Herbicide Resistant Weeds in Missouri: Sources and Solutions

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By

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The undersigned, appointed by the dean of the Graduate School,

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A candidate for the degree of

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And hereby certify that, in their opinion it is worthy of acceptance.

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Abstract

Herbicide resistant weeds continue to invade new territories each year. Two studies were designed to identify both the spread and the current status of herbicide resistant weeds in Missouri. In 2016 and 2017, 98 separate commercially available bird feed mixes were examined for the presence of weed seed. *Amaranthus* species were present in 94 of the 98 bags of bird feed examined and reached levels as high as 6,525 seeds kg^{-1}.. Results from linear regression and *t test* analysis indicate that when proso millet, grain sorghum, and corn were present in feed mixes, *Amaranthus* seed contamination was increased. The presence of proso millet and grain sorghum also increased contamination of grass weed species while sunflower increased *A. artemisiifolia* contamination and safflower increased contamination of *Bassia scoparia*. An additional study collected seed from 112 separate horseweed populations d from infested fields throughout Missouri just prior to soybean harvest in 2015 and 2016. A discriminating dose that represented twice the recommended field use rate of glyphosate, glufosinate, 2,4-D, dicamba, and cloransulam was applied to each population in order to determine the frequency and distribution of herbicide resistances in Missouri horseweed. A population was classified as resistant if visual control 28 days after application (DAA) was less than 60%. Glyphosate resistance was confirmed in all 112 populations while cloransulam resistance was confirmed in 89 of 112, or 79% of the populations. Two populations survived the application of 2,4-D while all populations were found to be susceptible to dicamba and glufosinate. The results of this survey suggest the use of glyphosate and cloransulam for controlling horseweed in Missouri is likely to result in unsatisfactory control and dicamba, glufosinate, and 2,4-D still provide adequate control of horseweed across the state. Both

studies draw attention to the distribution and spread of herbicide resistant weed species in the United States.

Herbicide Resistant Weeds in Missouri: Sources and Solutions

I. Investigating the Distribution of Herbicide Resistant Horseweed in Missouri

A survey conducted by the Weed Science Society of America has listed horseweed (*Conyza canadensis (L.) Cronq.*) as the third most troublesome and seventh most common weed species found in the United States (VanWychen 2016). Bruce and Kells (1990) found that 150 horseweed plants m^{-2} had the potential to reduce soybean yield by 83% while Holm et al. (1997) found that severe infestations of horseweed could reduce sugar beet yield by 64% and grape production by 28%. Horseweed is a winter or summer annual with seeds that germinate in either the fall or early spring, but can successfully germinate throughout the year (Buhler and Owen 1997). Each horseweed plant is capable of producing more than 200,000 seeds that use wind as their primary dispersal mechanism (Bhowmik and Bekech 1993; Shields et al. 2006). Shields et al. (2006) found that horseweed seed movement can occur in the planetary boundary layer, and that movement of 550 km is possible during a single flight. Seed movement of this distance can have severe implications on the distribution of herbicide-resistant horseweed (Shields et al. 2006). In fact, Owen and Zelaya (2005) indicated that herbicide resistance in a weed like horseweed should be considered a worst-case scenario, due to its autogamous nature and its ability to disperse seeds over long distances. Horseweed commonly grows in cultivated and abandoned fields, right of ways, and waste areas across the United States (Bryson and DeFelice 2009). Horseweed thrives in conservation or no-tillage systems but is controlled by common tillage practices (Vencill and Banks 1994). The increased adoption of glyphosate-resistant crops has resulted in corresponding increases in no-tillage crops like soybean [*Glycine max* (L.) Merr.], encouraging weeds like horseweed to thrive (Nandula et al. 2006).

Herbicide resistance is defined as “the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type” (WSSA 1998). As mentioned, high adoption of glyphosate-resistant crops increased no-till adoption, which also increased reliance on glyphosate for the control of horseweed (Nandula et al. 2006). Glyphosate-resistant horseweed was first discovered in soybean fields in Delaware in 2000 (Vangessel 2001). Currently, glyphosate-resistant horseweed has been identified in 25 states in the U.S. (Heap 2018). This increase in the number of glyphosate-resistant horseweed populations can most likely be attributed to the continuous selection pressure with multiple glyphosate applications during the same growing season, especially during the late 1990’s and early 2000’s. During that time, glyphosate was often used preplant, as well as post-emergence once or twice in-crop (VanGessel et al. 2009). In 2008, an Indiana survey found that 58% of horseweed populations collected and screened from Indiana soybean fields were resistant to glyphosate (Davis et al. 2008). Hanson et al. (2009) also classified 62% of the horseweed populations tested from the Central Valley of California as resistant to glyphosate. Additionally, Byker et al. (2013) reported that 147 of 168 horseweed populations tested in Ontario contained at least one plant that had the ability to survive a glyphostate application.

Several studies have been conducted to understand the mechanism(s) of glyphosate resistance in horseweed. Feng et al. (2004) found that the mechanism responsible for glyphosate resistance in horseweed populations from Delaware was impaired phloem loading resulting in reduced overall translocation of glyphosate. This mechanism was also transmitted genetically in crosses between susceptible and resistant biotypes. Ge et al. (2011) also found that a horseweed population sequestered glyphosate in the vacuoles when compared to a glyphosate-susceptible population. Page et al. (2018) reported the first known target site mutation in horseweed; a proline for serine substitution at position 106.

In addition to glyphosate, horseweed has evolved resistance to several other herbicide modes of action, including photosystem II inhibitors (group 5), acetolactate synthase (ALS) inhibitors (group 2), and photosystem 1 electron diverters (group 6) (Heap 2018). Horseweed biotypes resistant to ALS-inhibiting herbicides have been documented to occur frequently in the eastern corn belt, and ALS-resistant horseweed is now found in 6 states (Davis et al. 2009; Heap 2019). While one study suggests that resistance to ALS-inhibiting herbicides is likely due to increased herbicide metabolism (Christopher et al. 1991), most resistant populations are a result of mutations of the ALS gene (Tranel and Wright 2002) and these mutations confer high levels of resistance (Zheng et al. 2011). ALS-inhibiting herbicides are commonly tank mixed with glyphosate to enhance control of horseweed in soybean (Davis et al. 2009; USDA 2012). As a result, in recent years horseweed populations that exhibit multple resistance to glyphosate and ALS-inhibiting herbicides have been discovered in Ohio, Deleware, Indiana Mississippi, Missouri, and Ontario (Heap 2019; Kruger et al. 2009).

Currently there are no documented cases of 2,4-D or dicamba resistance in horseweed (Heap 2019; Kruger et al. 2008). Because of this, these herbicides are the most widely used synthetic auxin herbicides in the United States, and are commonly used for the control of horseweed prior to corn, soybean, or cotton planting (Crespo 2011). However, the recent introduction of 2,4-D- and dicamba-resistant crops is likely to increase the selection pressure on horseweed to evolve resistance to these herbicides due to the increase in application from preplant only to both preplant and in-crop use (Kruger et al. 2008). Increasing selection pressure for 2,4-d will likely cause problems as examples of variable control of horseweed with 2,4-D have already been observed (Mickelson et al. 2004; Sexsmith 1964). Kruger et al. (2008) investigated the likelhood of 2,4-D resistance in an Indiana horseweed population and concluded that due to the large standard deviation, it was reasonable to suspect that some of the horseweed would survive a field application of 2,4-D, especially when environmental conditions are not optimal for translocation and absorption. Similarly, Crespo et al. (2013) examined the potential for dicamba resistance in several Nebraska horseweed populations and found that, while none of the populations in their study could be considered resistant, certain individuals in the study survived and produced seed after an application of 140 g/ha^{-}1 of dicamba, which could be considered a “reduced rate”. Maintaining 2,4-D and dicamba as a viable tood for horseweed management will be critical in future agronomic production systems due to its effectivness and availability as an economical option for controlling horseweed prior to planting (Kruger et al. 2008; Thompson et al. 2007).

The objective of this research is to identify the geographical distribution, response and potential frequency of horseweed resistance to glyphosate, glufosinate, 2,4-D, dicamba and cloransulam. These types of surveys of resistance prevelance have been performed across a variety of other states and regions and provide important information pertaining to the reccomendations for the control of a troublesome weed species like horseweed within a given geographical area.

Part II: Examining Commercial Bird Feed as a Source of Viable Weed Seed Contaminants

The dispersal and spread of weeds is one of the most important factors that can affect the flora present within a given geography. In plants, seed dispersal is mainly passive and seeds are transported by animals, wind, or water (Ridley 1930). Darlington (1918) identified weed species within certain areas of Michigan over time and found that the flora grew form 47 to 147 species in less than 100 years. The ways in which weed seeds have spread both into and within the United States include immigrations in ship ballasts, deliberate introductions by governmental agencies, within packing materials, and as attachments to animals and in their feces (Mack 1991). Weed seeds can also be spread great distances by waterfowl. Farmer et al. (2017) found that waterfowl have the ability to spread troublesome weeds such as Palmer amaranth as far as 2,964 km from the original source. Although, one of the most common methods of weed seed dispersal is through contaminated crop seed and machinery (Blackshaw and Rode 1991; Mack 1981; Mack 1991).

Traded grain commodities have also been documented as a pathway for weed seeds to spread to new areas (Benvenuti 2007; Michael et al. 2010; Shimono and Konuma 2008). The International Standards of Phytosanitary Measures defines grain as 'seeds intended for processing or consumption and not for planting' (IPPC 2015). Many of the weeds that reside in the field during harvest are harvested with the crop and are usually not removed due to similarities in size and shape, or due to efficient gleaning of harvest equipment (Benvenuti 2007; Michael et al. 2010). Some of the factors that contribute to weed seed contamination of grain at harvest include weather, crop versus weed height, weed maturity, and combine settings (Forcella et al. 1996; Shimono and Konuma 2008). Weed seed presence in grain can be reduced with correct combine sieve and fan adjustments; however, this tends to be easier with large seeded crops like corn and soybeans than for smaller seeded crops like cereals and millet (Wilson et al. 2016). Forcella et al. (1996) found that most weed seed in harvested corn samples were free of weed seeds, indicating they had been dispersed during harvest. On the contrary, Shimono and Konuma (2008) discovered contamination of wheat with 42 species of weed seed from 14 families.

Seed cleaning is a grain handling method that can be used to remove dockage such as stones, straw, chaff, broken grains, and contaminant seeds (Wilson et al. 2016). This process uses aspirators, screens, and gravity tables to remove debris and weed seeds from the crop based on size, shape, or weight (Wilson et al. 2016). However, even when seed cleaning is implemented, many of these species have adapted to imitate crop seed characteristics and are easily overlooked (Benvenuti 2007). Crops such as wheat, rice, pulses, soybean, canola, sunflower,and flax are primarily used for human food products, while barley, oats, and sorghum are mainly used for livestock feed, and white millet grain is used for bird feed (Small 1999). AERC 2008; ANAC 2012). However, these grains are generally multi-purpose and there is crossover into other food sectors. Compared with grain used for human consumption, grain used for animal feed may not be cleaned as extensively. While this is implemented to reduce costs, it is concerning as animal feeds may contain weed seeds that could spread to other areas (Blackshaw et al. 2006;

Kurokawa 2001). It is also documented that millet, sunflower, and sorghum used in bird feed are unlikely to receive any processing at all, leaving this type of feed most susceptible to weed seed contamination (Wilson et al. 2016).

Many urban and suburban landownders use feeders around their homes to attract avian widlife (Henke et al. 2001). A survey performed by the United States Fish and Wildlife Service (2011) determined that 52.8 million homeowners have at least one bird feeder and the total annual expense for commercially purchased bird feed is 4 billion dollars (U.S. Department of the Interior 2011). Based on these statistics it can be estimated that 289,380 metric tons of bird feed are distributed annually in the United States (Henke et al. 2001). Although the intentions of bird feeding may be good, it may have unintended consequences. Hanson and Mason (1985) found that bird feed is often involved in the spread of alien weed species and discovered 438 different weed species present in bird seed mixes. Bird feed was also identified as an important route of the introduction of common ragweed (*Ambrosia artemisiifolia L.*) into England (Vitalos and Karrer 2008). This study reported levels as high as 531 ragweed seeds/kg of bird feed. In 2005, a Switzerland mandate required monitoring and control of bird feed for the presence of common ragweed seed. The results of their screening reported 56%, 57%, 39%, 50%, and 22% of samples were contaminated with common ragweed in 2005 through 2009 respectively. The highest levels found were 303 seeds/kg of feed mix (Frick et al. 2011).

The objectives of this research are to determine the abundance and viability of weed seed in commercially-available bird feed collected from eight states in he United

States. Additionally, weed seed contaminants will be tested to determine the likelihood of glyphosate resistance.

Summary and Objectives

Herbicide resistance in weed species is becoming increasingly problematic in agronomic production systems in the United States. The introduction of glyphosate-resistant crops increased the use of glyphosate and placed heavy selection pressure on common agronomic weeds to evolve resistance to that herbicide. Those weeds are continuing to evolve resistance to other herbicides now being used for the control of glyphosate-resistant species. In order for proper herbicide recommendations to be made, knowledge on the distribution and frequency of herbicide resistance in the target weed is important. Therefore the first objective of this study is to identify the frequency and distribution of herbicide resistance to 5 commonly used herbicides in Missouri horseweed populations.. As herbicide resistance continues to spread, identifying routes by which herbicide-resistant weeds can spread is important. The second objective of this research will include an evaluation of commercial bird feed mixes for the presence of weed seed contaminants. This research will also seek to identify geographic and compositional factors that could result in a increase or decrease in the quantity of weed seeds present in bird feed.

Literature Cited

Benvenuti S (2007) Weed seed movement and dispersal strategies in the agricultural environment. Weed Biology and Management 7:141-157

Bhowmik PC, Bekech MM (1993) Horseweed (*Conyza canadensis*) seed production, emergence, and distribution in no-tillage and conventional-tillage corn (*Zea mays*). Agron. Trends Agric. Sci 1:67-71

Blackshaw RE, O'DONOVAN JT, Harker K, Clayton GW, Stougaard RN (2006) Reduced herbicide doses in field crops: a review. Weed Biology and Management 6:10-17

Blackshaw RE, Rode LM (1991) Effect of ensiling and rumen digestion by cattle on weed seed viability. Weed Science 39:104-108

Bruce JA, Kells JJ (1990) Horseweed (*Conyza canadensis*) control in no-tillage soybeans (*Glycine max*) with preplant and preemergence herbicides. Weed Technology 4:642-647

Bryson CT, DeFelice MS (2009) Weeds of the South: University of Georgia Press

Buhler DD, Owen MD (1997) Emergence and Survival of Horseweed (*Conyza canadensis*). Weed Science 45:98-101

Christopher JT, Powles SB, Liljegren DR, Holtum JA (1991) Cross-resistance to herbicides in annual ryegrass (Lolium rigidum) II. chlorsulfuron resistance involves a wheat-like detoxification system. Plant Physiology 95:1036-1043

Crespo R, Bernards M, Kruger G, Lee D, Robert JW (2013) Response of Nebraska Horseweed (Conyza canadensis) Populations to Dicamba. Pp. 158-164

Crespo RJ (2011) Herbicide-resistant risk assessment: response of common Nebraska weeds to dicamba dose.

