Standard Error (SE).
[d]: 2,4-D-WR vs 2,4-D-WS *t*-statistics comparison of $ED_{50}$. All *P*-values were rounded to two significant digits.
[e]: Resistance level was calculated by dividing the $ED_{50}$ value of 2,4-D-WR by 2,4-D-WS for each treatment, rounded to $\pm$ 0.5.
[f]: 2,4-D-WR vs 2,4-D-WS *t*-statistics comparison of $ED_{90}$. All *P*-values were rounded to two significant digits.
[g]: Resistance level was calculated by dividing the $ED_{90}$ value of 2,4-D-WR by 2,4-D-WS for each treatment, rounded to $\pm$ 0.5.
[h]: herbicides tank mixtures values are based on herbicides rates (0, 0.125x, 0.250x, 0.5x, 0.75x, 1x, 1.5x, 2x, 3x, 4x, 6x and 9x) of recommended dose.

**Table 2.3 -** Estimated $I_{50}$ and $I_{90}$ values based on visual injury (%) in 2,4-D-resistant (2,4-D-WR) and susceptible (2,4-D-WS) waterhemp populations 21 d after treatment in a dose-response study with 16 phenoxy herbicides treatments, conducted under greenhouse conditions at the West Central Research and Extension Center, University of Nebraska-Lincoln in North Platte, Nebraska.

| Population [a] | Herbicide [b] | Regression parameters [c] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *b* | *c* | *d* | $I_{50}$ | *P*-value [d] | R:S [e] | $I_{90}$ | *P*-value [f] | R:S [g] |
| 2,4-D-WS | 2,4-D | -4.63 (4.92) | 58.79 (9.05) | 100 (1.63) | 111 (18) | <0.01 | 4 | 178 (74) | 0.19 | 20 |
| 2,4-D-WR | | -1.07 (0.33) | 6.36 (12.46) | 109.68 (9.75) | 455 (98) | | | 3532 (2118) | | |
| 2,4-D-WS | 2,4-D 2EHE | -1.66 (0.83) | -729.74 (1535.56) | 100.46 (1.47) | 8 (12) | 0.52 | 36 | 30 (35) | 0.42 | 25 |
| 2,4-D-WR | | -2.37 (0.61) | 22.86 (7.23) | 97.17 (1.95) | 287 (40) | | | 723 (117) | | |
| 2,4-D-WS | 2,4-D EE | -1.66 (0.84) | -784.84 [i] | 100.37 (1.72) | 9 [i] | NA | NA | 33 [i] | NA | NA |
| 2,4-D-WR | | -1.37 [i] | 1.04 [i] | 102.30 (1.55) | 265 [i] | | | 1308 (248) | | |
| 2,4-D-WS | 2,4-DB | -1.29 (0.53) | -98.10 (382.90) | 98.32 (2.32) | 26 (60) | 0.73 | 5 | 143 (240) | 0.62 | 6 |
| 2,4-D-WR | | -1.16 (0.39) | -17.55 (31.06) | 88.11 (4.45) | 133 (70) | | | 889 (253) | | |
| 2,4-D-WS | MCPA | -1.49 (0.72) | 28.98 (41.60) | 101.64 (2.56) | 98 (87) | <0.01 | 0.2 | 431 (142) | 0.53 | 0.5 |
| 2,4-D-WR | | -0.78 (0.17) | -158.50 (146.96) | 101.51 (4.60) | 17 (19) | | | 276 (226) | | |
| 2,4-D-WS | MCPA 2EHE | -7.47 (5.83) | 51.02 (4.01) | 99.41 (1.49) | 176 (30) | 0.56 | 0.5 | 236 (29) | 0.24 | 18 |
| 2,4-D-WR | | -0.55 (0.32) | -62.29 (120.57) | 122.13 (19.50) | 80 (165) | | | 4273 (3407) | | |
| 2,4-D-WS | MCPB | -1.77 (0.87) | 58.70 (7.66) | 96.38 (2.75) | 197 (61) | <0.01 | 0.04 | 684 (327) | 0.71 | 2 |
| 2,4-D-WR | | -0.43 (0.12) | -167.81 (107.40) | 104.10 (13.11) | 9 (13) | | | 1388 (1791) | | |
| 2,4-D-WS | CMPP | -2.44 (0.76) | 59.96 (6.41) | 100.20 (1.35) | 192 (41) | 0.06 | 1.5 | 474 (101) | 0.04 | 2 |
| 2,4-D-WR | | -2.15 (0.30) | 12.34 (4.30) | 98.49 (1.77) | 337 (26) | | | 936 (112) | | |
| 2,4-D-WS | CMPP-p | -2.19 (1.13) | 51.47 (11.39) | 101.07 (2.37) | 264 (95) | 0.55 | 0.5 | 719 (200) | 0.29 | 1.5 |
| 2,4-D-WR | | -1.18 (0.37) | -11.36 (28.99) | 104.89 (5.45) | 196 (88) | | | 1252 (363) | | |
| 2,4-D-WS | 2,4-DP | -7.17 (12.05) | 45.86 (5.23) | 100.01 (1.69) | 160 (18) | <0.01 | 2 | 218 (134) | 0.27 | 3.5 |
| 2,4-D-WR | | -2.35 (0.46) | 16.01 (6.24) | 100.67 (2.42) | 287 (32) | | | 731 (119) | | |
| 2,4-D-WS | 2,4-DP-p | -4.21 (0.94) | 35.34 (4.44) | 100.19 (1.38) | | 0.51 | 1 | 314 (43) | 0.04 | 2 |

Continue

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 2,4-D-WR | | -2.28 (0.51) | 26.59 (7.95) | 99.74 (1.78) | 210 (32) | | | 552 (85) | | |
| 2,4-D-WS | Corasil | -1.73 (0.45) | -215.64 [i] | 100.24 (0.64) | 0.55 [i] | NA | 12.5 | 7 (2) | NA | 10 |
| 2,4-D-WR | | -1.97 (0.58) | 73.68 (5.51) | 100.07 (0.79) | 7 (2) | | | 21 (4) | | |
| 2,4-D-WS | Dicamba DGA | -1.75 (0.78) | -856.18 [i] | 100.18 (0.53) | 4 [i] | NA | 5.5 | 12.65 [i] | NA | NA |
| 2,4-D-WR | | -3.90 (1.17) | -1076.34 [i] | 100.01 (0.47) | 19 [i] | | | 33.80 [i] | | |
| 2,4-D-WS | Dicamba DMA | -1.66 (0.28) | -140.87 [i] | 100.34 (0.80) | 14 [i] | NA | 1 | 51.89 [i] | NA | NA |
| 2,4-D-WR | | -2.18 (0.57) | -388.08 [i] | 100.21 (0.73) | 16 [i] | | | 45.09 [i] | | |
| 2,4-D-WS | 2,4-D + 2,4-DP [h] | -1.46 (0.60) | -447.55 (2287.16) | 100.60 (1.61) | 0.01 (0.05) | 0.78 | 19.5 | 0.06 (0.20) | 0.78 | 8 |
| 2,4-D-WR | | -3.62 (1.21) | 47.28 (5.77) | 99.66 (1.42) | 0.28 (0.03) | | | 0.51 (0.10) | | |
| 2,4-D-WS | 2,4-D + CMPP [h] | -1.45 (0.51) | -978.76 [i] | 100.46 (1.19) | 0.006 [i] | 0.19 | 8 | 0.03 [i] | NA | NA |
| 2,4-D-WR | | -1.09 (0.32) | -142.16 (307.61) | 101.42 (2.31) | 0.05 (0.09) | | | 0.38 (0.48) | | |

[a] Waterhemp populations were designated as 2,4-D-WR, 2,4-D-dichlorophenoxy acetic acid-resistant collected from a grass seed production field situated in Nebraska in 2011; 2,4-D-WS, 4-D-dichlorophenoxy acetic acid-susceptible collected from a field in Lancaster county, NE in 2012.
[b]: Phenoxy herbicides formulations (g ae $ha^{-1}$).
[c]: Regression parameters: four-parameter log logistic model $y = c + \frac{d-c}{1+exp[b(logx-loge)]}$ was used, where: *b* is the relative slope around *e*, *c* is the lower limit, *d* is the upper limit, and *e* is the $I_{50}$, $\pm$ Standard Error (SE).
[d]: 2,4-D-WR vs 2,4-D-WS *t*-statistics comparison of $ED_{50}$. All *P*-values were rounded to two significant digits.
[e]: Resistance level was calculated by dividing the $ED_{50}$ value of 2,4-D-WR by 2,4-D-WS for each treatment, rounded to $\pm$ 0.5.
[f]: 2,4-D-WR vs 2,4-D-WS *t*-statistics comparison of $ED_{90}$. All *P*-values were rounded to two significant digits.
[g]: Resistance level was calculated by dividing the $ED_{90}$ value of 2,4-D-WR by 2,4-D-WS for each treatment, rounded to $\pm$ 0.5.
[h]: herbicides tank mixtures values are based on herbicides rates (0, 0.125x, 0.250x, 0.5x, 0.75x, 1x, 1.5x, 2x, 3x, 4x, 6x and 9x) of recommended dose.
[i]: data lack of convergence in the four-parameter log logistic model.

**Figure 2.1 -** Herbicides chemical structure. [A]2,4-D, [B]2,4-D 2EHE, [C]2,4-D ethyl ester, [D] 2,4-DB, [E] MCPA, [F] MCPA 2 EHE, [G]MCPB, [H]CMPP, [I]CMPP-p, [J]2,4-DP, [K]2,4-DP-p, [L] Dichlorprop-p 2 EHE, [M]Dicamba DGA, [N]Dicamba DMA, and [O] IAA.

