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EFFECT OF PPO-INHIBITING HERBICIDES ON MALE-TO-FEMALE SEX RATIO OF AMARANTHUS PALMERI

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by

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Approved by:

Dr. Karla L. Gage, Chair

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TITLE: EFFECT OF PPO-INHIBITING HERBICIDES ON MALE-TO-FEMALE SEX RATIO OF *AMARANTHUS PALMERI*

MAJOR PROFESSOR: Dr. Karla L. Gage

CO-ADVISOR: Dr. David J. Gibson

Background: *Amaranthus palmeri* S. Wats. (Palmer amaranth) is a dicotyledonous, dioecious species having separate male and female plants that forces outcrossing, ensures genetic diversity, and is recognized as one of the most noxious, invasive agricultural weed pests in the Mid-West. It can be characterized by extended emergence periodicity, aggressive growth habit, high fecundity, and high water use efficiency as well as high competitive ability. Fisher (1930) predicted 1: 1 primary sex ratios after the period of parental investment, but initial field studies indicated that *A. palmeri* populations were female-biased, departing from the expected 1:1 sex ratio. Therefore, managing population sex ratios would be an important consideration for controlling *A. palmeri* populations, as this species has become resistant to several herbicide modes of action.

Objective: This study was conducted to investigate the male-to-female sex ratio of *Amaranthus palmeri* following exposure to PPO-inhibiting herbicides, to gain a better understanding of potential effects of herbicide application on the population sex ratio.

Methods: A greenhouse experiment and a two-year field experiment were conducted at the Horticultural Research Center, Southern Illinois University Carbondale in spring 2016 and Collinsville, Illinois in the summer season of 2015 and 2016, respectively. The greenhouse experiment was conducted by applying two protoporphyrinogen oxidase (PPO)-inhibiting herbicide treatments of either lactofen (Cobra) or fomesafen (Flexstar) on four different Illinois populations (Cahokia, Collinsville, Rend Lake, and Massac). The field experiment was conducted for two years in a soybean field throughout the growing season of 2015 and 2016 in Collinsville, Illinois, USA. This study included 12 pre- and post-emergence PPO-inhibiting herbicide treatments of 10 herbicides with 3 replicates to investigate the variation among sex ratios by treatment.

Results: For the greenhouse experiment, depending on the population, herbicide treatments expressed a male-to-female sex ratio of either 1:1 or male-biased in contrast to the female-biased field observations. This study also suggested that these PPO-inhibiting herbicide treatments may have an influence on the growth and sex ratio of *A. palmeri* populations.

The field experiment indicated that *A. palmeri* populations have a female-biased sex ratio in untreated controls. The pre-emergence application of sulfentrazone (Spartan) at rates of 226.8 to 340.19 g a.i./ha provided the highest control efficacy, as compared to other treatments. Post-emergence-only applications provided limited control over the population. Fomesafen (Flexstar) was the only PPO-inhibiting herbicide which led to a male-based population in both years. In the future, and with increased understanding of the mechanism behind sex expression in *A. palmeri*, knowledge of plant-environment relationships such as these could provide an opportunity to reduce seed production in populations by favoring the production of males.

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INTRODUCTION

This chapter consists of five parts: i) Variation of sex ratios in dioecious species, ii) Dioecious weed species, iii) Use of protoporphyrinogen oxidase (PPO)-inhibiting herbicides for managing *Amaranthus palmeri*, iv) Objective, and v) Hypotheses.

1.1 Variation of sex ratios in dioecious species:

Dioecy is a sexually dimorphic phenomenon generally found in some flowering species which has become a significant consideration for evolutionary biology (Nicotra, 1998). Dioecious species are characterized by having separate male and female reproductive parts on separate male and female plants of the same species. Dioecious species have received considerable attention since the work of Charles Darwin's book "*The Different Forms of Flowers on Plants of the Same Species*" (1877).

Fisherian theory predicted 1:1 primary sex ratios as a consequence of parental investment due to the effect of deleterious incidence, and reliance on selection to maintain unbiased sex ratios (Fisher, 1930; Fisher, 1958). Thus, the sex ratio of dioecious species should generally be close to unity when the male and female reproduction costs are equal. In case of dioecious angiosperms, departures from equilibrium have been reported. The origin of these departures remains a long-standing problem in biology related to ecology and evolutionary systems determined by complex ecological (biotic, abiotic factors that influence living organisms), demographic (socioeconomic characteristics of a population) and genetic factors (Charlesworth, 2016).

Species with heteromorphic sex chromosomes, having allelic differences at the locus, which determine the sex of the species, often possess a female-biased sex ratio (Lloyd and

Webb, 1977). In these species, both X and Y chromosomes are present. These sex chromosomes are larger than autosomes, and are common in all plant families. Heterogametic sex is present in males due to the lack of combination among loci that determines the sex ratio of plant populations. More deleterious mutations are observed in Y chromosomes than in X chromosomes, and this mutational deletion process is most prominent and accelerated, because Y chromosomes are only present in male populations. On the other, hand females have only X chromosomes. The mutational differences among the sex chromosomes could be a reason for noticeable reduction of the active population size of Y chromosomes, compared to the autosomes present of that specific population (Smith, 1963; Stehlik et al., 2008). Degeneration of Y chromosomes could be a possible reason for biased sex ratios between populations, and also provides evidence for inferior performance of male-determining micro gametophytes in comparison to female-determining micro gametophytes (Charlesworth, 2002; Smith, 1963).

Dioecious plant populations can exhibit deviations of sex ratio from an expected equilibrium of 1:1 males-to-females (Charlesworth, 2016). Such biased sex ratios are documented in different plants such as *Carica* spp., *Silene* spp., *Rumex* spp., *Fraxinus mandshurica* Rupr., and *Salix* spp. (Delph and Meagher, 1995). To explain these deviations, a large number of genetic and ecological proposals have been suggested (Delph et al., 2004; Sinclair et al., 2012). The factors that contribute to biased sex ratios are still not well understood. The evolutionary basis of biased sex ratios for dioecious plant populations is a topic of interest, as sex ratios can influence the reproductive success of a population and could influence the development of diverse plant behavioral changes due to the sex specific adaptation to the environment (Charnov, 1982; Graff et al., 2013).

Limited numbers of mating groups are present in dioecious plant populations. Neighborhood interactions and competition for limited resources are common mechanisms that operate at different life history stages causing dioecious species to be sensitive to spatial structure and arrangement. In the case of dioecious species, reproductive success of a population is particularly dependent on the mechanism of pollen dispersal. Reproductive success is generally influenced by the flowering density of the population. Populations with female-biased sex ratios produce offspring having frequencies less female-biased, whereas, male-biased populations produce female biased offspring and these biased behavior could be associated with the rate of mortality of the dioecious species (e.g. *Salix sachalinensis* Trautv. & C. A. Mey.) (Stehlik and Barrett, 2006; Zhang et al., 2014).

In some dioecious flowering plants, competition between pollen tubes (certation) is found to be a contributing mechanism to female-biased sex ratios. Quality of offspring and sex ratio of the progeny depends on the stigmatic pollen load through selective fertilization processes along with certation (Stehlik and Barrett, 2006). The evolution of sexual dimorphisms is a common phenomenon that evolved from sex-based differences, often resulting in biased sex ratios within populations (Barrett and Hough, 2013). The non-random dissemination of the genders, spatial segregation, with the consequent differing availability of a preventive resource and physical space for each gender, causes biased sex ratios. These biases generally favor more male individuals than females (Gao et al., 2012).

In the case of many dioecious species, females occur less frequently than males, and the competition among populations of one sex to the other sex for the reproductive access may also be an outcome of a biased sex ratio (Freeman et al., 1976). In case of dioecious flowering plants, a biased sex ratio may occur if sex-biased mortality happens subsequently after maternal investment or if uncertainty or dissimilarities occur within the cost of reproduction of both the sexes. Male-biased sex ratios are also expressed in long-lived species due to the differences exhibited for the costs of rearing between male and female individuals (Delph and Meagher, 1995; Lloyd and Webb, 1977). Due to limited availability of resources allocated to reproduction, plant populations may experience a fitness cost for reproduction. Females need more resources than males, which generally favors higher fitness costs for females than males for reproductive allocation. Stress is also imposed by higher reproductive investment and can lead to higher mortality rates among females, causing a male-biased sex ratio. The frequency of higher male reproduction is likely related to the low reproductive allocation of male populations (Bierzychudek and Eckhart, 1988). With increasing environmental stress and pressure or when there are limited resources, higher female reproductive costs can lead to vulnerability to stress, leading to a condition of higher male-biased populations in dioecious species (Dawson and Ehleringer, 1993). Female populations use a greater percentage of resources for their reproductive allocation than male populations, and this can cause greater ecological stress and pressure for females than males. These differences in resource allocation result in dissimilarities in the rate of recurrence of flowering, which may cause sex-biased mortality (Antos and Allen, 1999).

In most of the cases, dioecious plant populations exhibit male or female-biased sex ratios if the cost of production or reproductive fitness cost between male and female populations are not equal over time. However, natural selection could be an important mechanism to balance a biased sex ratio at 1:1 (García and Antor, 1995). Differences in expenditures of reproduction between the sexes can determine sex-ratio variation among species inhabiting dissimilar environments. Higher reproductive allocation by female populations is often accompanied by flowering sex ratios that are male-biased (Field et al., 2013a; Field et al., 2013b). These processes sometimes lead to an association between life-history characters that are related to gender-specific variations, in the cost of reproduction of the population. Earlier and more frequent flowering of males than females and higher mortality of females could be the primary cause for these biased sex ratios. Sex chromosomes, pollen and seed dispersal mechanisms, and gender-based variances in cost of reproductive allocation can play significant roles that affect flowering sex ratios in dioecious species (Field et al., 2013b). Although the evolutionary basis for biased sex-ratio-determining systems has been worked out, the exact mechanisms behind this process have received less consideration.

1.2 Dioecious weed species:

There are several dioecious weed species in agroecology. Some of the dioecious weed species from the Amaranthaceae family are: *Amaranthus palmeri* S. Watson (Palmer amaranth), *A. tuberculatus* (Moq.) J. D. Sauer (common waterhemp), and *A. arenicola* I. M. Johnst (sandhills amaranth) (Pratt and Clark, 2001). In this study, I will focus on *A. palmeri*.

Amaranthus palmeri is a fast-growing, invasive agricultural weed species belonging to Amaranthaceae, the pigweed family. The plant is highly competitive and native to the southern and southwestern parts of the US. It is one of the most common broadleaf weeds found in the pigweed family and has become a significant weed problem in the Midwest (Sauer, 1967). *Amaranthus palmeri* is a dioecious species having separate male and female individuals with a reported sex ratio of 1:1 (Giacomini et al., 2014) that forces outcrossing and ensures maintenance of genetic diversity (Webster and Grey, 2015). Sex-determining chromosomes are absent in the karyotypes of *A. palmeri* (Marisa et al., 2013). It is a wind pollinated plant, and studies have demonstrated that pollen grains can travel up to 46 km from the mother plant(Sosnoskie et al., 2012). Seeds of *A. palmeri* are 1 to 2 mm in size and are primarily dispersed with gravity. However, secondary dispersal through wind, machinery, humans (shoes, clothing), birds, water etc. are mechanisms responsible for infesting other non-infested habitats and agricultural fields (DeVlaming and Proctor, 1968; Menges, 1987). *Amaranthus palmeri* can be characterized by extended emergence periodicity, aggressive growth habit, high fecundity, and high water use efficiency as well as high competitive ability (Norsworthy et al., 2008). It has gradually spread to the southeast, Midwest, and northeast parts of the US and has become a problematic weed for cotton and soybean growers. It was ranked the most problematic weed species in the US (Price et al., 2016).

Amaranthus palmeri invades crop lands in the hot and moist climates and has become a major agricultural weed pest for growers (Berger et al., 2015). It is one of the most invasive and aggressive weeds in soybean, corn and cotton crops in the southern part of US. This species is usually found in dry prairies, roadsides, waste places, cultivated or fallow fields, and industrial land. Eradication of this species from agricultural fields like soybean and corn in the northcentral US has proven difficult (Beckie, 2006). *Amaranthus palmeri* is a summer annual and reaches a height of 182.9 to 304.8 cm or more, having a season-long interference with crops like soybean and cotton and reducing yield up to 79 and 91 percent, respectively (Massinga et al., 2003). It is a prolific seed producer. A single female plant within a population can produce 600,000 seeds/plant. It is a short-lived herbaceous plant adapted to relatively hot and moisture-limited areas (Ehleringer, 1983). It is a C_4 plant and can tolerate extreme heat with a large root volume, seed viability ranged from 9% (1-cm depth) to 22% (40-cm depth) (Sosnoskie et al., 2013), high germination rate and fast growth of seedlings (Steckel et al., 2006). *Amaranthus palmeri* easily establishes and rapidly grows to a height of 1 m in 6 weeks (Cahoon et al., 2015b). One female plant has the potential to accumulate 2.5 kg of biomass (Chandi et al., 2012). It can grow 0.18 to 0.21 cm per growing degree-day, reaching heights greater than 3.0 m. (Horak and Loughin, 2000).

Amaranthus palmeri is a destructive weed for summer season crops and regrows after manual pulling and chopping (Jhala et al., 2014). *Amaranthus palmeri* has allopathic effects, i.e. it can release natural growth inhibitors which are responsible for suppressing crop growth, and can the reduce seedling growth of sorghum, onion, and cabbage crops (Menges, 1987). *Amaranthus palmeri* interferes with harvesting, compromising harvest efficiency.

Amaranthus palmeri is a common and problematic invasive weed species in sweet potato production (Webster and Grey, 2015). Season-long *A. palmeri* interference in sweet potato reduced total marketable sweet potato yield 36 to 81% at densities of 0.5 to 6.5% plants per meter of crop row (Meyers et al., 2013). *Amaranthus palmeri* displays photosynthetic and morphological plasticity under 87% or less shade condition, on the other hand soybean canopy formation had also a minimal potential to affect temporal *A. palmeri* emergence (Jha and Stougaard, 2013; Norsworthy et al., 2008). *Amaranthus palmeri* is tremendously competitive with crops like sorghum and can decrease grain yields by 30 to 50% (Wiggins et al., 2015). A density of 0.33 to 10 *A. palmeri* plants per meter reduced soybean yields by 17 to 68% (Tracy and Lawrence, 1994). The presence of 0.5 to 8 plants/row of corn can reduce 11 to 91% of corn yields, and also 392 kg ha^{-1} sorghum grain yield can be reduced with the presence of each kg of *A. palmeri* dry biomass per 15 cm row (Reddy et al., 2014).

Research has focused on creating disturbance in habitats with germinating *A. palmeri* plants to reduce inputs to the seedbank and prevent seedling establishment (Sosnoskie et al., 2012). The accumulation of seeds in the soil seedbank depends on plant density for interspecific and intraspecific interference, growing season length, edaphic and environmental factors and the relative competitiveness of the crop in which *A. palmeri* is growing. Growers and weed scientists are now concerned about the high rate of spread of this problematic weed species into new geographic regions. Its weedy characteristics, as well as its evolution of resistance to several herbicide modes of action, make it a management concern. More effective control options are needed to minimize the cost and challenge of managing this species (Stehlik et al., 2008).

Amaranthus palmeri has become resistant to several herbicide modes of action (the way in which herbicides affects the biological process or enzymatic system of susceptible plants, and interrupts the normal growth and development of the plants, resulting in plant death): microtubule-inhibitors (Group 3), photosystem II inhibitors (Group 5), acetolactate synthase inhibitors (Group 2), 5-enolpyruvylshikimate-3-phosphate synthase inhibitors (Group 9), hydroxyphenylpyruvate dioxygenase inhibitors (Group 27), and more recently to protoporphyrinogen oxidase (Group 14) inhibiting herbicides (Heap, 2017).

There are two primary principles to minimize the risk of developing herbicide-resistant weeds (Beckie, 2006), i.e. prevent reproduction through dispersal of pollen or propagules and their survival in the soil seedbank, and reduce the intensity of the selection pressure (Norsworthy et al., 2012; Wiggins et al., 2015). *Amaranthus palmeri* has become resistant to glyphosate and was first confirmed in 2004 and remains the primary weed of concern for soybean and cotton producers. Glyphosate-resistant biotypes have been confirmed in a number of US states and growers use a lot of resources to manage this problematic weed species in agricultural fields (Merchant et al., 2014).

1.3 Use of protoporphyrinogen oxidase (PPO)-inhibiting herbicides for managing *Amaranthus palmeri*:

Amaranthus palmeri has become resistant to several herbicide modes of action. As a result, growers are now shifting their management practices towards soil-applied residual herbicides, such as protoporphyrinogen oxidase-inhibiting herbicides (group 14) and long chain fatty acid inhibitors (group 15) to control glyphosate-resistant biotypes. Herbicides that inhibit the protoporphyrinogen oxidase enzyme are called PPO-inhibiting herbicides. Protoporphyrinogen oxidase (PPO)-inhibiting herbicides have the potential to manage glyphosate-resistant *A. palmeri* populations and have once again become foundational for effective weed control for most of the broadleaf weed species in large-scale agricultural crop production systems, following the evolution of glyphosate-resistant weed species.

Protoporphyrinogen oxidase (PPO) is an important enzyme that is present in the plant cell. The protoporphyrinogen oxidase enzyme is the last enzyme in the tetrapyrrole biosynthesis pathway, in the chloroplast of the plant cell. This enzyme is responsible for the oxidation of protoporphyrinogen IX to protoporphyrin IX, where protoporphyrinogen IX is called protogen and protoporphyrin IX is called proto. Proto is an important molecule for biosynthesis of chlorophyll and heme, as chlorophyll is needed for the photosynthesis of plants and heme is needed for electron transfer system (Lermontova and Grimm, 2000; Sherman et al., 1991). Protoporphyrin is a very active photosensitizing molecule and its concentration remains very low in the cell.

In the biosynthesis pathway of plants, the two isoforms (protoporphyrinogen IX and protoporphyrin IX) of the protoporphyrinogen enzymes are encoded through *PPX1* and *PPX2*, two different nuclear genes. These two different nuclear genes share a minute sequence identity.

These genes are functionally classified and dissimilar in subcellular targeting, as *PPX1* are targeted to the plastids. On the other hand, *PPX2* are targeted to the mitochondria of the plant cell, however some *PPX2* are targeted on both plastids and mitochondria of the cell organelles. These protoporphyrin enzymes are directed through the production sites of the cell chloroplast to the other sites of action, where they are needed for biosynthesis of chlorophyll and heme. Light acts as an important factor that directly influences the protoporphyrinogen enzymes. When light is present, the protoporphyrin concentration begins to increase and accumulate within the cell organelles.

However, the accumulated protoporphyrin IX is unable to be utilized for further synthesis. Light absorption by protoporphyrin IX produces triplet state protoporphyrin IX, which is highly efficient and further interacts with oxygen molecules to produce singlet oxygen. Singlet oxygen is a form of reactive oxygen species, which, along with triplet state protoporphyrin IX, can take a hydrogen atom from the unsaturated bond of lipids, creating a lipid radical and causing subsequent lipid peroxidation. When lipids are attacked, cell membranes become leaky and disintegrate quickly by producing necrotic lesions, finally resulting in plant death (Salas et al., 2016; Wuerffel et al., 2015).

A number of PPO-inhibiting pre-emergence and post-emergence herbicides are now used to control broadleaf weeds in large-scale agronomic crop production systems. PPO-inhibiting herbicides that are used in the US belong to eight different chemical classes: diphenylethers, N-phenylphthalimides, oxadiazoles, oxazolidinediones, phenylpyrazoles, pyrimidinediones, thiadiazoles, and triazolinones. These herbicides are widely used to control broadleaf weeds in the agriculture field crops, vegetables, fruits, vines, nurseries, lawns, and industries. Glyphosate-resistant *A. palmeri* populations are common, and PPO-inhibiting herbicides are widely used in weed management programs in the US (Riggins and Tranel, 2012). For example, Sharpen, Spartan, Valor, Cobra, and Flexstar are PPO-inhibiting herbicides containing saflufenacil, sulfentrazone, flumioxazin, lactofen, fomesafen active ingredients, respectively (Corn and Soybean Herbicide Chart).

Fomesafen is a soil-applied pre-emergence and foliar-applied post-emergence PPO-inhibiting diphenylether herbicide (Peachey et al., 2012). Fomesafen applied pre-emergence (PRE) provided up to 95% control in the case of common waterhemp; 60% at one study location and 95% at another location, 2 and 8 weeks after herbicide treatment respectively (Duff et al., 2008). Fomesafen used pre-emergence provided 92 to 100% control of *Amaranthus retroflexus* L., *Amaranthus powellii* S. Wats., and *Abutilon theophrasti* Medik., in cucurbits (Peachey et al., 2012). In the case of sweet potato, fomesafen provided 97% control at 74 days after transplantation (Meyers et al., 2013).

Saflufenacil is a pyrimidinedione class herbicide used to control annual broadleaf weeds in several crops including corn, soybean, and cotton. Saflufenacil is used in preplant burndown applications (Grossmann et al., 2010). Absorption of saflufenacil through root and shoot tissue causes inhibition of PPO formation, resulting in rapid degradation of membrane function and integrity, causing rapid death of the cell constituents (Crow et al., 2015; Geier et al., 2009).

Flumioxazin is a soil-applied pre- and post-emergence herbicide. The herbicide may bind tightly to soil with a high amount of organic matter and is degraded in the presence of light. It is used to control annual broad-leaved weeds in a variety of crops like soybean, cotton, and several others with a reported half-life in soil of 10 to 32 days under field conditions and 12 to 18 days under laboratory conditions. Pre-emergence application of flumioxazin provides 90% residual Palmer amaranth control in North Carolina (Meyers et al., 2013; Meyers et al., 2010).

Flumioxazin provides 90% residual control of annual broadleaf weeds found in sweet potato like *Amaranthus spinosus* L.(Steckel et al., 2006). Pre-emergence application of Flumioxazin provides 99 to 100% control of *Abutilon theophrasti* Medik. (velvetleaf), pigweed species i.e. *Amaranthus retroflexus* L. (redroot pigweed) and *Amaranthus hybridus* L. (smooth pigweed) , *Chenopodium album* L. (common lambsquarters), *Ambrosia artemisiifolia* L. (common ragweed), *Setaria viridis* (L.) P. Beauv. (green foxtail) and *Setaria faberi* Herrm. (giant foxtail) (Kelly et al., 2006; Mahoney et al., 2014).

Lactofen is a widely used post-emergence herbicide for controlling annual broad-leaf weed species in soybean and corn fields (Grichar and Dotray, 2012). It is a new member of the diphenylether class of PPO-inhibiting herbicides. Application of lactofen results in leaf bronzing and leaf spotting as well as reduction of growth and development of plants conveyed by rapid death of the cell (Graham, 2005; Grichar and Dotray, 2011). Lactofen provides a good control option for both glyphosate-resistant and -susceptible *A. palmeri* populations in addition to other annual broadleaf weed control in soybean and cotton fields as a post-emergence herbicide (Aulakh et al., 2016; Chandi et al., 2013). In the case of peanut, pre-emergence application of lactofen results in the satisfactory yield of the peanut crop as a result of control of *A. palmeri* and *Tribulus terrestris* L. populations (Grichar and Dotray, 2011; Jordan et al., 1993).