Darlington HT (1918) Weed immigration into Michigan: Michigan Academy of Science

Davis VM, Gibson KD, Johnson WG (2008) A field survey to determine distribution and frequency of glyphosate-resistant horseweed (*Conyza canadensis*) in Indiana. Weed Technology 22:331-338

Davis VM, Kruger GR, Stachler JM, Loux MM, Johnson WG (2009) Growth and seed production of horseweed (Conyza canadensis) populations resistant to glyphosate, ALS-inhibiting, and multiple (glyphosate+ ALS-inhibiting) herbicides. Weed science 57:494-504

Farmer JA, Webb EB, Pierce RA, Bradley KW (2017) Evaluating the potential for weed seed dispersal based on waterfowl consumption and seed viability. Pest management science 73:2592-2603

Feng PC, Tran M, Chiu T, Sammons RD, Heck GR, CaJacob CA (2004) Investigations into glyphosate-resistant horseweed (Conyza canadensis): retention, uptake, translocation, and metabolism. Weed Science 52:498-505

Forcella F, Peterson DH, Barbour JC (1996) Timing and measurement of weed seed shed in corn (Zea mays). Weed Technology 10:535-543

Frick G, Boschung H, Schulz-Schroeder G, Russ G, Ujčič-Vrhovnik I, Jakovac-Strajn B, Angetter D, John I, Jørgensen JS (2011) Ragweed (*Ambrosia* sp.) seeds in bird feed. Biotechnologie, Agronomie, Société et Environnement 15:39-44

Ge X, d'Avignon DA, Ackerman JJ, Duncan B, Spaur MB, Sammons RD (2011) Glyphosate-resistant horseweed made sensitive to glyphosate: low-temperature suppression of glyphosate vacuolar sequestration revealed by 31P NMR. Pest management science 67:1215-1221

Hanson BD, Shrestha A, Shaner DL (2009) Distribution of glyphosate-resistant horseweed (*Conyza canadensis*) and relationship to cropping systems in the Central Valley of California. Weed Science 57:48-53

Hanson C, Mason J (1985) Bird seed aliens in Britain. Watsonia 15:237-252

Heap (2019) The International Survey of Herbicide Resistant Weeds. weedscience.org. Accessed March 27 2019

Henke SE, Gallardo VC, Martinez B, Bailey R (2001) Survey of aflatoxin concentrations in wild bird seed purchased in Texas. Journal of Wildlife Diseases 37:831-835

Kruger GR, Davis VM, Weller SC, Johnson WG (2008) Response and survival of rosette-stage horseweed (*Conyza canadensis*) after exposure to 2, 4-D. Weed Science 56:748-752

Kruger GR, Davis VM, Weller SC, Stachler J, Loux M, Johnson WG (2009) Frequency, distribution, and characterization of horseweed (*Conyza canadensis*) biotypes with resistance to glyphosate and ALS-inhibiting herbicides. Weed Science 57:652-659

Kurokawa S (2001) Invasion of exotic weed seeds into Japan, mixed in imported feed grains. Extension Bulletin-Food & Fertilizer Technology Center

Mack RN (1981) Invasion of Bromus tectorum L. into western North America: an ecological chronicle. Agro-ecosystems 7:145-165

Mack RN (1991) The Commercial Seed Trade: An Early Disperser of Weeds in the United States. Economic Botany 45:257-273

Michael PJ, Owen MJ, Powles SB (2010) Herbicide-resistant weed seeds contaminate grain sown in the Western Australian grainbelt. Weed Science 58:466-472

Mickelson JA, Bussan AJ, Davis ES, Hulting AG, Dyer WE (2004) Postharvest kochia (Kochia scoparia) management with herbicides in small grains. Weed technology 18:426-431

Nandula VK, Eubank TW, Poston DH, Koger CH, Reddy KN (2006) Factors affecting germination of horseweed (Conyza canadensis). Weed Science 54:898-902

Owen MD, Zelaya IA (2005) Herbicide-resistant crops and weed resistance to herbicides. Pest Management Science 61:301-311

Page ER, Grainger CM, Laforest M, Nurse RE, Rajcan I, Bae J, Tardif FJ (2018) Target and Non–target site Mechanisms Confer Resistance to Glyphosate in Canadian Accessions of Conyza canadensis. Weed Science 66:234-245

Ridley H (1930) The dispersal of plants throughout the world. The Dispersal of Plants throughout the World.

Sexsmith J (1964) Morphological and herbicide susceptibility differences among strains of hoary cress. Weeds:19-22

Shields EJ, Dauer JT, VanGessel MJ, Neumann G (2006) Horseweed (Conyza canadensis) seed collected in the planetary boundary layer. Weed Science 54:1063-1067

Shimono Y, Konuma A (2008) Effects of human-mediated processes on weed species composition in internationally traded grain commodities. Weed Research 48:10-18

Small E (1999) New crops for Canadian agriculture. Perspectives on new crops and new uses. ASHS Press, Alexandria, VA. USA:15-52

Thompson MA, Steckel LE, Ellis AT, Mueller TC (2007) Soybean tolerance to early preplant applications of 2, 4-D ester, 2, 4-D amine, and dicamba. Weed Technology 21:882-885

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Science 50:700-712

U.S. Department of the Interior USFaWS, U.S. Department of Commerce, U.S. Census Bureau (2011) National Survey of Fishing, Hunting, and Wildlife-Associated Recreation

USDA (2012) National Agricultural Statistics Service. www.pestmanagement.info/nass/apppuseage.cfm.

VanGessel MJ, Scott BA, Johnson QR, White-Hansen SE (2009) Influence of Glyphosate-Resistant Horseweed (Conyza Canadensis) Growth Stage on Response to Glyphosate Applications. Weed Technology 23:49-53

VanWychen L (2016) 2015 Baseline Survey of the Most Common and Troublesome Weeds in the United states and Canada: Weed Science Society of America. http://wssa.net/wp-content/uploads/2015-Weed-Survey_Baseline.xlsx

Vencill WK, Banks PA (1994) Effects of tillage systems and weed management on weed populations in grain sorghum (*Sorghum bicolor*). Weed Science 42:541-547

Vitalos M, Karrer G (2008) Distribution of *Ambrosia artemisiifolia* L.-is birdseed a relevant vector. Journal of Plant Diseases and Protection 21:345-348

Wilson CE, Castro KL, Thurston GB, Sissons A (2016) Pathway risk analysis of weed seeds in imported grain: A Canadian perspective. NeoBiota 30:49-74

WSSA (1998) Herbicide Resistance and Herbicide Tolerance Definitions. Weed Technology 12:789

Zheng D, Kruger GR, Singh S, Davis VM, Tranel PJ, Weller SC, Johnson WG (2011) Cross-resistance of horseweed (Conyza canadensis) populations with three different ALS mutations. Pest management science 67:1486-1492

Examination of Commercially-Available Bird Feed for Weed Seed Contaminants

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Abstract

In 2016 and 2017, 98 separate commercially available bird feed mixes were examined for the presence of weed seed. All weed seed contaminants were counted and identified by species. *Amaranthus* species were present in 94 of the 98 bags of bird feed examined and reached levels as high as 6,525 seeds kg^{-1}. *Amaranthus* species present in bird feed mixes included *Amaranthus tuberculatus* (Moq.), *Amaranthus retroflexus* (L.), *Amaranthus palmeri* (S. Wats), *Amaranthus hybridus* (*L*.), and *Amaranthus albus* (L.). *Amaranthus palmeri* was present in 27 of the 98 mixes. Seed of *Ambrosia artemisiifolia* (L.), *Bassia scoparia* (L.) *Sorghum bicolor* (L.), *Fallopia convolvulus* (L.), *Chenopodium album* (L.), *Digitaria sanguinalis,* and *Setaria* species were also present in bird feed mixes. A greenhouse assay to determine *Amaranthus* species seed viability and resistance to glyphosate revealed that approximately 19% of *Amaranthus* seed in bird feed mixes remain viable, and five mixes contained *A. tuberculatus and A. palmeri* seed that were resistant to glyphosate. Results from linear regression and *t test* analysis indicate that when proso millet, grain sorghum, and corn were present in feed mixes, *Amaranthus* seed contamination was increased. The presence of proso millet and grain sorghum also increased contamination of grass weed species while sunflower increased *A. artemisiifolia* contamination and safflower increased contamination of *Bassia scoparia.*

Introduction:

A survey conducted by the United States Fish and Wildlife Service (USFWS) in 2016 reported that 56.8 million homeowners own a bird feeder as an attractant for avian wildlife. Henke et al. (2001) also estimated that 289,000 metric tons of bird feed were distributed across the United States in 1999. However, this number is likely even higher today as the number of people that feed birds around their home has increased by 8.8 million in the most recent USFWS survey (U.S. Department of the Interior et al. 2016). While homeowners may have good intentions with bird feeding, this popular hobby may have unintended consequences. For decades, bird feed has been examined as a source for weed seed introduction into new areas (Chauvel et al. 2004; Frick et al. 2011; Hanson and Mason 1985; Vitalos and Karrer 2008). Hanson and Mason (1985) surveyed bird feed mixes in Britain and reported on 438 weed species that they believed to have been introduced through the bird feed industry. Bird feed was also identified as a vector in the introduction of common ragweed *(Ambrosia artemisiifolia* L.*)* into England at levels as high as 531 seeds kg^{-1} mix (Vitalos and Karrer 2008). (Brandes and Nitzsche 2006) also reported that bird feed was the main source for the establishment of *A. artemisiifolia* in Germany. Additionally, a Swiss study found that 22 to 57% of samples screened from 2005 through 2009 were contaminated with *A. artemisiifolia*, and that contamination reached as high as 303 seeds kg^{-1} of bird feed mix (Frick et al. 2011). Chauvel et al. (2004) found a correlation between the presence of *A. artemisiifolia* in bird feed mixes when sunflower (*Helianthus annuus* L.) was used as an ingredient while Wilson et al. (2016) reported that any millet (*Panicum miliaceum* L.), sunflower, and grain sorghum [*Sorghum bicolor (*L) Moench *ssp. Arundinaceum* (Desv.) de Wet & Harlan] used in bird feed is unlikely to undergo any processing to remove weed seed or alter its ability to germinate. The amount of weed seed that is present in grain after harvest can depend on a multitude of factors including end of season weed control, and combine sieve and fan adjustments (Clay et al. 2009; Davis 2008). Wilson et al. (2016) indicated that separating weeds from crops will likely be more difficult in small cereal grains, flax, and proso millet than in larger seeded crops such as corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.].

In Australia, the Queensland Agricultural Merchants have developed standards for bird feed that require all bird feed to be examined for weed seed contamination; they established that any presence of noxious seeds or weed seeds in amounts that exceed the tolerance levels are rejected (QAM 1998). In Switzerland, screening of bird feed mixes also resulted in regulations against *A. artemisiifolia* contamination, with a 10 seeds kg^{-1} threshold being established for bird feed producers. Additional members of the European Union (Germany, Denmark, and Slovenia) followed suit in the implementation of this threshold, and noticeable reduction of contaminated seed mixtures occurred in just 2 years (Frick et al. 2011). In the United States, however, the Federal Seed Act enforced by the Department of Agriculture regulates agricultural seeds, which are defined as grass, forage, and field crop seeds which the Secretary of Agriculture finds useful for seeding purposes (United States Department of Agriculture 1940). By definition, bird feed is not covered in the Federal Seed Act, and bird feed manufacturers are not required to reveal seed composition percentages such as the percentage of weed seed contamination, including noxious species. The Federal Drug Administration Center for Veterinary Medicine is the primary regulator of animal feed in the United States, but imposes regulations that are primarily directed towards contamination of pesticides or harmful foreign material such as metal shavings. Therefore, these regulations do not address weed seed contamination of bird feed. Additional guidelines were set forth by the Wild Bird Feed Industry in the Wild Bird Feed Industry Standards, which were adopted in 2004 and set guidelines for bird feed to be of a consistent quality (WBFI 2004). However, these standards also make no mention of weed seed contamination thresholds for bird feed distributed within the United States and Canada.

Palmer amaranth (*Amaranthus palmeri* S. Wats) has been documented as the most troublesome weed species present in agroecosystems today (VanWychen 2016). *A. palmeri* was historically a weed of the southwestern United States and Mexico, however, over time it has moved into more northern and eastern geographies (Heap 2019; Webster and Nichols 2012). The successful establishment of *A. palmeri* into new areas can be attributed to many factors, including its prolific seed production, highly competitive nature, and distinct ability to evolve resistance to herbicides (Legleiter and Johnson 2013). Human-mediated activities during the 20th and 21st century such as movement of machinery contaminated with seed, animal feed and manure, as well as contaminated pollinator planting seed mixes are largely to blame for the spread of this troublesome weed species across the country (Chahal et al. 2015). However, Farmer et al. (2017) reported that waterfowl were capable of spreading *A. palmeri* long distances, and other studies have found *Amaranth* species seeds in water runoff, so natural dissemination is possible as well (Wilson 1980). Human-mediated dispersal will often result in a more rapid dispersal of a new species into a geography than natural introduction due to multiple introductions occurring across large areas simultaneously (Taylor et al. 2012).

No previous research has examined the potential for weed seed contamination of bird feed mixes that are commercially available in the United States, or more specifically focused on the possibility of *A. palmeri* contamination in these mixes. Therefore, the objectives of this research were to: 1) identify contamination levels of weed species in commercially available bird feed in the United States, 2) to determine the viability and glyphosate-resistance status of any *Amaranthus* seed present in commercial bird feed mixes, and 3) to determine the effects of ingredient composition and location of purchase on the presence and abundance of weed species in bird feed mixes.

Materials and Methods:

Bird Feed Collection

Bird feed mixes were purchased from a variety of common retail locations in Missouri, Kentucky, Tennessee, Arkansas, Illinois, Virginia, and North Carolina in 2016 and 2017 (Table 2.1). Mixes were selected at random from these retail locations similar to Henke et al. (2001). The majority of bird feed mixes were purchased in Columbia, Missouri (n=62). Mixes ranged in size from 1 to 9 kg and ranged from single ingredient mixes to combinations of multiple ingredient feed mixtures (Table 2.1).

Bird Feed Screening

In all cases, the entire bag was examined to be certain all weed seed contaminants were extracted from the mix. All bird feed mixes were poured through a series of sieves to separate seeds by size for a more accurate assessment of contaminants. Large seeded ingredients like sunflower and safflower (*Carthamus tinctorius* L.) were initially separated with a 10 mm^2 sieve followed by the separation of medium-sized seeds like grain sorghum, proso millet, cracked corn, wheat (*Triticum araraticum* L.), and nyjer thistle [*Guizotia abyssinica* (L. f.)] with a 5 mm^2 sieve. All remaining ingredients were passed through a 1 mm^2 sieve which allowed primarily for the passage of smaller-sized weed seeds like the *Amaranthus* species. Lastly, remaining seeds and residue were placed in a 0.5 mm^2 sieve which allowed for the removal of dust and powder residues from larger seeds that could interfere with *Amaranthus* seed detection. Each stage of seed separation was examined for weed seed contaminants, which were removed for further identification. For this experiment, all seeds that were not listed as an ingredient of the mix were considered weed seed. Bird feed ingredients commonly used in bird feed that were included in the analysis include sunflower, proso millet, grain sorghum, safflower, wheat, Nyjer thistle and annual canarygrass (*Phalaris canariensis* L.). Certain mixes contained dried fruits and hulled nuts, which were categorized as processed ingredients due to a significantly higher level of handling prior to incorporation into the final commercial bird feed mix.

Amaranthus Identification and Resistance Screening

For all weed species except the *Amaranthus* species, identification was possible without the necessity of seed germination. All *Amaranthus* species seeds collected from bird feed mixes were broadcast in 54- by 27- by 6- cm greenhouse flats (Hummert International; Earth City Missouri) containing a commercial potting medium (Pro-Mix BX; Premier Tech Horticulture, Quakertown, PA) and were maintained in a greenhouse at 30 degrees C. Natural light was supplemented with metal-halide lamps (600 µmol photon m^{-2} s^{-1}) providing a 14 hour photoperiod and flats were watered as needed. Approximately 14 days after planting, a germination percentage was recorded to ensure any plants that did not survive until identification were accounted for. When *Amaranthus* species reached 5 cm in height, they were identified by species and transplanted into individual 10- by 10- cm diameter pots with a 1:1 ratio of the same commercial potting medium and field topsoil. Once plants reached 10 cm in height, a discriminating dose of 3.3 kg ha^{-1} of glyphosate (Roundup PowerMax®, 540 g ai L^{-1}, Monsanto; St. Louis, Missouri) was applied to all plants using a CO_2-pressurized backpack sprayer applying 140 L ha^{-1} water volume at 144 kPa with a XR 8002 flat fan nozzle (TeeJet®, Spraying Systems Co., PO Box 7900, Wheaton, IL 60187). Visual injury was estimated 21 days after application on a 0 to 100% scale with 0 indicating no phytotoxic effects present and 100% indicating complete plant death. If any mixes contained any plants that survived this application of glyphosate, that mix was marked as containing glyphosate resistant seed. Survival was determined visually in a subjective evaluation of each plants ability to survive and reproduce following the application of glyphosate.

Statistical Analysis

Results were compiled from 98 bird feed mixes from 22 brands and seven states collected throughout 2016 and 2017. A linear regression (PROC REG; SAS® 9.4 Institute Inc., Cary, NC, USA) was performed to determine what assortment of ingredients fit the model to predict weed seed contamination. Feed mix ingredients were used as predictor variables to determine relationships between ingredients and weed species abundance. The response variables were quantity of weed seeds detected. These analyses were performed on all weed species found in feed mixes, and weed species with significant regression models were analyzed further. Of the 29 weed species extracted from bird feed mixes, a significant model was developed for *Amaranthus* species, grass weed species, *A. artemisiifolia*, and kochia [*Bassia scoparia* (L.) A. J. Scott]. For each model, an equation could be developed to predict the abundance of each of these four weed species based on what ingredients are present in the feed mix. An example equation for each weed species is represented by: [$y = x(ingredient) + Intercept$], wherein y = weed species x=parameter estimates for seed abundance, and $(ingredient)$=1 if present and =0 if absent from feed mix. When ingredient parameter estimates were significant, the prediction of the increase or decrease in overall weed seed abundance from that ingredient is considered significantly different from zero. However, to predict contamination levels, all factors must be included in the equation regardless of significance. *B. scoparia* and *A. artemisiifolia* datasets included a large number of zeros as they were a less common ingredient found in feed mixes than *Amaranthus* and grass weed species. The datasets could not be normalized through the use of data transformations, so to support linear regression an additional binomial logistic regression was conducted for each weed species. The binomial logistic regression predicts only the probability that a response variable is grouped into one of two categories, which in this case was weed seed presence or absence. The binomial logistic regression also does not require the assumption that data is normally distributed, so significance is not impacted by outliers.