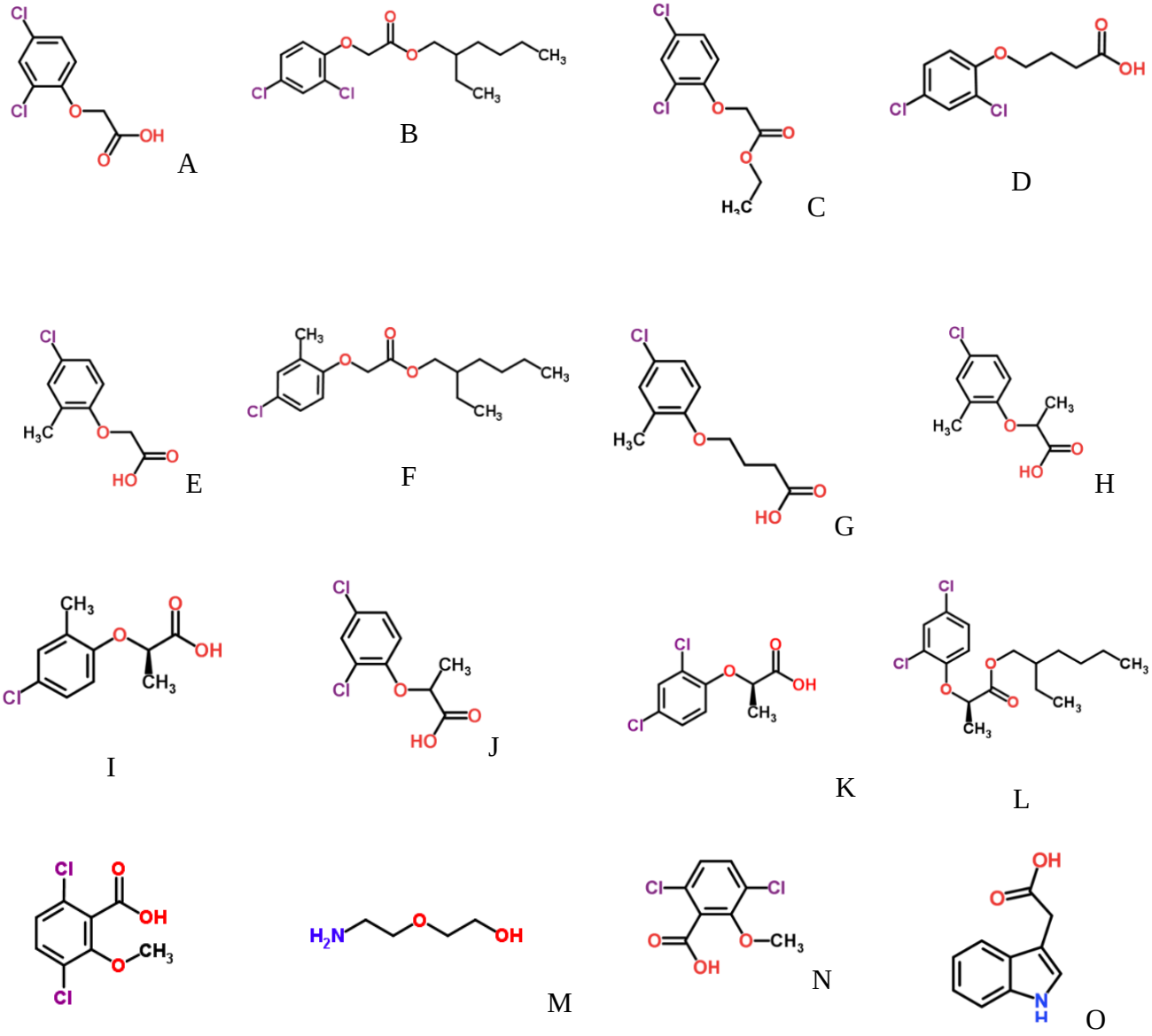
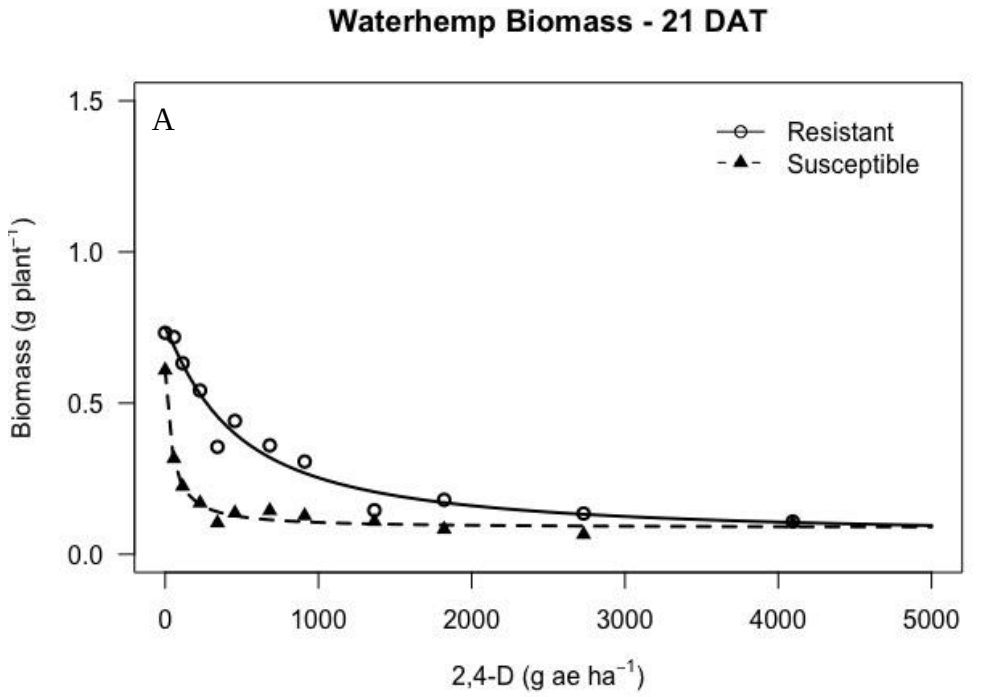


**Figure 2.2 -** Biomass dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]2,4-D and [B] 2,4-D 2 EHE.



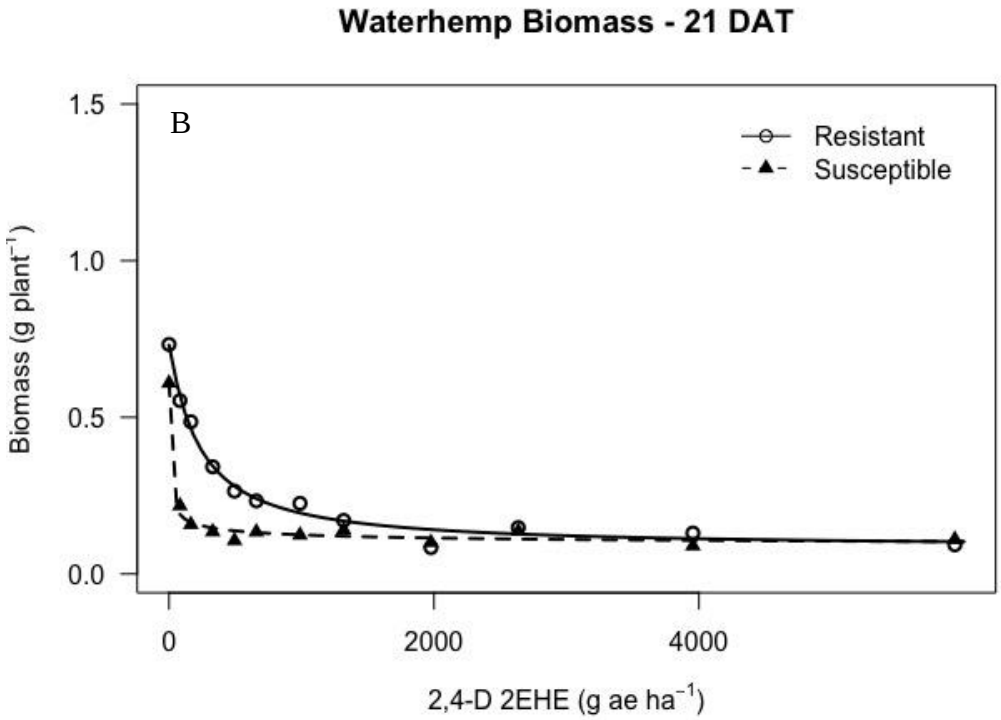
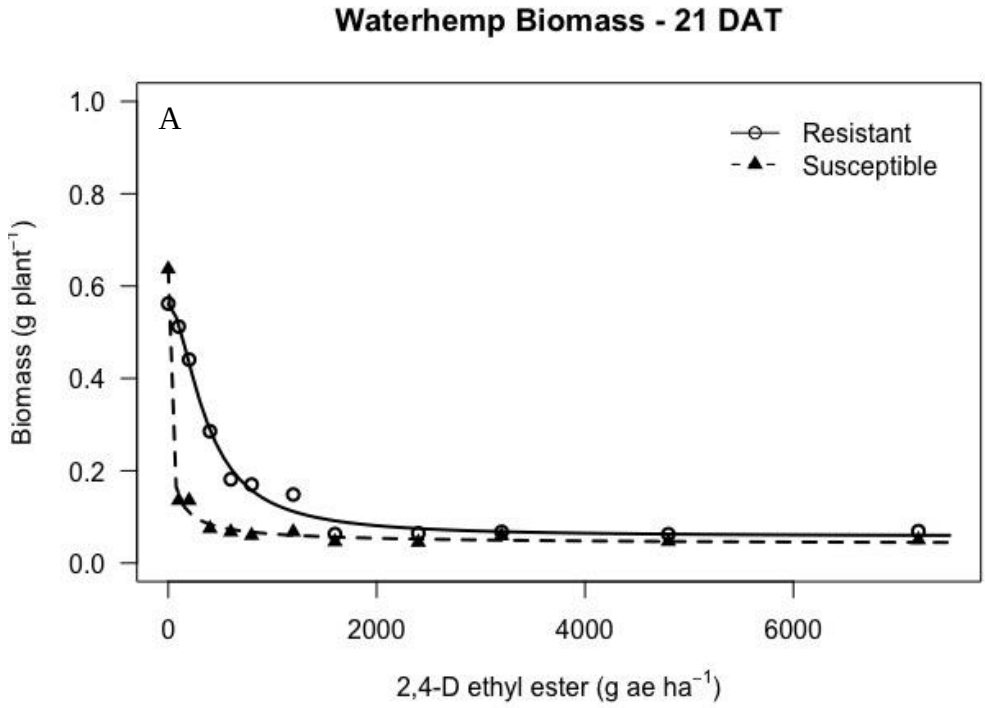



**Figure 2.3 -** Biomass dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]2,4-D ethyl ester and [B]2,4-DB.



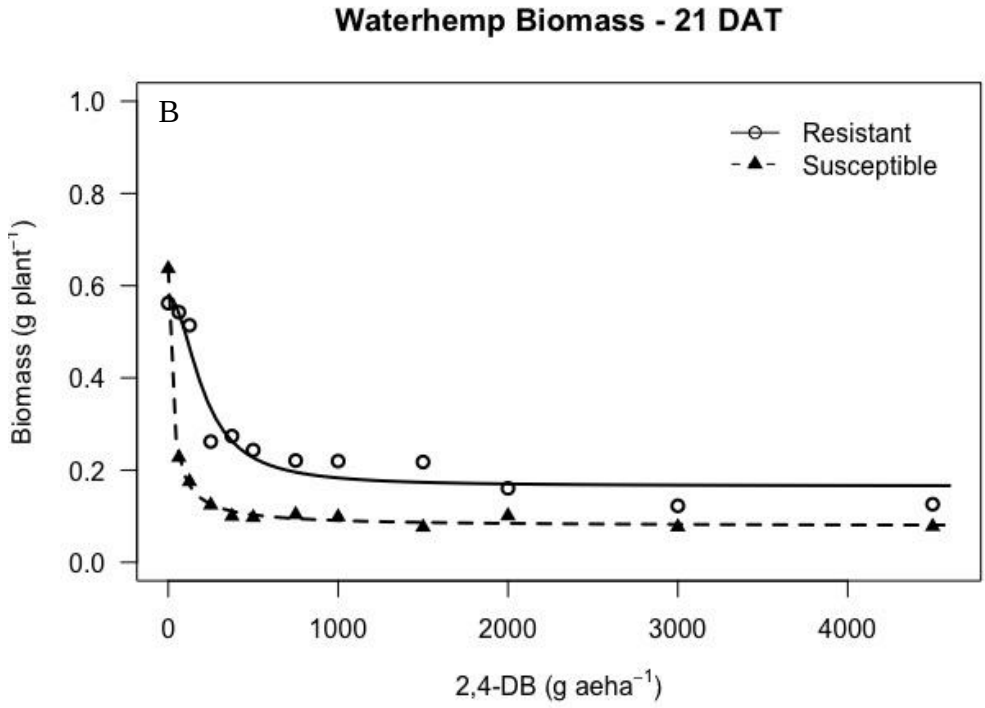
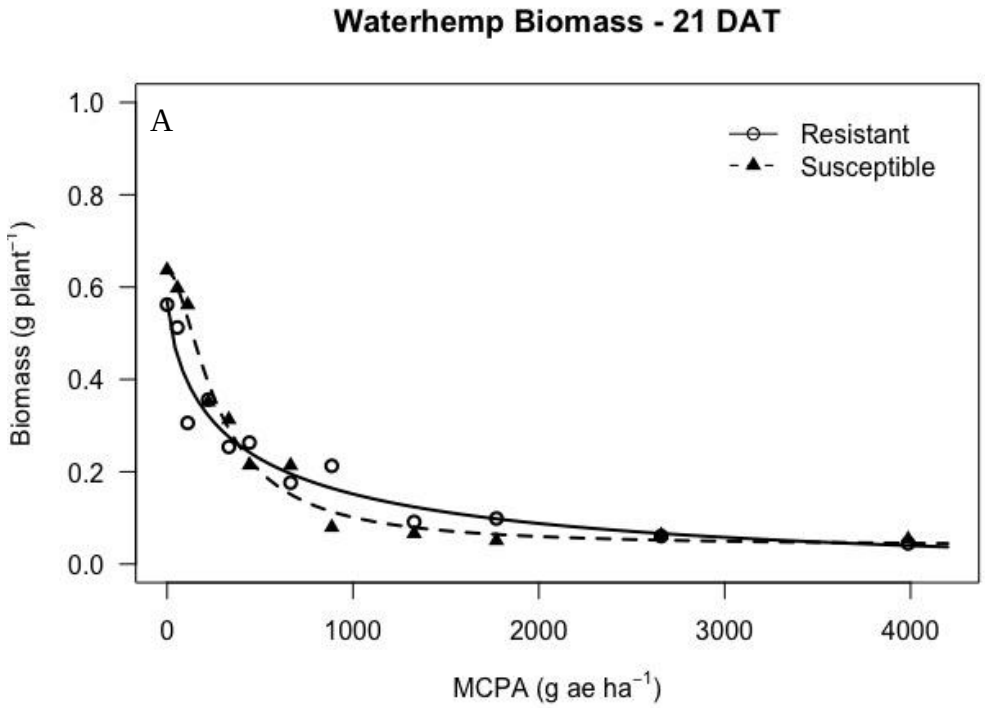