Sulfentrazone is a post-emergence PPO-inhibiting herbicide used for controlling broad-leaf weeds in crop fields including soybean and corn. Sulfentrazone provides 69 to 90% Palmer amaranth control (Reddy et al., 2015). Sulfentrazone provides 97, 46, 60, 71, and 100% control of *Chenopodium album* L. (common lambsquarters), *Ambrosia artemisiifolia* L. (common ragweed), *Setaria viridis* (L.) P. Beauv. (green foxtail) and *Setaria faberi* Herrm. (giant foxtail) respectively at 2 weeks after treatment as well as improved soybean yield (Wuerffel et al., 2015).

Preliminary studies in the field and greenhouse suggest that male-to-female sex ratios may be altered by exposure to PPO-inhibiting herbicides. A better understanding of the underlying causes affecting sex ratios may provide insight into improved management practices for *A. palmeri* populations in agricultural fields.

1.4 Objective:

The objective of the study is to investigate the male-to-female sex ratio of *A. palmeri* after exposure to PPO-inhibiting herbicides.

1.5 Hypotheses:

Question 1: Does the application of PPO-inhibiting herbicides affect the male-to-female sex ratio of *A. palmeri*?

Hypothesis 1: Application of PPO-inhibiting herbicides affect the male-to-female sex ratio.

Question 2: Do PPO-inhibiting herbicides have any effect on the performance of male and female plants?

Hypothesis 2: PPO-inhibiting herbicides differentially affect the growth characteristics of male and female plants.

CHAPTER 2

EFFECT OF PPO-INHIBITING HERBICIDES ON GROWTH CHARACTERISTICS AND SEX RATIO OF A DIOECIOUS WEED SPECIES *AMARANTHUS PALMERI* (PALMER AMARANTH)

Abstract

Amaranthus palmeri S. Watson (Palmer amaranth) is a fast-growing, highly competitive agricultural weed species which is spreading across the US Midwest. It is a dioecious species, having separate male and female plants, which makes it an obligate outcrosser and ensures the maintenance of genetic diversity. Population sex ratios are an important consideration in the management of *A. palmeri* populations as this species has become resistant to several herbicide modes of action, and there is need to minimize seed production by female plants. Preliminary field observations have indicated that *A. palmeri* populations were female-biased, departing from the expected 1:1 sex ratio. A greenhouse experiment was conducted to investigate the effect of two protoporphyrinogen oxidase (PPO)-inhibiting herbicide treatments of either lactofen (Cobra) or fomesafen (Flexstar) on four different Illinois populations (Cahokia, Collinsville, Rend Lake, and Massac). Plants from Massac populations were tallest, and both males and females from this populations also had more vegetative biomass. Female plants from the Collinsville populations had more reproductive biomass than male plants. Depending on the population, herbicide treatments altered the male-to-female sex ratio to 1:1 or towards a male-biased ratio, in contrast to the female-biased ratio of field populations. This study suggests that the PPO-inhibiting herbicide treatments may influence the growth and sex ratio of *A. palmeri* populations, potentially providing the opportunity to reduce seed production in populations by shifting sex ratios towards a male bias.

Introduction

In some flowering species, dioecy is a sexually dimorphic phenomenon (Nicotra, 1998). Dioecious species have separate male and female reproductive parts on separate male and female plants. Male-to-female primary sex ratios considered 1:1 because of parental investment due to the effect of deleterious incidence, and reliance on selection to maintain unbiased sex ratios (Fisher, 1930; Fisher, 1958). Thus, the sex ratio of dioecious species should generally be close to unity when the male and female reproduction costs are equal. The origin of departures from this ratio remains a long-standing problem in biology related to ecology and evolutionary systems determined by complex ecological (biotic, abiotic factors that influence living organisms), demographic (socioeconomic characteristics of a population) and genetic factors (Charlesworth, 2016). Some of these biotic and abiotic factors including physical and chemical characteristics of the environment reported to shift sex expression in plant populations include day length, light intensity, air chemistry, mineral nutrients, soil moisture, and ambient temperature (Freeman et al., 1980).

Sexual expression of an individual may be strongly correlated with environmental stress. A number of previous studies reported that sexual expression of a species can be altered through environmental factors, such as in the case of *Amaranthus rudis* (Moq.) J. D. Sauer populations where application of composted swine manure produced more males in the population than females (Liebman et al., 2004). Higher nitrogen content in the soil can change the sex expression towards more males for *Zea mays* L. (Heslop-Harrison, 1957). On the other hand, higher nitrogen rate (200 Vs 80 kg ha^{-1} N) can produce a more female-biased population for *Cannabis sativa* L. (Van der Werf et al., 1995). Sexual expression can also be altered through the application of plant hormones, such as in the case of *Spinacea oleracea* L. populations where the application of gibberellins and kinetin produced more female-biased populations in long days. On the other hand, AMO 1618 (a plant growth retardant) and abscisic acid application in long day photoperiod produced more male-biased populations (Culafic et al., 1983; Culafic and Neskovic, 1980). Physical and chemical characteristics of the environment including soil moisture, air chemistry, soil fertility are also often responsible for sex expression of some species, such as *Amaranthus rudis* produced more female-biased populations in Illinois, whereas Ontario populations yielded 1:1 male-to-female sex ratios (Costea et al., 2005; Lemen, 1980).

In case of dioecious flowering plants, a biased sex ratio may result if sex-biased mortality happens subsequently after maternal investment or if dissimilarities occur with the cost of reproduction of each sex (Delph and Meagher, 1995; Lloyd and Webb, 1977). Due to limited availability of resources allocated to reproduction, plant populations may experience a fitness cost for reproduction. Females need more resources than males and therefore, generally express a higher fitness cost than males for reproductive allocation. The rate of recurrence of higher male reproduction is more likely related to the lower reproductive allocation of male plants (Bierzychudek and Eckhart, 1988). With environmental stress or when there are limited resources, higher female reproductive costs can lead to vulnerability to stress, and result in male-biased populations in dioecious species (Dawson and Ehleringer, 1993). These differences in resource allocation result in dissimilarities in the rate of recurrence of flowering, which causes sex-biased mortality. Generally, dioecious plant populations exhibit male- or female-biased sex ratios if the cost of production or reproductive fitness between male and female populations are not equal over time; and these altered or biased sex ratios can impact population growth rates over time (Dawson and Ehleringer, 1993).

Amaranthus palmeri is a dioecious, obligate outcrossing, agricultural weed. Native to the southern and southwestern part of the USA in California, Texas and New Mexico in the Amaranthaceae family. Under ideal conditions and without competition, this species can produce 200,000-600,000 seeds per female plant (Keeley et al., 1987; Sauer, 1950; Steckel, 2007). Its high photosynthetic rates, aggressive growth pattern, abundant seed production, prolonged periods of germination, and capability to adapt to a wide range of environmental conditions make it a competitive and successful weed in cropping systems of the US. Depending on its density and the crop growth stage, the presence of *A. palmeri* results in yield losses by competing with the crop plants for water, light and nutrients. An *A. palmeri* infestation with a density of 0.5 to 8 plants m^{-1} of corn row can reduce the crop yield by 11 to 91% (Massinga et al., 2003). On the other hand, season-long competition with the sorghum plants can reduce yields up to 38 to 63% (Moore et al., 2004). *Amaranthus palmeri* densities of 10 plants m^{-1} can result in 68% to 78% yield losses in case of soybean (Bensch et al., 2003; Klingaman and Oliver, 1994). Likewise, due to the infestation of *A. palmeri,* biomass of cotton was also decreased to more than 50% and season-long interference can reduce yields by 13 to 54% (Morgan et al., 2001). In the case of peanut, season-long interference of *A. palmeri* with a density of 1 to 5.5 plants m^{-1} row can reduce yield by 28 to 68%, respectively (Burke et al., 2007). A density of 0.5 and 6 *A. palmeri* plants m^{-2} also can reduce the yield of sweet potatoes by 56% and 94% (Meyers et al., 2013; Meyers et al., 2010). Along with competitive interference, *A.palmeri* can also affect seedling growth of crops such as grain sorghum, carrot, cabbage, onion and tomato through allelopathic effects (Connick et al., 1987; Menges, 1987).

Crop protection problems posed by *A.palmeri* infestation are compounded by its evolution of resistance to several herbicide modes of action (the way in which herbicides affect the biological process or enzymatic system of susceptible plants, and interrupt the normal growth and development of the plants, resulting in death) including microtubule-inhibitors (Group 3), photosystem II inhibitors (Group 5), acetolactate synthase inhibitors (Group 2), 5-enolpyruvylshikimate-3-phosphate synthase inhibitors (Group 9), hydroxyphenylpyruvate dioxygenase inhibitors (Group 27), and more recently protoporphyrinogen oxidase (Group 14) inhibiting herbicides (Heap, 2017). Now glyphosate-resistant biotypes have become established in a number of states of the US and growers use a lot of resources to manage this problematic weed species in agricultural fields (Merchant et al., 2014). The plant is currently undergoing an extensive range expansion into other regions within the US, and the management challenges posed by *A. palmeri* support the importance of understanding the factors affecting sex ratio and the potential manipulation of these factors as another tool in integrated pest management scenarios.

In the case of many dioecious plant species, flowers are unisexual, having either stamens or pistils and the most common sex-determination system is XY sex chromosomes, in which males are heterogametic (XY) and females are homogametic (XX). However, some species, such as *A. palmeri*, lack sex chromosomes and may rely on other factors for sex determination, such as epigenetic changes in response to environmental stimuli. Clues towards elucidating the mechanisms underlying sex expression in cryptic species may be found in work on Cucurbitaceae (Martin et al., 2009). Studies exploring the genetic expression of sex in Cucurbitaceae showed that epigenetic changes of a transcription factor result in the conversion from male to female flowers in a gynoecious locus. These mutations affect plant growth and development and these epigenetic changes affect the plant reproductive system, which is important to sustain a species in a wide range of environmental conditions, adaptation to stress and long-term evolution (Martin et al., 2009). Dioecy may evolve from monoecy in Cucurbitaceae through an identified androecy gene which codes for a limiting enzyme in ethylene biosynthesis when expressed, leading to female flowers; repression of the androecy gene promotes the monoecious condition in *Cucumis melo* L. (Boualem et al., 2015). The mechanisms governing dioecy in *A. palmeri* are still unknown, i.e. sex-determining chromosomes are not present (Ward et al., 2013). A recent study examined potential gender-specific DNA sequences of a closely related species, *Amaranthus tuberculatus*, and found that gender-related tags (64-base-pair sequences) were heavily male-biased, suggesting that males are the heterogametic sex; only one-fifth of females had gender-specific tags, suggesting that a non-functional male locus may be present in some female plants (Sadeque et al., 2017). Better knowledge of dioecy in *A. palmeri* would have significant implications for managing herbicide-resistant biotypes of this species.

Due to the frequency of glyphosate and ALS-inhibitor herbicide-resistant *A. palmeri* species, growers have shifted their management practices towards long chain fatty acid and protoporphyrinogen oxidase-inhibiting herbicides. Protoporphyrinogen oxidase (PPO)-inhibiting herbicides have the potential to manage resistant weed populations and have become foundational to effective weed control for many of the broadleaf weed species in large-scale agricultural crop production systems, but these herbicides have now begun to fail due to the evolution of resistance in *A. tuberculatus* and *A. palmeri* populations to soil and foliar applied PPO-inhibiting herbicides (Dan Hess, 2000; Lee et al., 2008; Lermontova and Grimm, 2000; Schwartz-Lazaro et al., 2017; Wuerffel et al., 2015).

PPO-inhibiting herbicides interrupt the tetrapyrrole pathway in the chloroplast. Under the ideal conditions (without herbicide), the tetrapyrrole pathway starts in the chloroplast and after a series of reactions, glutamate is converted to δ-aminolevulinic acid, and protoporphyrinogen IX (protogen IX) is converted to protoporphyrin IX (proto IX), and heme or chlorophyll production follows (Riggins and Tranel, 2012; Roberson, 2012; Rousonelos et al., 2012). PPO-inhibiting herbicides inhibit the protoporphyrinogen oxidase (protox) enxyme and disrupt the oxidation of protoporphyrinogen IX to protoporphyrin IX. As a result protoporphyrinogen IX starts to accumulate and leak from the plastids into the cytoplasm and cell membranes (Wuerffel et al., 2015). Due to the interruption heme and chlorophyll production, the pathway is deregulated and large amounts of protoporphyrin IX are created. In the presence of light, protoporphyrin IX reacts with oxygen and light and produces massive amounts of singlet oxygen. This singlet oxygen triggers the lipid peroxidation process, damaging cell membranes, leading to rapid necrosis and finally death of the cell (Graham, 2005).

Very few studies have related herbicides to sex ratios in plant populations. In the case of *Cirsium arvense* (L.) Scop. (Canada thistle), males had greater vegetative regrowth following applications of glyphosate and these differences in vegetative reproduction might change the sex ratios in the field (Thomas et al., 1998). However, despite the aggressiveness of this species and need for management, no work has been published on sex ratios of *A. palmeri*. A preliminary field study in 2015 (unpublished) showed that the PPO-inhibiting herbicides lactofen (Cobra) and fomesafen (Flexstar) altered the sex ratio of *A. palmeri* populations.

Therefore, greenhouse research was initiated to investigate the male-to-female sex ratio of four different populations of *A. palmeri* after exposure to these two post-emergence PPO-inhibiting herbicides lactofen (Cobra) and fomesafen (Flexstar). Hypotheses were: 1) Application of PPO-inhibiting herbicides affects the male-to-female sex ratio, and (2) PPO-inhibiting herbicides differentially affect the growth characteristics of male and female plants.

Materials and methods

The greenhouse experiment was conducted at the Horticultural Research Centre and the Tree Improvement Center of Southern Illinois University Carbondale (SIUC) in March 2016 (APPENDIX A).The experiment was arranged in a completely randomized block design with three replicates and the experimental unit was a group of 10 pots each containing a single plant. The experiment investigated sex ratios among 4 different *A. palmeri* seed sources treated with post-emergence applications of PPO-inhibiting herbicides.

PPO-inhibiting herbicide applications included two different PPO herbicides, fomesafen and lactofen. The first year of the field study included 13 different pre-emergence and 2 post-emergence PPO herbicides, i.e. lactofen (Cobra) and fomesafen (Flexstar). These two post-emergence PPO herbicides led to a departure from the expected 1:1 sex ratio, with female-biased populations and, therefore, were chosen for investigation in this experiment.

Amaranthus palmeri seeds were collected from four fields in Illinois, i.e. glyphosate-resistant *A. palmeri* seeds from Cahokia, (latitude 38.563276' and longitude -90.131007') and Collinsville, (latitude 38.688847' and longitude -90.016194') and glyphosate-susceptible seeds from Rend Lake, (latitude 38.131023' and longitude -88.915100') and Massac (latitude 37.509131'and longitude -88.593149') (APPENDIX B). Cahokia and Collinsville populations were collected during 2014 and 2015 respectively. Rend Lake and Massac populations were collected during 2013 and 2010 respectively. After collection, all seeds were preserved by refrigeration at 2.2 C.

Seeds were germinated on a flat and grown in greenhouse growing media containing 70-80% Canadian sphagnum peat moss, perlite, vermiculite, dolomite lime pH adjuster, and a wetting agent (Professional growing mix, SunGro Horticulture Sunshine, 4180 S Moorland Ave, Santa Rosa, CA 95407). After germination, the seedlings were initially allowed to grow in the flats. When the seedlings were between the 1st and 3rd true leaf stage they were transplanted into 10.2 cm pots (one seedling per pot). Pots were placed on two benches in the greenhouse at the SIUC Horticultural Research Center (HRC); and then three weeks after herbicide application, they were transferred to a greenhouse at the SIUC Tree Improvement Center (TIC) on three benches (experimental blocks), which act as replicates in the experiment. Plants were maintained in a 16 h photoperiod with 430 W sodium lighting providing 250 μmol m^{-2} s^{-1} of supplemental photosynthetically-active radiation. The air temperature was maintained near 32(±5) C during the course of the experiment in both greenhouses. The temperature of both rooms was recorded with a data logger. Once plants reached 10 to 12 cm in height, herbicide applications were made in a spray chamber using an XR 8002 nozzle applying 15 GPM at 30 psi with lactofen (Cobra) and fomesafen (Flexstar) at 255.15 g a.i./ha and 283.50, g a.i./ha respectively.

After the application of herbicides, plants were returned from the spray chamber to greenhouse benches. After the 1st week following herbicide application, plants were visually rated for herbicide injury. Injury ratings were conducted at 7, 14, and 28 days after treatment (DAT). For each treatment, 10 plants were selected on the basis of herbicide injury. Severely injured plants were selected for monitoring and assessment of their sex determination and growth characteristics. The most severely injured plants (99% to 95% injury) were selected based on injury ratings for additional measures. Three replications of 360 plants were monitored including untreated plants. Growth characteristics were assessed by monitoring leaf number during spraying, the height of the plant at application, the height of the plant at 2, 4, and 6 weeks (14, 28, and 42 DAT) after herbicide application, and the date of 1st flowering. Also, the biomass of reproductive parts was measured. Upon flowering, when anthers and the stigma were prominently visible, plants were examined to determine sex. Once the sex of a plant was confirmed, the above-ground biomass was collected by cutting the plant from the soil surface and weighing the whole plant after oven drying at 48.9 C for 3 days. The reproductive parts were separated by collecting inflorescences and clipping all the flowers from the stem and weighing them after oven drying (APPENDIX C).

Statistical analysis:

In order to determine the relationship between performance measures in the greenhouse experiments, a 3-way ANOVA with mixed model analysis was performed to identify the effects of four different *A. palmeri* populations, sex, and herbicide treatment on height over time. Biomass of reproductive and vegetative parts and flowering date were analyzed with 3-way ANOVA. Separate paired t-tests on the proportion of males-to-females in each replicate group of ten pots were performed testing deviation from a 1:1 sex ratio; all analyses were conducted in SAS 9.4.

Results

Effect of population sources and herbicide treatments on the height of *A. palmeri*:

Three-way Repeated Mixed Model Analysis ($P < 0.05$) indicated that there were significant differences among population sources with sex ($F_{3,955} = 5.49$, $p < 0.001$) (Figure 2.1); population sources with herbicide treatments ($F_{6,949} = 8.23$, $p < 0.0001$) (Figure 2.2); population sources with time ($F_{6,871} = 5.01$, $p < 0.0001$) (Figure 2.3); and herbicide treatments with time ($F_{4,871} = 25.11$, $p < 0.0001$) (Figure 4) on *A. palmeri* plant height (Table 2.1). Male and female plants from the Massac populations were significantly taller than plants grown from all other population sources. There was no difference in height between male and female plants from either the Massac or Cahokia population. Female plants from the Collinsville population were taller than the male plants from this population, whereas male plants from Rend Lake were taller than females from this population (Figure 2.1).

Not surprisingly, all the untreated control plants from each of the four population sources were taller than the populations treated with both herbicides (Figure 2.2). There were no differences in height in response to lactofen (Cobra) or fomesafen (Flexstar) herbicide in plants from the Collinsville, Massac and Rend Lake populations. Cahokia plants treated with lactofen (Cobra) were shorter than plants from this population treated with fomesafen (Flexstar) (Figure 2.2). Plant height increased over time for all the population sources after 2, 4 and 6 weeks after herbicide application. Plants from the Massac populations were the tallest plants throughout, and plants from Collinsville populations were the shortest by six weeks. The height of plants from Cahokia and Rend Lake populations were intermediate in height between the Massac and Collinsville plants by six weeks (Figure 2.3). Herbicide treatments led to significant differences in height through time. All the untreated control populations were taller than the populations treated with lactofen (Cobra) and fomesafen (Flexstar) herbicides. The height of lactofen (Cobra) and fomesafen (Flexstar) treated populations increased through time but did not differ from each other (Figure 2.4).

Effect of population sources and herbicide treatments on vegetative biomass of *A. palmeri*: There was a significant effect of population sources and herbicide treatments on vegetative biomass at flowering stage ($F_{6, 316} = 3.68$, $p<0.0015$) (Table 2.2). The mean value for vegetative biomass showed that the untreated control plants from the Massac populations had greater vegetative biomass than the untreated controls of other populations. Rend Lake treated plants had the lowest vegetative biomass than treated plants from other populations. There was no significant difference between Cahokia plants treated with lactofen (Cobra) and fomesafen

(Flexstar) in terms of vegetative biomass. Collinsville plants treated with fomesafen (Flexstar) had greater vegetative biomass than plants treated with lactofen (Cobra) or untreated control plants (Figure 2.5).

Effect of population sources and herbicide treatments on reproductive biomass of *A. palmeri*: There was a significant interaction between sex and population sources but not herbicide treatments on reproductive biomass at flowering stage ($F_{3, 224} = 2.74$, $p<0.04$) (Table 2.2). The mean value for reproductive biomass showed that only male and female plants from the Collinsville population were significantly different from each other for reproductive biomass (females with higher reproductive biomass than males). However, there was no significant difference in reproductive biomass between males and females from Cahokia, Massac, and Rend Lake populations (Figure 2.6).

Effect of population sources and herbicide treatments on the flowering date of *A. palmeri*: There was a significant interaction between sex and herbicide treatments, but not population sources on flowering dates ($F_{2, 333} = 3.02$, $p<0.05$) (Table 2.3). The mean value for the flowering date (days) showed that flowering date was significantly different between male and female plants. All the untreated control populations produced earlier flower induction and were significantly different from all other populations. There were no significant differences within lactofen- (Cobra) and fomesafen- (Flexstar) treated male populations, as well as no significant differences within lactofen- (Cobra) and fomesafen- (Flexstar) treated female populations for flowering date (Figure 2.7).

Effect of population sources and herbicide treatments on the male-to-female sex ratio of *A. palmeri*:

Paired t-tests on the proportions of males to females (testing deviations from 1:1 sex ratio, i.e. 0.5 proportions for males and females) indicated that Cahokia untreated controls, Cahokia populations treated with lactofen (Cobra), all the treated and non-treated Collinsville populations, Massac untreated controls, and Massac populations treated with fomesafen (Flexstar) herbicide gave a significant departure from a 1:1 male-to-female sex ratio. Four out of 7 treatments had male-biased sex ratios, i.e. Cahokia untreated control, Collinsville untreated control, Collinsville population treated with fomesafen, and Massac population treated with fomesafen. Cahokia populations treated with fomesafen (Flexstar), Massac populations treated with lactofen, (Cobra) and all the treated Rend Lake populations regardless of treatment (herbicides or controls) were not significantly different from a 1:1 male-to-female sex ratio (Figure 2.8, Table 2.4).