Certain bird feed ingredients are more commonly used in feed mixes than others. For example, proso millet was used in 60 mixes, and grain sorghum was used in 38 mixes. However, grain sorghum was only present in 3 feed mixes in which proso millet was not. Because of this, it could be possible for a more common ingredient such as proso millet to conceal the true weed seed contribution of an ingredient like grain sorghum when all ingredients are included in the model. Therefore, an additional analysis was performed using independent sample *t- tests* to evaluate how individual ingredients affect weed seed quantity found in bird feed mixes (PROC TTEST; SAS®). Variance equality was assessed using the folded *F* method and for instances when variances were unequal, the Satterthwaite method was used to calculate *t* values (Satterthwaite 1946). The Satterthwaite method is appropriate when variances of two groups are unequal.

Finally, differences in *Amaranthus* species abundance from feed mixes purchased from different states were tested through a linear mixed-effects model utilizing the PROC GLIMMIX procedure (SAS® 9.4 Institute Inc., Cary, NC, USA).

Results

Bird Feed Screening

There was not a significant effect of the state from which bird feed mixes were purchased (P=0.98), therefore mixes from all locations were combined for analysis. From the 98 bird feed mixes evaluated in this research, 29 different species of weeds were identified (Table 2.2). The most frequently identified weed species were *Amaranthus* species that were found in levels as high as 6,525 seeds kg^{-1} of feed mix and were found in 94 of 98, or 96%, of all bird feed mixes screened. Collectively, grass weed species were the second most abundant weed seeds found in the bird feed mixes, and these consisted of foxtail species (*Setaria faberi* Herrm.), [*Setaria pumila* (Poir.) Roem. & Schult.], [*Setaria viridis* (L.) P. Beauv.], large crabgrass [*Digitaria sanguinalis* (L.) *Scop*]., barnyardgrass [*Echinochloa crus-galli* (L.) *P*. Beauv.], shattercane [*Sorghum bicolor* (L.) Moench *ssp. verticilliflorum* (Steud.) de Wet ex Wiersema & J. Dahlb.], johnsongrass [*Sorghum halepense* (L.) Pers.], and longspine sandbur [*Cenchrus longispinus* (Hack.) Fernald]. Grass weed species were found in 76% of the bird feed mixes at levels ranging from 0 to 3,896 seeds kg^{-1} of feed mix. The third most frequently identified species found in bird feed mixes *was A. artemisiifolia*. Seed of this species was found in 43% of feed mixes and was found at levels as high as 296 seeds kg^{-1} of feed mix. In a similar study, Vitalos and Karrer (2008) found *A. artemisiifolia* seeds in 37% of bird feed mixes screened at levels as high as 531 seeds kg^{-1}. The next most common and abundant weed screened in our study was wild buckwheat (*Fallopia convolvulus* (L.). *F. convolvulus* was found in 30% of mixes and reached levels of 56 seeds kg^{-1} of feed mix. Additional weeds that were found in bird feed mixes that have relevance as troublesome species (VanWychen 2016) include *B. scoparia*, morningglory species (*Ipomoea* spp.), common lambsquarters (*Chenopodium album* L.) and velvetleaf (*Abutilon theophrasti* Medik.) which were found in 13%, 17%, 10% and 13% of mixes, respectively. Hanson and Mason (1985) also found each of these same weed species in a bird feed screening conducted in Great Britain, however they did not report on the exact quantities present in each mix.

Amaranthus Species Identification and Resistance Screening

Amaranthus species germination ranged from 0 to 78% with an average viability of 19% across all seeds planted (Table 2.2). Five different *Amaranthus* species were identified. These included redroot pigweed (*Amaranthus retroflexus* L.), common waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer], smooth pigweed *(Amaranthus hybridus* L.), A. *palmeri*, and tumble pigweed (*Amaranthus albus* L.). We were unable to identify *Amaranthus* seed at the species level when seed present in a mix was not viable.

Of the 94 seed mixes that contained *Amaranthus* seed, 71% contained viable seed. *A. retroflexus* was the *Amaranthus* species that was most common in seed mixes (50%), however only 16 mixes contained *Amaranthus* seed of only one species. Bird feed mixes are most often comprised of seed collected from more than one field and often from multiple crop species, so weed seed contamination was shown to vary greatly even within the same mix. *A. albus* was the second most common *Amaranthus* species identified (34%), followed by *A. palmeri* (28%) and *A. tuberculatus* (23%). The least common *Amaranthus* species found was *A. hybridus* which was present in only 4% of mixes screened. These results are consistent with previous research that reported *A. retroflexus* as the most common *Amaranthus* species present in a Canadian grain sampling program that took place from 2007 through 2015 (Wilson et al. 2016). While *A. retroflexus* and *A. albus* were the two most common *Amaranthus* species identified in these experiments, neither of these species exhibited resistance to glyphosate in any of the bird feed mixes tested. All *A. hybridus* plants were also controlled by the discriminating dose of glyphosate and were not deemed resistant. To date, there are no known cases of glyphosate resistance in *A. retroflexus* or *A. albus*, and only three known cases of glyphosate resistance in *A. hybridus* in Argentina, therefore these results seem consistent with the status of glyphosate resistance in these species in the United States (Heap 2019). However, of the 26 bird feed mixes that contained viable A. *palmeri* seed, four contained glyphosate-resistant *A. palmeri* plants. Similarly, of the 23 bird feed mixes that contained viable *A. tuberculatus* seed, three contained glyphosate-resistant plants. An additional two mixes contained both *A. palmeri* and *A. tuberculatus* seed that were resistant to glyphosate. It is important to note that in two of the three mixes that contained glyphosate-resistant *A. tuberculatus* and in all four mixes that contained glyphosate-resistant *A. palmeri*, all plants screened were found to be resistant. This segregation in resistance suggests that in some cases, the *Amaranthus* species that are present in a bird feed mix could be originating from one source. To date, glyphosate-resistant *A. palmeri* has been documented in 26 states in the U.S., as well as Argentina and Brazil while glyphosate-resistant *A. tuberculatus* occurs in 18 states in the U.S. and also in Canada (Heap 2019). These results not only demonstrate another possible avenue for the spread of *Amaranthus* species, but also another route for the spread of glyphosate-resistant *Amaranthus* species throughout the United States.

Prediction of Amaranthus seed contamination

The equation [$Amaranthus\ \mathrm{seed} = 66.1(\mathrm{proso\ millet}) + 2.9(\mathrm{grain\ sorghum}) + 1.4(\mathrm{corn}) + 2.3(\mathrm{sunflower}) - 1.5(\mathrm{safflower}) + 1.1(\mathrm{wheat}) - 7.5(\mathrm{nyjer\ thistle}) - 4.8(\mathrm{processed}) + 4.0(\mathrm{canarygrass}) + 977.2$] (Table 2.4) best predicted the likelihood of *Amaranthus* contamination (P=<0.0001). Additionally, from the *t test* analysis (Figure 1), it was determined that when proso millet, grain sorghum, and corn were present in seed mixes, there was an overall increase in *Amaranthus* seed presence. While the results from the *t test* analysis suggests several ingredients could potentially increase *Amaranthus* seed contamination, proso millet is the only ingredient that demonstrated a significant effect in both analyses. *Amaranthus* seed size varies from 0.32 to 0.63 mm^2 (Farmer et al. 2017) and since proso millet is a small-seeded crop, mechanical separation will be especially difficult (Duary 2014; Wilson et al. 2016). Additionally, proso millet that is used for bird feed is unlikely to undergo any additional processing or cleaning to reduce weed seed contamination (Wilson et al. 2016). Corn and grain sorghum also increased the contamination of *Amaranth* seeds in the *t test* analysis (Figure 1). These results are in agreement with previous research in that grain sorghum was found to be a major source of weed seed contamination, including contamination of *Amaranthus* species, in Japanese feed imports (Kurokawa 2001). Another study found *Amaranthus* species were one of six species that were consistently present at harvest time in Illinois corn fields (Davis 2008). In contrast, when processed ingredients were present in the feed mix, there was a decrease in *Amaranthus* seed contamination (Figure 1). The decrease in contamination as a result of the presence of processed ingredients is likely explained by the reduction in weed seed that will inevitably occur when grain products are subject to processing practices such as milling, shelling, or seed cleaning (Hoseney 1994). Additionally, it is expected that processed ingredients such as raisins and nuts would be free of A*maranthus* seed contaminants due to the differences in harvesting methods and processing elements in comparison with raw agronomic grain. These results indicate that contamination of *Amaranthus* species in bird feed mixes could be originating from proso millet, grain sorghum, and corn and that further processing of these feed ingredients to remove these seeds may have the potential to reduce *Amaranthus* seed contamination.

Prediction of grass weed species seed contamination

The equation [grass weed species seed = 9.84(proso millet) + 2.67(grain sorghum) - 2.81(corn) – 1.20(sunflower) – 2.81(safflower) + 630.95(wheat) + 3.75(nyjer thistle) – 10.47(processed) + 3.16(canarygrass) + 358.3] (*P*=<0.0001) best predicted contamination of grass weed species (Table 2.5). The *t test* analysis demonstrated a significant increase in grass weed seeds when wheat, grain sorghum and proso millet were present in the mix (Figure 2.1). Shimono and Konuma (2008) found similar results with *Poaceae* species appearing the most often in wheat grain samples. Historically, the control of grass weeds in monocotyledonous crops like these has proven difficult due to the limited availability of selective herbicides used for grass control and lack of herbicide-resistant cultivars. Shimono and Konuma (2008) found that in-field abundance of weeds and weed height were two factors that correlated to the number of weed seeds that contaminated wheat. Processed ingredients decreased weed seed in the model (p=.0470) as well as in the *t test* analysis (p=.0027), similar to results with the *Amaranthus* species. Grass weed seed was also lower when safflower was in the mix. The relatively large seed size of safflower would allow for more effective mechanical separation of the desired crop and weed seed by harvesting equipment. Additionally, previous research has shown that the cyclohexanedione and aryloxyphenoxypropionate (WSSA Group 1) herbicides are highly effective in controlling grass weed species in safflower production (Blackshaw et al. 1990). These results suggest that the monocotyledonous crop species commonly found in bird feed mixes like wheat, grain sorghum, and proso millet are primary contributors to grass weed seed contamination in feed mixes. Therefore, bird feeders placed directly in homeowner yards could be responsible for the introduction of weeds such as *D. sanguinalis* and *S. viridis*. It is also worth noting that the amount of glyphosate- and multiple-resistant grass weed species continues to increase (Heap 2019). To reduce the amount of grass weed seed transported in bird feed, mixes that incorporate processed ingredients or safflower should be promoted.

Prediction of A. artemisiifolia contamination

The model [$A.\ artemisiifolia = -3.97(\text{proso millet}) - 3.90(\text{grain sorghum}) - 11.48(\text{corn}) + 19.95(\text{sunflower}) + 14.45\ (\text{safflower}) + 3.32(\text{wheat}) - 1.12(\text{nyjer thistle}) - 3.14(\text{processed}) + 3.55(\text{canarygrass}) + 25.15$] ($P$=0.0017) best predicted contamination of *A. artemisiifolia*. The logistic regression supported these results for all species except safflower (Table 2.6). Safflower was also not found to decrease *A. artemisiifolia* in the *t test* analysis, suggesting that the linear regression may have overestimated its contribution to *A. artemisiifolia* contamination due to large variance in the data set for this species. Corn decreased *A. artemisiifolia* levels in all analyses. This can likely be explained by the variety of corn herbicides that are effective in controlling *A. artemisiifolia* as well as the ability of most harvesting machines to mechanically separate corn grain from *A. artemisiifolia* seeds (Heap 2019; Wilson et al. 2016). Sunflower increased contamination in both regression analyses but was not a factor in the *t test* analysis *(P=0.0769)*. Many other studies have found sunflower to be an important factor in *A. artemisiifolia* contamination. Vitalos and Karrer (2008) found that all samples that were contaminated with *A. artemisiifolia* seed contained sunflower. They also found the highest levels of *A. artemisiifolia* seed (531 seeds kg^{-1}) in bird feed mixes that contained only sunflower as an ingredient. Bohren et al. (2006) also found that *A. artemisiifolia* was commonly found in imported sunflower and deemed it nearly impossible to separate *A. artemisiifolia* from the desired crop. Brandes and Nitzsche (2006) also proposed that sunflower should routinely be checked for *A. artemisiifolia* contamination, and also proposed a certified *Ambrosia*-free bird feed classification. *A. artemisiifolia* has been found resistant to four classes of herbicides in the United States (WSSA Groups 2, 5, 9, and 14) (Heap 2019) and the presence of this seed in feed mixes could provide a route for herbicide resistant *A. artemisiifolia* seed to spread into new geographies.

Prediction of B. scoparia contamination

B. scoparia was found in bird feed mixes at quantities much lower than any of the other weed species discussed, but is also an economically-important weed on WSSA's list of top ten most troublesome weeds in the United States (VanWychen 2016). The model [$B.\ scoparia = -1.04(\text{proso millet}) + 2.18(\text{grain sorghum}) - 1.59(\text{corn}) - 4.79(\text{sunflower}) + 3.80(\text{safflower}) - 1.05(\text{wheat}) - 2.23(\text{nyjer thistle}) - 1.36(\text{processed}) - 1.34(\text{canarygrass}) + 1.23$] (*P=0.0203*) best predicted *B. scoparia* contamination in feed mixes (Table 2.7). Factors in the model of the linear regression align with results from the logistic regression suggesting the linear regression was not affected by the non-normalized data set for this species (Table 2.8). The *t test* analysis found canarygrass and nyjer thistle to reduce *B. scoparia* contamination. The control of *B. scoparia* in canarygrass production would likely be achievable with WSSA group 4 herbicides, reducing any seeds present at harvest. The reduction observed with nyjer thistle is likely due to the fact that this species is often harvested by hand and not by mechanical means, which could allow for manual separation of *B. scoparia* from nyjer thistle plants (Duke 1983). Safflower increased contamination in both analyses. Several previous studies have noted the problematic nature of *B. scoparia* in safflower production (Anderson 1987; Berglund et al. 2007; Blackshaw et al. 1990). These results indicate that *B. scoparia* contamination in bird feed mixes originates primarily from safflower. In fact, *B. scoparia* was the only weed species analyzed that did not result in a significant intercept in the model which indicates that *B. scoparia* is not expected to be present in the mix unless safflower was used as an ingredient.

The results of this research draw attention to what may be an overlooked and underestimated pathway of seed spread of troublesome weed species. Many weed seeds are being transported in bird feed mixes including *Amaranthus* species, which are some of the most troublesome weeds in the United States. Our screening has also proven that glyphosate-resistant *Amaranthus* seed is being transported in bird feed mixes. In a much earlier similar study, Watts and Watts (1979) suggested that the series of chance events that would make it possible for a component of a bird feed mix to escape and ultimately settle in an area conducive for its germination may happen more than expected. Others doubt bird feed plays much more than possibly a minor role in the introduction of weed species into new territories (Vitalos and Karrer 2008). Regardless, endozoochory may be involved, as several studies have found weed seeds to remain viable after alimentary excretion in cattle, waterfowl, and other avian species (Dowsett-Lemaire 1988; Farmer et al. 2017; Lhotska and Holub 1989; Powers et al. 1978). When this issue was exposed in Europe, European agencies imposed regulations for bird feed contamination; which the data suggests has decreased overall contamination levels. Nevertheless, across our entire screening, we found an average of 363 *Amaranthus* seeds kg^{-1} of bird feed. Using the results from our study in conjunction with data from the USFWS survey, it is possible that 105 million *Amaranth* seeds are transported in bird feed mixes each year.