**Figure 2.4 -** Biomass dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]MCPA and [B]MCPA 2EHE.



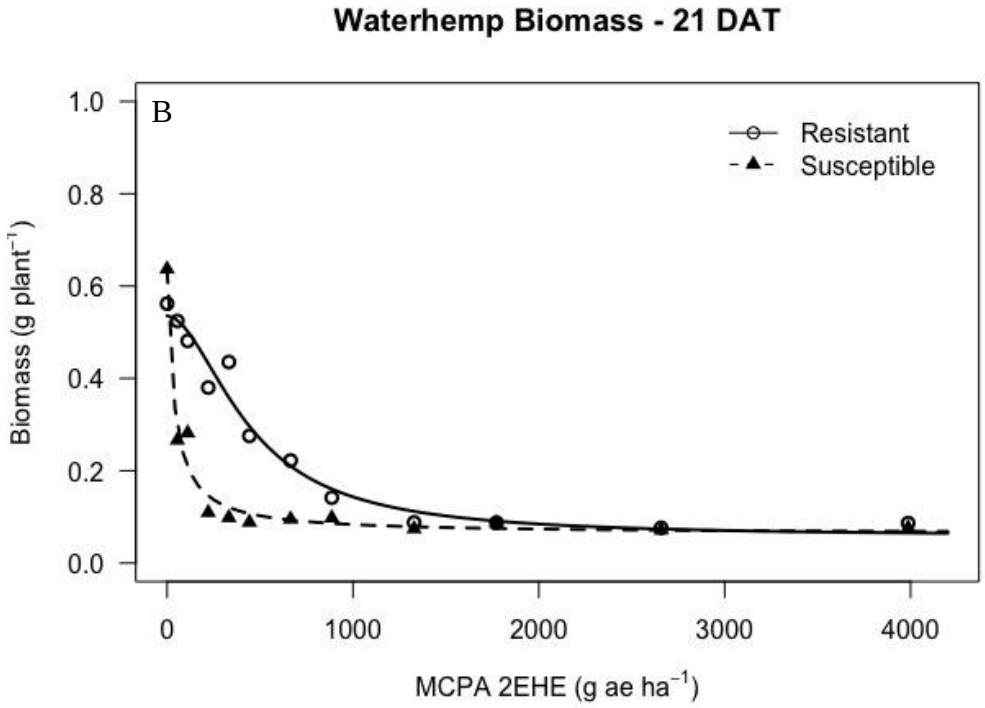
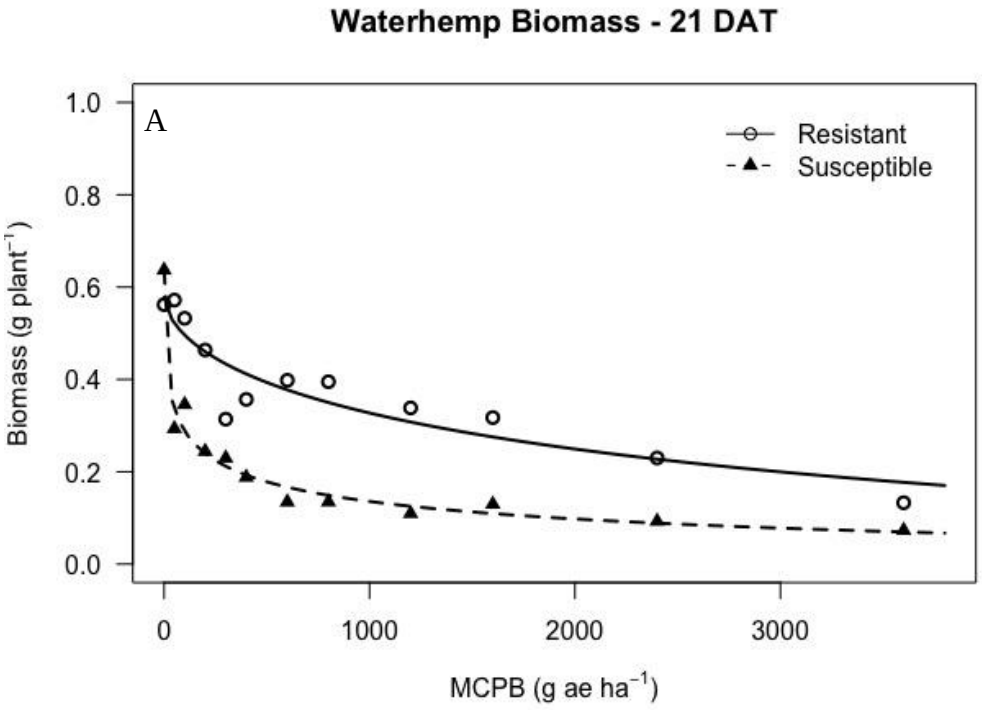



**Figure 2.5 -** Biomass dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]MCPB and [B]CMPP.



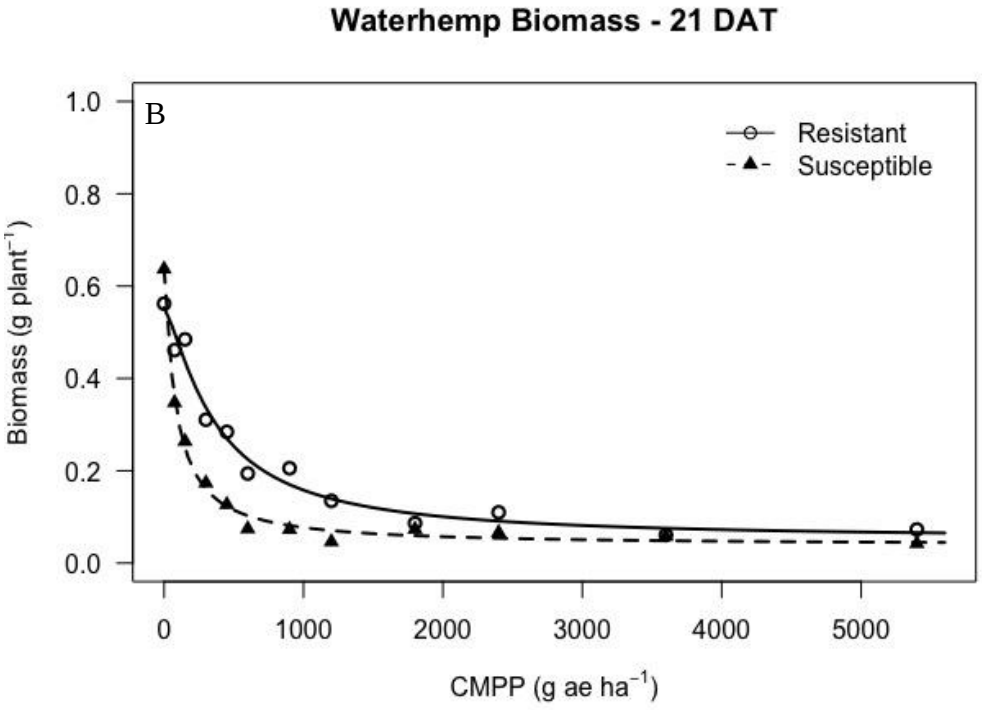
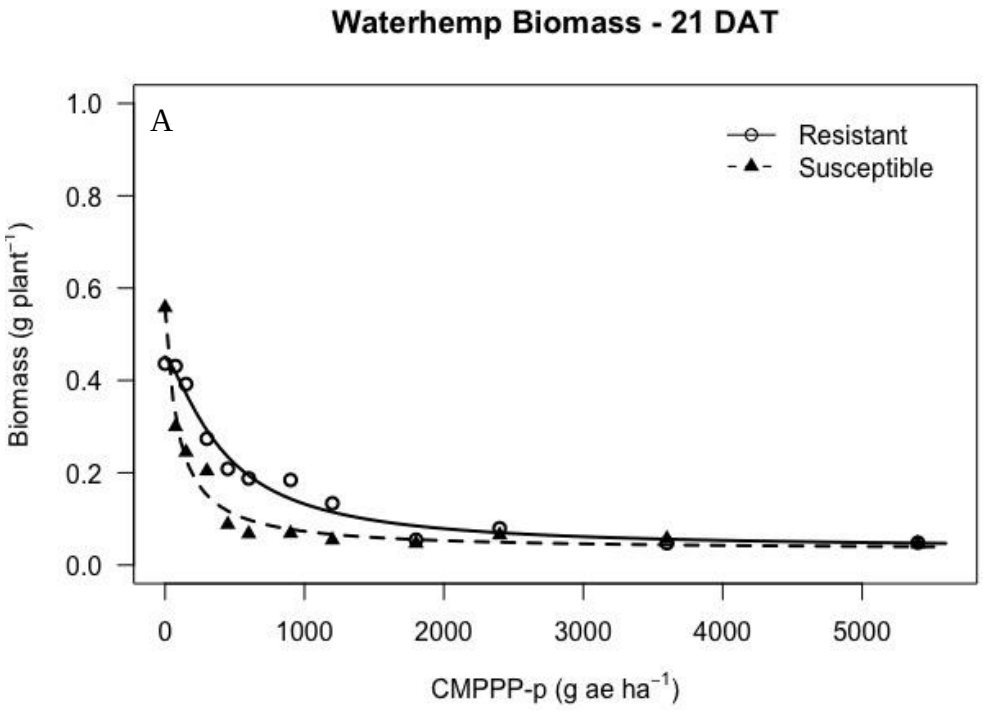