Discussion

Height:

In this study, height, vegetative and reproductive biomass, flowering date and male-to-female sex ratios of four *A. palmeri* populations in response to two PPO-inhibiting herbicides lactofen (Cobra) and fomesafen (Flexstar) were examined. The invasive potential of *A. palmeri* populations was assessed based on these parameters, considering height, biomass, and flowering date of *A. palmeri* as measures of this species' competitive effect on agricultural crops. Male-to-female sex ratio appears to be a key factor to emphasize in the management of this invasive species in agricultural fields.

The four different *A. palmeri* populations exhibited variations in height in response to PPO-inhibiting herbicides. In the case of short-lived species, females are often taller than males, although this variation may depend on the timing when the comparisons were made (Obeso, 2002). In the present study, one population was consistently taller than the other three. Plants from the Massac population were consistently larger when treated with herbicides and might have size-asymmetric competition for resource uptake and resource utilization (Schwinning and Weiner, 1998). Plant height is considered to be an important factor in considering competition with neighboring species including crops, as taller plants can capture more resources than the shorter plants (Nagashima and Hikosaka, 2011). Crops growing under the shade of neighboring plants will receive reduced light intensity and altered light quality that can inhibit growth and development indicating approaching competition during canopy development (Schwartz et al., 2016a). On the other hand, tall male plants are beneficial for wind-pollinated species to maximize propagule dispersion by the wind (Barrett et al., 2010). Hence, plants from the Rend Lake populations may have a greater impact on long-distance pollen dispersal and a greater contribution to cross-pollination among populations than plants from the shorter growing populations.

Biomass:

Sex-specific growth structure and resources for reproductive allocation are well-defined evidence for dynamic patterns of dioecious species. In most of the cases, vegetative growth is similar for both male and female plants prior to flowering stage (Putwain and Harper, 1972). Comparatively, when the reproductive stages begin, females grow larger than males and live longer. This difference in size and longevity between males and females provides good evidence for sexual dimorphism (Zluvova et al., 2010).

Biomass is an indicator of competitiveness (Gough et al., 1994). Untreated control plants from the Massac populations had greater vegetative biomass than other populations. Therefore, plants from the Massac populations may have greater competitive ability than other populations. On the other hand, Collinsville plants treated with fomesafen had greater vegetative biomass than untreated control plants and plants treated with lactofen. That may be due to following herbicide application; Collinsville plants treated with fomesafen may had to accumulate more biomass to have enough energy for reproduction.

In the case of dioecious species, size dimorphism is a natural phenomenon that is due to the sexually different allocation patterns for both sexes. Only Collinsville males and females differed in reproductive biomass, and Collinsville females produced more reproductive biomass than the males. Females may be investing more resources in the production of larger leaves for photosynthesis, supplying more carbon to fruits for better and higher seed sets. Environmental heterogeneity could also affect sexual differences and allocations for reproduction (Delph and Herlihy, 2012)

Flowering date:

There are sex-specific differences in growth and reproduction consistent with life history traits that affect the longevity of male and female populations. There is a senescence strategy for males, which tend to exhibit narrower phenological windows compared to females (Obeso, 2002). In this study I observed that all the untreated control male plants, as well as lactofen (Cobra), and fomesafen (Flexstar) treated male plants produced flowers (and thus could be sexed) earlier than the females produced flowers. Male populations invested more resources for root growth that may be due to the high early investment of nitrogen rich pollen, leading to decreased photosynthetic rates. These processes restrict the vegetative growth for above-ground structure of males, although it is challenging to determine the proper resource currencies to evaluate reproductive expenditure in dioecious species (Delph et al., 1993). Male-biased sex ratios could be associated with the higher reproductive allocation of females as well as a consequence of earlier and more frequent onset and flowering of males. Variation in environmental factors can also influence the flowering patterns of males and females in dioecious species (Antos and Allen, 1999). *Amaranthus palmeri* is a C_4 species and light is a limiting factor for growth and reproduction of this species (MacRae et al., 2013). I observed our study in controlled greenhouse condition with a maintained temperature and photoperiod, and this could be a reason for early male flowering, as flowering intensity depends on the day length (Bonduriansky et al., 2008).

Sex ratio:

Dioecious plant populations commonly exhibit deviations from the expected 1:1 primary sex ratio after parental investment (Field et al., 2013a; Field et al., 2013b). As *A. palmeri* is developing resistance mechanisms to survive herbicide applications, effective tools to manage this invasive species in agricultural fields need to be developed. Intentional manipulation of sex ratios via herbicides could also be an effective way to manage *A. palmeri* if relative densities of seed-producing female plants can be reduced. Therefore, sex-ratio-specific management practices implemented by the growers have the potential to become an integrated tactic in managing herbicide-resistant *A. palmeri* populations. Both herbicides applied in the present study resulted in different effects on these *A. palmeri* populations. Herbicide treatments changed the female-biased sex ratios observed in the field to 1:1 or male-biased. Lactofen (Cobra) and fomesafen (Flexstar) are both PPO-inhibiting herbicides but the induced sex ratio expression was not same for all the populations. The differential effect of fomesafen may be due to the presence of the adjuvant system in the Flexstar® herbicide, which increases herbicide absorption and translocation. Populations with a female-biased sex ratio may need more effective management practices than populations having more male-biased sex ratios where few seeds will be produced.

The greenhouse-controlled conditions of this experiment produced male-biased populations. As PPO-inhibiting herbicides have the ability to destroy the enzymatic pathway by damaging cell constituents; therefore, there is a possibility that differences in sex expression could be related to imbalances of hormonal systems. Previous studies have shown that the sex expression of some dioecious species (*Mercurialis* spp., *Vitis* spp., *Spinacia* spp., *Cannabis* spp.) was related to the pathways of the hormone system (Chailakhyan and Khryanin, 1978; Fechter et al., 2012; Louis et al., 1990; Negi and Olmo, 1971). Cytokinin, a growth hormone, produces more females in normal growth conditions and produces more males in stressful conditions. Male sterility of *Mercurialis annua* L. is controlled by sterile S cytoplasm when S cytoplasm interacts with nuclear genes I, R1 and R2. The feminizing hormone cytokinin alters the sex of the male sterile mutant to female (Louis and Durand, 1978). So, in this research, there is the possibility that due to the stressful conditions placed upon the plants by the herbicides, plants could not produce enough cytokinins, and produced more male-biased populations. On the other hand, environmental factors, such as photoperiod, day length, soil moisture, and soil fertility, are also responsible for affecting and shifting the sex ratio of a variety of species (Freeman et al., 1980; Janousek et al., 1996; Martin et al., 2009). Further study is needed to investigate whether any of these or other factors determine sex ratio expression in *A. palmeri*.

Table 2.1: *Statistical results (F and p values) from the 3-way ANOVA repeated mixed model analysis of four different A. palmeri population sources (pop), herbicide treatments (treat), time (timen) and sex for plant height (cm). Significant values (p ≤ 0.05) highlighted in bold*

Effect	**DF**		**F value**	**Pr > F**
pop	3	960	**76.05**	**<.0001**
treat	2	54.4	**83.58**	**<.0001**
pop*treat	6	949	**8.23**	**<.0001**
sex	1	902	0	0.9519
pop*sex	3	955	**5.49**	**0.001**
treat*sex	2	875	0.29	0.7492
pop*treat*sex	6	945	1.72	0.1128
timen	2	871	**1386.57**	**<.0001**
pop*timen	6	871	**5.01**	**<.0001**
treat*timen	4	871	**25.11**	**<.0001**
pop*treat*timen	12	871	1.26	0.2385
sex*timen	2	871	0.44	0.6467
pop*sex*timen	6	871	0.27	0.9524
treat*sex*timen	4	871	0.01	0.9996

Table 2.1(Continued)

Effect	**DF**		**F value**	**Pr > F**
pop*treat*sex*timen	12	871	0.65	0.8011

Table 2.2: *Statistical results (F and P values) from the 3-way ANOVA of four different A. palmeri population sources (pop), herbicide treatments (treat), and sex for plant biomass measures (vegetative and reproductive). Significant values ($p \leq 0.05$) highlighted in bold.*

Biomass:

Vegetative biomass

Effect	DF		F value	Pr > F
pop	1	1	29.38	0.1161
treat	2	31.6	**5.54**	**0.0087**
pop*treat	6	316	**3.68**	**0.0015**
sex	1	1	**Infty**	**<.0001**
pop*sex	3	330	0.56	0.6413
treat*sex	2	333	1.18	0.3095
pop*treat*sex	6	330	0.66	0.6815

Table 2.2(Continued)

Reproductive biomass

Effect	DF		F value	Pr > F
pop	3	222	0.5	0.6824
treat	2	18.5	0.38	0.6914
pop*treat	6	215	1.84	0.0934
sex	1	267	0.29	0.5889
pop*sex	3	224	**2.74**	**0.0443**
treat*sex	2	255	0.03	0.9693
pop*treat*sex	6	220	0.96	0.4505

Table 2.3: *Statistical results (F and P values) from the 3-way ANOVA of four different A. palmeri population sources (pop), herbicide treatments (treat), and sex for plant flowering date. Significant values ($p \leq 0.05$) highlighted in bold.*

Effect	DF		F value	Pr > F
pop	3	326	**36.28**	**<.0001**
treat	2	49.9	**115.20**	**<.0001**
pop*treat	6	324	0.10	0.9963
sex	1	333	**26.89**	**<.0001**
pop*sex	3	333	1.54	0.2052
treat*sex	2	333	**3.02**	**0.050**
pop*treat*sex	6	333	0.70	0.6487

Table 2.4: *Results from paired t-tests, testing the deviation from a 1:1 sex ratio of four A. palmeri populations following the effects of two PPO-inhibiting herbicides (lactofen and fomesafen). Values ($p \leq 0.05$) highlighted in bold give no departure from 1:1 male-to-female sex ratio.*

Treatments	**Population**	**DF**	**t-value**	***p*-value**
Cobra	Cahokia	2	-3.59	0.0696
Cobra	Collinsville	2	-3.59	0.0696
Cobra	Massac	2	-4.35	**0.0491**
Cobra	Rendlake	2	-6.50	**0.0229**
Control	Cahokia	2	-0.69	0.5598
Control	Collinsville	2	-2.17	0.1628
Control	Massac	2	-2.50	0.1296
Control	Rendlake	2	-4.25	**0.050**
Flexstar	Cahokia	2	-4.33	**0.0494**
Flexstar	Collinsville	2	-1.70	0.2312
Flexstar	Massac	2	-2.80	0.1732
Flexstar	Rendlake	2	-6.06	**0.0261**

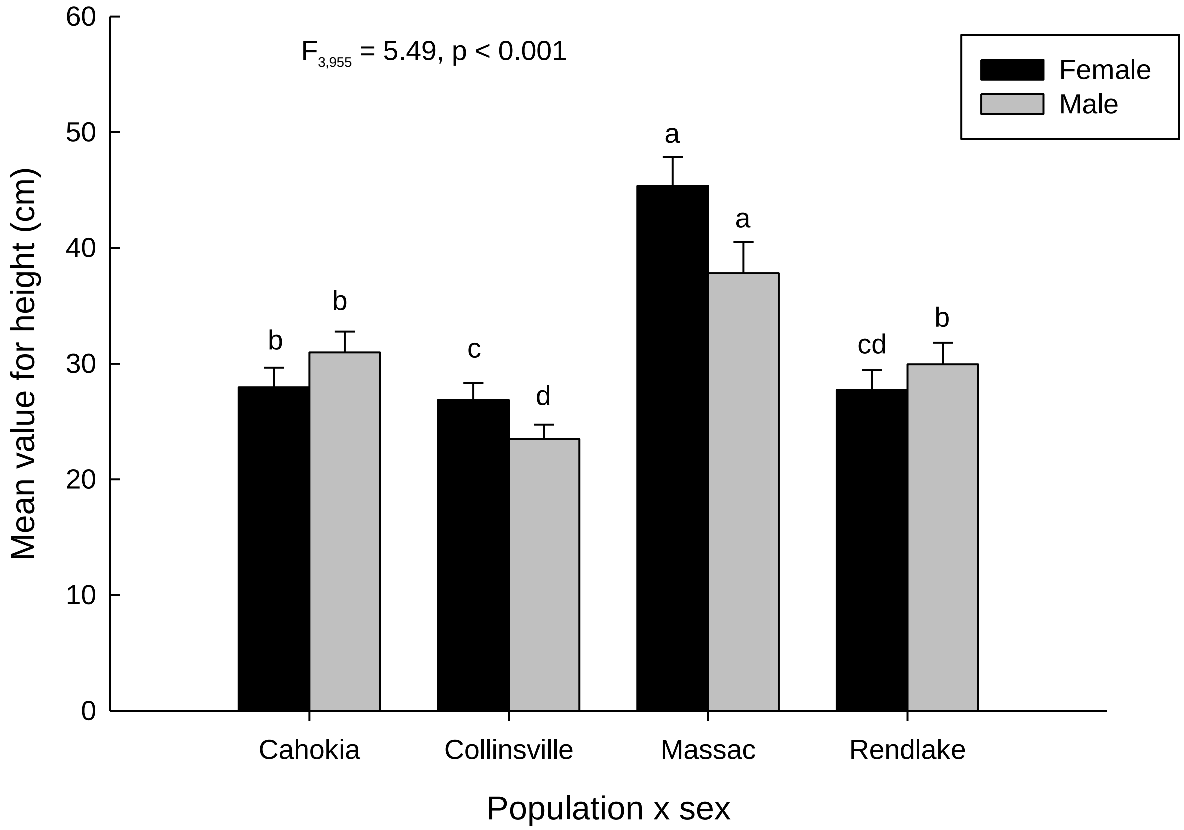

Figure 2.1: Height (cm) of *Amaranthus palmeri* plants from population sources (Cahokia, Collinsville, Massac, and Rend Lake) by sex (female and male) following application of PPO-inhibiting herbicides. Bars with different letters are significantly different ($p \leq 0.05$).

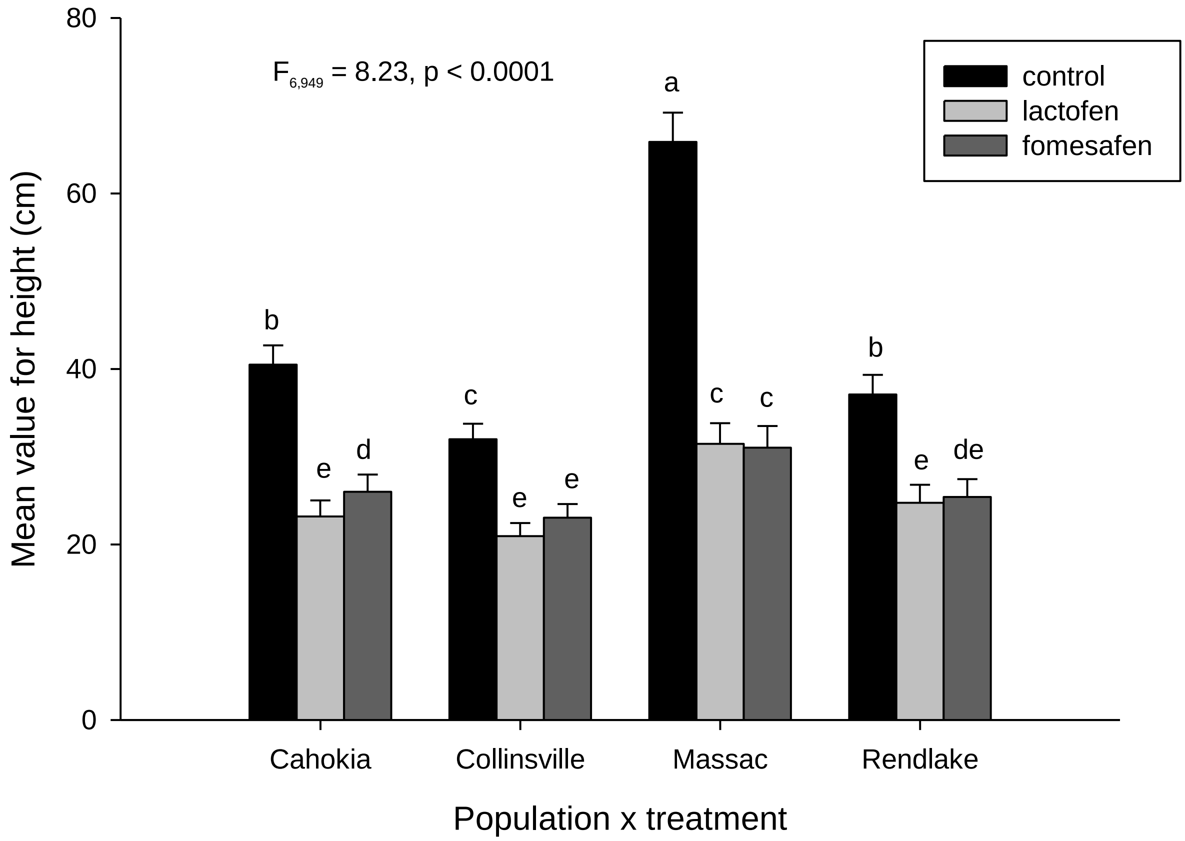

Figure 2.2: Height (cm) of *Amaranthus palmeri* plants from population sources (Cahokia, Collinsville, Massac, and Rend Lake) by treatments following application of PPO-inhibiting herbicides (lactofen and fomesafen). Bars with different letters are significantly different ($p \leq 0.05$).

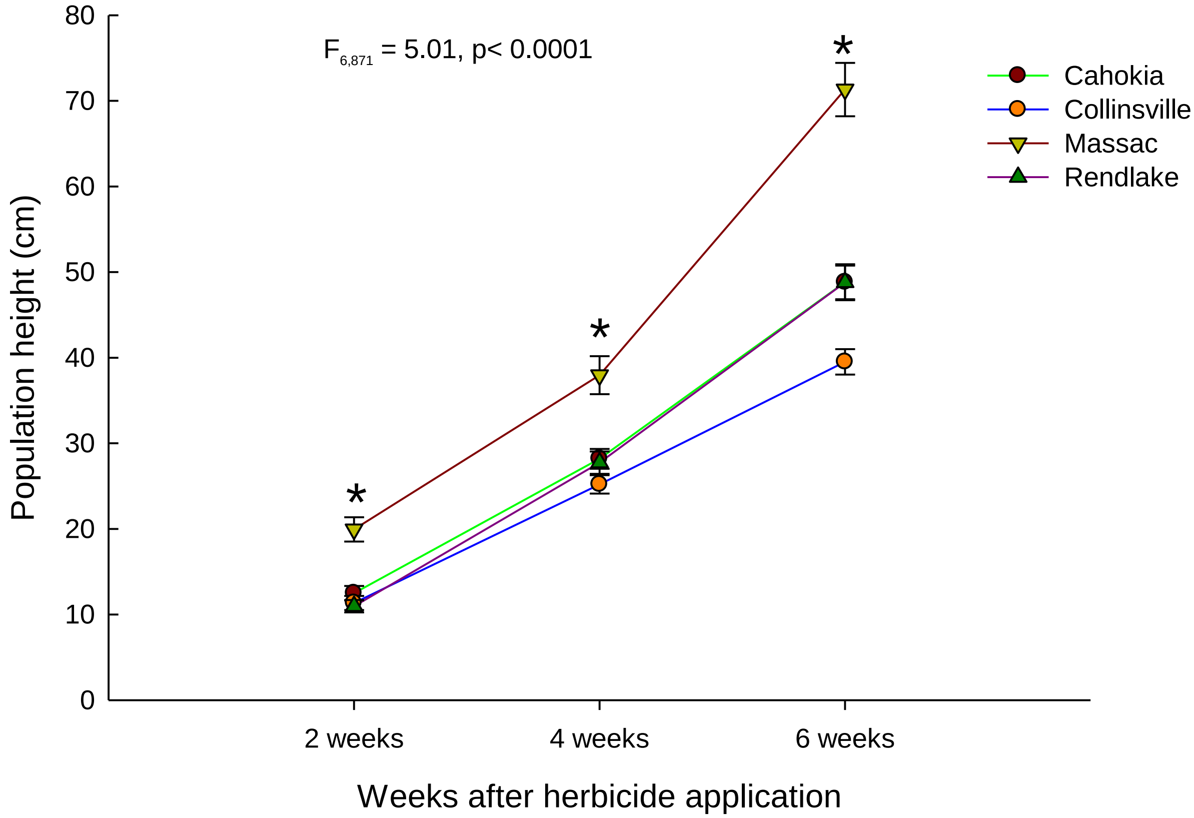

Figure 2.3: Population source (Cahokia, Collinsville, Massac and Rend Lake) by height (cm) at 2, 4, and 6 weeks after herbicide application. Asterisk (*) represents significantly different ($p \leq 0.05$).

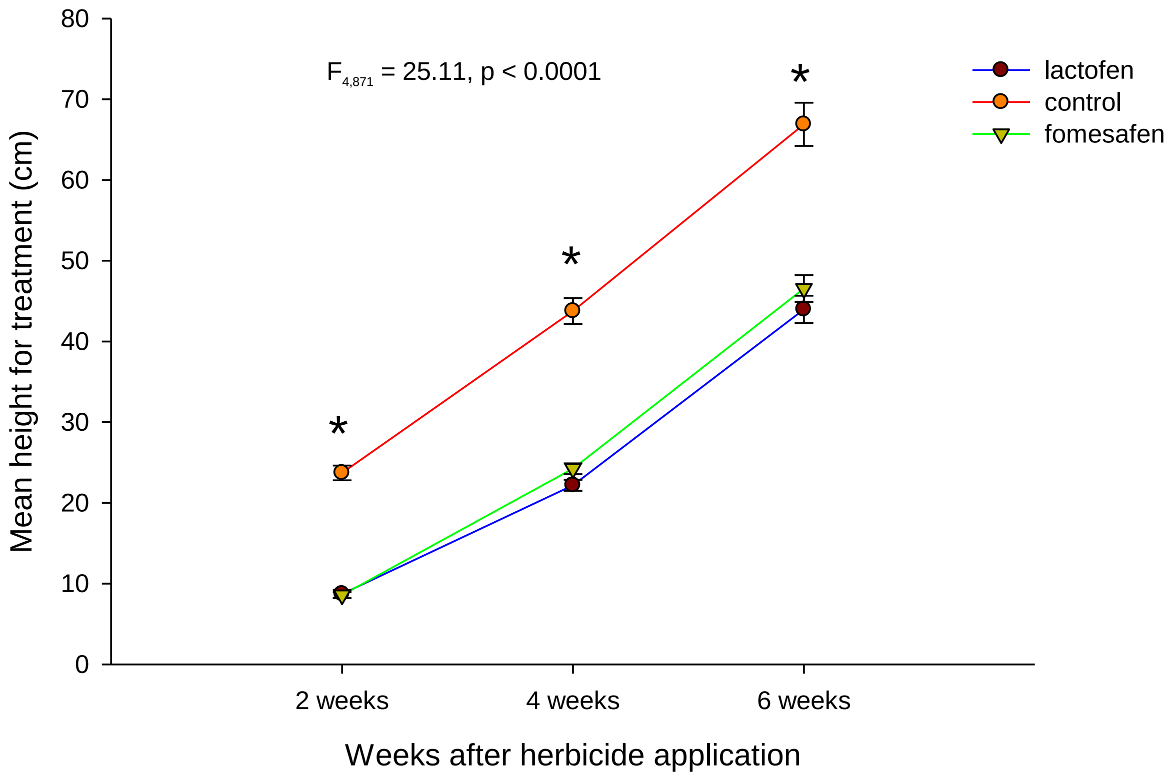

Figure 2.4: The effect of PPO-inhibiting herbicides (lactofen and fomesafen) on population height (cm) at 2, 4, and 6 weeks after herbicide application. Asterisk (*) represents significantly different ($p \leq 0.05$).