Literature Cited

Anderson RL (1987) Broadleaf weed control in safflower (*Carthamus tinctorius*) with sulfonylurea herbicides. Weed Technology 1:242-246

Berglund D, Reveland N, Bergman J (2007) Safflower Production. North Dakota State University https://library.ndsu.edu/ir/bitstream/handle/10365/9154/A870_2007.pdf?sequence=1

Blackshaw RE, Derksen DA, Muendel H-H (1990) Herbicide combinations for postemergent weed control in safflower (*Carthamus tinctorius*). Weed Technology 4:97-104

Bohren C, Mermillod G, Delabays N (2006) Common ragweed *(Ambrosia artemisiifolia* L.) in Switzerland: development of a nationwide concerted action. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz-Sonderheft 20:497

Brandes D, Nitzsche J (2006) Biology, introduction, dispersal, and distribution of common ragweed (*Ambrosia artemisiifolia* L.) with special regard to Germany. Nachrichtenblatt-Deutschen Pflanzenschutzdienstes Braunschweig 58:286

Chahal PS, Aulakh JS, Jugulam M, Jhala AJ (2015) Herbicide-Resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.) in the United States—mechanisms of resistance, impact, and management. InTech:10.5772/61512

Chauvel B, Vieren E, Fumanal B, Bretagnolle F (2004) Spread possibility of *Ambrosia artemisiifolia* L. by way of seed of sunflower. Pages 445-452 *in* The Proceedings of the 12th International Symposium on the Biology of Weeds. Dijon, France: National Association of Plant Protection

Clay D, Reitsma K, Clay S (2009) Best management practices for corn production in South Dakota SDSU Extentension Circulars 95-96 p https://openprairie.sdstate.edu/extension_circ/490

Davis AS (2008) Weed seed pools concurrent with corn and soybean harvest in Illinois. Weed Science 56:503-508

Dowsett-Lemaire F (1988) Fruit choice and seed dissemination by birds and mammals in the evergreen forests of upland Malawi. Rev. Ecol (Terre et Vie) 43:251-285

Duary B (2014) Weed Prevention for Quality Seed Production of Crops. SATSA Annual Technical Issue 18:48-57

Duke JA (1983) Handbook of Energy Crops *Guizotia abyssinica* (L.f) Cass. Purdue University Online

Farmer JA, Webb EB, Pierce RA, Bradley KW (2017) Evaluating the potential for weed seed dispersal based on waterfowl consumption and seed viability. Pest management science 73:2592-2603

Frick G, Boschung H, Schulz-Schroeder G, Russ G, Ujčič-Vrhovnik I, Jakovac-Strajn B, Angetter D, John I, Jørgensen JS (2011) Ragweed (*Ambrosia* sp.) seeds in bird feed. Biotechnologie, Agronomie, Société et Environnement 15:39-44

Hanson C, Mason J (1985) Bird seed aliens in Britain. Watsonia 15:237-252

Heap (2019) The International Survey of Herbicide Resistant Weeds. weedscience.org. Accessed March 27 2019

Henke SE, Gallardo VC, Martinez B, Bailey R (2001) Survey of aflatoxin concentrations in wild bird seed purchased in Texas. Journal of Wildlife Diseases 37:831-835

Hoseney RC (1994) Principles of cereal science and technology. American Association of Cereal Chemists. Inc., St. Paul:170

Kurokawa S (2001) Invasion of exotic weed seeds into Japan, mixed in imported feed grains. Extension Bulletin-Food & Fertilizer Technology Center

Legleiter TR, Johnson B (2013) Palmer amaranth biology, identification, and management: Purdue Extension, West Lafayette, IN. https://www.extension.purdue.edu/extmedia/WS/WS-51-W.pdf

Lhotska M, Holub M (1989) Influence of the digestive tract of cattle on the germination of seeds of selected plant speceis Biológia (Bratislava) 44:433-439

Powers KD, Noble RE, Chabreck RH (1978) Seed distribution by waterfowl in southwestern Louisiana. The Journal of Wildlife Management:598-605

QAM (1998) Bird Seeds Purchasing and Export Market Standards. https://www.graintrade.org.au/sites/default/files/file/Commodity%20Standards/2015_2016/Section%2006%20-%20Bird%20Seed%20Standards%20201516.pdf. Accessed March 27, 2019,

Satterthwaite FE (1946) An approximate distribution of estimates of variance components. Biometrics bulletin 2:110-114

Shimono Y, Konuma A (2008) Effects of human-mediated processes on weed species composition in internationally traded grain commodities. Weed Research 48:10-18

Taylor K, Brummer T, Taper ML, Wing A, Rew LJ (2012) Human-mediated long-distance dispersal: an empirical evaluation of seed dispersal by vehicles. Diversity and Distributions 18:942-951

U.S. Department of the Interior, U.S. Fish and Wildlife Service, U.S. Department of Commerce, U.S. Census Bureau (2016) National Survey of Fishing, Hunting, and Wildlife-Associated Recreation, .38 p

United States Department of Agriculture (1940) Federal Seed Act Agricultural Marketing Service. 1 p https://www.ams.usda.gov/sites/default/files/media/Federal%20Seed%20Act.pdf

VanWychen L (2016) 2015 Baseline Survey of the Most Common and Troublesome Weeds in the United states and Canada: Weed Science Society of America. http://wssa.net/wp-content/uploads/2015-Weed-Survey_Baseline.xlsx

Vitalos M, Karrer G (2008) Distribution of *Ambrosia artemisiifolia* L.-is birdseed a relevant vector. Journal of Plant Diseases and Protection 21:345-348

WBFI (2004) Wild Bird Feeding Industry Standards http://www.ectownusa.net/wbfi/docs/2018_Standards_Slides_for_June.pdf. Accessed March 27, 2019

Webster TM, Nichols RL (2012) Changes in the prevalence of weed species in the major agronomic crops of the Southern United States: 1994/1995 to 2008/2009. Weed Science 60:145-157

Wilson CE, Castro KL, Thurston GB, Sissons A (2016) Pathway risk analysis of weed seeds in imported grain: A Canadian perspective. NeoBiota 30:49-74

Wilson R (1980) Dissemination of weed seeds by surface irrigation water in Western Nebraska. Weed Science 28:87-92

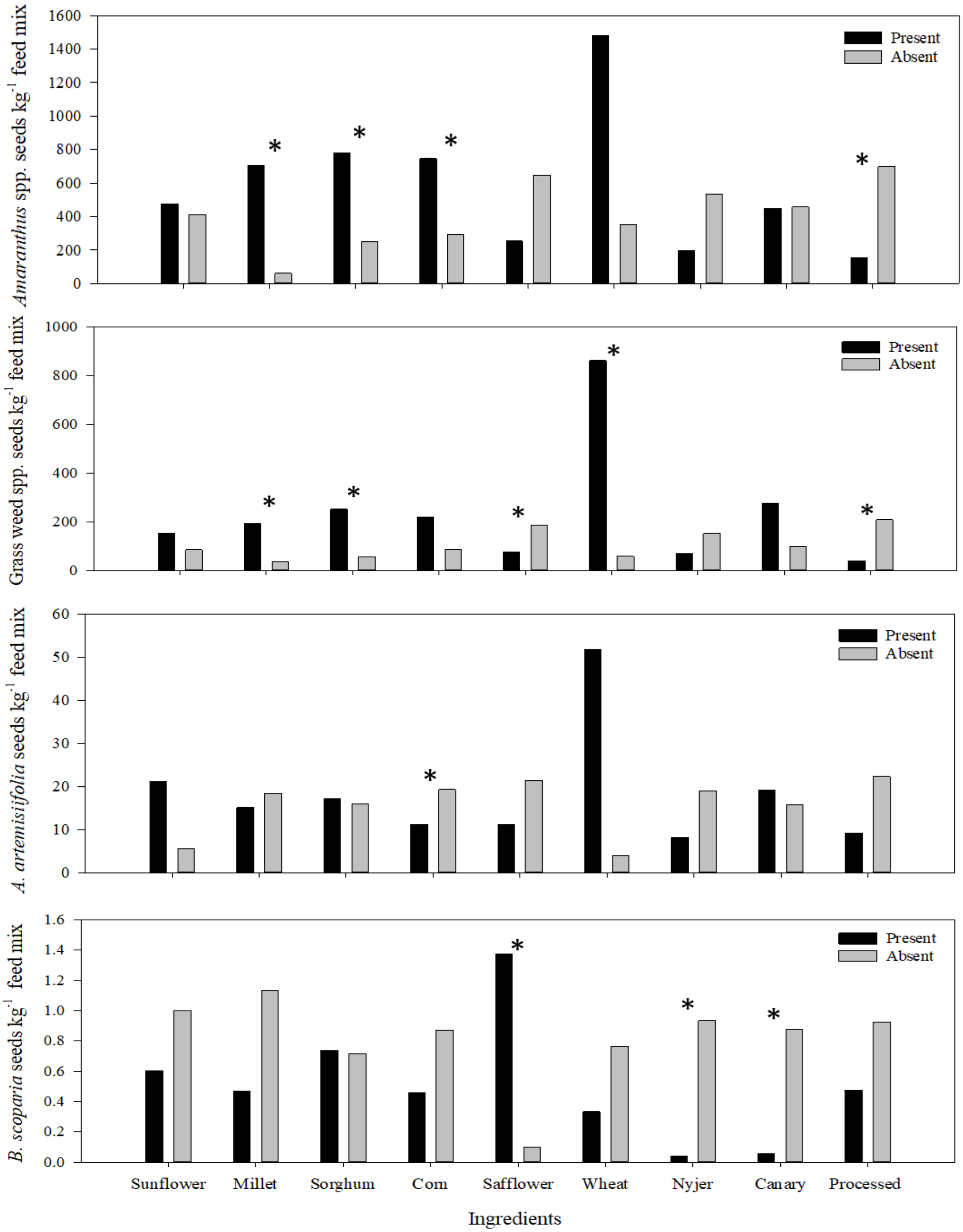

Figure 2.1. Weed seed contamination based on the presence and absence of common bird feed ingredients. Dark bars illustrate weed seed contamination when a given ingredient is present, lighter bars illustrate weed seed contamination when that ingredient is absent from bird feed mixes. Asterisks indicates significant difference between paired bars based on t test analysis.

Table 2.1 Ingredient composition and purchase location of feed mixes used in the experiment.

Brand	Variety	Ingredients	State Purchased
3D	Nut & Berry	processed, safflower, sunflower	MO
	Premium Songbird	processed, safflower, sunflower	IL
	Premium Woodpecker	processed, safflower, sunflower	IL; MO
Ace	Safflower Seed	safflower	MO
Audubon Park	Cardinal Supreme	millet, processed, safflower, sunflower	MO
	Colorful Bird Blend	millet, nyjer, processed, safflower, sunflower	NC; VA
	Patio and Garden Blend	corn, millet, processed	MO
	Premium Nut and Fruit	millet, nyjer, sunflower	MO
	Signature Harvest	processed, sorghum	MO
	Songbird Selections	millet, safflower, sorghum, sunflower	VA
	Wild Bird Food	corn, millet, sorghum, sunflower	NC; VA
Cole’s	Blue Ribbon Blend	corn, millet, processed, sunflower	MO
	Critter Munchies	corn, sunflower	MO
	White Millet	millet	MO
Enchanted Garden	Midwest Blend	millet, nyjer, safflower, sunflower	MO
	No Waste	corn, millet, processed, safflower, sorghum	MO
Feathered Friend	Birdsnack	canary, corn, millet, sorghum, sunflower, wheat	MO
	Economy Bird Feed	corn, millet, sorghum, sunflower, wheat	MO
	Finch Delight	canary, nyjer, processed	MO
Garden Treasures	Cardinal Blend	corn, millet, safflower, sunflower	KY; MO
	Finch Blend	canary, millet, nyjer	KY; MO
	Songbird Blend	corn, millet, safflower, sorghum, sunflower	KY

	Wild Bird Food	corn, millet, sorghum, sunflower	KY; TN
Harvest Seed	No Waste	canary, millet, processed	TN
Kaytee	Birder's Blend	corn, millet, safflower, sorghum, sunflower, wheat	MO
	Southern Blend	millet, processed, safflower, sorghum, sunflower	MO
	Waste Free	canary, corn, millet, processed	MO
	Wild Finch	canary, millet, nyjer, processed	MO
Kroger	Wild Bird Seed	millet, sorghum, sunflower	MO
National Audubon Society	Cardinal Mix	safflower, sunflower	MO
	Deluxe Blend	processed, safflower, sunflower	TN
	Finch Blend	millet, nyjer, processed	TN
	Wild Bird Food	corn, millet, sorghum, sunflower	KY
Nature's Own	Cracked Corn	corn	MO
	Finch Food	millet, nyjer, sunflower	VA
	Fruit and Nut	corn, millet, processed, sunflower	MO
	Safflower Seed	safflower	MO
Nature's Song	Cardinal Blend	processed, safflower, sorghum, sunflower	MO
	Safflower Bird Seed	safflower	MO
	Thistle Seed	nyjer	MO
	Wild Bird Seed	millet, sorghum, sunflower	MO
	Wild Finch	millet, nyjer	MO
Orschlen's	Bulk Bird Seed	corn, millet, safflower, sorghum, sunflower	MO
Pennington	Birder's Blend	millet, safflower, sorghum, sunflower, wheat	MO
	Black Oil Sunflower	sunflower	IL
	Classic Wild Bird Feed	millet, sorghum, sunflower, wheat	IL
	Harvest Deluxe	processed, safflower, sunflower	TN
	Premium Select Blend	millet, safflower, sorghum, sunflower, wheat	NC
	Safflower	safflower	MO
	Songbird Blend	processed, safflower, sunflower	MO

	Supreme Wild Finch	canary, nyjer, millet	MO
	Ultra Fruit and Nut	corn, processed, safflower, sunflower	KY; MO
	Ultra Waste Free	canary, corn, processed	MO; TN
Petco	All Purpose Seed Mix	corn, millet, sorghum, sunflower	MO
Royal Wing	Cardinal Mix	canary, safflower, sunflower	AR
	Nut and Fruit Blend	processed, safflower, sunflower	AR
	Splendid Blend	sorghum, sunflower, wheat	AR; MO
	Wild Finch Blend	canary, millet, nyjer	AR
Shafer	White Millet	millet	VA
Stokes Select	Premium Cardinal	safflower, sunflower	IL
	Supreme Blend	safflower, sunflower	IL
Valley Splendor	Premium Blend	millet, safflower, sorghum, sunflower	MO
	Wild Bird Food	corn, millet, sorghum, sunflower	MO
Wagner's	Sunflower Seed	sunflower	NC
	Cardinal Blend	safflower, sunflower	MO
	Cracked Corn	corn	MO
	Deluxe Blend	canary, millet, nyjer, processed	MO; VA
	Finches Deluxe	nyjer, processed	IL
	Finches Supreme	canary, millet, nyjer, processed	NC
	Greatest Variety	canary, corn, millet, nyjer, processed, sorghum, safflower, sunflower	IL; MO
	Wild Bird Food	corn, millet, sorghum, sunflower	MO
Wild Delight	Buffet for Birds	millet, sorghum, sunflower	MO
	Nut and Berry	processed, safflower, sunflower	MO
	Songbird Food	processed, safflower, sunflower	VA
	Special Finch	processed, nyjer	MO

Table 2.2 *Amaranthus* species seed presence, viability, and abundance, and grass and other broadleaf weed species seed abundance in commercially available bird feed mixes.