**Figure 2.6 -** Biomass dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]CMPP-p and [B]2,4-DP.



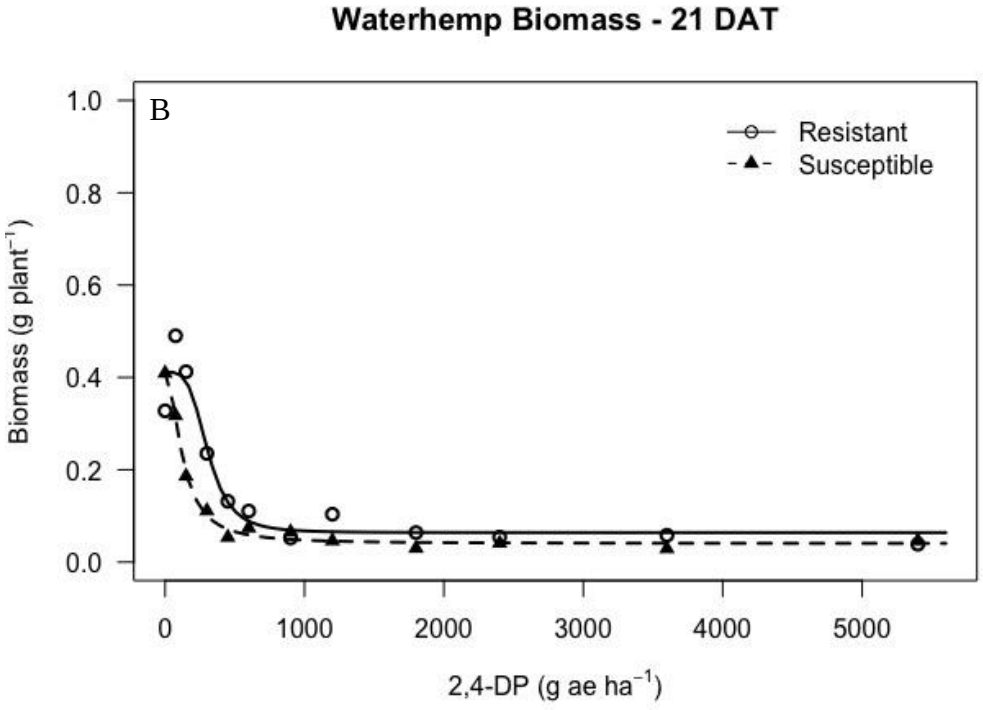
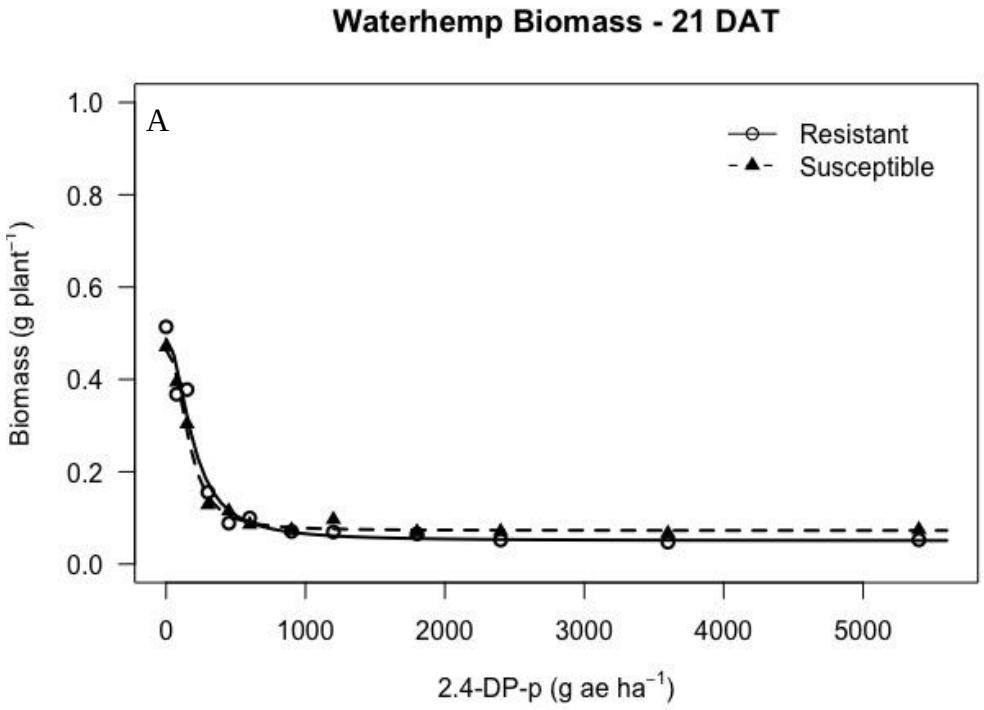



**Figure 2.7** - Biomass dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]2,4-D-p and [B]Corasil.



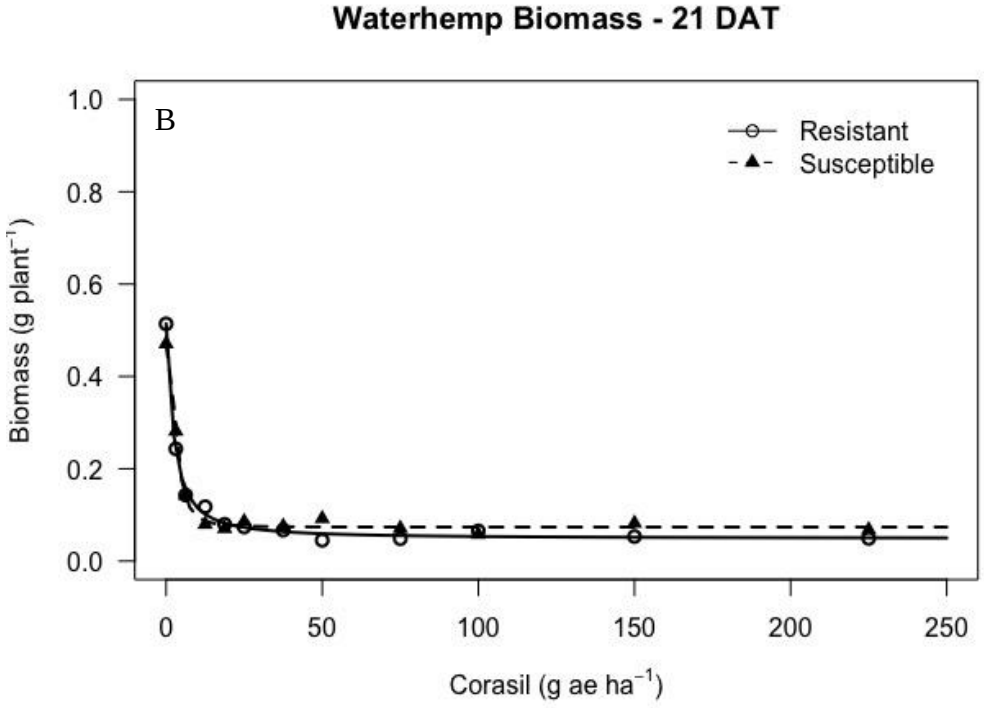
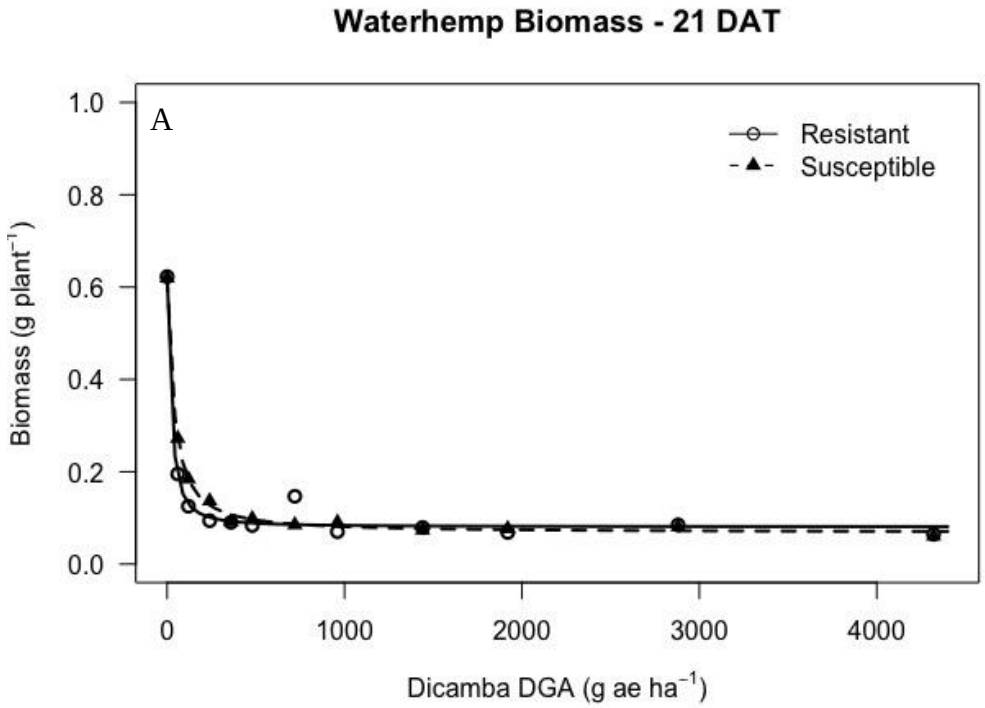



**Figure 2.8 -** Biomass dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]Dicamba DGA and [B]Dicamba DMA.



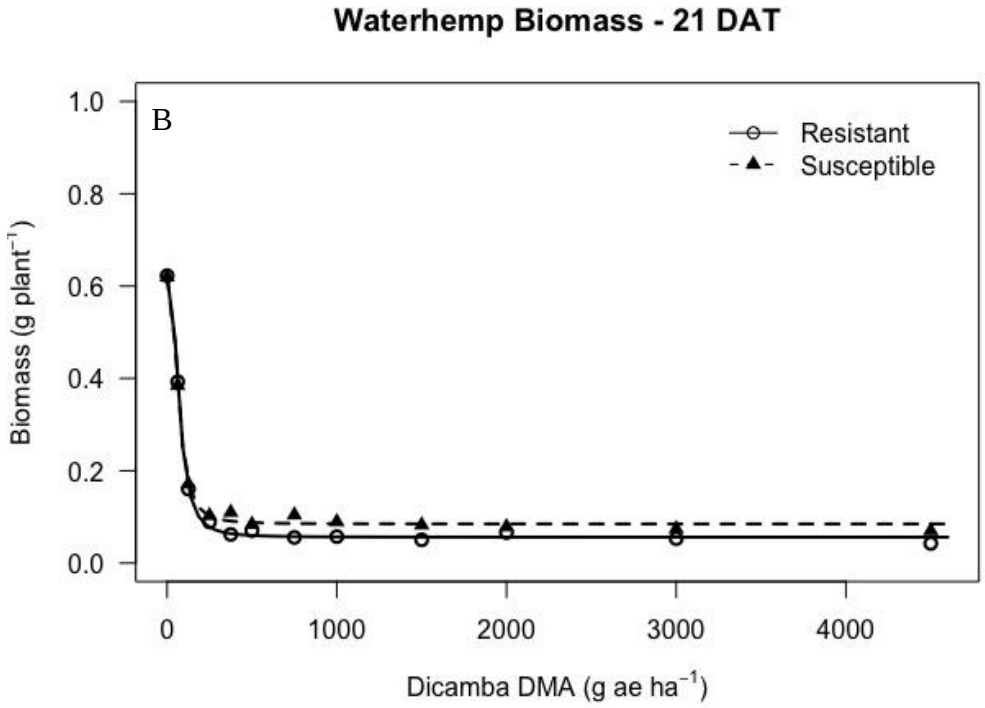
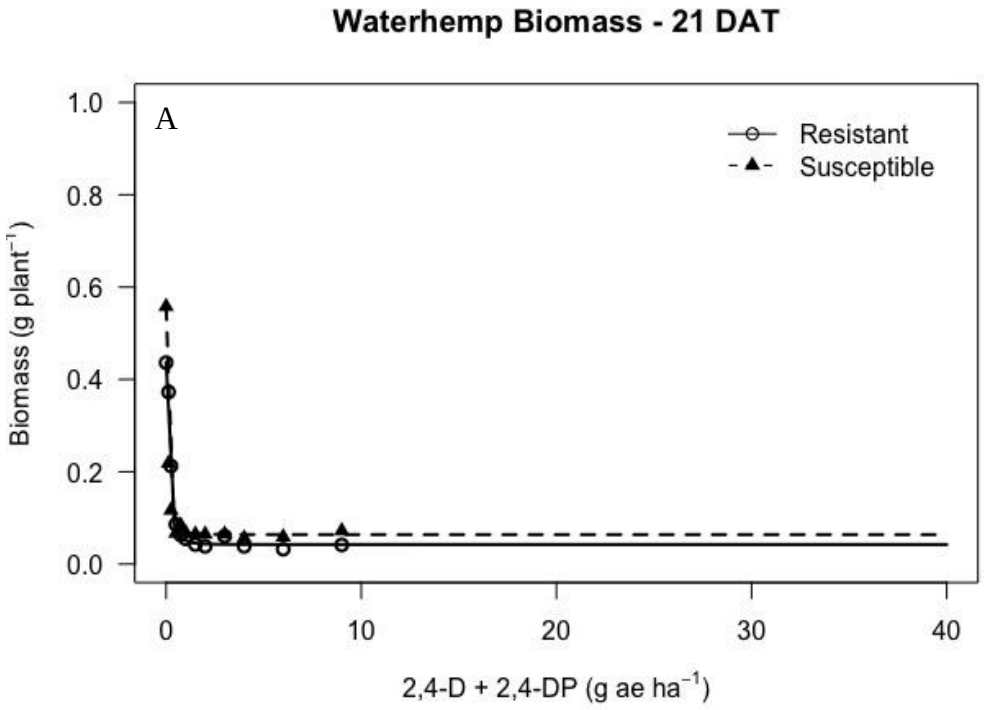