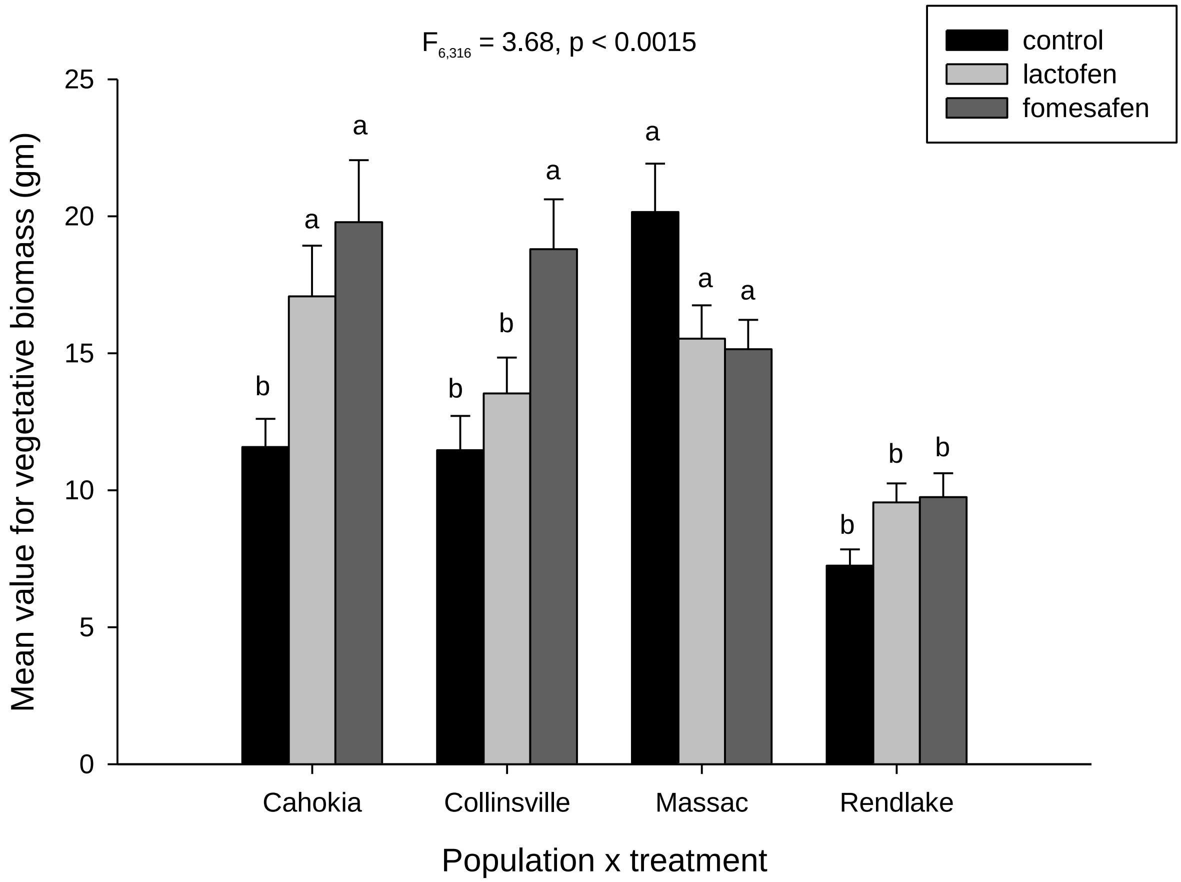

Figure 2.5: Vegetative biomass (mean ± se) at flowering stage of *Amaranthus palmeri* plants from four population sources (Cahokia, Collinsville, Massac and Rend Lake) by treatment following application of PPO-inhibiting herbicides (lactofen and fomesafen). Bars with different letters are significantly different from each other ($p \leq 0.05$).

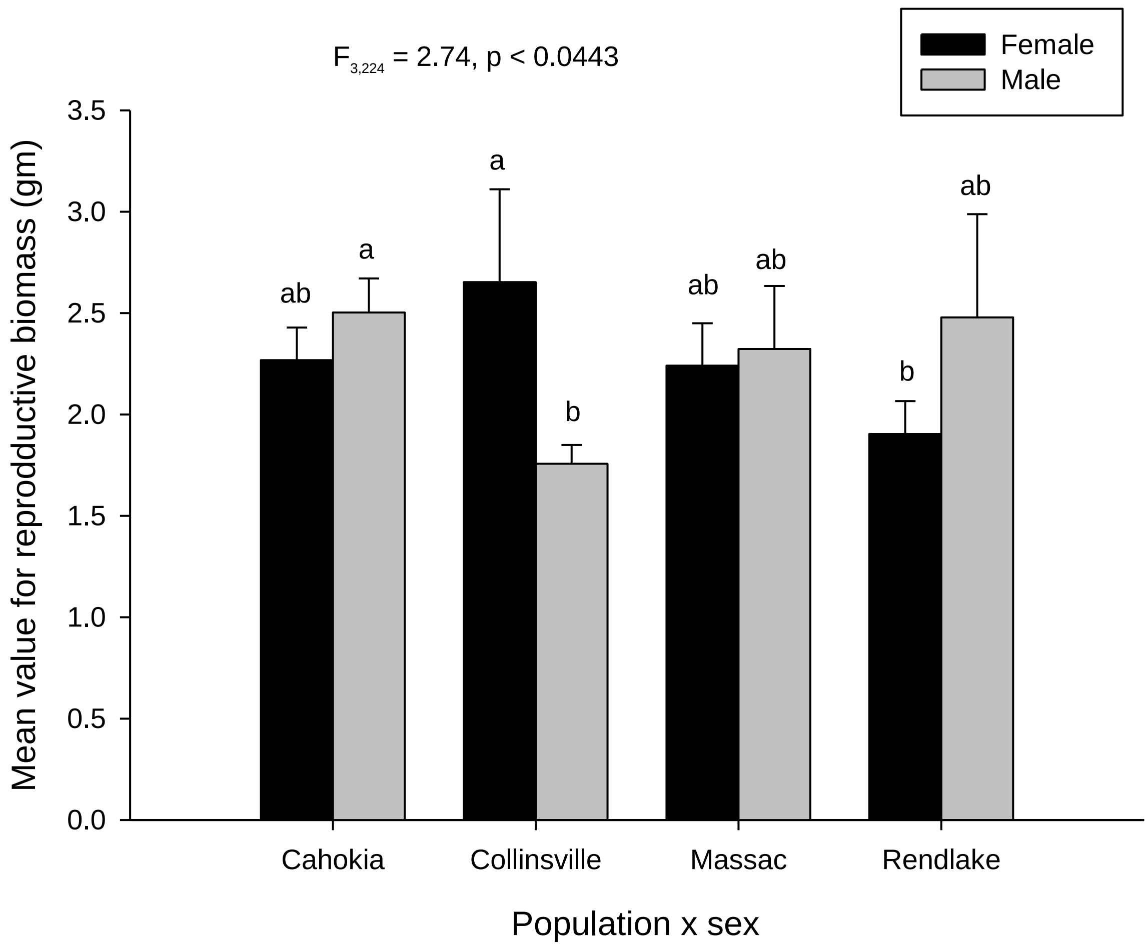

Figure 2.6: Reproductive biomass (mean ± se) of *Amaranthus palmeri* plants from four population sources (Cahokia, Collinsville, Massac, and Rend Lake) by sex following application of PPO-inhibiting herbicides (lactofen and fomesafen). Bars with different letters are significantly different from each other ($p \leq 0.05$).

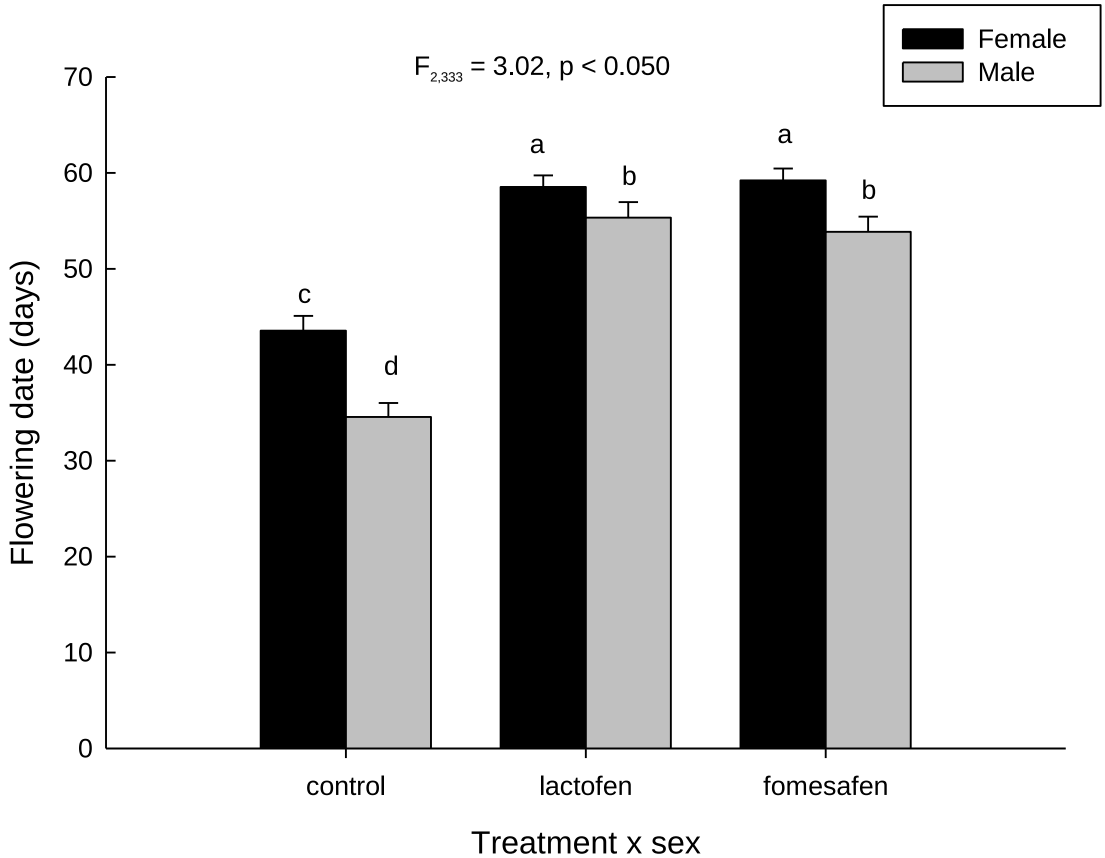

Figure 2.7: Effect of PPO-inhibiting herbicides (lactofen and fomesafen) by sex on flowering date (days). Bars with different letters are significantly different from each other ($p \leq 0.05$).

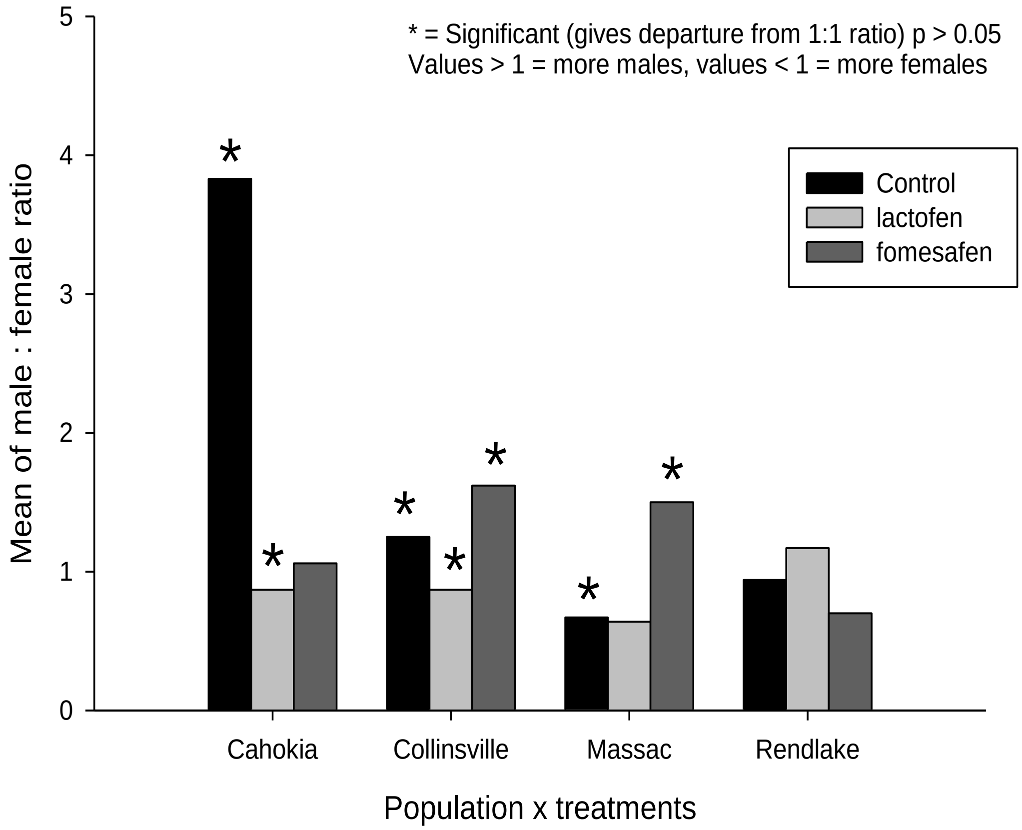

Figure 2.8: The male-to-female sex ratio of *Amaranthus palmeri* plants from four population sources (Cahokia, Collinsville, Massac, and Rend Lake) by treatment following application of PPO-inhibiting herbicides (lactofen and fomesafen). Asterisk (*) represents significant deviation from 1:1 male-to-female sex ratio.

CHAPTER 3

DOES EXPOSURE TO PPO-INHIBITING HERBICIDES ALTER THE MALE-TO-FEMALE SEX RATIO OF *AMARANTHUS PALMERI*?

Abstract

Amaranthus palmeri is a highly competitive weed in crops like cotton and soybean due to its rapid growth and germination and high fecundity. In addition this species has evolved resistance to multiple herbicides. *Amaranthus palmeri* is a dioecious species, with separate male and female plants, which results in obligate outcrossing and increased genetic diversity, allowing potentially rapid evolution of herbicide resistance. Initial studies in the field and greenhouse suggests that male-to-female sex ratios may be altered by exposure to protoporphyrinogen oxidase (PPO)-inhibiting herbicides. In this study, a two-year field experiment was conducted in a soybean field throughout the growing season of 2015 and 2016 in Collinsville, Illinois, USA. This study included 12 pre-emergence (PRE) and 2 post-emergence (POST) PPO-inhibiting herbicide treatments of 10 herbicides with 3 replicates to investigate the variation among sex ratios by treatment. The experiment indicated that *A. palmeri* populations have a female-biased sex ratio in untreated controls. The PRE applications of sulfentrazone (Spartan) at rates of 226.8 to 340.19 g a.i./ha provided the highest control efficacy of *A. palmeri* populations compared to other treatments, with 97 to 70% control at 15 to 56 days after treatment (DAT). On the other hand, POST-only applications had a limited control over the population, with control dropping to 30% at 56 DAT. Application of sulfentrazone (Spartan) and flumioxazin (Valor) herbicide treatments caused the greatest decrease in the density of *A. palmeri* throughout the growing season (less than 2 plants m^{-2}) compared to other treatments. Populations in all treated and non-treated plots for cohorts of plants tagged in early and mid-June exhibited type III survivorship

curves except the early-June cohort of plants treated with pyroxasulfone +fluthiacet-ethyl (Anthem). These type III survivorship curves reflect high early-season mortality of *A. palmeri* seedlings that was exacerbated when treated with pyroxasulfone +fluthiacet-ethyl (Anthem). The survivorship curves for an early July cohort were type II, reflecting a constant mortality risk throughout the life time. Applications of lactofen (Cobra), fomesafen (Flexstar), and flumioxazin (Valor) at 56.70 g a.i. ha^{-1} led to higher female density and biomass and lower male biomass than other treatments. Some of the herbicide treatments expressed a female-biased sex ratio, rather than male-biased or 1:1. Therefore, an understanding of sex determination in *A. palmeri* may lead to more effective control options in the future.

Introduction

The control of *Amaranthus palmeri* is a challenge due to its rapid growth and germination throughout the growing season, high fecundity, and ability to capture more resources than neighboring plants (Keeley et al., 1987; Norsworthy et al., 2012). In one growing season this species can substantially increase the soil seedbank by producing 200,000-600,000 seeds / female plant under ideal conditions and without competition (Keeley et al., 1987; Steckel, 2007). *Amaranthus palmeri* is highly competitive in agricultural cropping systems and can reduce yield by 78 to 91% respectively, at densities of 9 plants m^{-2} for crops like soybean (*Glycine max*) and corn (*Zea mays*) (Bensch et al., 2003; Massinga et al., 2003).

Amaranthus palmeri has become resistant to several herbicide modes of action (the way in which herbicides affects the biological process or enzymatic system of susceptible plants, and interrupt the normal growth and development of the plants, resulting in cell death) including microtubule-inhibitors (Group 3), photosystem II-inhibitors (Group 5), acetolactate synthase-inhibitors (Group 2), 5-enolpyruvylshikimate-3-phosphate synthase-inhibitor (Group 9; glyphosate), hydroxyphenylpyruvate dioxygenase-inhibitors (Group 27), and more recently protoporphyrinogen oxidase- (Group 14) inhibiting herbicides (Heap, 2017).

Overreliance on a single mode of action in the past decade, i.e. glyphosate, has led to a high selection pressure and increased the rate of resistance, as well as altered the effectiveness and success of weed management programs (Riggins and Tranel, 2012). Consequently, pre-emergence (PRE) application of soil residual herbicides, along with integrated weed management tactics, are becoming increasingly important, where herbicide application has limited post-emergence (POST) options, especially for row crops like soybean (*Glycine max*) and cotton (*Gossypium hirsutum*) (Norsworthy et al., 2012) (Table 3.1, 3.2).

Table 3.1: *Herbicide evaluated in field studies for Amaranthus palmeri control.*

Herbicide	Trade name	Manufacturer	Manufacturer location
pyroxasulfone + fluthiacet-ethyl	Anthem®	FMC Corporation	Philadelphia, PA
s-metolachlor	Dual Magnum®	Syngenta Crop Protection	Research Training Park, NC
acetochlor	Warrant®	Monsanto Company	St. Louis, MO
pendimethalin	Prowl® H2O	BASF Corporation	Florham Park, NJ
pyroxasulfone	Zidua®	BASF Corporation	Florham Park, NJ
saflufenacil	Sharpen®	BASF Corporation	Florham Park, NJ
sulfentrazone	Spartan®	FMC Corporation	Philadelphia, PA
flumioxazin	Valor®	Valent U.S.A. Corp.	Walnut Creek, CA
lactofen	Cobra®	Valent U.S.A. Corp.	Walnut Creek, CA
fomesafen	Flexstar®	Syngenta Crop Protection	Research Training Park, NC

Table 3.2: *Pre-emergence (PRE) and post-emergence (POST) percent control of Amaranthus palmeri with chemical name and source of herbicide used.*

Application timing	Chemical	Control efficacy	Reference
PRE	pyroxasulfone + fluthiacet-ethyl	85 to 95 % of *A. palmeri*	(Currie and Geier, 2015)
PRE	s-metolachlor	>95% for all weed species and 92 to 96% of *A. palmeri*	(Clewis et al., 2006; Meyers et al., 2010)
PRE	acetochlor	80% of all weed species and 84% of *A. palmeri*	(Armel et al., 2003; Cahoon et al., 2015a)
PRE	pendimethalin	64% of *A. palmeri*	(Cahoon et al., 2015a)
PRE	pyroxasulfone	>97% of *A. palmeri*	(Meyers et al., 2013)
PRE	saflufenacil	>94% of *A. palmeri*	(Montgomery et al., 2014)
PRE	sulfentrazone	69 to 90% of *A. palmeri*	(Reddy et al., 2015)
PRE	flumioxazin	>90% of *A. palmeri*	(Meyers et al., 2010)
POST	lactofen	>90% of *A. palmeri*	(Berger et al., 2014)
POST	fomesafen	>90% of *A. palmeri*	(Ahmed and Holshouser, 2012)

Dioecious plant populations (having separate male and female plants) commonly exhibit deviation from the expected 1:1 primary sex ratio after parental investment, predicted by Fisher (1930). As *A. palmeri* has evolved herbicide resistance to 6 modes of action (Heap, 2017), there are fewer effective tools left to eradicate this invasive species from the field. Overuse of a single mode of action and evolution of herbicide-resistance has led to increases in the use of protoporphyrinogen oxidase (PPO)-inhibiting herbicides for controlling *A. palmeri* (Sosnoskie et al., 2012). Understanding the population sex ratio via herbicide applications could be an effective way to manage it in the future by controlling the density of seed producing females. Therefore, sex ratio-specific management practices implemented by the growers could have a significant benefit for managing herbicide-resistant *A. palmeri* populations.

Thus, the objective of this study was to investigate the male-to-female sex ratio of *A. palmeri* populations after exposure to PPO-inhibiting herbicides as well as other commonly used herbicides. Hypotheses were: 1) Application of PPO-inhibiting herbicides affect the male-to-female sex ratio; and 2) PPO-inhibiting herbicides differentially affect the growth characteristics of male and female plants.

Materials and methods

The field experiment was conducted throughout the growing seasons of 2015 and 2016 in Collinsville, Illinois (latitude 38.688847’ and longitude -90.016194’) on soil classified as an Orion silt loam (coarse-silty, mixed, super active, mesic Aquic Udifluvents) with 2.5 % organic matter content, CEC of 14, and pH 7.1. Plots were located in the same field, same population of *A. palmeri*, and different areas of the soybean field (APPENDIX D).