	***Amaranthus* spp.**			**Grass weed species**						**Other broadleaf weed species**														
Brand/Variety	Species Present [AB]	Germination	Abundance	*Cenchurus longispinus*	*Digitaria sanguinalis*	*Echinocloa crus-galli*	*Setaria* spp.	*Sorghum bicolor*	*Sorghum halpense*	*Abutilon theophrasti*	*Ambrosia artemisiifolia*	*Ambrosia trifida*	*Brassica* species	*Chenopodium album*	*Cirsium* spp.	*Convolvulus arvensis*	*Fagopyrum esculentum*	*Ipomoea* spp.	*Bassia Scoparia*	*Portulaca oleracea*	*Rumex crispus*	*Salsola tragus*	*Tribulus terrestris*	*Xanthium strumarium*
	-----	%		# of seeds kg^{-1} bird feed mix																				
3D																								
Nut and Berry	R[A]	30	4	-	-	-	5	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Woodpecker	W*[c]	78	300	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Woodpecker	R	20	4	1	39	-	-	8	-	-	-	-	3	-	-	-	-	-	9	-	-	-	-	-
Premium Songbird	n/a	0	7	-	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Woodpecker	n/a	0	1	-	-	-	-	-	-	1	51	-	6	-	-	-	-	-	-	-	-	-	-	-
Ace																								
Safflower Seed	R,S,T	33	45	4	-	-	-	-	3	-	3	-	-	-	-	-	12	3	7	-	-	6	-	-
Audubon Park																								
Signature harvest	n/a	0	6	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Nut & Fruit	R,T	29	75	-	19	-	-	2	2	-	10	-	-	-	-	-	15	3	-	-	-	-	-	-
Patio and Garden	R,T	61	1208	-	-	-	337	10	1	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-
Songbird Select	R,T,W	44	953	-	-	18	90	-	-	-	1	-	-	2	-	-	-	8	-	-	-	-	-	-

Bird Food	R,W	30	391	-	19	1	-	-	-	9	-	-	-	-	-	-	8	4	-	-	-	-	-	-
Bird Food	R,W	16	911	-	-	-	-	-	-	20	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Colorful Bird	n/a	0	675	-	-	-	61	-	1	-	1	4	-	-	-	-	3	-	-	-	4	-	-	-
Cole's																								
Blue Ribbon Blend	P,R	10	13	-	-	-	-	50	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
Critter Munchies	P	45	27	-	-	-	-	23	-	-	10	-	-	-	-	-	21	-	-	-	-	-	-	-
White Millet	P,W	27	250	-	-	-	18	-	-	-	2	-	7	-	-	-	-	1	-	-	-	-	-	-
Enchanted Garden																								
Midwest Blend	R	17	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-
No Waste	n/a	0	250	-	15	77	71	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	2
Feathered Friend																								
Economy Feed	P*,R,S,T	46	699	-	15	-	652	80	-	-	15	-	5	6	-	-	56	9	-	-	-	-	3	-
Finch Delight	P,T	25	17	-	10	-	85	-	-	-	8	-	-	-	-	-	-	-	-	3	-	-	-	-
Birdsnack	P,R,T,W	19	2012	-	1078	5	2768	50	-	-	296	-	-	52	-	-	6	-	-	-	-	-	-	-
Garden Treasures																								
Cardinal Blend	R,T, W*	27	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Cardinal	P,R,T,W	13	378	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Finch Blend	n/a	0	80	-	-	-	-	-	2	-	9	1	-	-	4	-	-	-	-	-	-	-	-	-
Finch Blend	P,T,W	39	512	-	5	-	372	-	-	-	19	-	-	-	-	-	-	-	-	-	-	-	-	-
Wild Bird Food	R,W	49	80	-	-	-	15	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Wild Bird Food	P,R,T	26	1608	-	-	-	-	-	-	-	-	-	-	8	-	-	-	5	-	-	-	-	-	-
Songbird Blend	n/a	0	15	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Harvest Seed and Supply																								

No Waste	n/a	0	75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kaytee																								
Birder's Blend	R,T	73	315	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	3	-	-	-
Waste Free	n/a	0	40	-	.	-	-	60	1	-	-	-	-	-	-	-	10	14	-	-	5	-	-	-
Southern Regional	n/a	0	17	-	-	-	-	10	-	14	-	-	-	-	3	-	18	-	-	-	-	-	-	-
Wild Finch	P	13	75	-	18	-	-	50	-	-	-	-	-	-	5	5	-	-	-	-	-	-	3	-
Kroger																								
Wild Bird Seed	P,R	23	45	-	-	-	-	25	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Morning Song																								
Deluxe Bird	P,R	16	924	-	-	-	15	-	-	-	17	-	-	-	-	-	2	-	-	-	-	2	-	-
Deluxe Bird	R,T	9	624	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	2	-	-
Dove and Ground	P,R,	41	100	-	-	-	221	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wild Finch	n/a	0	25	0	26	-	-	28	-	-	-	-	-	-	-	-	1	-	-	2	-	-	-	-
Wild Finch	n/a	0	50	-	-	-	20	-	-	-	8	-	-	-	-	-	-	-	1	-	1	-	-	-
Birdwatcher's	P,R,T,W	35	1419	-	-	-	86	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-
National Audubon Society																								
Cardinal Mix	P,R	29	624	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Deluxe Blend	n/a	0	90	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
Finch Blend	n/a	0	23	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Wild Bird Food	n/a	0	1121	-	-	-	-	26	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
Nature's Own																								
Cracked Corn	P,R	40	10	-	18	-	-	55	-	1	-	-	-	-	-	-	6	-	-	-	-	-	-	-
Finch Food	R,T,W	69	64	-	-	-	-	92	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Fruit and Nut	R,T,W	8	75	-	22	-	-	5	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-

Safflower Seed	n/a	0	2	-	-	-	-	5	-	-	-	-	-	-	15	-	15	-	8	2	-	-	-	-
Cardinal Songbird	n/a	0	464	-	20	-	-	3	-	-	3	-	-	-	-	1	-	2	6	-	-	1	-	-
Thistle Seed	R	1	18	3	22	-	-	5	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Wild Finch	P,R,T	28	6525	-	32	-	800	62	-	-	82	-	-	-	-	-	15	-	1	-	-	-	-	-
Safflower Seed	n/a	0	0	-	10	3	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	3	-	-
Orschlen's																								
Birdseed Mix	P,R,T,W	24	1948	-	-	-	40	50	-	-	-	-	-	-	-	-	-	-	15	-	-	-	-	-
Pennington																								
Bird Feed	R,T	61	1006	-	-	-	5	52	13	-	146	-	-	-	-	-	-	-	2	-	-	-	-	-
Nut and Fruit	n/a	0	18	2	7	-	-	-	-	-	21	-	-	-	9	-	13	-	-	-	-	-	-	-
Birders Blend	n/a	0	156	-	-	-	-	5	-	-	-	-	-	6	-	-	6	-	-	-	-	-	-	-
Nut and Fruit	W	25	21	-	-	-	-	-	-	-	5		-	-	-	-	-	-	-	-	-	-	-	-
Select Blend	R,W	19	684	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	1	-	-	-	-	-
Safflower Seed	T	21	14	-	-	-	14	8	-	-	5	-	-	-	-	-	-	-	13	-	-	-	-	-
Sunflower	R,T	47	413	-	-	-	-	14	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
Wild Finch	R	8	140	-	-	-	12	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-
Nut and Fruit	R	13	20	-	-	3	-	-	-	-	-	-	1	-	-	1	18	-	-	-	-	-	-	-
Waste Free	T,W	20	40	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Waste Free	P*,W*	9	300	-	8	-	15	-	-	-	-	-	9	-	-	-	3	-	-	-	-	-	-	-
Songbird Blend	n/a	0	0	-	8	-	15	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Nut and Fruit	R	10	41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Harvest Deluxe	P	15	89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	1	-	-
Petco																								

All Purpose Mix	R	5	21	-	28	-	60	63	-	-	-	-	-	5	2	10	1	5	-	-	-	-	-	-
Royal Wing																								
Nut & Fruit	n/a	0	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Splendid Blend	n/a	0	11	-	-	-	78	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardinal Mix	n/a	0	26	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-
Splendid	R,S,T	15	47	1	-	-	-	-	-	-	10	-	-	-	-	-	2	-	-	-	-	-	-	-
Wild Finch	P*,R,	41	271	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shafer																								
White Millet	P,R	17	2380	-	3	-	-	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stokes																								
Supreme Blend	P*,R,T	58	50	-	50	-	-	10	-	-	-	-	-	-	-	-	22	-	-	-	1	-	-	-
Cardinal	R,T,W	39	9	-	-	-	38	-	-	1	12	-	28	-	-	-	-	-	-	-	-	-	-	-
Tractor Supply																								
Bird Food Value	P*,R,T	59	20	-	20	2	10	10	-	-	-	-	2	-	-	-	-	6	-	-	-	-	-	-
Valley Splendor																								
Premium Blend	R,T	35	248	-	-	72	45	-	-	22	10	3	-	-	-	-	2	-	2	-	-	-	-	-
Bird Food	R,W	22	70	-	-	-	225	4	-	-	-	-	2	-	-	-	-	3	-	-	-	-	-	-
Wagner's																								
Greatest Variety	P,R,T,W	30	1432	-	-	113	65	80	2	2	1	-	41	-	-	-	-	-	-	-	-	-	-	-
Wild Bird Food	P*,T,W*	27	31	-	-	-	18	8	-	-	1	-	-	-	6	-	3	3	-	-	-	-	-	-
Greatest Variety	P	20	20	-	-	-	19	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-
Deluxe	n/a	0	21	-	24	-	-	-	-	13	-	-	-	-	-	-	1	1	-	-	-	-	-	-
Sunflower	R	4	62	-	16	-	18	-	-	-	-	2	-	-	-	-	-	-	-	6	-	-	-	-

Cardinal Blend	P,R	21	50	-	18	-	-	35	-	15	1	-	10	-	-	18	-	1	-	-	-	-	-	-
Cracked Corn	R,T, W	10	40	1	-	-	-	3	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-
Deluxe Blend	R,T, W*	48	850	-	-	19	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-
Finch	n/a	0	46	-	-	-	6	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Greatest Var.	R,S,T	19	562	-	4	-	-	5	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-
Songbird	R	4	27	-	-	-	229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wild Bird	n/a	0	1974	-	1	-	75	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-
Finches Deluxe	n/a	0	0	1	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wild Delight																								
Special Finch	n/a	0	4	-	153	-	10	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
Buffet for Birds	R,T	13	154	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nut and Berry	n/a	0	0	-	-	-	-	-	-	-	2	-	-	-	-	-	2	-	-	-	-	-	-	-
Songbird	n/a	0	19	-	3	-	-	-	-	-	-	-	-	-	2	-	-	-	3	-	-	-	-	-

Abbreviations: [A] R, redroot pigweed; P, palmer amaranth; T, tumble pigweed; W, waterhemp; S, smooth pigweed
[B] Asterisks indicate species determined to be glyphosate-resistant

Table 2.3 Sources of materials used in the experiment.

Brand	Company	Address
3D	D & D Commodities, Ltd.	P.O. Box 359, Stephen, Minnesota 56757
Ace	Ace Hardware Inc.	2200 Kensington Court, Oak Brook, Illinois 60523
Audubon Park	Audubon Park	16000 Christensen Rd, Seattle, Washington 98188
Cole's	Cole's Wild Bird Products	P.O. Box 2227, Kennesaw, GA, 30156
Enchanted Garden	n/a	n/a
Feathered Friend	CHS Sunflower	220 Clement Ave, Grandin, North Dakota 58038
Garden Treasures	n/a	n/a
Harvest Seed and Supply	Global Harvest Foods	16000 Christensen Rd Seattle Washington 98188
Kaytee	Kaytee Products Inc.	521 Clay Street, Chilton, Wisconsin 53014
Kroger	The Kroger Co.	1014 Vine Street, Cincinnati, Ohio 65202
National Audubon Society	National Audubon Society	225 Varick Street, New York, New York 10014
Nature's Own	Performance Seed	PO Box 7126, St. Cloud, Minnesota 56302
Nature's Song	The Kroger Co.	1014 Vine Street, Cincinnati, Ohio 65202
Orschlen's	Orschlen's Farm & Home	1800 Overcenter Drive, Moberly, Missouri 65270
Pennington	Pennington Seed Inc.	1280 Atlanta Highway, Madison, Georgia 30650
Petco	Petco Animal Supplies Stores, Inc	9125 Rehco Rd, San Diego, California 92121
Royal Wing	Tractor Supply Co.	5401 Virginia Way, Brentwood, Tennessee 37027
Shafer	Shafer Seed	P.O. Box 170, Oakes, North Dakota 58474
Stokes Select	Classic Brands, LLC	3600 S Yosemite St., Denver, Colorado 80237
Valley Splendor	Red River Commodities, Inc,	501 42nd Street NW, Fargo, North Dakota 58102
Wagner's	Wagner's LLC	P.O. Box 54, Jericho, New York 11753
Wild Delight	D & D Commodities Ltd.	P.O. Box 359, Stephen, Minnesota 56757

Table 2.4 Prediction of *Amaranthus* species seed contamination in commercially available bird feed mixes based on linear regression analysis.

Ingredient	Parameter Estimate	Standard Error	T-Value	P-Value
Intercept	977.23	2.95	6.34	<.0001*
Proso millet	66.06	2.75	4.11	<.0001*
Grain sorghum	2.88	2.88	1.00	0.3215
Corn	1.40	0.36	0.72	0.4347
Sunflower	2.34	3.23	0.72	0.4759
Safflower	-1.47	2.57	-0.41	0.6808
Wheat	1.13	4.57	0.08	0.9344
Nyjer thistle	-2.03	3.52	-0.57	0.5732
Processed	-4.81	2.62	-1.63	0.1074
Canarygrass	3.95	3.71	1.05	0.2967

Model is significant *P=<.0001*. When p-value from individual ingredient is significant, the parameter estimate from that ingredient is different from zero

Table 2.5 Prediction of grass weed species seed contamination in commercially available bird feed mixes based on linear regression analysis.

Ingredient	Parameter Estimate	Standard Error	T-Value	P-Value
Intercept	358.9	3.63	4.55	<.0001*
Proso millet	4.57	3.31	1.26	0.2126
Grain sorghum	1.27	3.54	0.19	0.8507
Corn	1.62	3.02	0.44	0.6585
Sunflower	-2.29	3.09	-0.59	0.5592
Safflower	-1.37	3.14	-0.28	0.7838
Wheat	2802	6.21	4.35	<.0001*
Nyjer thistle	7.76	4.46	1.37	0.1735
Processed	-10.15	3.15	-2.01	0.0470*
Canarygrass	1.04	4.67	0.03	0.9780

Model is significant $P=<.0001$. When p-value from individual ingredient is significant, the parameter estimate from that ingredient is different from zero

Table 2.6 Prediction of *A artemisiifolia* seed contamination in commercially available bird feed mixes based on linear regression analysis.

Ingredient	Parameter Estimate	Standard Error	T-Value	P-Value
Intercept	37.15	3.09	3.19	0.0019*
Proso millet	-5.88	2.89	-1.69	0.0953
Grain sorghum	-6.72	3.03	-1.72	0.0891
Corn	-20.89	2.67	-3.09	0.0027*
Sunflower	89.12	3.44	3.64	0.0005*
Safflower	-15.48	2.72	-2.75	0.0073*
Wheat	1.58	4.94	0.29	0.7727
Nyjer thistle	1.43	3.71	0.27	0.7843
Processed	-4.09	2.73	-1.40	0.1647
Canarygrass	3.33	3.92	0.88	0.3836

Model is significant $P=<.0017$. When p-value from individual ingredient is significant, the parameter estimate from that ingredient is different from zero

Table 2.7 Prediction of *B. Scoparia* seed contamination in commercially available bird feed mixes based on linear regression analysis.

Ingredient	Parameter Estimate	Standard Error	T-Value	P-Value
Intercept	1.23	1.62	-0.45	0.6510
Proso millet	-1.04	1.54	-0.12	0.9049
Grain sorghum	2.18	1.61	1.64	0.1043
Corn	-1.59	1.51	-1.10	0.2740
Sunflower	-4.79	1.69	-2.95	0.0041*
Safflower	3.80	1.53	3.11	0.0025*
Wheat	-1.05	1.98	-0.09	0.9317
Nyjer thistle	-2.23	1.74	-1.46	0.1490
Processed	-1.36	1.51	-0.72	0.4756
Canarygrass	-1.34	1.79	-0.54	0.5922

Model is significant $P=<.0203$. When p-value from individual ingredient is significant, the parameter estimate from that ingredient is different from zero

Table 2.8 Logistic regression analysis of bird feed ingredient effects on *A. artemisiifolia* and *B. scoparia* seed contamination.

Bird feed ingredient	*A. artemisiifolia*	*B. scoparia*
	------------------------ Pr>F ------------------------	
Proso millet	0.0619	0.5779
Grain sorghum	0.6000	0.0926
Corn	0.0331	0.1360
Sunflower	0.0279	0.0182
Safflower	0.1064	0.0592
Wheat	0.5849	0.6973
Nyjer Thistle	0.4166	0.1020
Processed	0.4331	0.9102
Canarygrass	0.8512	0.9981

A Survey to Determine the Distribution and Frequency of Herbicide Resistant Horseweed (*Conyza canadensis*) in Missouri

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Abstract:

Horseweed (*Conyza canadensis*) has been classified as one of the ten most troublesome and common weeds in the U.S according to a recent Weed Science Society of America survey. Quantitative data regarding the distribution and frequency of herbicide resistance in horseweed populations in Missouri is lacking. Seed from 112 separate horseweed populations was collected from infested fields throughout Missouri just prior to soybean harvest in 2015 and 2016. A discriminating dose that represented twice the recommended field use rate of glyphosate, glufosinate, 2,4-D, dicamba, and cloransulam was applied to each population in order to determine the frequency and distribution of herbicide resistances in Missouri horseweed. A population waso classified as resistant if visual control 28 days after application (DAA) was less than 60%. Glyphosate resistance was confirmed in all 112 populations while cloransulam resistance was confirmed in 89 of 112, or 79% of the populations. Two populations survived the application of 2,4-D while all populations were found to be susceptible to dicamba and glufosinate. The results of this survey suggest the use of glyphosate and cloransulam for controlling horseweed in Missouri is likely to result in unsatisfactory control and dicamba, glufosinate, and 2,4-D still provide adequate control of horseweed across the state. These results will provide Missouri producers with valuable data regarding the distribution of herbicide-resistant horseweed populations in the state.