**Figure 2.9 -** Biomass dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]2,4-D + 2,4-DP [B] 2,4-D + CMPP.



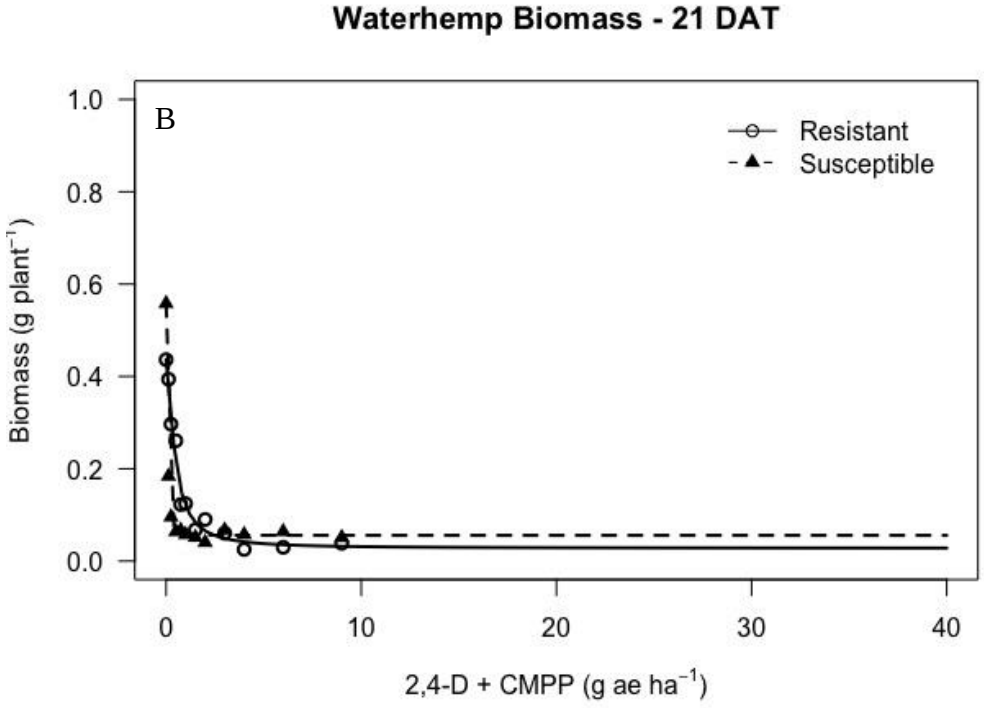
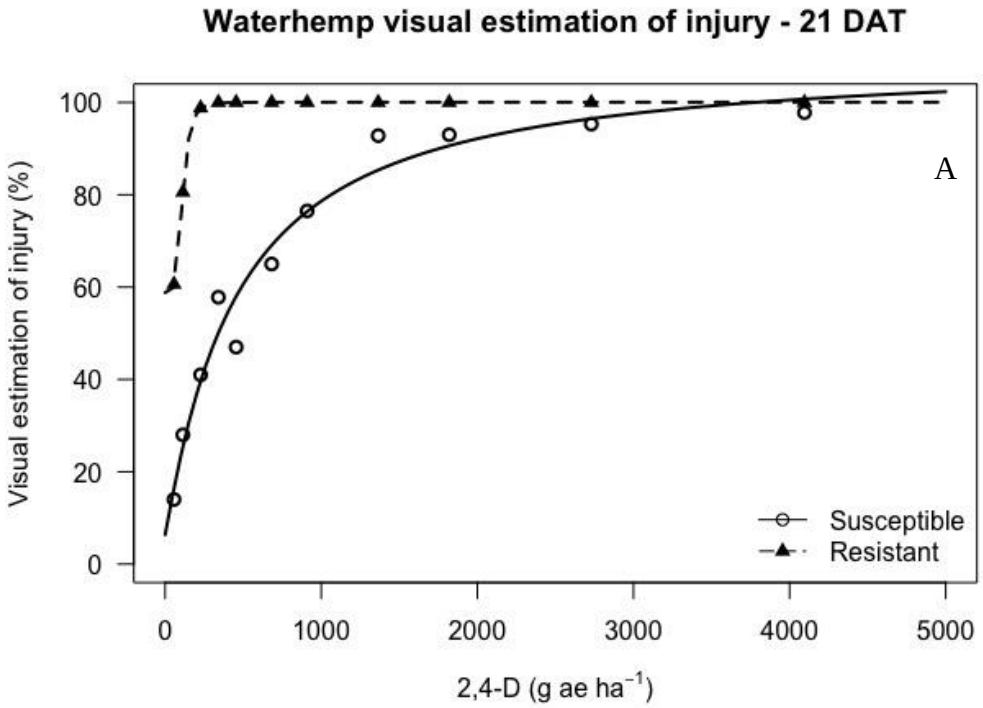



**Figure 2.10 -** Visual estimation of injury dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]2,4-D and [B] 2,4-D 2 EHE.



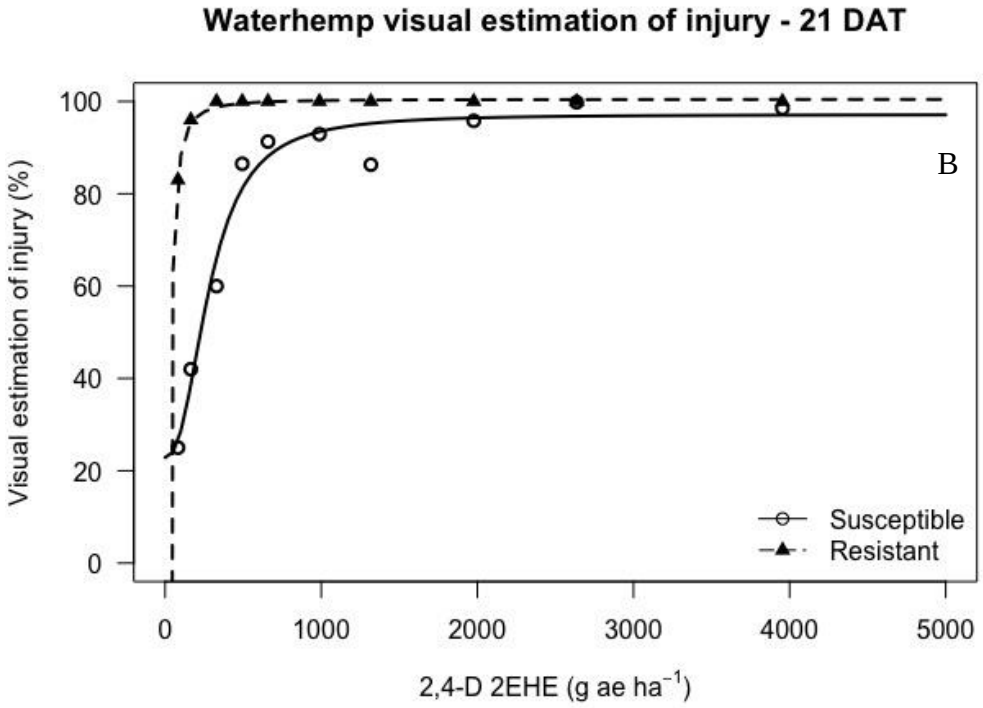
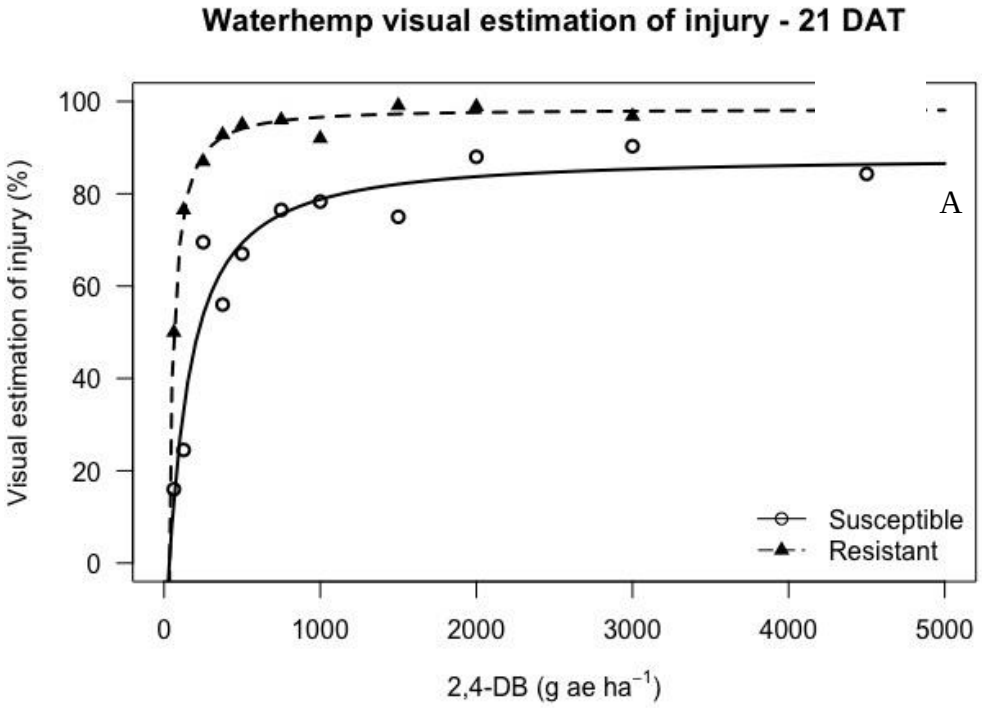



**Figure 2.11 -** Visual estimation of injury dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]2,4-DB and [B] MCPA.