The experiment was arranged in a completely randomized block design with three replicates. This study included 12 pre-emergence (PRE) and 2 post-emergence (POST) long chain fatty acid inhibiting and PPO-inhibiting herbicide treatments of 10 herbicides with 3 replicates to investigate the variation among sex ratios by treatment. The experiment was conducted in the presence of soybeans (*Glycine max*, variety P 37T09L) planted in 15 cm row spacing. Experimental sites were selected because they were previously planted to corn and had a high *A. palmeri* density with confirmed resistance to glyphosate. Plots were 3 m wide by 10 m long. Herbicide applications were made using a CO_2 backpack sprayer with a 2 m boom, equipped with XR8002 flat fan nozzles spaced 50 cm apart, delivering 15 gallons per acre at 30 PSI.

Experiments were initiated in a high density Palmer amaranth population within a tilled field. Thirteen PRE and two POST herbicide treatments were applied on 5-22-2016 and 6-13-2016 (Table 3.3).

Table 3.3: *List of 15 different herbicide treatments along with herbicide rates and application timings.*

No.	Herbicide treatment	Rate (g a.i. [a] /ha)	Other rate	Application timing
1	Non treated			
2	pyroxasulfone+fluthiacet-ethyl (Anthem)	226.80	8 fl oz/a	PRE[a]
3	s-metolachlor (Dual magnum)	755.79	1.33 pt/a	PRE
4	acetochlor (Warrant)	1704.78	3 pt/a	PRE
5	pendimethalin (Prowl H2O)	1420.65	2.5 pt/a	PRE
6	pyroxasulfone (Zidua)	70.87	2.5 oz/a	PRE
7	saflufenacil (Sharpen)	28.35	1 fl oz/a	PRE
8	sulfentrazone (Spartan 4F)	226.80	8 fl oz/a	PRE
9	sulfentrazone (Spartan 4F)	283.50	10 fl oz/a	PRE
10	sulfentrazone (Spartan 4F)	340.19	12 fl oz/a	PRE
11	flumioxazin (Valor SX)	56.70	2 oz/a	PRE
12	flumioxazin (Valor SX)	70.87	2.5 oz/a	PRE
13	flumioxazin (Valor SX)	85.05	3 oz/a	PRE
14	Lactofen (Cobra)	340.19	12 fl oz/a	POST[a]
15	Fomesafen (Flexstar)	680.39	24 fl oz/a	POST

[a]Abbreviations: a.i = Active ingredients, PRE = Pre-emergence application, POST = Post-emergence application. Note: Herbicide treatments lactofen and fomesafen were applied with COC (prime oil) amd N-PAK AMS at 1% and 5% volume/volume.

Following the PRE and POST emergence herbicide applications, the plots were monitored at two- week intervals at 15, 29, 44, 57, 71, 85 days after treatment (DAT). Every visit, 20 newly emerged plants were randomly selected and marked with toothpicks. To identify the specific plant, 20 flags were placed in such a position so that flags would not harm the marked plant. Previously marked plants were checked to record mortality. After checking for mortality, toothpicks and flags of dead plants were removed and surviving plants were marked with a loose plastic tag on the stem. This tagging was done in a way that if any other individual emerged beside the marked plant, the tags could easily differentiate that specific plant. Different colored plastic tags were used to differentiate cohorts every time the plots were sampled. The first set of tagged plants were designated as “cohort 1”, the second set two weeks later as “cohort 2”, and the third, final set as “cohort 3” (APPENDIX H).

During the first visit, two 0.5m^2 quadrats were placed in a randomly selected location in each treated and non-treated plot. The corners of each quadrat were marked with permanently with wire flags until the completion of the experiment. All the plants in each quadrat were counted at each sampling (APPENDIX G). When seeds on the plants were mature, data were collected by randomly selecting a 1m^2 area in each plot. Performance was assessed by counting total number of male and female plants within the quadrats, collecting fresh weights of male and female biomass, and seed weight per plot after seed cleaning. Reproductive biomass was collected by separating inflorescences from the stem and weighing. Floral material was hand-stripped from the plant stems and seeds were removed from the terminal and axillary flowers by using sieves of 0.5 to 3.35 mm mesh size. Then the total seed weight was measured (APPENDIX E, F).

Statistical analysis:

A 1-way ANOVA was used to analyze the visual injury ratings with a rating scale of 0 = no injury and, 100 = plant death. Following the application of herbicides, a 3-way repeated mixed model was used to analyze density, a 3-way mixed model analysis was used to analyze the biomass and for testing deviation from a 1:1 sex ratio and, separate paired t-tests on the proportion of males-to-females in each plot were performed. Survivorship was analyzed with a Cox proportional hazard test in SAS 9.4 (Proc phreg) setting the non-treated control as a baseline for comparison. A preliminary proportional hazard test showed that the observed residual was too large relative to randomly generated processes ($p < 0.05$) meaning that a non-proportional model was needed (i.e., survivorship curves for the different cohorts crossed) (Fox, 2000). An appropriate non-proportional model was achieved by fitting Cox proportional hazard models separately for each cohort.

Results

***Amaranthus palmeri* control:**

Timing of application affected the activity of herbicides on *A. palmeri* (Table 3.2). Ninety-five to 97% control of *Amaranthus palmeri* was provided by PRE application of herbicides by 15 DAT except pyroxasulfone (Zidua), which was lower at 92% (Figure 3.1).

All the PRE applications provided 80 to 95% control of *A. palmeri* at the 29th DAT. Sulfentrazone (Spartan 4F) showed the most effective activity against *A. palmeri* at the rate of 12 fl oz/acre. On the other hand, 7 days after all the POST application provided 90% control of *A. palmeri*. Again, 21 days after POST application, control efficacy had been reduced to 20% for all herbicides (Table 3.3, Figure 3.1). Fifty-six days after PRE herbicide application, all the PRE herbicides provided 40 to 77% control of *A. palmeri*, and again sulfentrazone (Spartan 4F) provided the best control (77%). On the other hand, all the POST herbicide applications provided only 33% control of *A. palmeri* by 42 DAT (Table 3.3, Figure 3.1).

***Amaranthus palmeri* density:**

A 3-way repeated measures mixed model analysis ($P < 0.05$) was used to describe the effect of herbicides on *A. palmeri* density. There were significant effects of PRE and POST herbicide treatments with six consecutive time periods (15, 29, 44, 57, 71, 85 DAT) on *A. palmeri* density ($F_{70, 150} = 7.23$, $p < 0.0001$). In case of non-treated control plots, *A. palmeri* density was highest 594.5 +/- 89.1 plants m^{-2} during the very first period of sampling decreasing to 107.0 +/- 18.7 plants m^{-2} by the last sampling period. Density in the POST herbicide treatments fomesafen (Flexstar) and lactofen (Cobra) was 27.0 +/- 6.2 and 31.0+/- 3.6 plants m^{-2}, respectively, which was 4 to 15 times higher than in all the PRE herbicide applied plots (Figure 3.2, Table 3.4) at the last sampling period.

Density varied among the PRE herbicide applied plots. The highest density of *A.palmeri* occurred in pendimethalin (Prowl H_20) and pyroxasulfone
(Zidua) treated plots (32.5 +/- 24.3 and 33.3 +/- 14.8 plants m^{-2}) with the lowest density in sulfentrazone (Spartan 4F) and flumioxazin (Valor SX) at the rate of 12 fl oz/a and 3 oz/a (0.8+/- 0.8 and 2.5 +/- 1.4 plants m^{-2}) at the first sampling period. At the last sampling period, density was 2 to 8 plants m^{-2} for all the pre-emergence applied plots with the lowest density 1.66 +/- 1.66 and 1.66 +/- 0.83 plants m^{-2} in sulfentrazone (Spartan 4F) and flumioxazin (Valor SX) at the rate of 12 fl oz/a and 3 oz/a plots (Figure 3.3, Table 3.5).

Survivorship of *Amaranthus palmeri* after the application of PRE and POST herbicides application:

Survivorship of *Amaranthus palmeri* after the application of PRE and POST herbicides application was investigated by using a non-proportional Cox hazard regression model for each cohort. The test was significant for cohort-1 (Wald Chi-Square = 28.8, $p = 0.004$ at $df = 12$), but not later cohorts 2 and 3 (both $p > 0.05$). Analysis of Maximum Likelihood Estimates for cohort 1 showed that hazard ratios were significantly different to the non-treated control for only treatment 5, (pendimethalin (Prowl H_2O) (microtubule inhibiting herbicides)) indicating that the treatment 5 plants had an increased likelihood of mortality compared to the non-treated control plants (Table 3.6, 3.7, Figure 3.4). No other treatments differed significantly from the non-treated control plants, although hazard ratios of several treatments differed from Prowl and the middle Valor rate (Table 3.6, Figure 3.4).

The survivorship curves for cohort-1 and cohort-2 were type-III, except pyroxasulfone +fluthiacet-ethyl (Anthem) treated plots in cohort-2. Type-III curves reflected a high mortality and rapid decrease in the numbers of seedlings during the first couple of weeks after emergence with subsequent mortality rates decreasing with time (Figure 3.4, 3.5). No survivors were found in sulfentrazone (Spartan 4F) at the rate of 8 and 10 fl oz/a treated plots for cohort-2 by August 1st (Figure 3.5). The survivorship curves for the cohort-3 were type-II, reflecting a constant mortality risk throughout the life time. There were no survivors of cohort-3 plants by August 1st except in acetochlor (Warrant), lactofen (Cobra) and fomesafen (Flexstar) treated plots (Figure 3.6).

Biomass of *Amaranthus palmeri* after the application of PRE and POST herbicides:

Biomass in 2015. There was a significant PRE and POST herbicide treatments by sex interaction effect on biomass of *Amaranthus palmeri* at maturity ($F_{12, 40}$= 2.32, p<0.0234) (Table 3.8). The mean value of female and male biomass (kg) from $1m^2$ area showed that the s-metolachlor (Dual magnum) treated male and female plants had greater biomass than other treated populations but not statistically different from them. On the other hand, pyroxasulfone (Zidua) treated male population had lower biomass than other treated plants (Figure 3.7).

Biomass in 2016. There was a significant PRE and POST herbicide treatments by sex interaction effect on biomass of *Amaranthus palmeri* at maturity ($F_{9, 22}$ = 2.95, p<0.0186) (Table 3.8). The mean value of female and male biomass (kg) from $1m^2$ area showed that the female populations of lactofen (Cobra) treated female plants had greater biomass than lactofen (Cobra) male, fomesafen (Flexstar) male and female, saflufenacil (Sharpen) male, flumioxazin (Valor) 2 oz male, and pyroxasulfone (Zidua) male plants. However, lactofen (Cobra) treated male plants had the lowest biomass and were not significantly different from the biomass of s-metolachlor (Dual magnum) females, fomesafen (Flexstar) males, pendimethalin (Prowl H_2O) females, sulfentrazone (Spartan) 10 oz female, sulfentrazone (Spartan) 8 oz female and flumioxazin (Valor) 2.5 oz female plants (Figure 3.8).

Effect of PRE and POST emergence herbicide treatments on male-to-female sex ratio of *A. palmeri*:

Population sex ratio in 2015. Paired t-tests on the proportions of males to females (testing deviations from 1:1 sex ratio) indicated that non-treated control plots, plots treated with Anthem (pyroxasulfone +fluthiacet-ethyl), Prowl H_2O (pendimethalin), Sharpen (saflufenacil), and Cobra (lactofen) gave a significant departure from 1:1 male-to-female sex ratio (female > male) (Table 3.9, Figure 3.9).

Population sex ratio in 2016. Paired t-tests on the proportions of males to females (testing deviations from 1:1 sex ratio) indicated that non-treated control, Prowl H_2O (pendimethalin), Valor (flumioxazin) at a rate of 2 and 2.5 oz, and Cobra (lactofen) gave a significant departure from 1:1 male-to-female sex ratio (female > male) (Table 3.10, Figure 3.10).

Discussion

***Amaranthus palmeri* control:**

Soil applied PRE treatments provided good control efficacy of *A. palmeri*. This research supports the use of best management practices, including the use of soil residual herbicides, as well as proper herbicide selection, to control at least 90 % of *A. palmeri* (Jonathan et al., 1998). On the other hand, PRE applications of herbicides were more variable and showed insufficient control efficacy that may be due to the environmental conditions or improper timing of herbicide application. The Collinsville population was recently documented to have multiple herbicide resistance to glyphosate and PPO-inhibiting herbicides. The site history does not reveal high selection pressure for PPO-inhibitor-resistance, and it is thought that the resistant biotype may have been brought in by waterfowl traveling along the Mississippi Flyway route for migratory birds. A random selection of 16 plants from the site was sent for quantitative PCR tests in 2016, and the result showed 3 plants (18.8%) were heterozygous for PPO-inhibitor-resistance i.e. possessing the glycine 210 deletion (ΔG210) (Heap, 2017). Therefore, the efficacy of the PRE applications of lactofen (Cobra) and fomesafen (Flexstar) may have been impacted by PPO-inhibitor resistance at the site. Growers need to place greater importance on developing robust weed management programs, operating as if PPO-inhibitor-resistant *Amaranthus palmeri* is already present onsite to avoid control failures (Jenkins et al., 2017).

***Amaranthus palmeri* density:**

Significant self-thinning was observed in the plots, eliminating the smaller plants and suppressing the growth of other individuals in high densities. Self-thinning was more noticeable in the non-treated control plots, which may be due the increasing temperature throughout the summer season, as plants that grow in high densities under high temperatures may be moisture limited (Bazzaz and McConnaughay, 1992). *Amaranthus palmeri* density was lower in PRE herbicide treated plots than the non-treated plots. From all of the PRE treatments, plots treated with pendimethalin (Prowl H_2O) and pyroxasulfone (Zidua) had the highest density of plants by 15 DAT and 85 DAT, which may be due to their short persistence (pendimethalin = 44 days and pyroxasulfone = 16-26 days) in the soil, depending on the soil characteristics and weather conditions. On the other hand, sulfentrazone (Spartan)-treated plots provided good control efficacy and had the lowest density of *A. palmeri* that may be due to the herbicides long persistence in the soil (sulfentrazone = 121-302 days) (Shaner, 2014).

Survivorship:

All the treated, non-treated control plots at the sites appeared to exhibit type II and type III survivorship curves. The type II curves indicated a constant mortality risk throughout the life time (Schwartz et al., 2016b). Type II survivorship curves are characteristic of some perennial herbaceous species, reflecting seasonal oscillations of mortality rates and risk of high mortality during growth and reproductive stages (Schwartz and Gibson, 2014). The type III survivorship curves reflected high mortality with rapid decrease of seedlings during the juvenile stage like other annual species (Klemow and Raynal, 1983). Most of the survivors were found from the first cohorts, individuals that germinate first were generally able to completing their life cycle. These observations provided an estimation of pre-reproductive mortality like other herbaceous species (Hawthorn and Cavers, 1976; Thomas and Dale, 1975). Although the reason for mortality was not recorded but herbicide persistence in the soil, herbivory, shade, competition from neighboring plants, or drought could be the reason associated with high mortality of individuals in different plots (Schwartz et al., 2016c). An extreme drought condition were observed at the early season and the mortality of the species could be a reason of drought condition at that early growing season in the Collinsville, Illinois (APPENDIX I).

Biomass:

Only post-emergence application of lactofen (Cobra), fomesafen (Flexstar), and pre-emergence application of flumioxazin (Valor at 2 oz) had more female biomass than male biomass like the non-treated control plants. It is well known that dioecious species exhibit size dimorphism due to resource allocation with female plants investing more resources than male plants due to the production of reproductive parts and seed (Delph and Herlihy, 2012). However, PPO-inhibitor-resistance was documented in *A. palmeri* from the Collinsville, Illinois study site (Heap, 2017; Jenkins et al., 2017). Resistant plants were confirmed heterozygous for the resistance trait, the glycine 210 deletion, by quantitative PCR on 16 plants randomly selected from plots treated with lactofen or fomesafen. Three out of 16 plants were confirmed resistant for an estimated frequency of 18.8 % of the population. Additionally, two new possible mutations were recently found in *A. palmeri* i.e. *PPX2* (R98G, R98M), conferring resistance to the PPO-inhibitor fomesafen (Giacomini et al., 2017). These data showed 20% control efficacy for *A. palmeri* for these two POST PPO-inhibiting herbicides lactofen and fomesafen. This observed reduced efficacy, as compared to other herbicides, may be the reason for higher biomass in the lactofen treatment.

Sex ratio:

Amaranthus palmeri has become resistant to several herbicide modes of action (Heap, 2017). As a result, effective management practices need to be developed to manage this invasive species in agricultural fields. Manipulation of weed population sex ratios by application of herbicides could be an integrated method of control if the density of female plants could be reduced in the field via herbicides or other environmental influences. This research suggests that different herbicides affect the sex ratio of this *A. palmeri* population in different ways. Some herbicide treatments changed the female-biased population sex ratios to 1:1 by producing a greater male population as compared to non-treated control plants. Production of more male plants through exposure to herbicide application may be the result of decreased cytokinins in plant cells. Cytokinin is a plant growth regulator and is associated with sexually differentiated tissues, producing more females in normal growth conditions and producing more males in stressful conditions. This change has been observed in dioecious plants, such as *Mercurialis annua* L. and *Gymnocladus dioica* (L.) K. Koch, and may give evidence for sex determination of other dioecious plants like *A. palmeri* (Hautala et al., 1986). In conclusion, understanding the factors related to the expression of sex ratio of *A. palmeri* in various management scenarios and in response to various environmental factors may lead to the ability to manipulate the sex ratio of these populations. The ability to create a population without females may improve management scenarios.

Table 3.4: *Influence of pre- and post-emergence herbicides on A. palmeri control.*

Trade name	Herbicide treatment (active ingredient)	Rate (g a.i./ha)	Application timing	Control % 15 DAT[a]	29 DAT	56 DAT
Non treated						
Anthem	pyroxasulfone+fluthiacet-ethyl	226.80	PRE[a]	96ab	88bcd	47defg
Dual magnum	s-metolachlor	755.79	PRE	96ab	87bcde	42fg
Warrant	acetochlor	1704.78	PRE	95ab	84def	57bcde
Prowl H2O	pendimethalin	1420.65	PRE	95ab	85def	58bcd
Zidua	pyroxasulfone	70.87	PRE	92c	85def	40g
Sharpen	saflufenacil	28.35	PRE	95b	80f	58bcd
Spartan 4F	sulfentrazone	226.80	PRE	96ab	91abc	68abc
Spartan 4F	sulfentrazone	283.50	PRE	97ab	92ab	70ab

Table 3.4(Continued)

Trade name	Herbicide treatment (active ingredient)	Rate (g a.i./ha)	Application timing	Control %		
				15 DAT[a]	29 DAT	56 DAT
Spartan 4F	sulfentrazone	340.19	PRE	97a	95a	77a
Valor SX	flumioxazin	56.70	PRE	95b	82ef	43efg
Valor SX	flumioxazin	70.87	PRE	96ab	87bcdef	42fg
Valor SX	flumioxazin	85.05	PRE	96ab	86 cdef	55cef
				7 DAT	15 DAT	35 DAT
Cobra	lactofen	340.19	POST[a]	90c	20g	33g
Flexstar	fomesafen	680.39	POST	90c	20g	33g

[a]Abbreviations: PRE= Pre-emergence application, POST = Post-emergence application, , DAT=days after treatment for PRE and POST applications. [b]*Amaranthus palmeri* control was evaluated based on a visual scale of 0 (no control) to 100 (complete control). Means followed by the same letters are not significantly different ($p \leq 0.05$).

Table 3.5: *Amaranthus palmeri density in 1 m^2 plots over the course of the study.*

Herbicide treatment	15 DAT	29 DAT	44 DAT	57 DAT	71 DAT	85 DAT
Non treated	594.5 +/- 89.1	320.0 +/- 10.0	230.0 +/- 19.4	148.3 +/- 29.8	139.1 +/- 29.0	107.5 +/- 18.7
pyroxasulfone+fluthiacet-ethyl (Anthem)	23.3+/- 20.8	10.0 +/- 5.0	15.0 +/- 9.0	9.1 +/- 4.4	5.8 +/- 3.3	5.0 +/- 3.8
s-metolachlor (Dual magnum)	14.1 +/- 4.16	6.6 +/- 3.3	15.8 +/- 8.7	13.3 +/- 5.4	11.6 +/- 5.8	7.5 +/- 3.8
acetochlor (Warrant)	17.5 +/- 11.4	15.0 +/- 5.2	10.8 +/- 4.4	10.0 +/- 6.2	5.0 +/- 3.8	4.1 +/- 4.1
pendimethalin (Prowl H2O)	32.5 +/- 24.2	25.0 +/- 5.2	21.6 +/- 8.3	11.6 +/- 3.6	11.6 +/- 3.6	7.5 +/- 2.5
pyroxasulfone (Zidua)	33.3 +/- 14.8	18.3 +/- 4.6	14.1 +/- 8.2	10.8 +/- 4.6	9.1 +/- 5.4	6.6 +/- 4.1
saflufenacil (Sharpen)	26.6 +/- 21.6	30.8 +/- 6.0	18.3 +/- 4.4	10.8 +/- 0.8	5.8 +/- 1.6	5.8 +/- 1.6
sulfentrazone (Spartan 4F)	9.1 +/- 6.6	8.3 +/- 4.6	8.3 +/- 3.3	5.8 +/- 0.8	4.1 +/- 0.8	4.1 +/- 0.8
sulfentrazone (Spartan 4F)	4.1 +/- 1.6	9.1 +/- 1.6	5.0 +/- 3.8	4.1 +/- 3.0	4.1 +/- 3.0	4.1 +/- 3.0

Table 3.5(Continued)

Herbicide treatment	15 DAT	29 DAT	44 DAT	57 DAT	71 DAT	85 DAT
sulfentrazone (Spartan 4F)	0.8 +/- 0.8	0.0000	1.6 +/- 1.6	1.6 +/- 1.6	1.6 +/- 1.6	1.6 +/- 1.6
flumioxazin (Valor SX)	13.3 +/- 2.2	4.1 +/- 1.6	8.3 +/- 2.2	8.3 +/- 4.6	6.6 +/- 3.0	5.0 +/- 2.8
flumioxazin (Valor SX)	3.3 +/- 3.3	4.1 +/- 0.8	7.5 +/- 1.4	5.0 +/- 1.4	5.0 +/- 1.4	5.0 +/- 1.4
flumioxazin (Valor SX)	2.5 +/- 1.4	10.8 +/- 5.8	10.0 +/- 3.8	2.5 +/- 0	1.6 +/- 0.8	1.6 +/- 0.8
lactofen (Cobra)			204.1 +/- 79.0	85.8 +/- 19.5	46.6 +/- 2.2	31.6 +/- 3.6
fomesafen (Flexstar)			196.6 +/- 67.7	82.5 +/- 2.5	43.3 +/- 9.6	27.5 +/- 6.2

Table 3.6: *Statistical results from the Cox proportional hazard regression model. Hazard ratios (± 95% confidence limits) provide a comparison of survivorship compared with non-treated control plants (Hazard Ratio = 1.0, $p \leq 0.05$).*