Introduction:

Horseweed (*Conyza canadensis (L.)* Cronq.) is an erect winter or summer annual native to North America commonly found in pastures, right-of-ways, roadsides, and cultivated and abandoned fields (Cronquist 1943; Kapusta 1979). Horseweed has the potential to produce 200,000 seeds per plant, which are aerially dispersed and have a discontinuous emergence pattern (Bhowmik and Bekech 1993; Buhler and Owen 1997; Koger et al. 2004). Shields et al. (2006) collected horseweed seed from the plasenetary boundary layer and reported that horseweed seed movement can reach up to 550 km, while Dauer et al. (2007) indicated that heavily-infested fields could likely disperse seed 1 to 5 km from the source and impact the weed seedbanks of hundreds of surrounding farms. Dense infestations of horseweed (150 plants m^{-2}) have been shown to reduce soybean [*Glycine max* (L.) Merr.] yields up to 83% (Bruce and Kells 1990).

Horseweed is particularly troublesome in no-till soybean production (Bruce and Kells 1990), and approximately 40% of the soybean acres in the U.S. were produced using no-till practices in 2012 (USDA 2012). Historically, producers who have adopted no-till practices have had to rely primarily on herbicides, rather than tillage, for the control of this species (Bhowmik and Bekech 1993; Kapusta 1979; Vencill and Banks 1994). This has led to the selection for herbicide resistance in many horseweed populations across the United States. To date, horseweed populations in the United States have been documented with resistance to EPSPS Inhibitors (group 9) ALS inhibitors (acetolactate synthase inhibitors-group 2), photosystem II inhibitors (group 5), and photosystem I electron diverter (group 22) herbicides (Heap 2019) .VanGessel (2001) first reported glyphosate resistance in horseweed in Delaware in the year 2000, but resistance to this herbicide has since been documented in Tennessee, Kentucky, Ohio, Indiana, Maryland, New Jersey, Missouri, Arkansas, Mississippi, North Carolina, Pennsylvania, California, Illinois, Kansas, Virginia, Nebraska, Oklahoma, South Dakota, Iowa, Wisconsin, and Montana (Heap 2019). Glyphosate resistance has also been documented in horseweed populations outside of the United States in twelve additional countries. While glyphosate resistance has been confirmed in Missouri, little work has been conducted to determine the extent of horseweed resistance throughout the state, and also to determine the extent of resistance to herbicides other than glyphosate that are commonly utilized for the control of this species.

Previous surveys of waterhemp (*Amaranthus tuberculatus (Moq.)* J. D. Sauer) populations in Missouri have provided useful information pertaining to the distribution of herbicide resistances in this species across the state (Rosenbaum and Bradley 2013; Schultz et al. 2015). Similar surveys have been performed in other geographies to determine the distribution of herbicide resistance in annual ryegrass (*Lolium perenne L.* ssp. *multiflorum* (Lam.) Husnot), rigid ryegrass *(Lolium rigidum Gaudin)*, wild oat (*Avena fatua* L.), kochia (*Bassia scoparia* (L.) A. J. Scott), horseweed, and others (Beckie et al. 1999; Davis et al. 2008; Hall et al. 2014; Hanson et al. 2009; Légère et al. 2000; Llewellyn and Powles 2001; Owen et al. 2007). Surveys of this nature allow management strategies to be adjusted to further combat the spread of herbicide-resistant weed populations (Beckie et al. 2000; Johnson and Gibson 2006). In a survey of Indiana horseweed populations, Davis et al. (2008) found that 78% of all populations collected were resistant to glyphosate. Additionally, 7%, 68%, and 88% of horseweed plants collected in the northern, central, and southern regions of the central valley of California, respectively, were classified as resistant to glyphosate (Okada et al. 2013). Another survey found that 94% of horseweed samples collected in Ohio and 39% of samples collected in Iowa were able to survive a glyphosate application with some populations surviving as much as 40 times the normal field use rate (Beres et al. 2018). Byker et al. (2013b) also determined that 147 of 168 horseweed populations collected in Canada in 2011 and 2012 were able to survive a discriminating dose of glyphosate. All of these studies were conducted in order to provide producers with information pertaining to the regional distribution of horseweed resistance so that management and mitigation practices could be adjusted accordingly. Similarly, the objective of this research was to determine the distribution and frequency of resistance to glyphosate, glufosinate, cloransulam, 2,4-D, and dicamba in Missouri horseweed populations.

Materials and Methods:

Mature horseweed seed samples were collected from 112 soybean fields across 47 counties in Missouri approximately 2 weeks after soybean senescence during the months of September and October in 2015 and 2016. The primary soybean-producing regions of the state were targeted during both years of the survey. At each sample location, approximately 15 seedheads were harvested from the field in question and combined to created one population sample. Horseweed plants can grow to heights of at least 2 m and often protrude through the soybean canopy. Therefore, our sampling method was similar to Bourgeois and Morrison (1997) in that horseweed patches observed from the field perimeter during roadside scouting were the primary factor for inclusion of a sample in the survey. As with many previous surveys (Davis et al. 2008; Rosenbaum and Bradley 2013), the intent was not to determine the frequency of horseweed in Missouri soybean fields, or even to determine the level of resistance present in Missouri horseweed populations, but rather to determine the likelihood of herbicide resistance in horseweed that remains at soybean harvest. Following collection, seed heads were dried at room temperature for 28 days and then threshed to create a uniform seed sample representative of each field location. Samples were then stored at -4 degrees Celsius for 6 months until experiments were initiated.

Horseweed seed was weighed to equal one gram and was sown in individual 54- by 27- by 6- cm plastic flats (Hummert International; Earth City Missouri) filled with commercial potting medium (Premier Tech Horticulture, Quakertown, PA) and were maintained in a greenhouse at 30 degrees Celsius. Natural light was supplemented with metal-halide lamps (600 µmol photon m^{-2} s^{-1}). After germination, plants grew for approximately 30 days at which time young seedlings were transplanted to 10 by 10 cm square pots with two plants per pot composing one experimental unit. Growing media consisting of a 2:1 mixture of the same commercial potting medium and field top soil. Plants were watered and fertilized as needed throughout the course of the experiment. Once plants reached 10 cm in width, herbicide treatments were applied using a CO_2 pressurized backpack sprayer equipped with XR 8002 flat-fan nozzle tips (TeeJet®, Spraying Systems Co., PO Box 7900, Wheaton, IL 60187) that delivered 140 L ha^{-1} at 144 kPa. All herbicides were applied at a discriminating dose of twice the labeled field use rate similar to previous studies (Beckie et al. 2000; Davis et al. 2008; Rosenbaum and Bradley 2013). The treatments evaluated included 1.68 kg ae ha^{-1} glyphosate, 0.07 kg ae ha^{-1} cloransulam, 1.19 kg ae ha^{-1} glufosinate, 1.12 kg ae ha^{-1} dicamba, and 1.12 kg ae ha^{-1} 2,4-D amine (Table 3.1). A non-treated control of each horseweed population was included for comparison. Treatments were arranged factorially in a randomized complete block with six replications, where the factors were population and treatment. Experiments were separated by the year the population was collected, and each experiment was repeated once.

Visual estimates of percent control were determined on a scale of 0 to 100%, with 0% indicating no phytotoxic effects present, and 100% indicating complete plant death. These estimates were determined at 28 days after treatment (DAT). Injury estimates considered chlorosis, necrosis, growth inhibition, and biomass reduction in comparison to the non-treated controls. A population was classified as resistant if the visual control was less than 60% across all replications, in accordance with a previous survey conducted by Davis et al. (2008). Fresh weights were collected 28 DAT and fresh weight biomass reduction (FWBR) was calculated using the equation [$\% \, FWBR = 100 - \frac{Fresh\ weight\ of\ treated\ plant}{Average\ fresh\ weight\ of\ non-treated\ plants} x100$]. Data from each population was combined from the two repetitions and were displayed using box and whisker plots using Sigma Plot (Systat Software Inc., San Jose, CA). By displaying the data in this format, the variability in response to each herbicide within and among horseweed populations can be observed. An additional mixed linear effects model was performed to determine the most effective herbicides for Missouri horseweed populations using SAS (PROC GLIMMIX; SAS® 9.4 Institute Inc., Cary, NC, USA).

Results and Discussion:

Glyphosate

Across both years of horseweed collection, 100% of the horseweed populations screened were classified as resistant to glyphosate (Figure 3.1 & 3.2). While there was significant inter-population variability suggesting possibility of segregation of resistant plants within fields, overall none of the horseweed populations were controlled more than 59% by the 2X rate of glyphosate (Figure 3.2). Across all Missouri horseweed populations collected, the average visible control in response to a 2X rate of glyphosate was 19% while horseweed biomass was reduced by an average of 25% (Table 3.2). When compared to the other herbicides tested in the survey, glyphosate averaged the lowest in visual injury and biomass reduction across all horseweed populations (Table 3.2). These results are consistent with the findings of Davis et al. (2008), who reported that 78% of horseweed populations in Indiana were resistant to glyphosate, and Byker et al. (2013b) who reported 87% of horseweed populations to be resistant to glyphosate in Canada in 2011 and 2012. Beres et al. (2018) also reported that resistance to glyphosate in horseweed has become more widespread across the state of Ohio over the past decade. Previous surveys have also reported on differences in horseweed resistance across different regions of the state (Davis et al. 2008; Okada et al. 2013). The populations collected throughout Missouri showed little variability in the level of control by region. These results suggest that in Missouri, glyphosate should no longer be considered an effective site of action for the control of horseweed.

Cloransulam

Overall, Missouri horseweed populations were also highly resistant to cloransulam; 89 of 112, or 79%, of the populations were not controlled by this ALS-inhibiting herbicide (Figure 3.3). Across all populations, cloransulam provided an average of 34% visual control and 44% biomass production (Table 3.2). Similar to glyphosate, there was high variability within populations, suggesting that there could be segregation of resistance occurring within the populations collected. Similar results were found in Ohio in 2001 where 82% of horseweed populations were found to be resistant to cloransulam (Trainer et al. 2005). In contrast, a survey in Indiana found only 20% of populations screened with cloransulam contained resistant plants (Kruger et al. 2009). Both the study from Ohio and Indiana found that when glyphosate and cloransulam were applied in combination there was usually an additive effect and, in some cases, a synergistic effect. Budd et al. (2017) also reported that 73% of horseweed populations in specific regions of Canada were resistant to glyphosate, however only 21% of these populations were resistant to a cloransulam and glyphosate mixture. Further testing of these combinations would be necessary to confirm whether a similar response would occur in the Missouri populations.

2,4-D

All but two populations were controlled greater than 60% by the 2X application of 2,4-D. The average level of visual control and biomass reduction of the Missouri horseweed populations with 2,4-D was 90% and 74%, respectively (Figure 3.4). The two populations that were controlled below the 60% resistance threshold were both from the northwestern portion of Missouri, which is a cause of concern for producers in this area (Figure 3.1). While there are no officially documented 2,4-D-resistant horseweed populations in the United States (Heap 2019), this would not be the first report of horseweed being unsatisfactorily controlled with 2,4-D (Kruger et al. 2008). In fact, in recent years extension weed scientists are recommending alternate herbicides other than 2,4-D for controlling horseweed because of the variability in control that it provides (Hager 2016; Loux 2014). Similar to glyphosate, 2,4-D has great utility in controlling broadleaf weeds prior to planting as well as in 2,4-D-resistant crops (Craigmyle et al. 2013). However, producers must be cautious of the variability in horseweed control with 2,4-D in certain regions of Missouri. The two populations with an average level of control below 60% will require additional dose-response experiments to further characterize the likelihood of resistance.

Dicamba

Dicamba effectively controlled all 112 horseweed populations tested, and provided an average of 97% visual control across all the horseweed populations tested (Figure 3.5; Table 3.2). When comparing the response of the horseweed populations to dicamba compared to 2,4-D, the box and whisker plots indicate much less variability in control with dicamba than with 2,4-D. To date, there are no documented cases of horseweed resistant to dicamba in the United States, but few studies have compared the responses of horseweed populations to dicamba across wide geographies. These results indicate that dicamba is a very effective herbicide for controlling horseweed across Missouri, and should be used in conjunction with other effective herbicide modes of action as well as cultural practices for the management and mitigation of resistant horseweed populations (Loux 2014).

Glufosinate

Similar to dicamba, glufosinate was successful in controlling all 112 of the horseweed populations tested (Figure 3.6). Glufosinate resulted in an average of 91% visual control of the horseweed populations, which was similar to the level of visual control caused by 2,4-D (Table 3.2). Also, as with 2,4-D, there was greater variability in the control of horseweed populations with glufosinate compared to dicamba. While there are no documented cases of horseweed resistance to glufosinate (Heap 2019), there are conflicting reports of the efficacy it has on horseweed. Byker et al. (2013a) found that glufosinate was one of the most effective herbicides in controlling glyphosate-resistant horseweed populations from Ontario. In contrast, Steckel et al. (2006) found the control of horseweed with glufosinate to be inconsistent and suggested that adding 2,4-D or dicamba would increase the consistency.

By understanding the distribution of resistance in Missouri, decisions can be more informed and effective strategies can be implemented to slow the evolution of herbicide resistance. The results of this survey suggest that in Missouri, producers have lost the utility of glyphosate for effective control of horseweed populations. Most Missouri horseweed populations are also resistant to cloransulam and so it is likely that other ALS-inhibiting herbicides like chlorimuron that are commonly utilized for the control of horseweed will not be effective on this species either. Figure 3.7 illustrates that there are numerous horseweed populations resistant to more than one herbicide site of action in Missouri. These resistances, coupled with the fact that horseweed is able to aerially disperse its seed great distances, illustrates the need for an even greater emphasis on resistance management with this species. The results of this survey also indicate that producers still have effective herbicide options for horseweed such as dicamba, glufosinate, and 2,4-D but that these herbicides should be integrated with other cultural weed management strategies where possible in order to reduce the likelihood of further herbicide resistance evolution.

Literature Cited:

Beckie HJ, Heap IM, Smeda RJ, Hall LM (2000) Screening for herbicide resistance in weeds. Weed Technology 14:428-445

Beckie HJ, Thomas AG, Légère A (1999) Nature, occurrence, and cost of herbicide-resistant green foxtail (*Setaria viridis*) across Saskatchewan ecoregions. Weed Technology:626-631

Beres ZT, Ernst EE, Ackley BA, Loux MM, Owen MD, Snow AA (2018) High Levels of Glyphosate Resistance in *Conyza canadensis* from Agricultural and Non-Agricultural Sites in Ohio and Iowa. Scientific Reports 8:10483

Bhowmik PC, Bekech MM (1993) Horseweed (*Conyza canadensis*) seed production, emergence, and distribution in no-tillage and conventional-tillage corn (*Zea mays*). Agron. Trends Agric. Sci 1:67-71

Bourgeois L, Morrison IN (1997) A survey of ACCase inhibitor resistant wild oat in a high risk township in Manitoba. Canadian Journal of Plant Science 77:703-708

Bruce JA, Kells JJ (1990) Horseweed (*Conyza canadensis*) control in no-tillage soybeans (*Glycine max*) with preplant and preemergence herbicides. Weed Technology 4:642-647

Budd CM, Soltani N, Robinson DE, Hooker DC, Miller RT, Sikkema PH (2017) Distribution of glyphosate and cloransulam-methyl resistant Canada fleabane [*Conyza canadensis* (L.) Cronq.] in Ontario. Canadian Journal of Plant Science 98:492-497

Buhler DD, Owen MD (1997) Emergence and Survival of Horseweed (*Conyza canadensis*). Weed Science 45:98-101

Byker HP, Soltani N, Robinson DE, Tardif FJ, Lawton MB, Sikkema PH (2013a) Control of glyphosate-resistant Canada fleabane [*Conyza canadensis* (L.) Cronq.] with preplant herbicide tankmixes in soybean [*Glycine max* (L). Merr.]. Canadian Journal of Plant Science 93:659-667

Byker HP, Soltani N, Robinson DE, Tardif FJ, Lawton MB, Sikkema PH (2013b) Occurrence of glyphosate and cloransulam resistant Canada fleabane (*Conyza canadensis* L. Cronq.) in Ontario. Canadian Journal of Plant Science 93:851-855

Craigmyle BD, Ellis JM, Bradley KW (2013) Influence of herbicide programs on weed management in soybean with resistance to glufosinate and 2, 4-D. Weed Technology 27:78-84

Cronquist A (1943) The separation of *Erigeron* from *Conyza*. Bulletin of the Torrey Botanical Club:629-632

Davis VM, Gibson KD, Johnson WG (2008) A field survey to determine distribution and frequency of glyphosate-resistant horseweed (Conyza canadensis) in Indiana. Weed Technology 22:331-338

Hager A (2016) Dicamba and Soybean: What to expect in 2017 University of Illinois http://bulletin.ipm.illinois.edu/?p=3765.