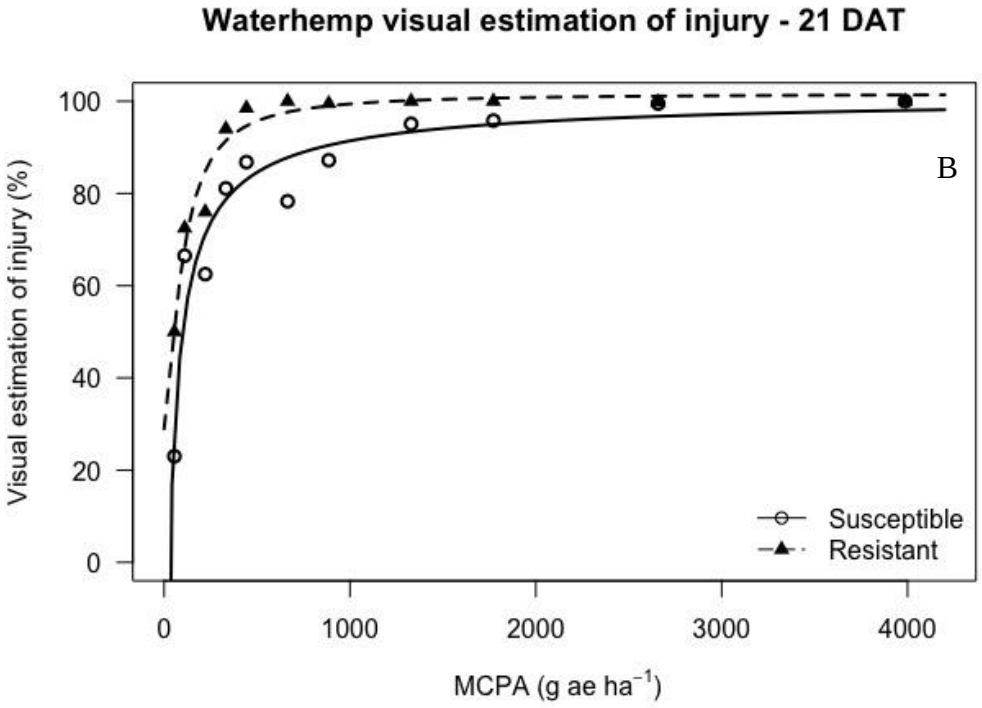
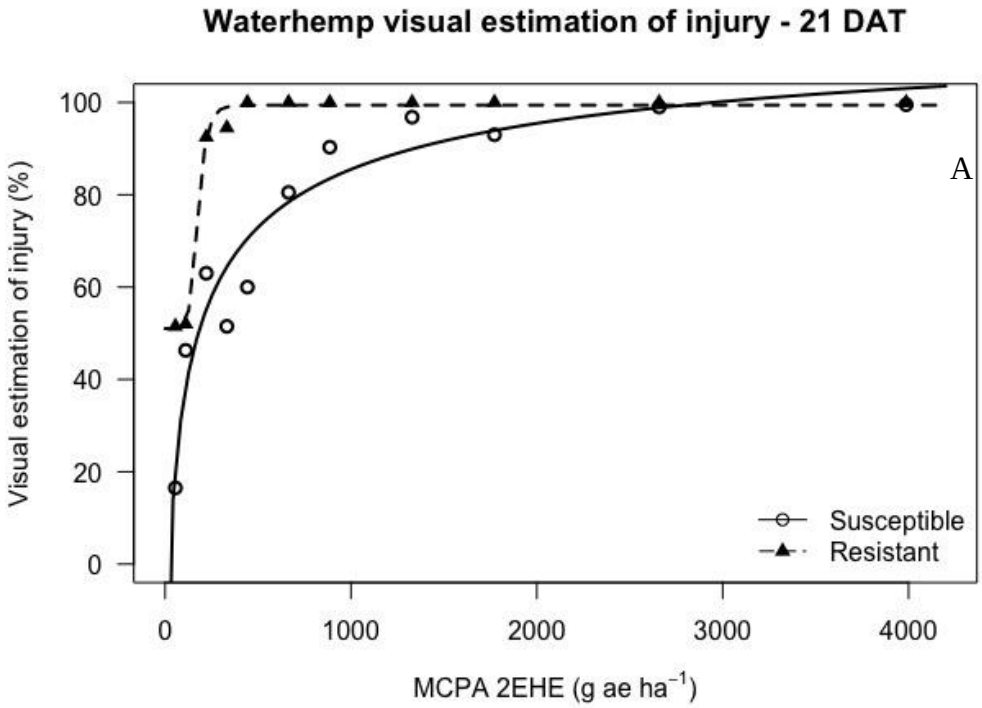



**Figure 2.12 -** Visual estimation of injury dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]MCPA 2 EHE and [B] MCPB.



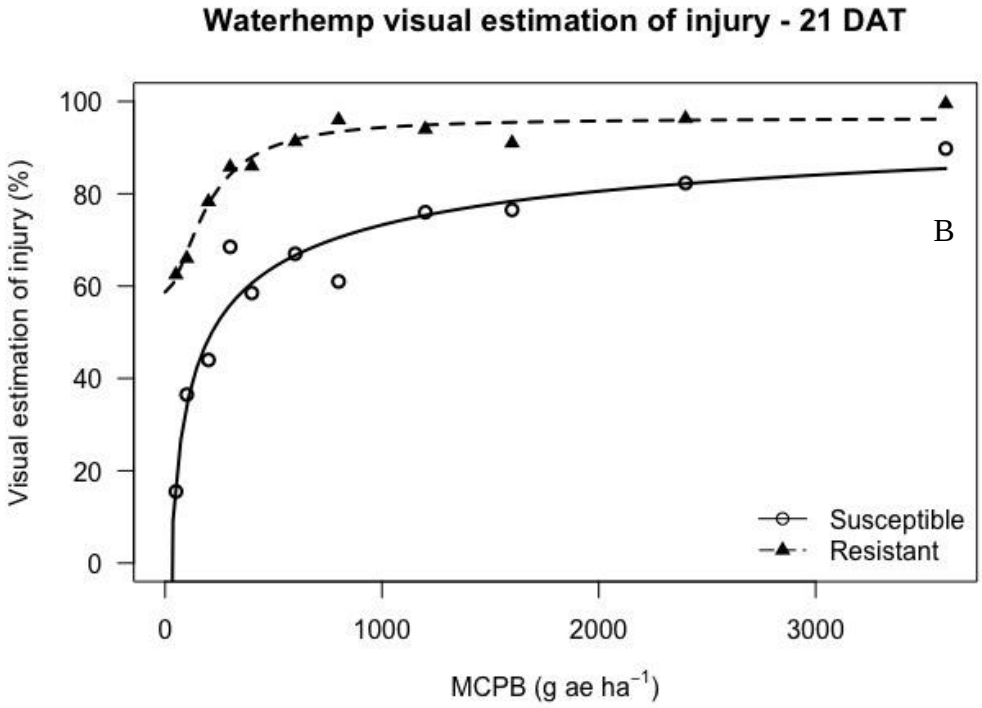
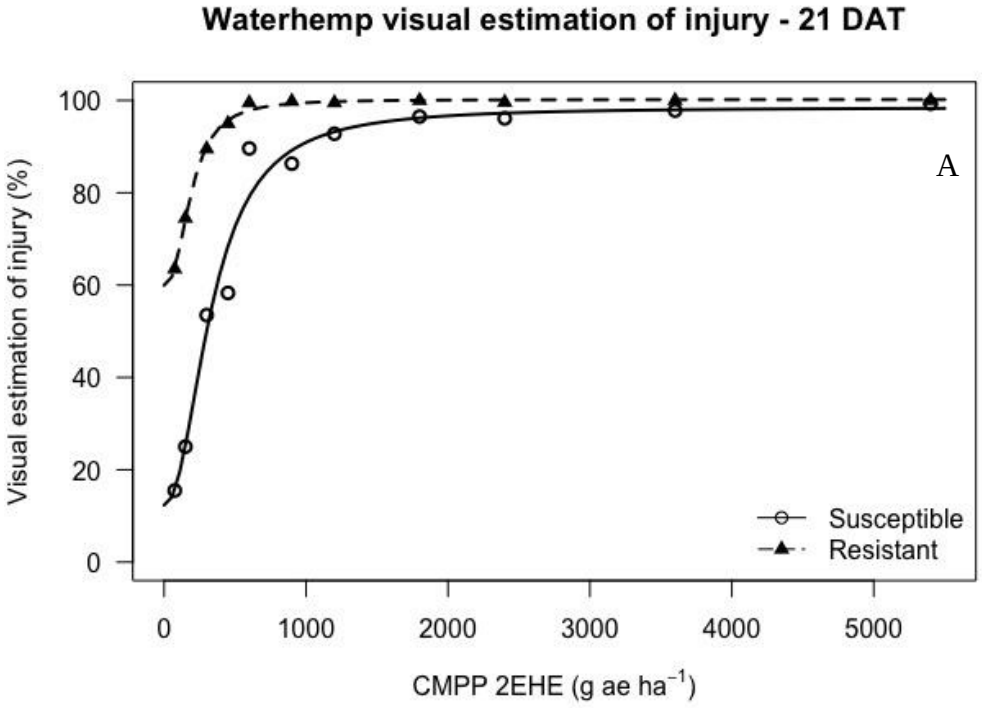



**Figure 2.13 -** Visual estimation of injury dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]CMPP and [B] CMPP-p.



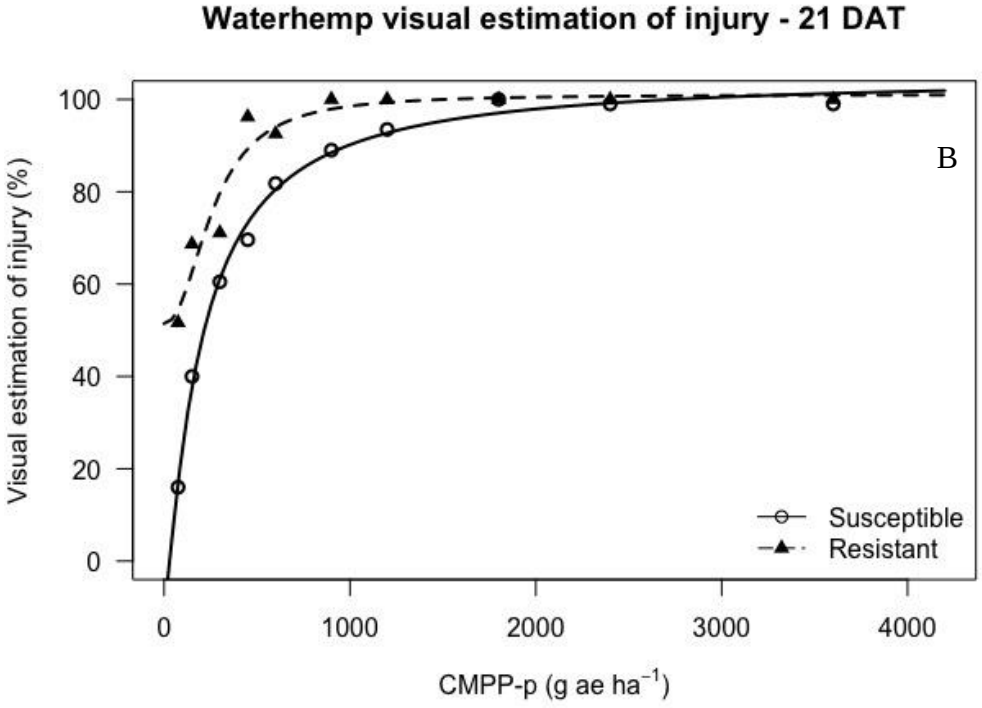
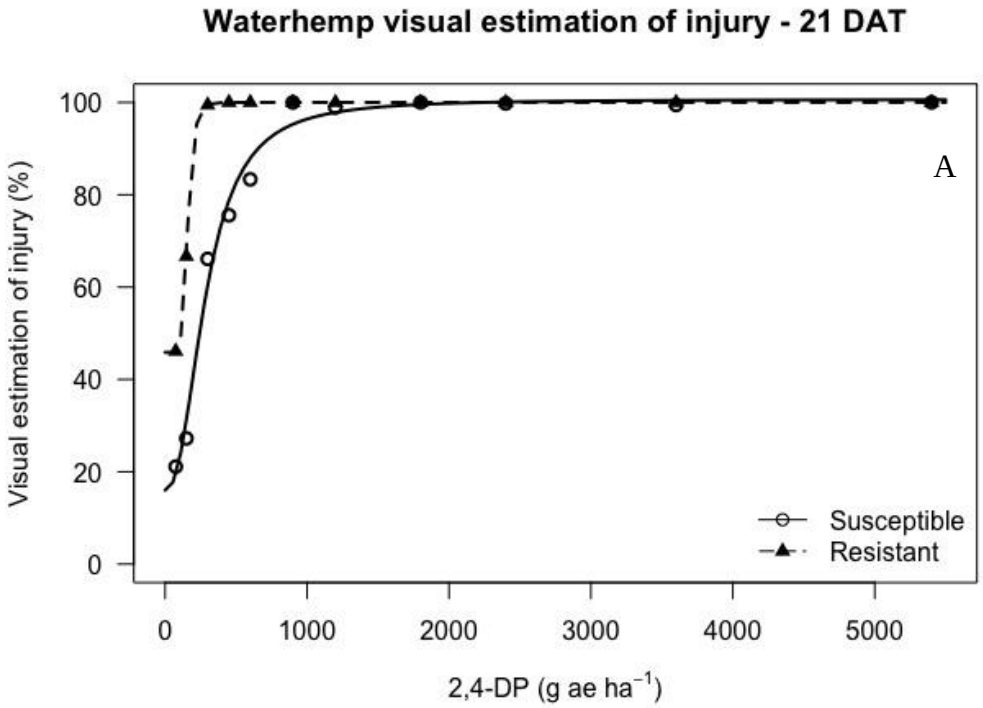



**Figure 2.14 -** Visual estimation of injury dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]2,4-DP and [B] 2,4-DP-p.