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio
sulfentrazone (Spartan 12 oz)	1	0.20	0.33	0.36	0.54	1.22	0.63/2.35
flumioxazin (Valor 2 oz)	1	0.08	0.24	0.10	0.74	1.08	0.66/1.76
flumioxazin (Valor 2.5 oz)	1	-0.35	0.27	1.73	0.18	0.69	0.41/1.19
flumioxazin (Valor 3 oz)	1	0.30	0.24	1.51	0.21	1.35	0.83/2.18
pyroxasulfone+ fluthiacet-ethyl (Anthem)	1	0.11	0.24	0.19	0.65	1.11	0.68/1.81
s-metolachlor (Dual magnum)	1	-0.41	0.26	2.39	0.12	0.66	0.39/1.11
acetochlor (Warrant)	1	0.09	0.26	0.13	0.71	1.10	0.65/1.83
pendimethalin (Prowl H_20)	1	0.67	0.22	9.22	0.002	1.95	1.26/3.01

Table 3.6(Continued)

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio
pyroxasulfone (Zidua)	1	0.07	0.24	0.09	0.75	1.07	0.66/1.75
saflufenacil (Sharpen)	1	0.03	0.26	0.01	0.89	1.03	0.62/1.73
sulfentrazone (Spartan 8 oz)	1	0.27	0.28	0.967	0.32	1.31	0.76/2.28
sulfentrazone (Spartan 10 oz)	1	0.16	0.28	0.33	0.56	1.17	0.67/2.06

Table 3.7: *Survivorship of A. palmeri after the application of PRE and POST emergence herbicides as recorded over the course of the study. 1st sampling period (log survivors):*

Herbicide treatment	15 DAT	29 DAT	44 DAT	57 DAT	71 DAT
non treated	1.78	1.56	1.42	1.32	1.32
pyroxasulfone+fluthiacet-ethyl (Anthem)	1.65	1.47	1.2	1.14	1.14
s-metolachlor (Dual magnum)	1.73	1.64	1.5	1.5	1.5
acetochlor (Warrant)	1.58	1.45	1.12	1.12	1.11
pendimethalin (Prowl H2O)	1.74	1.56	1.18	0.71	0.71
pyroxasulfone (Zidua)	1.70	1.50	1.32	1.30	1.30
saflufenacil (Sharpen)	1.6	1.46	1.26	1.18	1.17
sulfentrazone (Spartan 4F)	1.4	1.28	0.95	0.71	0.71
sulfentrazone (Spartan 4F)	1.45	1.28	1.04	0.95	0.95
sulfentrazone (Spartan 4F)	1.25	0.9	0.78	0.78	0.78
flumioxazin (Valor SX)	1.70	1.46	1.34	1.30	1.3
flumioxazin (Valor SX)	1.72	1.56	1.49	1.49	1.47
flumioxazin (Valor SX)	1.67	1.56	1.18	1.18	1.17

2nd sampling period (log survivors):

Herbicide treatment	29 DAT	44 DAT	57 DAT	71 DAT
non treated	1.78	0.95	0.9	0.47
pyroxasulfone+fluthiacet-ethyl (Anthem)	1.74	0.84	0.6	0.49
s-metolachlor (Dual magnum)	1.70	1.00	0.3	0.30
acetochlor (Warrant)	1.60	0.84	0.49	0.49
pendimethalin (Prowl H2O)	1.78	1.66	0.64	0.48
pyroxasulfone (Zidua)	1.7	1.07	0.48	0.3
saflufenacil (Sharpen)	1.7	1.3	0.30	0.3
sulfentrazone (Spartan 4F)	1.56	0.78	-1.0	-1.0
sulfentrazone (Spartan 4F)	1.59	0.78	0.3	0.78
sulfentrazone (Spartan 4F)	1.28	0.90	0.78	1.3
flumioxazin (Valor SX)	1.70	1.46	1.34	1.48
flumioxazin (Valor SX)	1.72	1.56	1.48	1.48
flumioxazin (Valor SX)	1.67	1.56	1.18	1.18

3rd sampling period (log survivors):

Herbicide treatment	44 DAT	57 DAT	71 DAT
Non treated	1.88	1.05	-1.0
pyroxasulfone+fluthiacet-ethyl (Anthem)	1.71	0.8	-1.0
s-metolachlor (Dual magnum)	1.1	1.05	-1.0
acetochlor (Warrant)	1.3	0.7	0.41
pendimethalin (Prowl H2O)	1.8	0.77	-1.0
pyroxasulfone (Zidua)	1.3	0.7	-1.0
saflufenacil (Sharpen)	1.83	0.1	-1.0
sulfentrazone (Spartan 4F)	1.33	0.1	-1.0
sulfentrazone (Spartan 4F)	1.32	0.57	-1.0
sulfentrazone (Spartan 4F)	1.33	0.1	-1.0
flumioxazin (Valor SX)	1.6	0.88	-1.0
flumioxazin (Valor SX)	1.84	1.0	-1.0
flumioxazin (Valor SX)	1.80	0.58	-1.0
Lactofen (Cobra)	1.88	1.18	1.08

3rd sampling period (log survivors) (Continued)

Herbicide treatment	44 DAT	57 DAT	71 DAT
Fomesafen (Flexstar)	1.88	1.05	0.7

Table 3.8: *Statistical results (F and P values) from 2-way ANOVA testing the effects of herbicide treatments and sex on biomass of A. palmeri. Significant values (p ≤ 0.05) highlighted in bold.*

Biomass in 2015				
Effect	DF		F value	Pr > F
treat	12	40	1.71	0.1006
sex	1	40	11.99	0.0013
treat*sex	12	40	2.32	0.0234
Biomass in 2016				
Effect	DF		F value	Pr > F
treat	9	22	**2.25**	**0.05**
sex	1	22	2.41	0.1345
treat*sex	9	22	**2.95**	**0.0186**

Table 3.9: *Results from paired t-tests, testing the deviation from a 1:1 sex ratio following the effects of PRE and POST herbicide treatments in 2015. Values ($p \leq 0.05$) highlighted in bold were sigificantly different from a 1:1 male-to-female sex ratio.*

Treatments	DF	T value	Pr > t	Male: Female
Nontreated	2	**-6.78**	**0.0211**	**1:5**
pyroxasulfone+fluthiacet-ethyl (Anthem)	2	**-5.50**	**0.0315**	**1:1.3**
s-metolachlor (Dual magnum)	2	-3.30	0.0809	1:1.13
acetochlor (Warrant)	2	-3.31	0.0885	1:1
pendimethalin (Prowl H2O)	2	**-9.95**	**0.0099**	**1:2.6**
pyroxasulfone (Zidua)	2	-1.69	0.2326	1:1.6
saflufenacil (Sharpen)	2	**-7.12**	**0.0192**	**1:4.25**
sulfentrazone (Spartan 8 oz)	2	-2.95	0.0985	1:1.6
sulfentrazone (Spartan 10 oz)	2	-2.75	0.1107	1:3.3

Table 3.9(Continued)

Treatments	DF	T value	Pr > t	Male: Female
sulfentrazone (Spartan 12 oz)	2	-3.21	0.0848	1:4.3
flumioxazin (Valor 2 oz)	2	-2.75	0.1110	1:1.12
flumioxazin (Valor 2.5 oz)	2	-2.75	0.1645	1:1.17
flumioxazin (Valor 3 oz)	2	-3.21	0.0848	1:1.7
lactofen (Cobra)	2	**-18.81**	**0.0028**	**1:2.3**
fomesafen (Flexstar)	2	-1.00	0.4226	1:0.62

Table 3.10*: Results from paired t-tests, testing the deviation from a 1:1 sex ratio following the effects of PRE and POST herbicide treatments in 2016. Values ($p \leq 0.05$) highlighted in bold were sigificantly different from a 1:1 male-to-female sex ratio.*

Treatments	DF	T value	Pr > t	Male: Female
Nontreated	2	**-4.37**	**0.0485**	**1:2.93**
pyroxasulfone+fluthiacet-ethyl (Anthem)	2	-1.25	0.3377	1:2
s-metolachlor (Dual magnum)	2	-3.50	0.0728	0:4
acetochlor (Warrant)	2	-0.25	0.8259	1:1
pendimethalin (Prowl H2O)	2	**-Infty**	**<.0001**	**0:1.66**
pyroxasulfone (Zidua)	2	-0.69	0.5598	1:1
saflufenacil (Sharpen)	2	-0.25	0.8259	1:1
sulfentrazone (Spartan 8 oz)	2	-2.50	0.1296	0:0.33
sulfentrazone (Spartan 10 oz)	2	-2.50	0.1296	0:1.66

Table 3.10(Continued)

Treatments	DF	T value	Pr > t	Male: Female
sulfentrazone (Spartan 12 oz)	2	-0.87	0.4778	1:3
flumioxazin (Valor 2 oz)	2	**-17**	**0.0034**	**1:2.5**
flumioxazin (Valor 2.5 oz)	2	**-Infty**	**<.0001**	**0:2**
flumioxazin (Valor 3 oz)	2	-1.25	0.3377	1:1.5
lactofen (Cobra)	2	**-Infty**	**<.0001**	**1:3**
fomesafen (Flexstar)	2	-1.50	0.2724	1:0.62

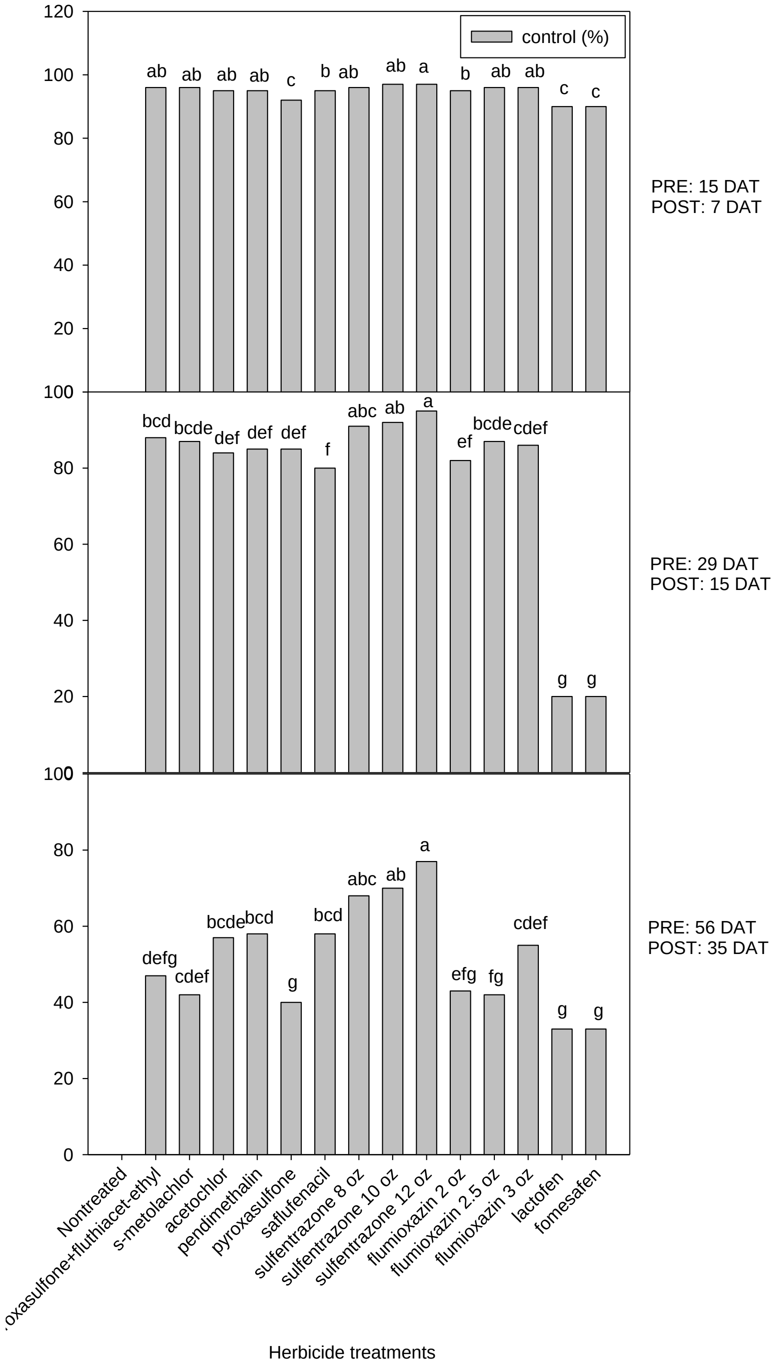

Figure 3.1: Visual injury ratings of *A. palmeri* following pre-emergence (PRE) and post-emergence (POST) herbicide applications. Twelve of the herbicide applications were PRE-only treatments. Two applications, lactofen and fomesafen, were POST-only treatments. Bars with different letters are significantly different from each other (($p \leq 0.05$).

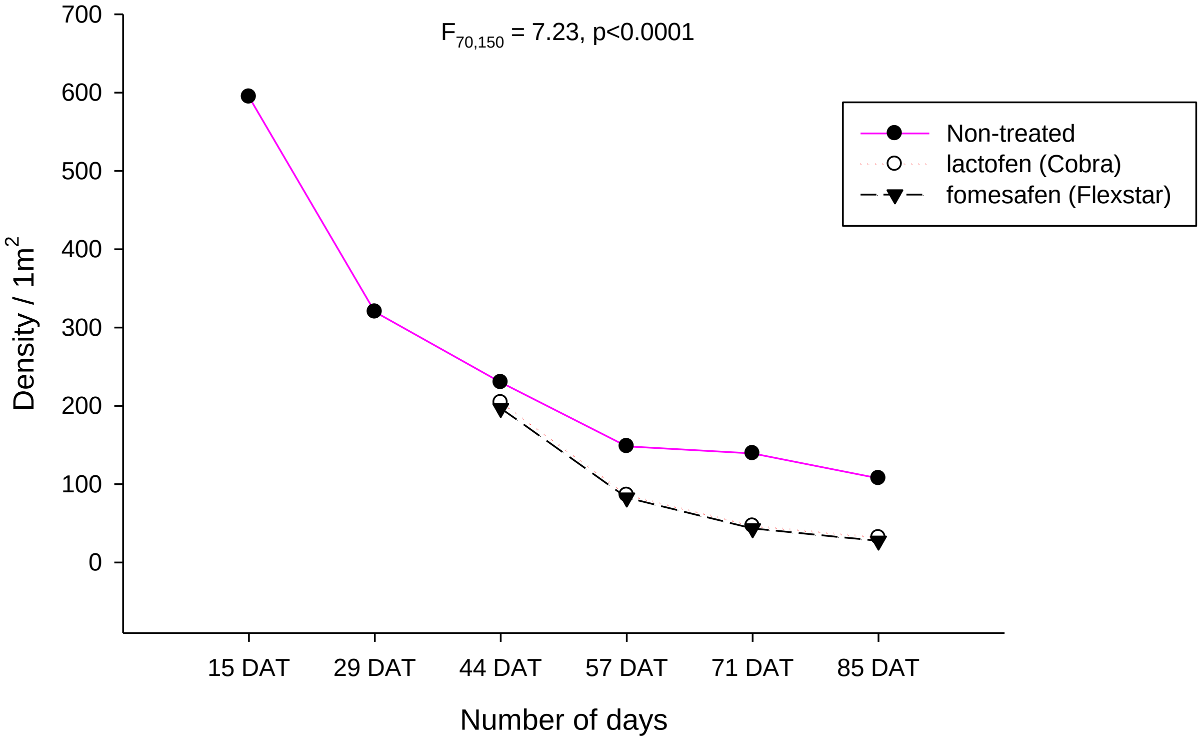

Figure 3.2: Density of *A. palmeri* plants per $1m^2$ in non-treated control plots and in plots treated with a post-emergence application of lactofen and fomesafen.

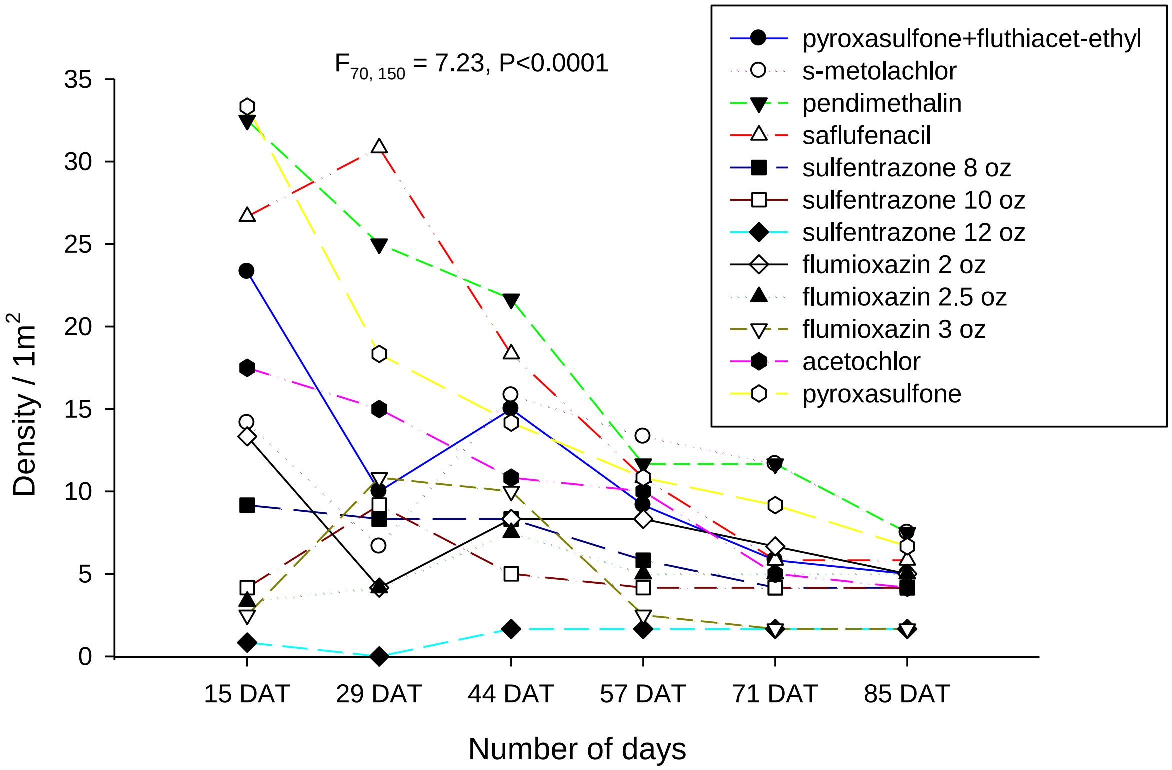

Figure 3.3: Density of plants per $1m^2$ in plots with a pre-emergence herbicide application.

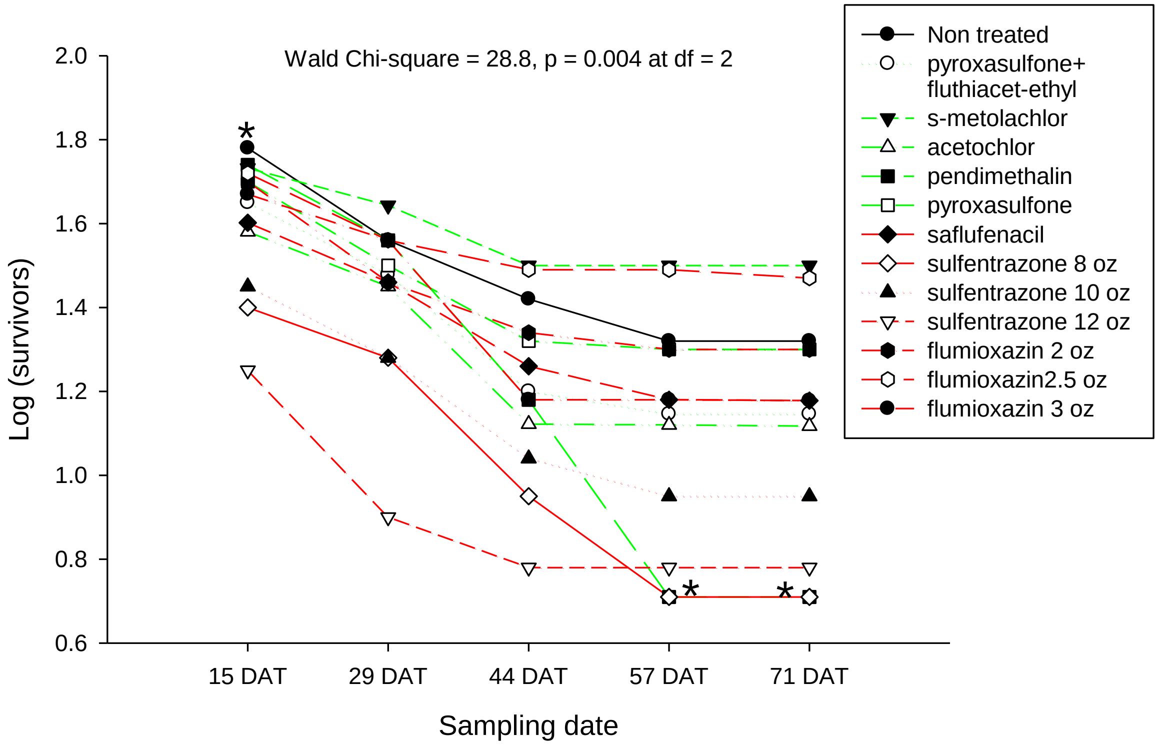

Figure 3.4: Survivorship curves of 1st cohort. Asterisk (*) represents significant likelihood of mortality of plants in the pendimethalin treatment compared to plants in the non-treated control plots.

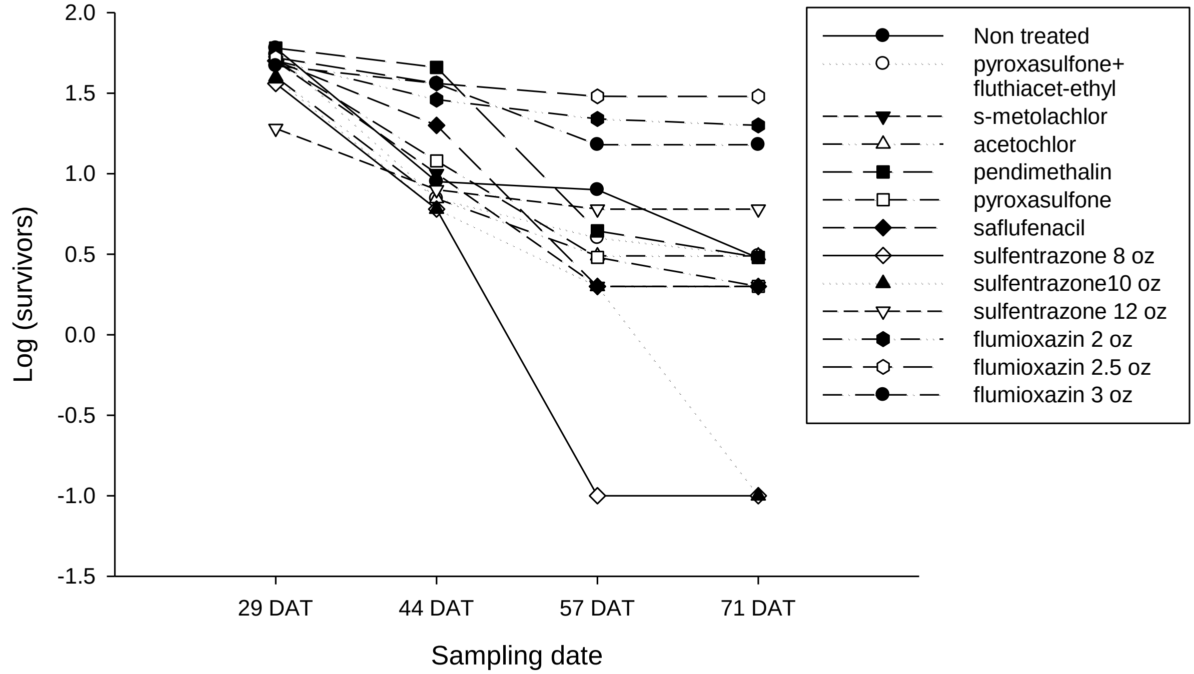

Figure 3.5: Survivorship curves of 2nd cohort.