Hall LM, Beckie HJ, Low R, Shirriff SW, Blackshaw RE, Kimmel N, Neeser C (2014) Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta. Canadian Journal of Plant Science 94:127-130

Hanson BD, Shrestha A, Shaner DL (2009) Distribution of glyphosate-resistant horseweed (*Conyza canadensis*) and relationship to cropping systems in the Central Valley of California. Weed Science 57:48-53

Heap (2019) The International Survey of Herbicide Resistant Weeds. weedscience.org. Accessed March 27 2019

Johnson WG, Gibson KD (2006) Glyphosate-resistant weeds and resistance management strategies: an Indiana grower perspective. Weed Technology 20:768-772

Kapusta G (1979) Seedbed tillage and herbicide influence on soybean (*Glycine max*) weed control and yield. Weed Science 27:520-526

Koger CH, Poston DH, Hayes RM, Montgomery RF (2004) Glyphosate-resistant horseweed (*Conyza canadensis*) in Mississippi. Weed Technology 18:820-825

Kruger GR, Davis VM, Weller SC, Johnson WG (2008) Response and survival of rosette-stage horseweed (*Conyza canadensis*) after exposure to 2, 4-D. Weed Science 56:748-752

Kruger GR, Davis VM, Weller SC, Stachler J, Loux M, Johnson WG (2009) Frequency, distribution, and characterization of horseweed (*Conyza canadensis*) biotypes with resistance to glyphosate and ALS-inhibiting herbicides. Weed Science 57:652-659

Légère A, Beckie HJ, Stevenson FC, Thomas AG (2000) Survey of management practices affecting the occurrence of wild oat (*Avena fatua*) resistance to acetyl-CoA carboxylase inhibitors. Weed Technology 14:366-376

Llewellyn RS, Powles SB (2001) High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheat belt of Western Australia. Weed Technology 15:242-248

Loux M (2014) Management of Herbicide-Resistant Horseweed (Marestail) in No-Till Soybeans The United Soybean Board pg, 2

Okada M, Hanson BD, Hembree KJ, Peng Y, Shrestha A, Stewart Jr CN, Wright SD, Jasieniuk M (2013) Evolution and spread of glyphosate resistance in *Conyza canadensis* in California. Evolutionary Applications 6:761-777

Owen MJ, Walsh MJ, Llewellyn RS, Powles SB (2007) Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations. Australian Journal of Agricultural Research 58:711-718

Rosenbaum KK, Bradley KW (2013) A survey of glyphosate-resistant waterhemp (*Amaranthus rudis*) in Missouri soybean fields and prediction of glyphosate resistance in future waterhemp populations based on in-field observations and management practices. Weed Technology 27:656-663

Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley KW (2015) Distribution of herbicide resistances and molecular mechanisms conferring resistance in Missouri waterhemp (*Amaranthus rudis* Sauer) populations. Weed Science 63:336-345

Steckel LE, Craig CC, Hayes RM (2006) Glyphosate-resistant horseweed (*Conyza canadensis*) control with glufosinate prior to planting no-till cotton (*Gossypium hirsutum*). Weed Technology 20:1047-1051

Trainer GD, Loux MM, Harrison SK, Regnier E (2005) Response of horseweed biotypes to foliar applications of cloransulam-methyl and glyphosate. Weed Technology 19:231-236

USDA (2012) National Agricultural Statistics Service. www.pestmanagement.info/nass/apppuseage.cfm.

VanGessel MJ (2001) Glyphosate-resistant horseweed from Delaware. Weed Science 49:703-705

Vencill WK, Banks PA (1994) Effects of tillage systems and weed management on weed populations in grain sorghum (*Sorghum bicolor*). Weed Science 42:541-547

Table 3.1 Sources of herbicides used in the experiments.

Active Ingredient	Formulation	Trade Name	Manufacturer	Address
Glyphosate[ab]	4.5 SC	Roundup PowerMax	Bayer	St. Louis, Missouri
Dicamba[b]	5 SL	Engenia	BASF	Raleigh, North Carolina
Glufosinate[a]	280 SL	Liberty	BASF	Raleigh, North Carolina
2,4-D Amine[b]	3.8 SL	Weedar 64	Nufarm Inc.	Alsip, Illinois
Cloransulam[ab]	3.8 SL	Enlist One	Corteva	Indianapolis, Indiana
AMS	3.4 SL	N-Pak AMS	Winfield United	St. Paul, Minnesota
NIS	90 L	Astute	MFA	Columbia Mo

[a] Treatment contained ammonium sulfate
[b] Treatment contained non-ionic surfactant
[c] Abbreviations: AMS, Ammonium sulfate; NIS, Non-Ionic Surfactant

Table 3.2 Average response of Missouri horseweed populations to five herbicide treatments.

Herbicide	Horseweed Population Response	
	% Visual Control	% Biomass Reduction
	-------------------- 28 Days after Application --------------------	
Dicamba	97 a	83 b
Glufosinate	91 b	88 a
2,4-D	90 b	74 c
Cloransulam	34 c	44 d
Glyphosate	19 d	25 e

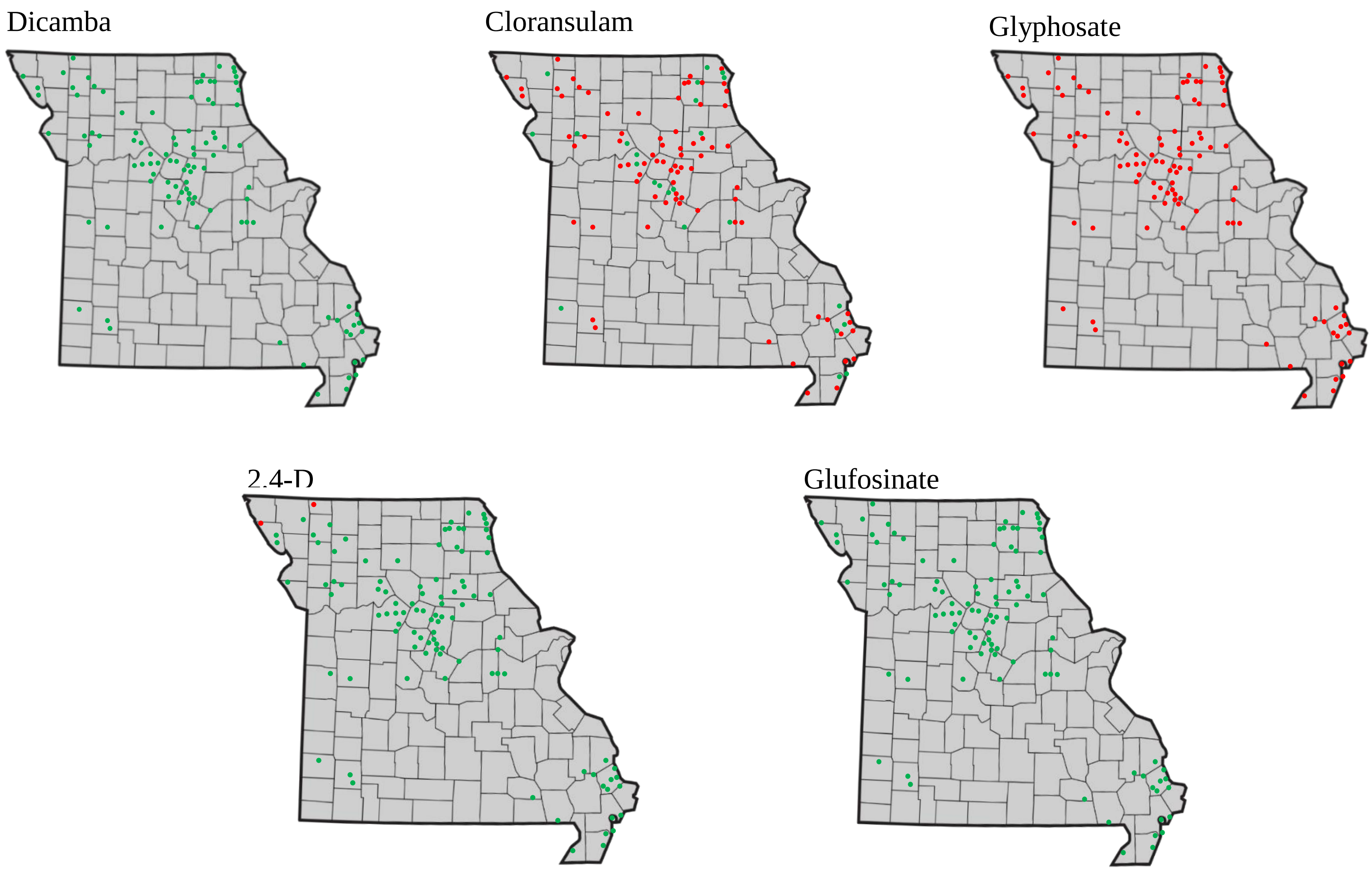

Figure 3.1. Geographical distribution of resistant and susceptible horseweed populations in Missouri. Denotes a susceptible population controlled > 60% by the respective herbicide while denotes a resistant population controlled <60% by the respective herbicide.

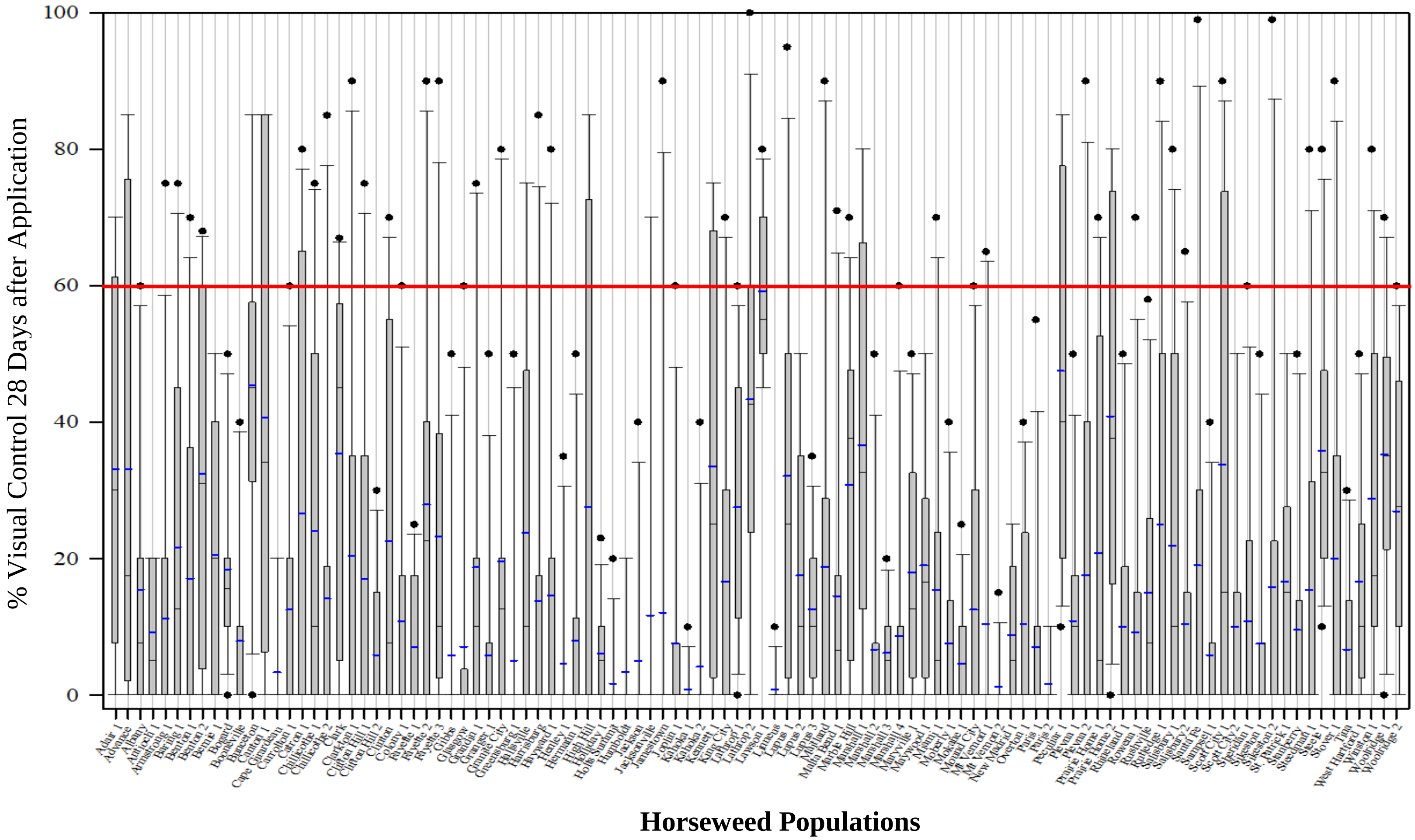

Figure 3.2. Visual control of horseweed populations 28 days after an application of glyphosate at twice the normal use rate (2X). Lower and upper boxes represent the second and third quartiles, respectively. Vertical lines represent the minimum and maximum data points. Black dots denote outliers. The red line spanning the length of the figure denotes the 60% threshold classifying resistance. When the population average is beneath the 60% threshold, the population is considered resistant.

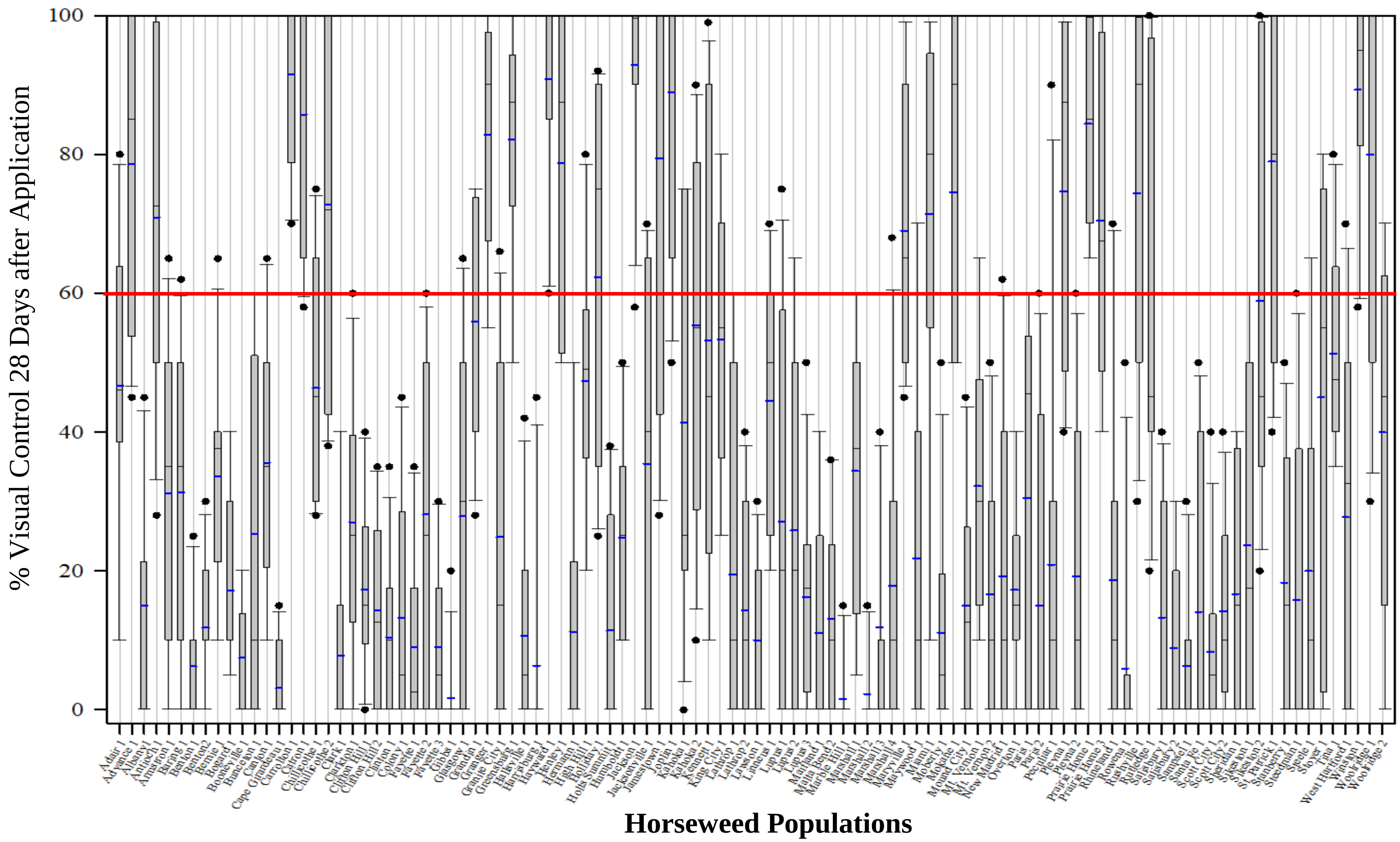

Figure 3.3. Visual control of horseweed populations 28 days after an application of cloransulam at twice the normal use rate (2X). Lower and upper boxes represent the second and third quartiles, respectively. Vertical lines represent the minimum and maximum data points. Black dots denote outliers. The red line spanning the length of the figure denotes the 60% threshold classifying resistance. When the population average is beneath the 60% threshold, the population is considered resistant.