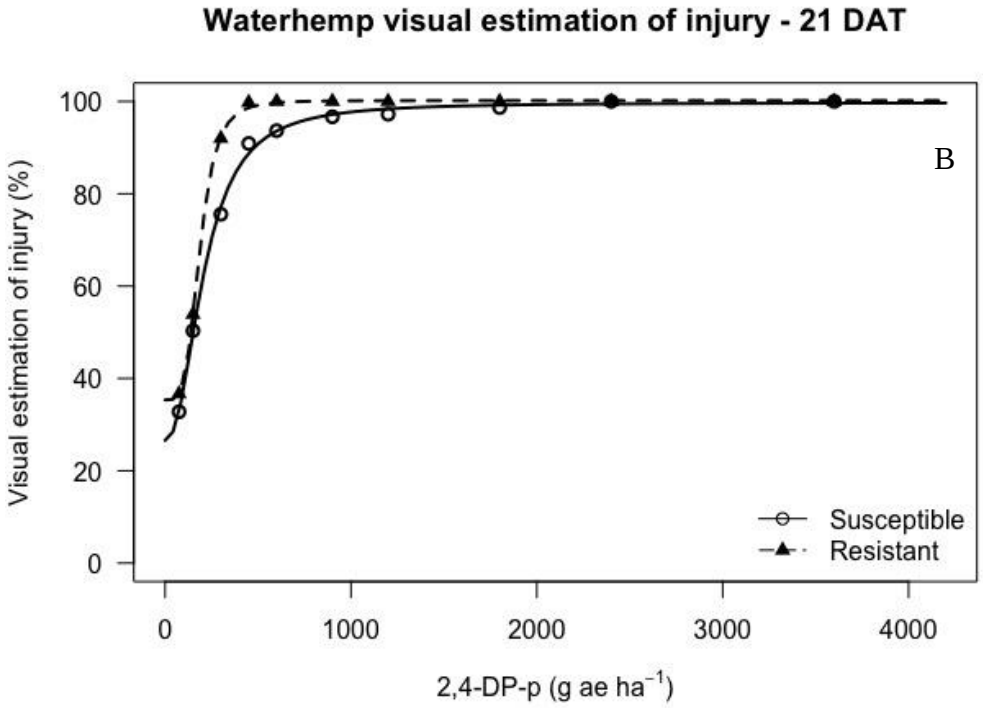
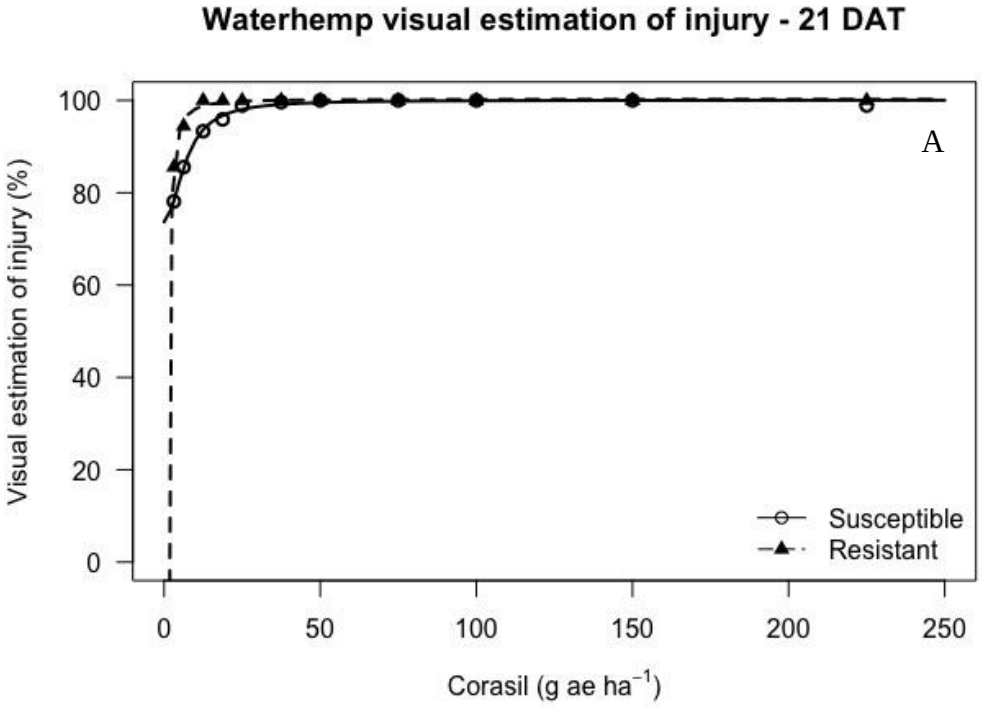



**Figure 2.15 -** Visual estimation of injury dose-response of 2,4-D waterhemp-resistant and susceptible at 21 d after treatments with phenoxy herbicides under greenhouse conditions. [A]Corasil and [B] 2,4-D + 2,4-DP.



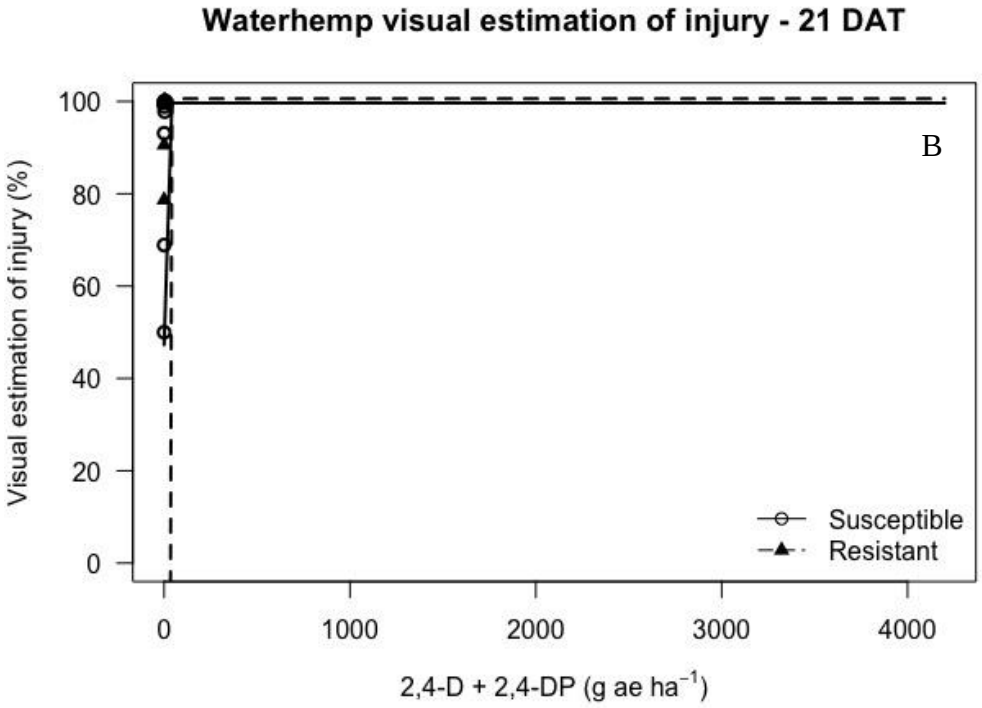



**Figure 2.16 -** 2,4-D-WS on top and 2,4D-WR on bottom populations treated with: [A] 2,4-D, [B] 2,4-D 2EHE, [C] 2,4-D EE, [D] 2,4-DB, [E] MCPA, [F] MCPA 2EHE, [G] MCPB and [H] CMPP.

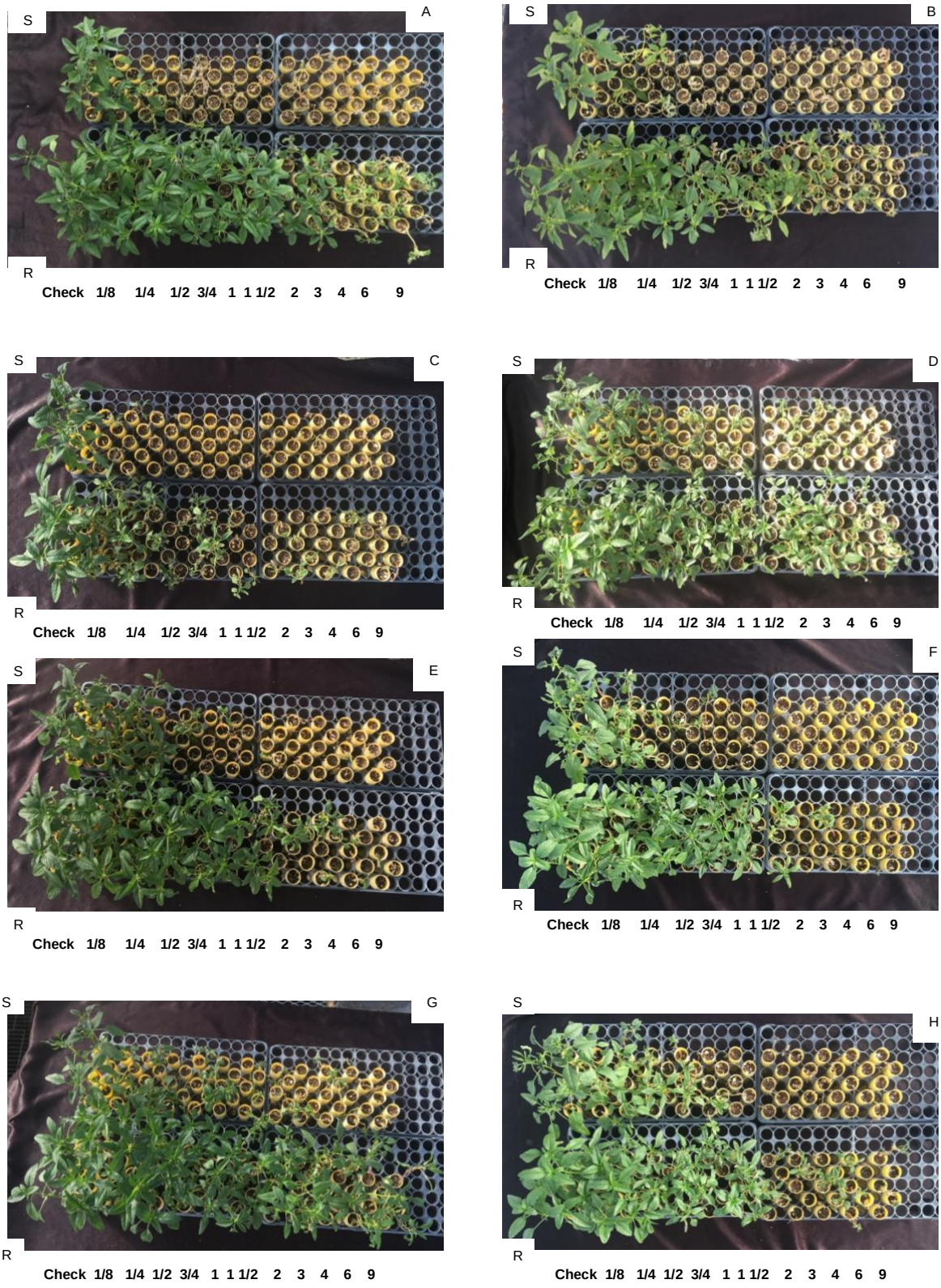
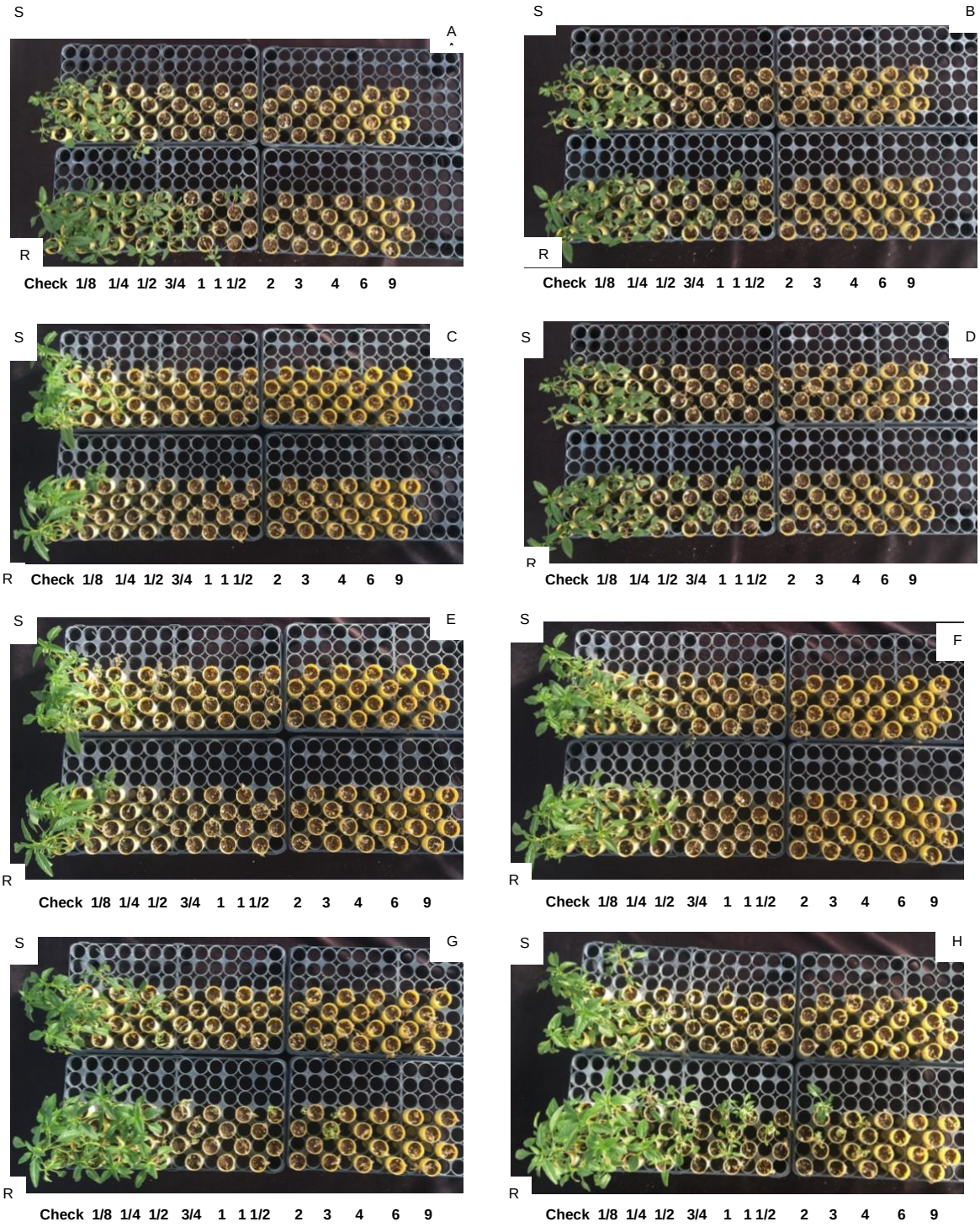