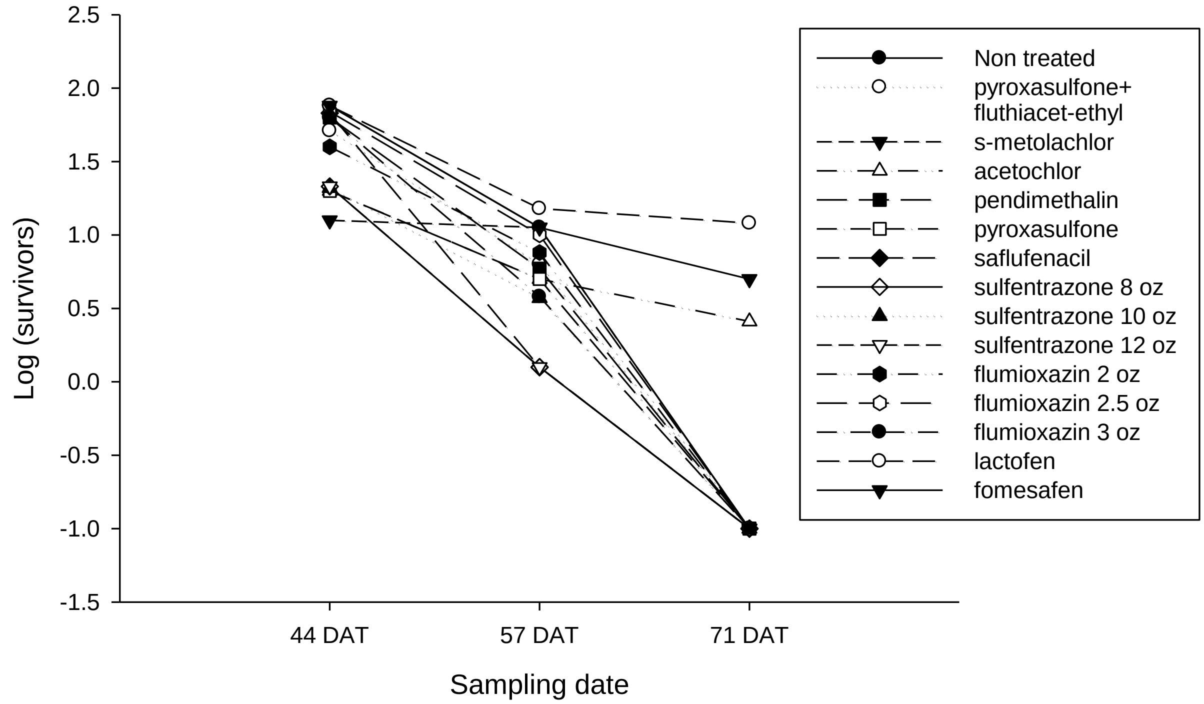

Figure 3.6: Survivorship curves of 3rd cohort.

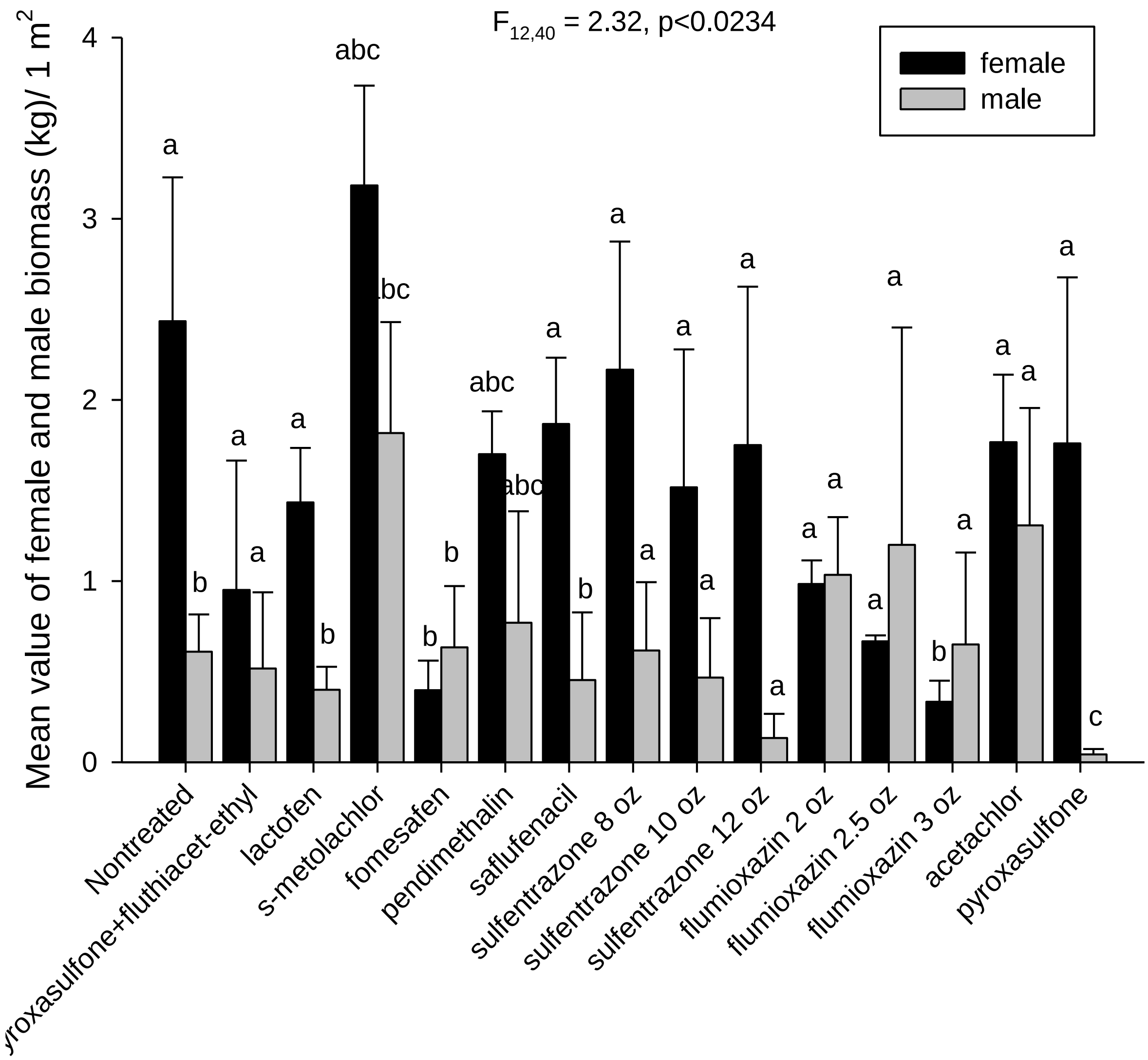

Figure 3.7: Biomass (kg) from $1m^2$ area by sex (female and male) following application of pre- and post-emergence herbicide treatments during the year 2015. Bars with different letters are significantly different from each other ($p \leq 0.05$).

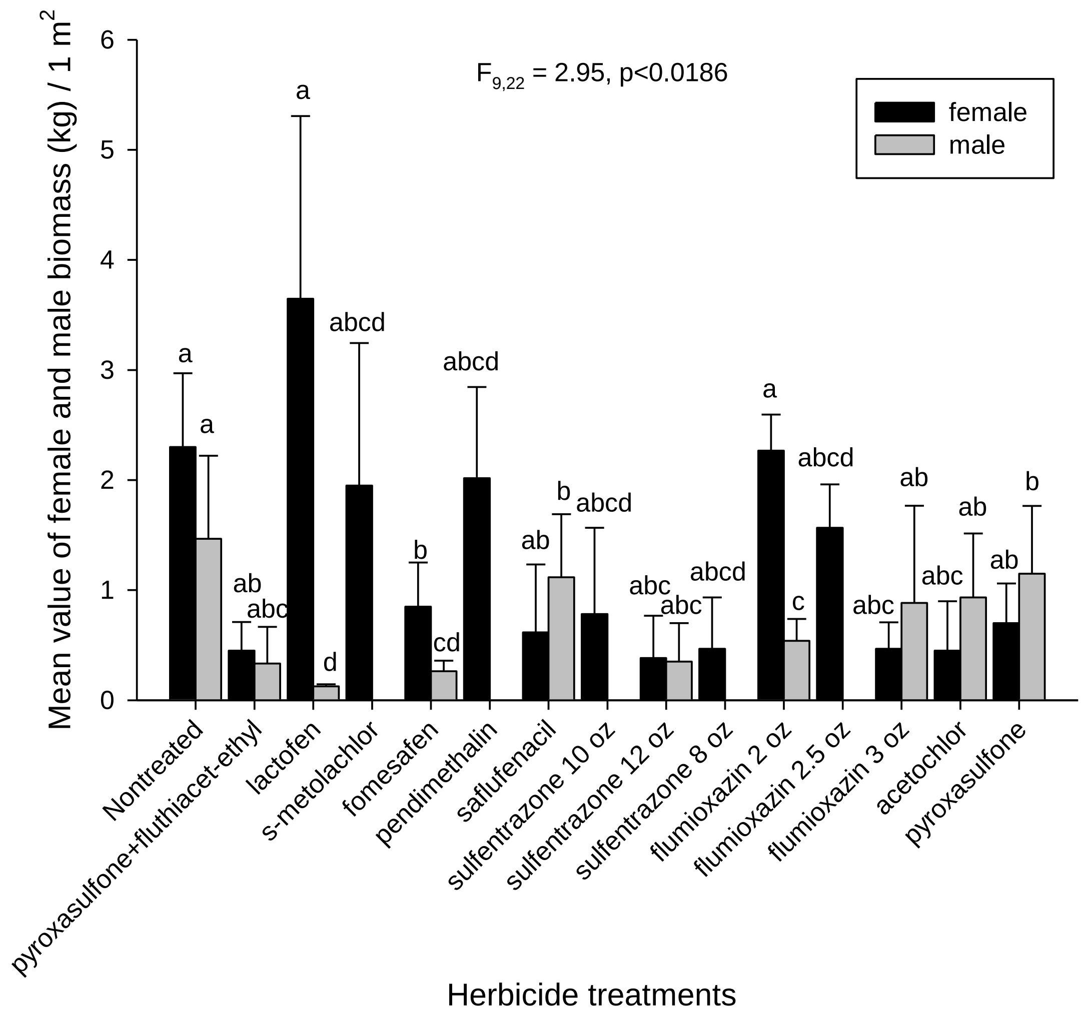

Figure 3.8: Biomass (kg) from 1m^2 area by sex (female and male) following application of pre- and post-emergence herbicide treatments during the year 2016. Bars with different letters are significantly different from each other ($p \leq 0.05$).

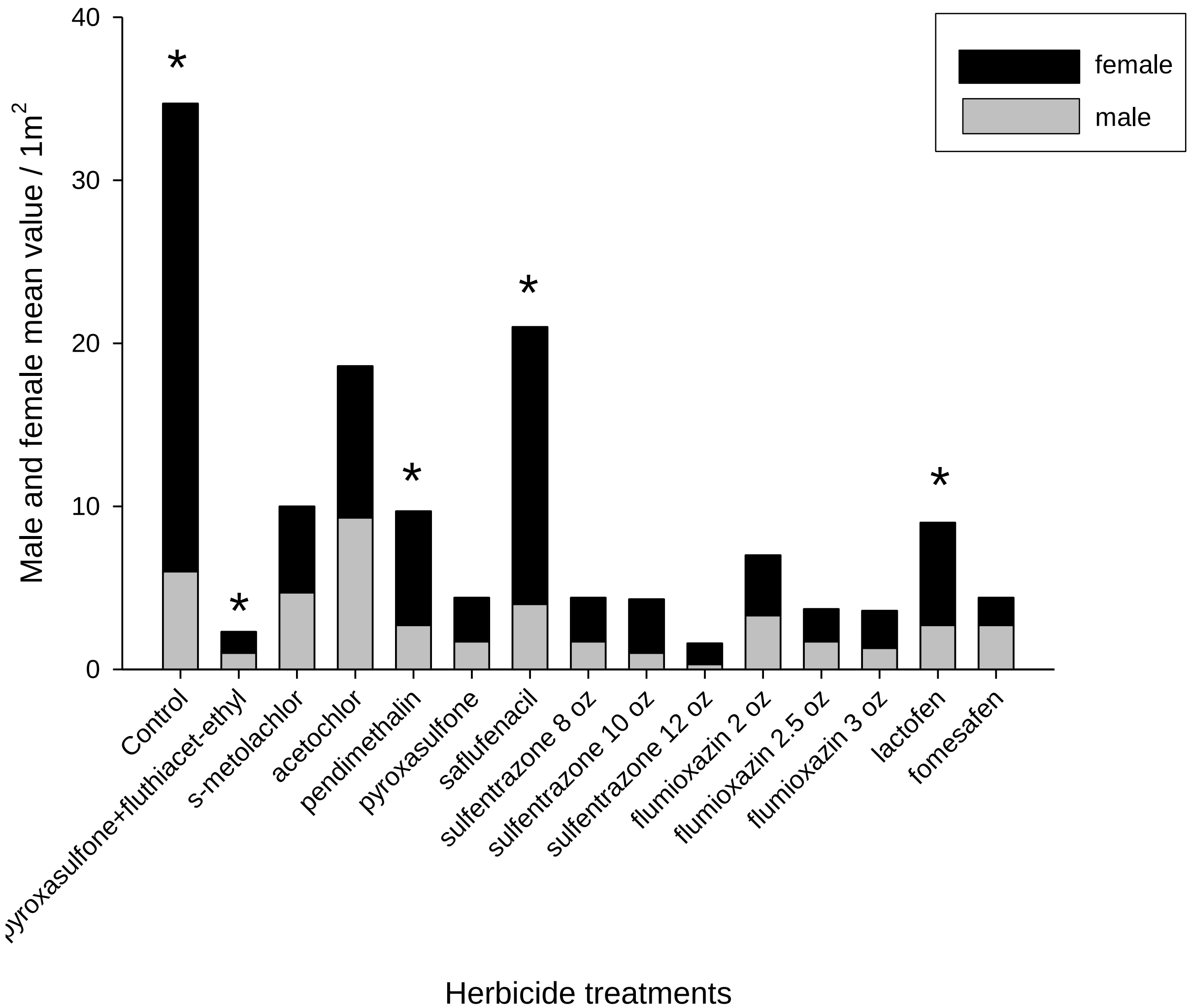

Figure 3.9: Male-to-female sex ratios of *A. palmeri* by herbicide treatments following pre- and post-emergence application in 2015. Asterisk (*) represents significant departure from 1:1 male-to-female sex ratio (Chi-square tests, $p \leq 0.05$).

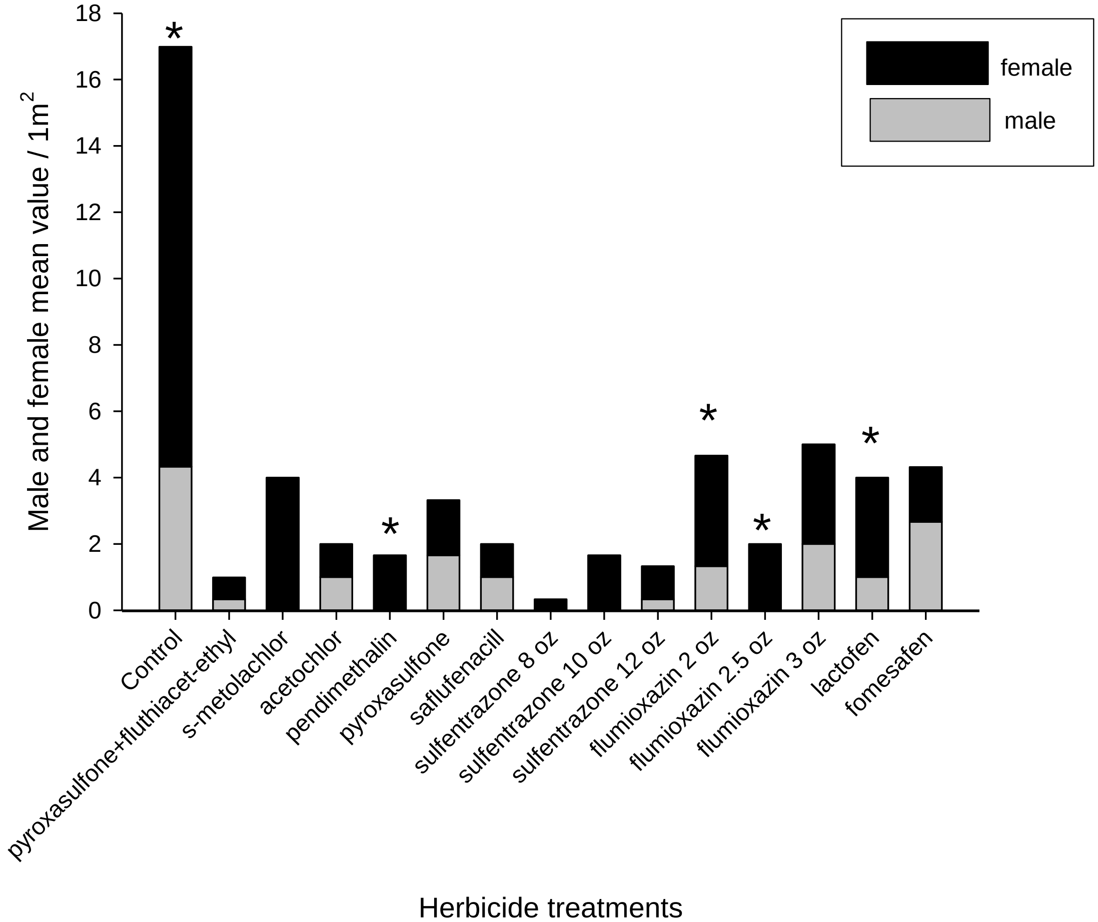

Figure 3.10: Male-to-female sex ratios of *A. palmeri* by herbicide treatments following pre- and post-emergence application in 2016. Asterisk (*) represents significant departure from 1:1 male-to-female sex ratio (Chi-square tests $p \leq 0.05$).

CHAPTER 4

CONCLUSION

The final chapter of this thesis consists of objectives and hypotheses that were proposed at the beginning of the document, a brief summarization of the data chapters (2 and 3), and an overall conclusion of the study.

Objective:

The objective of the study is to investigate the male-to-female sex ratio of *Amaranthus palmeri* after exposure to PPO-inhibiting herbicides.

Hypotheses:

Hypothesis 1: Application of PPO-inhibiting herbicides affects the male-to-female sex ratio of *Amaranthus palmeri*.

Hypothesis 2: PPO-inhibiting herbicides differentially affect the growth characteristics of male and female plants.

Chapter 2: Effect of PPO-inhibiting herbicides on growth characteristics and sex ratio of a dioecious weed species *Amaranthus palmeri* (Palmer Amaranth).

Summary:

This study was one of the first studies to look for an effect of herbicides on sex ratios in plant populations. This study investigates the effect of two protoporphyrinogen oxidase (PPO)-inhibiting herbicide treatments of lactofen (Cobra) and fomesafen (Flexstar) on four different Illinois populations (Cahokia, Collinsville, Rend Lake, and Massac) of *Amaranthus palmeri*. Depending on the population, herbicide treatments altered the male-to-female sex ratio to 1:1 or male-biased in contrast to the female-biased field observations. The results of this study provide a better understanding of the factors affecting sex ratio and the potential manipulation of these factors towards management protocols of *A. palmeri*.

Chapter 3: Does exposure to PPO-inhibiting herbicides alter the male-to-female sex ratio of *Amaranthus palmeri*?

Summary:

This study included 12 pre-emergence (PRE) and 2 post-emergence (POST) PPO-inhibiting herbicide treatments of 10 herbicides with 3 replicates to investigate the variation among sex ratios by treatment. The field study indicated that *A. palmeri* populations have a female-biased sex ratio in untreated controls. PRE application of sulfentrazone (Spartan) at rates of 226.8 to 340.19 g a.i./ha provided the highest control efficacy of *A. palmeri* populations compared to other treatments, with 97 to 70% control at 15 to 56 days after treatment (DAT). Seedling cohorts emerging in early and mid-June exhibited type-III survivorship curves (Cohort-1 and cohort -2) in all treated and non-treated plots, except pyroxasulfone +fluthiacet-ethyl (Anthem)- treated plots. Type-III survivorship curves reflect high early-season mortality of *A. palmeri* seedlings. The survivorship curves for early July emerging seedlings (cohort-3) were type-II, reflecting a constant mortality risk throughout time. Lactofen (Cobra), fomesafen (Flexstar), and flumioxazin (Valor) at 2 oz treated plots led to higher female density and biomass and lower male biomass than other treatments. These results suggest that understanding of sex ratio in *A. palmeri* could be a better control options for the future compared with traditional control methods.

Overall summary:

The findings of both the chapters were contradictory. The findings of greenhouse study (chapter 2) indicated more male-biased treatments in the four populations, while the findings of field study were more likely to indicate a female-biased population. *Amaranthus palmeri* has no sex chromosomes, and therefore, it is uncertain which factors or gene regions are responsible for sex expression.

Based on previous literature, several factors that might affect the sex of this plant are: photoperiod, day length, soil moisture, and soil fertility. Dioecious species showed sex-related differences in stress tolerance. A recent study showed that water stress, water logging condition, extreme acidity, high and low temperature, freezing, chilling, elevated UV-B radiation, nitrogen, and phosphorus deficiency, manganese, and copper stress could produce more male populations in dioecious species (Juvany and Munné-Bosch, 2015).

Herbicide is an important factor in the agricultural environment. Depending on the chemical used, herbicides are applied to the soil or plant foliage during the seedling stage to kill the unwanted plants. PPO-inhibiting herbicides control the weed population by disrupting enzymatic pathways, raising the possibility of a resulting hormonal imbalance following the herbicide application.