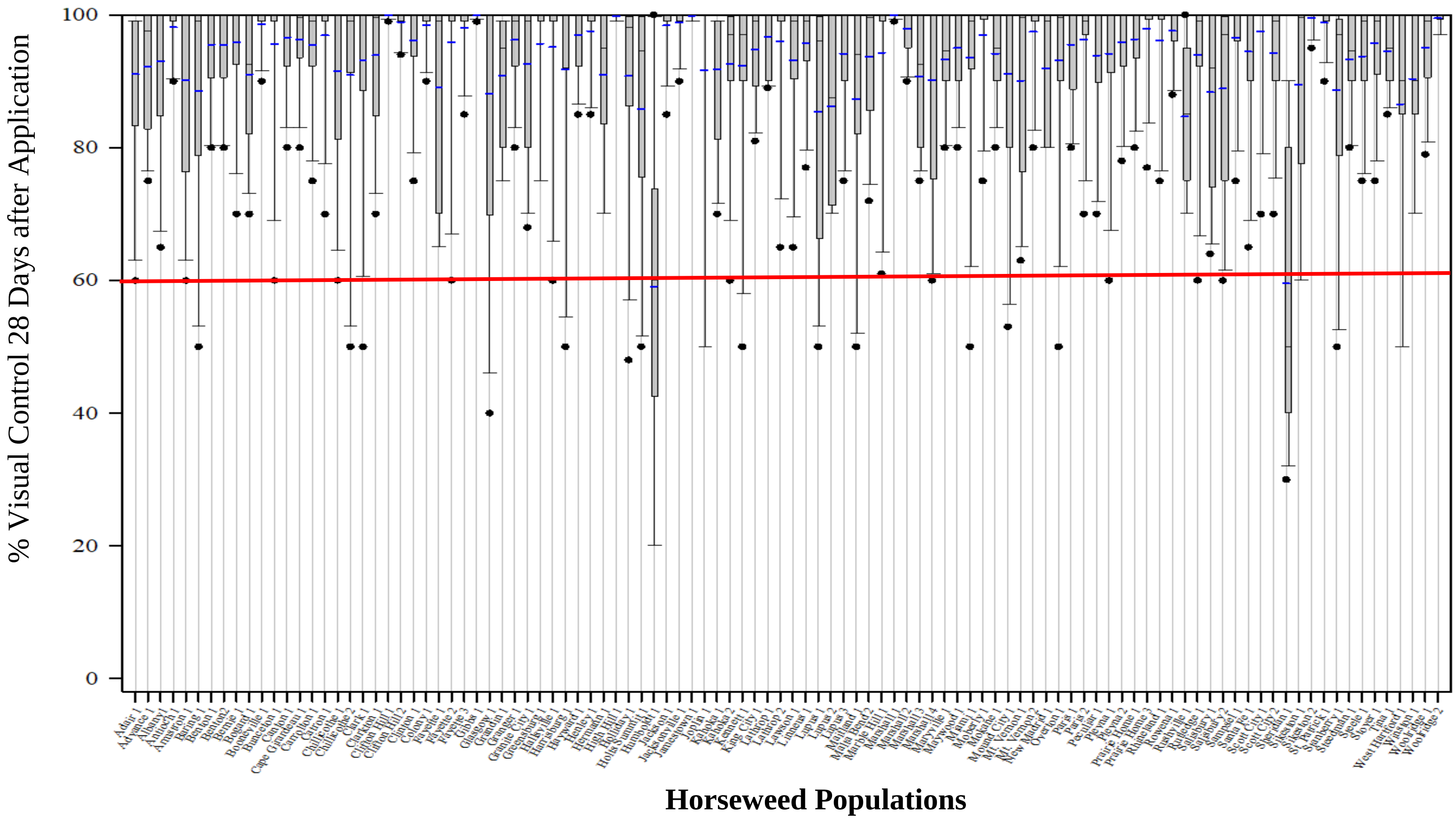

Figure 3.4. Visual control of horseweed populations 28 days after an application of 2,4-D at twice the normal use rate (2X). Lower and upper boxes represent the second and third quartiles, respectively. Vertical lines represent the minimum and maximum data points. Black dots denote outliers. The red line spanning the length of the figure denotes the 60% threshold classifying resistance. When the population average is beneath the 60% threshold, the population is considered resistant.

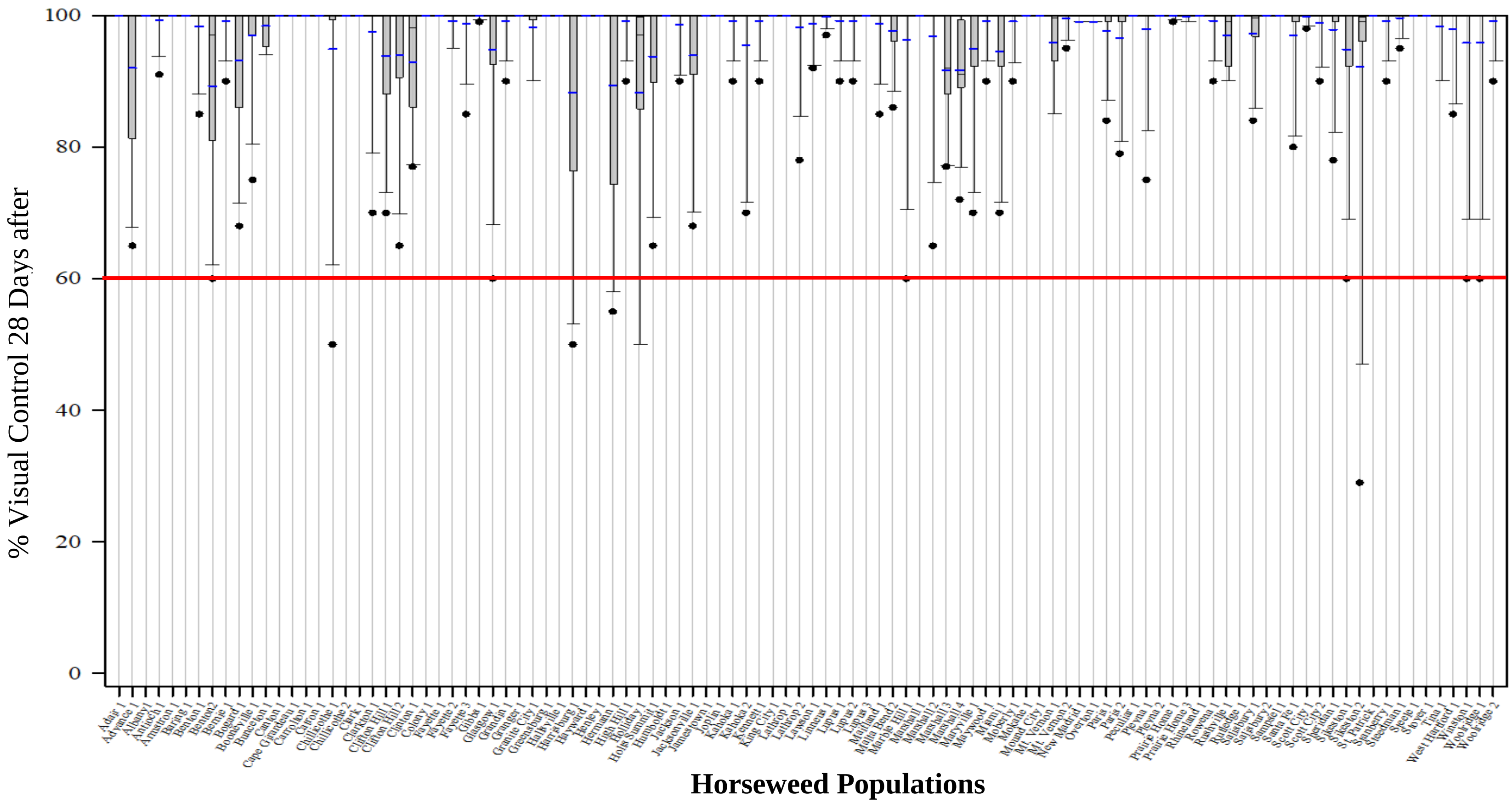

Figure 3.5. Visual control of horseweed populations 28 days after an application of dicamba at twice the normal use rate (2X). Lower and upper boxes represent the second and third quartiles, respectively. Vertical lines represent the minimum and maximum data points. Black dots denote outliers. The red line spanning the length of the figure denotes the 60% threshold classifying resistance. When the population average is beneath the 60% threshold, the population is considered resistant.

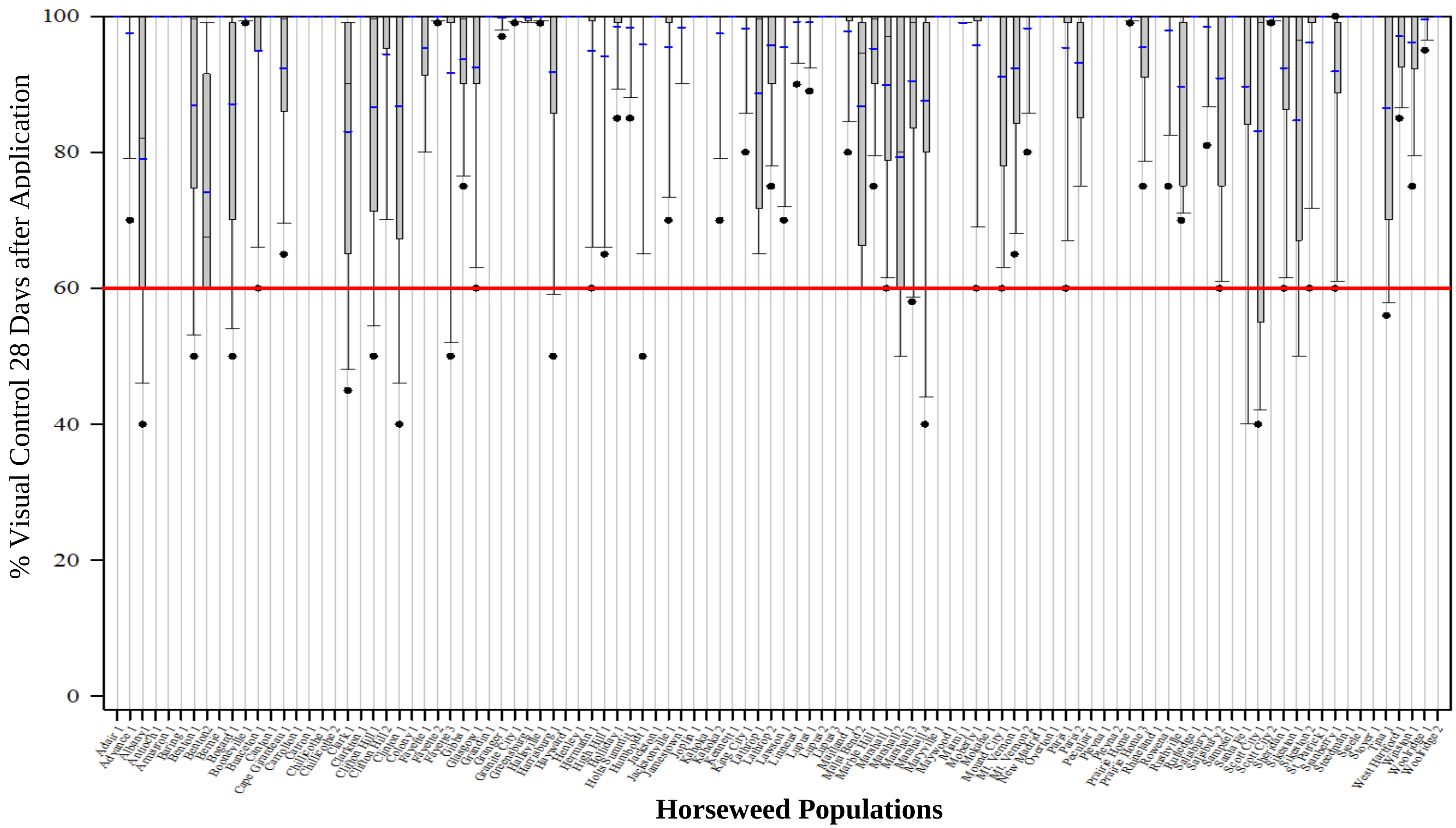

Figure 3.6. Visual control of horseweed populations 28 days after an application of glufosinate at twice the normal use rate (2X). Lower and upper boxes represent the second and third quartiles, respectively. Vertical lines represent the minimum and maximum data points. Black dots denote outliers. The red line spanning the length of the figure denotes the 60% threshold classifying resistance. When the population average is beneath the 60% threshold, the population is considered resistant.

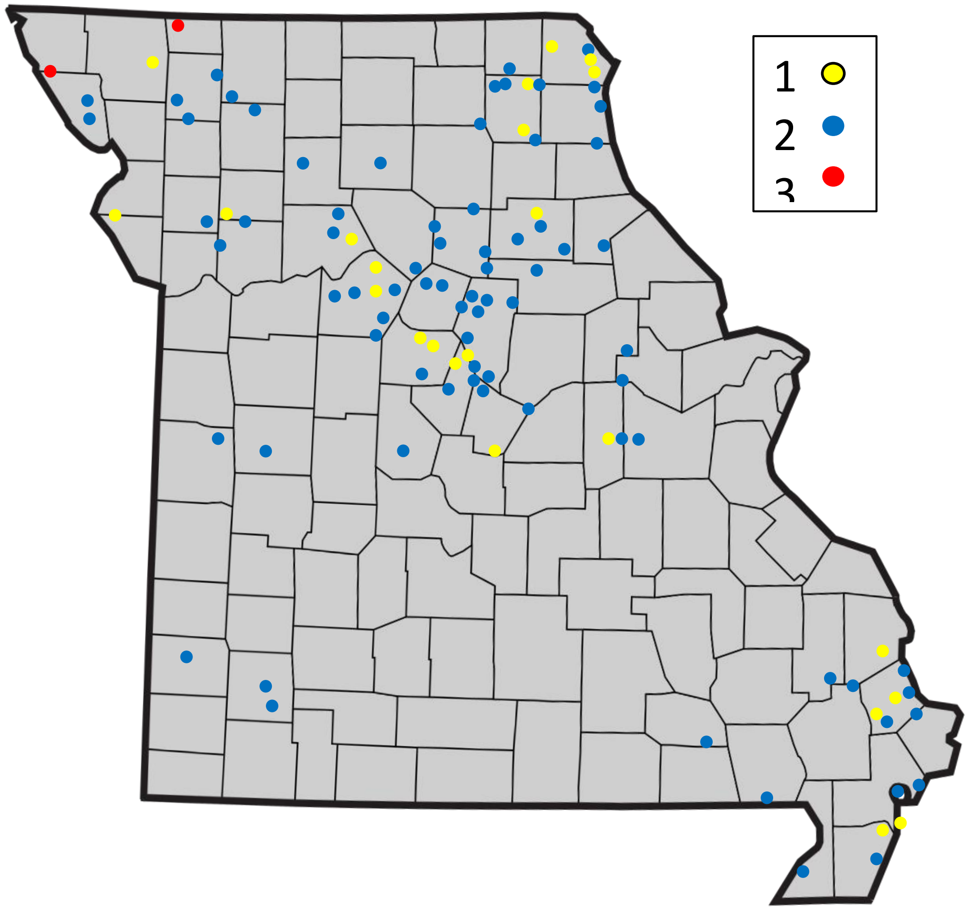

Figure 3.7. Geographical distribution of horseweed populations with resistance to multiple herbicide sites of action in Missouri