**Figure 2.17 -** 2,4-D-WS on top and 2,4D-WR on bottom populations treated with: [A]CMPP-p, [B]2,4-DP, [C]2,4-DP-p, [D]Corasil, [E]Dicamba DGA, [F]Dicamba DMA, [G] 2,4-D + 2,4-DP and [H] 2,4-D + CMPP.



biomass reduction (1.26 times higher than the label rate) (Table 2.2; Figure 2.3B), and 889 g ae $ha^{-1}$ to $I_{90}$ (1.78 times higher than the label dose) (Table 2.3; Figure 2.11A; Figure 2.16D).

The MCPA rate necessary to control 90% of 2,4-D-WR and 2,4-D-WS populations were correspondingly 7122 g ae $ha^{-1}$ and 1029 g ae $ha^{-1}$, 16 and 2.5 times higher than the recommended dose, respectively. The model estimated a 7-fold resistance to 2,4-D relative to the susceptible population. (Table 2.2; Figure 2.4A; Figure 2.16E).

The addition of a 2-ethylhexyl group in MCPA formulation improved waterhemp susceptible and resistant population control, as observed in 2,4-D 2EHE treatment. The label rate of MCPA 2EHE (443 g ae $ha^{-1}$) provided about 63% control of the 2,4-D-WR and 50% control of the 2,4-D-WR population (Table 2.2; Figure 2.4B). Visual injury for 2,4-D-WR population exceeded almost 10 times at MCPA 2EHE rate of 443 g ae $ha^{-1}$. In another hand, the maximum MCPA 2EHE rate applied (3987 g ae $ha^{-1}$) was not adequate to control the resistant population. Plants were showing symptom but not controlled (Table 2.3; Figure 2.12A; Figure 2.16F).

MCPB is a phenoxy herbicide structurally similar with 2,4-DB, with a methyl bonded to the phenyl ring instead of chlorine. The MCPB dose predicted to achieve 90% biomass based on $ED_{90}$, and 90% of visual estimated of injury was 167,474 g ae $ha^{-1}$ and 1,388 g ae $ha^{-1}$ for the 2,4-D-WR population, respectively, and 26,843 g ae $ha^{-1}$ and 684 g ae $ha^{-1}$ for the 2,4-D-WS population, respectively (Figure 2.5A; Table 2.2; Figure 2.12B; Table 2.3). Dose-response curves based on biomass (g $plant^{-1}$) suggested 2,4-D-WR population treated with 3,600 g ae $ha^{-1}$ presented less than 50% of control (Table 2.2; Figure 2.5A). The highest dose of this treatment was not sufficient to completely provide 90% of control in both waterhemp populations (Table 2.2; Table 2.3; Figure 2.16G).

Dose-response curves based on biomass (g $plant^{-1}$) suggested that recommended dose of CMPP (600 g ae $ha^{-1}$) provided 65.5% of control in 2,4-D-WR population and 89.26% of control em 2,4-D-WS population (Table 2.2; Figure 2.5B). There was a 2-fold increase in the CMPP dose required to achieve the $I_{90}$ in the 2,4-D-WR (Table 2.3; Figure 2.13A; Figure 2.16H).

The 2,4-D-WR population was approximately 2.5-fold and 1.5-fold more resistant to CMPP-p relative to the 2,4-D-WS population, based on $ED_{90}$ and $I_{90}$, respectively.

For the resistant population, an estimated dose of 2056 g ae $ha^{-1}$ would be predicted to result in 90% biomass reduction (3.43 times over than the recommended rate) (Table 2.2; Figure 2.6A), and 1252 g ae $ha^{-1}$ to $I_{90}$ (2.1 times over than the recommended rate) (Table 2.3; Figure 2.13B; Figure 2.17A).

2,4-DP is a phenoxy herbicide similar in structure to 2,4-D, with the methylation of carboxylic acid radical in the aromatic ring. The recommended dose of 2,4-DP (600 g ae $ha^{-1}$) provided about 93% of control in 2,4-D-WR, and 95% of control in 2,4-DS population (Table 2.2; Table 2.3; Figure 2.6B, Figure 2.14A; Figure 2.17B). 2,4-DP-p is the dextro isomer active as herbicide of 2,4-DP. The recommended dose of 2,4-DP-p (600 g ae $ha^{-1}$) reached around 90.95% of control in 2,4-D-WR, and 95.6% of control in 2,4-DS population. (Table 2.2; Table 2.3; Figure 2.7A, Figure 2.14B; Figure 2.17C). The herbicide control efficacy between regular and dextro isomer formulation did not show difference (Data no showed).

2,4-DP-p 2 EHE is the active component of Corasil, commercialized in low rates as a plant growth regulator for oranges and mandarins in Australia, and also registered as an herbicide. The herbicide recommended dose 25 g ae $ha^{-1}$ provided 95% of control in 2-4-D-WR population, while 21 g ae $ha^{-1}$ provided more than 99% of control in 2-4-D-WS population based on (Table 2.2; Figure 2.7B, Figure 2.15A; Figure 2.17D).

Dicamba is a benzoic acid herbicide in auxinic herbicide group, as phenoxy herbicides. Currently is commercialized in two formulations, diglycolamine salt (DGA) and dimethylamine salt (DMA). 2,4-D-WR and 2,4-D-WS plants were treated with both dicamba formulations. Resistant population was more sensitive to dicamba DGA than the susceptible population, a dose of 112 g ae $ha^{-1}$ and 257 g ae $ha^{-1}$ was necessary to achieve 90% of control in 2,4-D-WR and 2,4-D-WS population, respectively (Table 2.2; Figure 2.8A; Figure 2.17E). Dicamba DMA formulation provided comparable control in both waterhemp populations with 32% of the recommended dose (Table 2.2; Figure 2.8B; Figure 2.17F).

The phenoxy herbicide tank mixture of 2,4-D + 2,4-DP provided the most efficient control in 2,4-D-WR, where the $ED_{90}$ and $I_{90}$ value necessary to control 2,4-D-WR was about 50% of the recommended dose (2,4-D – 227.5 g ae $ha^{-1}$ and 2,4-DP – 300 g ae $ha^{-1}$), even though 2,4-D-WR presented 2-fold more resistance to this mixture than 2,4-D-WS population (Table 2.2; Figure 2.9A; Table 2.3; Figure 2.15B; Figure 2.17G).

Accordingly, with the dose-response curves based on biomass (g $plant^{-1}$) suggested that recommended dose of 2,4-D + CMPP herbicide tank mixture (455 +

600 g ae $ha^{-1}$) provided 77.5% of control in 2,4-D-WR population, while 98% of 2,4-D-WS population was controlted with 50% of the recommended dose (Table 2.2; Figure 2.9B; Figure 2.17H).

The results of this chapter showed that 2,4-D-WR population were more sensitive to Dicamba DGA, Dicamba DMA, Corasil, 2,4-DP, and 2,4-DP-p herbicides formulations. Conversely, the resistant population survived to the higher doses of 2,4-D 2EHE, 2,4-D EE, 2,4-DB, MCPB, MCPA, MCPA 2EHE, CMPP and CMPP-p and this demonstraded that 2,4-D-WR population exhibits cross-resistance to these herbicides (Figure 2.16 to Figure 2.17). Although, survided plants suffered epinasty without strong tissue necrosis, suggesting $SCF^{TIR/AFB}$ mechanisms of resistance (CHRISTOFFOLETI et al. 2015).

Cross-resistance between two or more herbicides can be defined as the expression of a single mechanism of resistance conferring the ability to endure to herbicides from the same or different chemical classes (BECKIE; TARDIF, 2012; POWLES; PRESTON, 1995). Cross resistance can occur among phenoxy, benzoic acid and carboxylic acid classes of auxinic herbicides, as well as active ingredients from each herbicide class (BECKIE; TARDIF, 2012). A *Carduus nutans* L. and *Ranunculus acris* L. population were reported with cross-resistance to 2,4-D, MCPA and MCPB herbicides (HARRINGTON; WOOLLEY, 2006; BOURDÔT et al. 1992). Van Eerd et al. (2005) related cross-resistance in a quinclorac-resistant population of *Galium spurium* L. to phenoxy herbicide (MCPA), benzoic acid (dicamba), and pyridine carboxylic acid (picloram, fluroxypyr and triclopyr) classes of auxinic herbicides whereas Craston et al. (2001) found *Kochia scoparia* resistant to 5 phenoxy herbicides (dicamba, dichlorprop, 2,4-D, mecoprop, MCPA), and to a pyridine carboxylic acid (picloram).

In brief, informations as concerns cross-resistance to herbicides are important to conceive in what way works the mechanism of resistance in weeds, and develop strategies to reduce occurence of resistance populations.

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## GENERAL CONSIDERATIONS

In the first study, even gene flow by pollen have been reported in *Conyza canadensis*, the majority of the horseweed populations collected in pasture/rangeland surrounding crop areas showed a low frequency of glyphosate resistanc

In the second study, the 2,4-D-WR population were significantly more sensitive to Dicamba DGA, Dicamba DMA, Corasil, 2,4-DP, and 2,4-DP-p herbicides formulations, whereas survived to the higher doses of 2,4-D 2EHE, 2,4-D EE, 2,4-DB, MCPB, MCPA, MCPA 2EHE, CMPP and CMPP-p and this suggests that 2,4-D-WR population exhibits cross-resistance to these herbicides.

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