Cytokinin is the most important hormone for the growth and development of plant populations. In optimal conditions, cytokinins produce more female population. Under stressful conditions, female plants perform worse than males. In the greenhouse, plants were grown in controlled environmental conditions; when herbicides were applied, plants experienced stress from herbicide injury at sub-lethal herbicide rates. This stress could be a possible reason for the production of more males.

On the other hand, field conditions were more variable than greenhouse conditions. In the field, there might be a possibility of overcoming the stress induced by the herbicide treatments, thereby preventing a hormonal effect and leading to a female bias. For example, *Mercurialis annua* (Euphorbiaceae) is a dioecious angiosperm in which male sterility is controlled by sterile cytoplasm. The male sterile mutants are converted to females by an increased level of cytokinins (Louis and Durand, 1978). A recent study provides a good evidence for this prediction, suggesting a link between ethylene signaling and DNA damage in anther specific female flowers in cucumber species (*Cucumis melo* L.). This study also suggests that male genes might be present prior-to or co-opted more than the female genes before flower development, and promotion of ethylene produced more females whereas, inhibition of ethylene is responsible for stamen development in males (Sun et al., 2010).

Amaranthus tuberculatus and *Amaranthus palmeri* are two problematic weeds from the Amaranthaceae family with no sex chromosomes. Recent research suggests that *Amaranthus tuberculatus* has non-functional male loci in some females, and provides some evidence of having male specificity (Sadeque et al., 2017). *Amaranthus palmeri* is an invasive weed, capturing more resources in favorable conditions, which may be more suitable for producing high levels of cytokinins, and therefore producing more females. If growers could manage for conditions in which cytokinin production would be decreased, then more plants would be males, leading to a decrease in population seed production. This research may increase the understanding of sex ratio as a novel management tool for the future.

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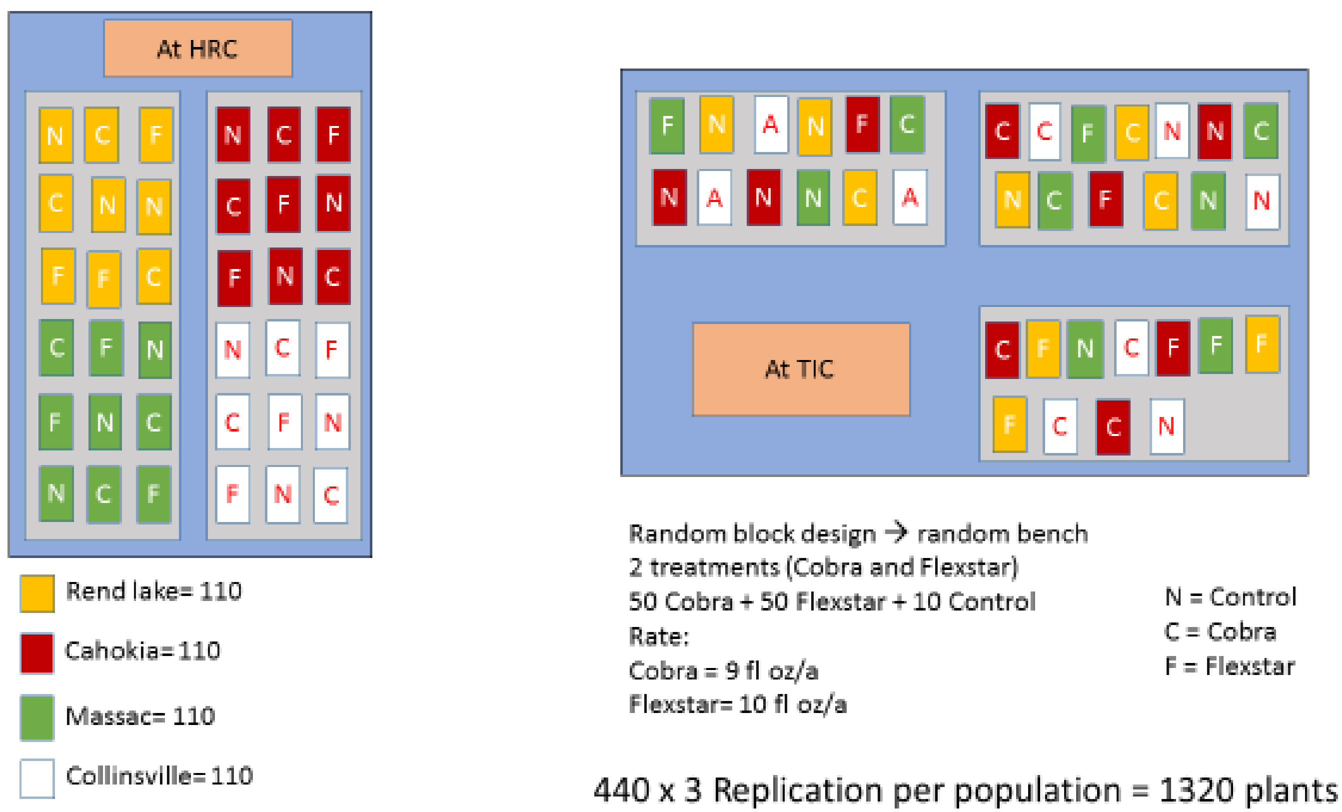
APPENDICES

APPENDIX A: Experimental design of greenhouse study (Randomized Complete Block Design) with 2 herbicides lactofen (Cobra) and fomesafen (Flexstar) at 9 and 10 fl oz/a, three replicates for four different Palmer amaranth populations from Illinois (each of the small square represents a group of 10 pots). ‘N’ represents non-treated control pots, ‘C’ represents lactofen (Cobra) treated pots and ‘F’ represents fomesafen (Flexstar) treated pots. Plants were germinated in HRC and three weeks after herbicide application, transferred to TIC).



APPENDIX B: Dates and growth stages of data collection for greenhouse study for four different *Amaranthus palmeri* populations

Replications	Populations	Leaf collection date	Leaf no. during spray	Height at spray (cm)
1	Rend lake	4/6/2016	7-11	7.62-11.43
2	Rend lake	4/4/2016	7-10	8.89-12.7
3	Rend lake	4/6/2016	7-13	8.89-15.24
1	Collinsville	4/6/2016	7-10	7.62-15.24
2	Collinsville	4/4/2016	7-10	7.62-11.43
3	Collinsville	4/7/2016	7-9	7.62-15.24
1	Massac	4/15/2016	7-9	7.62-12.7
2	Massac	4/18/2016	7-13	7.62-15.24
3	Massac	4/18/2016	7-13	7.62-15.24
1	Cahokia	4/6/2016	7-10	7.62-15.24

2	Cahokia	4/8/2016	7-9	7.62-15.24
3	Cahokia	4/7/2016	7-10	7.62-15.24

APPENDIX C: Greenhouse experiment: Replication (Rep), Population (Pop), Identification number of the pot plant (ID), Treatment (Treat), Height at 2nd week after treatment (Ht at 2 wk), Height at 4th week after treatment (Ht at 4 wk), Height at 6th week after treatment (Ht at 6 wk), Height at flowering condition (Ht at fl), Flowering date (Fl date), Sex of the plant (Sex), Reproductive biomass (Rep bio), Vegetative biomass (Veg bio), Female (F), Male (M). Height in inches and biomass in grams.

Rep	Pop	ID	Treat	Ht at 2 wk	Ht at 4 wk	Ht at 6 wk	Ht at fl	Fl date	Sex	Rep bio	Veg bio
1	Rend lake	2	lactofen	4	12	27.5	17	5/11/2016	M	2.83	8.93
1	Rend lake	3	lactofen	1.25	11	22	27	5/25/2016	F	1.15	11.25
1	Rend lake	9	lactofen	1.25	6	12	46.5	6/2/2016	F	0.91	11.64
1	Rend lake	12	lactofen	4	8	22.5	12.5	5/7/2016	M	1.29	5.71
1	Rend lake	13	lactofen	2	11	30	19	5/11/2016	M	22.36	9.7
1	Rend lake	17	lactofen	3.75	11	23.5	13	5/11/2016	M	1.43	8.62
1	Rend lake	18	lactofen	4.5	14	25.5	17	11-May	M	0.72	7.59
1	Rend lake	21	lactofen	2	10	13.5	33.5	5/25/2016	F	0.49	10.1
1	Rend lake	31	lactofen	1.75	10	18	13.5	5/11/2016	M	2.42	7.13
1	Rend lake	32	lactofen	2.5	13	23.5	18	5/5/2016	F	5.57	2.17
1	Rend lake	56	fomesafen	5.25	10	20.5	13.5	5/5/2016	M	0.84	5.09

1	Rend lake	57	fomesafen	4.5	9.5	17.5	11.5	5/11/2016	F	1.38	8.59
1	Rend lake	58	fomesafen	5	12	17	13	5/6/2016	M	2	5.21
1	Rend lake	61	fomesafen	3.5	15.5	25	17	5/4/2016	M	6.68	1.59
1	Rend lake	73	fomesafen	1.5	7	9.5	28.5	6/2/2016	F	1.21	17.75
1	Rend lake	82	fomesafen	1.75	15.5	26	17	5/5/2016	F	4.8	2.25
1	Rend lake	84	fomesafen	2.5	13	22	18	5/16/2016	F	1.27	15.09
1	Rend lake	86	fomesafen	1	8.5	13.5	22	5/26/2016	F	1.66	12.48
1	Rend lake	93	fomesafen	1.5	13	19.5	16	5/11/2016	M	3.04	13.72
1	Rend lake	96	fomesafen	2.5	16	40	27.5	5/5/2016	M	1.15	8.43
1	Rend lake	101	control	9	17.5	22	16.5	29-Apr	M	2.38	7.12
1	Rend lake	102	control	7	17	28.5	11	4/29/2016	F	2.05	7.53
1	Rend lake	103	control	8	20.5	32	14.5	4/29/2016	F	2.65	7.92
1	Rend lake	104	control	12	25.5	39.5	22	4/29/2016	F	1.83	9.87
1	Rend lake	105	control	7.25			7.25	4/20/2016	M	2.49	3.8
1	Rend lake	106	control	8.5	16	25	13	5/16/2016	M	2.13	19.21
1	Rend lake	107	control	8.5			8.5	4/20/2016	M	2.52	3.05

1	Rend lake	108	control	6.5	14.5	20	12	4/26/2016	M	1.9	6.42
1	Rend lake	109	control	7.5			7.5	4/25/2016	M	2.02	3.76
1	Rend lake	110	control	5.5	12	17	8	4/29/2016	F	3.27	4.75
2	Rend lake	3	lactofen	2.75	9	21.5	17	5/11/2016	F	1.13	10.79
2	Rend lake	5	lactofen	4.5	10	18	11	5/5/2016	M	1.86	6.48
2	Rend lake	6	lactofen	3	7.5	12	30	5/25/2016	M	1.42	9.38
2	Rend lake	20	lactofen	1.5	5	8	28	6/3/2016	F	2.16	13.49
2	Rend lake	33	lactofen	2.5	5	12	17	5/25/2016	F	1.05	5.46
2	Rend lake	34	lactofen	1	2	8	29	6/1/2016	F	1.4	11.25
2	Rend lake	36	lactofen	3.5	4.5	10	28.5	5/27/2016	M	1.59	10.61
2	Rend lake	42	lactofen	4.25	8	19	3.5	5/5/2016	M	0.52	3.42
2	Rend lake	45	lactofen	2.25	7	13	9	5/16/2016	F	0.68	11.63
2	Rend lake	47	lactofen	2	4	10	14	5/25/2016	M	3.5	10.52
2	Rend lake	51	fomesafen	2.55	8	21	6.5	5/5/2016	M	1.29	4.67
2	Rend lake	53	fomesafen	2.75	10	26.5	9.5	5/5/2016	M	2.9	6.43
2	Rend lake	61	fomesafen	2.5	7	12	14	5/25/2016	F	1.25	7.16

2	Rend lake	68	fomesafen	3.5	8	16.5	14.5	5/16/2016	F	1.05	12.71
2	Rend lake	74	fomesafen	4.5	9	25.5	17	5/11/2016	F	1	6.88
2	Rend lake	83	fomesafen	2.5	9	16	21	5/25/2016	F	1.41	7.47
2	Rend lake	85	fomesafen	2.5	8	15	13	5/17/2016	F	1.77	9.22
2	Rend lake	92	fomesafen	2.75	9	13	28	6/1/2016	F	1.47	14.26
2	Rend lake	97	fomesafen	2	7	5	27.5	6/3/2016	F	0.75	10.96
2	Rend lake	100	fomesafen	6	10.5	22.5	13.5	5/11/2016	M	1.86	8.62
2	Rend lake	101	control	9.5	20	29.5	11	4/29/2016	F	2.06	10.2
2	Rend lake	102	control	11.25			11.25	4/20/2016	M	2.22	2.62
2	Rend lake	103	control	5.5	10	17.5	12	5/11/2016	F	1.441	8.92
2	Rend lake	104	control	9.5			12.5	4/25/2016	F	2.05	4.18
2	Rend lake	105	control	8.5	17	22.5	13	4/29/2016	M	2.67	8.25
2	Rend lake	106	control	7	17		10	4/29/2016	F	2.46	7.46
2	Rend lake	107	control	7.5	16	23	11	4/29/2016	M	2.03	7.09
2	Rend lake	108	control	4.5	15	21	4	4/25/2016	M	2.27	6.87
2	Rend lake	109	control	7	10	17	22	6/3/2016	F	2.08	7.23

2	Rend lake	110	control	7.25	12	20	9.5	5/5/2016	F	0.93	7.51
3	Rend lake	10	lactofen	4	11	25	21.5	5/16/2016	F	0.98	6.89
3	Rend lake	15	lactofen	4	12	35	12	5/6/2016	M	1.15	5.85
3	Rend lake	27	lactofen	2	11	19	24.5	5/23/2016	F	1.34	14.46
3	Rend lake	29	lactofen	2	4	10	33	6/1/2016	F	2.38	18.22
3	Rend lake	30	lactofen	3	8	16	13	5/11/2016	M	1.61	5.62
3	Rend lake	32	lactofen	3	9	18	22	5/25/2016	F	2.87	18.2
3	Rend lake	33	lactofen	3.5	8	13	20	5/25/2016	M	1.85	11.23
3	Rend lake	36	lactofen	3	8	22	19	5/16/2016	F	0.76	8.35
3	Rend lake	39	lactofen	1.5	7	10	11	5/19/2016	M	1.37	8.06
3	Rend lake	42	lactofen	3	10	20	23.5	5/21/2016	M	1.93	13.92
3	Rend lake	57	fomesafen	1.5	11	23	20	5/16/2016	F	2.31	19.01
3	Rend lake	61	fomesafen	1.5	7	11	15	5/25/2016	M	0.99	6.88
3	Rend lake	69	fomesafen	2	6	13	27	6/3/2016	F	1.42	14.28
3	Rend lake	76	fomesafen	2	11	19	23	5/25/2016	F	2.41	18.02
3	Rend lake	80	fomesafen	2	6	12	23	6/3/2016	M	2.17	15.6

3	Rend lake	93	fomesafen	2.5	8	13	21	6/4/2016	F	2.24	12.43
3	Rend lake	96	fomesafen	2.5	7	23.5	6	5/5/2016	M	0.8	4.18
3	Rend lake	98	fomesafen	1.5	7	14	16	5/25/2016	F	2.32	10.43
3	Rend lake	99	fomesafen	3	6	11	8	5/11/2016	M	1.05	5.03
3	Rend lake	100	fomesafen	3	7.5	13	15	5/25/2016	F	2.02	8.06
3	Rend lake	101	control	6.75	13.5	20	8	4/25/2016	F	1.86	6.31
3	Rend lake	102	control	8	11	15	9.5	4/25/2016	F	2.23	3.71
3	Rend lake	103	control	3.5	7	13	16	5/25/2016	F	1.32	12.87
3	Rend lake	104	control	3.5	8	13.5	6	5/5/2016	F	1.05	8.11
3	Rend lake	105	control	4.5	12	16.5	6.5	4/25/2016	M	1.69	6.14
3	Rend lake	106	control	6	15.5	22	6.5	4/25/2016	M	2.9	6.6
3	Rend lake	107	control	5	15.5	19	6	4/25/2016	M	2.16	5.06
3	Rend lake	108	control	8	18	24.5	10	4/29/2016	M	2.11	9.15
3	Rend lake	109	control	9.5	27	36	11	4/25/2016	F	4.2	8.66
3	Rend lake	110	control	11.25	22.5	26	13	4/25/2016	F	5.26	7.08
1	Collinsville	1	lactofen	1.75	3.5	8	12	5/25/2016	M	1.66	5.92

1	Collinsville	12	lactofen	1.75	9	13	16	5/25/2016	F	1.3	9.54
1	Collinsville	17	lactofen	2.25	8	12	14	5/25/2016	F	1.08	7.35
1	Collinsville	18	lactofen	3.5	9.5	14.5	42	6/13/2016	F	2.17	33.23
1	Collinsville	21	lactofen	3	6.25	21.5	18	5/16/2016	F	0.4	4.96
1	Collinsville	29	lactofen	2	9	16.5	12	5/16/2016	F	0.85	7.53
1	Collinsville	30	lactofen	2	3	3	19	6/10/2016	M	2.25	8.89
1	Collinsville	31	lactofen	2.5	10.5	17	12	5/12/2016	M	1.71	7.62
1	Collinsville	34	lactofen	3.5	8	10	35	6/9/2016	F	22.57	23.92
1	Collinsville	37	lactofen	2	8	14	17	5/25/2016	M	1.38	7.14
1	Collinsville	51	fomesafen	2.25	6	11	25	6/3/2016	F	1.41	14.41
1	Collinsville	54	fomesafen	2	11	16	43.5	6/7/2016	M	2.34	45.08
1	Collinsville	66	fomesafen	2.5	10.5	18	22	5/25/2016	F	2.06	23.53
1	Collinsville	67	fomesafen	2	5.5	5.5	28	5/31/2016	F	4.3	24.85
1	Collinsville	70	fomesafen	4	12	20	27	5/25/2016	F	1.77	31.3
1	Collinsville	71	fomesafen	3.5	13	18.5	15	5/19/2016	M	1.67	12.25
1	Collinsville	72	fomesafen	3.5	7.5	14	48	6/10/2016	F	5.82	40.12

1	Collinsville	76	fomesafen	3.5	10	16	19.5	5/25/2016	M	2.76	13.08
1	Collinsville	82	fomesafen	2.5	10	16.5	23	5/25/2016	F	1.51	15.87
1	Collinsville	88	fomesafen	3	13	22	17.5	5/16/2016	F	2.37	15.52
1	Collinsville	101	control	8	13	16	11.5	5/5/2016	F	1.36	7.81
1	Collinsville	102	control	7	13	18	21	5/25/2016	F	1.81	15.82
1	Collinsville	103	control	15	27	41	19.5	4/29/2016	F	0.67	13.03
1	Collinsville	104	control	9	14	21	16	5/7/2016	F	2.1	17.75
1	Collinsville	105	control	8	13	16	27	5/25/2016	M	1.78	29.19
1	Collinsville	106	control	11	21	29.5	15	4/29/2016	F	2.48	10
1	Collinsville	107	control	7	11	16	7	4/20/2016	M	1.39	3.57
1	Collinsville	108	control	5.5	12	17.5	10	4/29/2016	F	2.02	5.6
1	Collinsville	109	control	8	20.5	25.5	13.5	4/25/2016	F	4.18	7.09
1	Collinsville	110	control	5.5	10	14	16	5/19/2016	M	1.93	12.9
2	Collinsville	1	lactofen	3	5	8	51.5	5/31/2016	F	2.86	10.07
2	Collinsville	7	lactofen	3.5	10	14	11.5	5/16/2016	M	1.3	12.15
2	Collinsville	15	lactofen	1.75	7	11	54	5/31/2016	F	3	16.53

2	Collinsville	16	lactofen	4	12	17	54	6/1/2016	F	1.77	17.11
2	Collinsville	22	lactofen	3.75	8	11.5	19.5	5/26/2016	F	2.51	12.71
2	Collinsville	24	lactofen	4.75	10	16	25	5/29/2016	F	2.15	15.91
2	Collinsville	26	lactofen	2	4	13	29	5/27/2016	F	1.99	22.99
2	Collinsville	29	lactofen	3	7.5	15	22	5/23/2016	F	1.46	15.44
2	Collinsville	36	lactofen	3.25	5.75	14	12	5/16/2016	M	1.52	12.25
2	Collinsville	47	lactofen	5	9.5	14	18	5/23/2016	M	1.95	8
2	Collinsville	51	fomesafen	3.5	9	12	19	5/23/2016	M	1.44	8.22
2	Collinsville	62	fomesafen	3	8	16	14.5	5/16/2016	M	2.92	11.93
2	Collinsville	66	fomesafen	5.5	13	25.5	24	5/16/2016	F	1.72	13.81
2	Collinsville	67	fomesafen	3.5	8	13	49	6/8/2016	F	4.8	38.79
2	Collinsville	74	fomesafen	3	8	14	14.5	5/19/2016	M	1.87	8.59
2	Collinsville	75	fomesafen	4	9	14	26	5/31/2016	M	0.97	16.16
2	Collinsville	78	fomesafen	1.5	7.5	13	16	5/23/2016	M	1.54	7.9
2	Collinsville	79	fomesafen	4	9	14	26	5/31/2016	M	0.97	16.16
2	Collinsville	88	fomesafen	3.5	4	5	21	5/23/2016	M	0.97	4.42

2	Collinsville	98	fomesafen	2.75	8	18.5	7.5	5/5/2016	M	1.84	4.86
2	Collinsville	101	control	6	10	15	16	5/19/2016	F	1.7	17.97
2	Collinsville	102	control	4	2	12	17	5/24/2016	M	1.46	8.58
2	Collinsville	103	control	4.5	7	9	5.5	4/29/2016	M	1.45	2.84
2	Collinsville	104	control	3.75	9	14	6.5	4/29/2016	M	2.11	5.97
2	Collinsville	105	control	5	5	13	32.5	5/29/2016	F	2.74	14.45
2	Collinsville	106	control	7	12.5	16	10	4/29/2016	M	2.59	7.08
2	Collinsville	107	control	7	12	22	12.5	5/7/2016	F	0.92	6.82
2	Collinsville	108	control	4	10.5	15	20	5/27/2016	F	2.55	13.86
2	Collinsville	109	control	6.25	7		6.25	4/20/2016	M	1.4	2.81
2	Collinsville	110	control	6.5	11	18	36.5	5/27/2016	F	2.04	20.28
3	Collinsville	1	lactofen	4	6	8	33	5/30/2016	M	1.01	16.54
3	Collinsville	10	lactofen	22	3.5	9	19.5	6/10/2016	F	1.98	11.54
3	Collinsville	12	lactofen	4	16	29.5	16	5/6/2016	F	6.14	2.17
3	Collinsville	19	lactofen	4	9	13	27	6/12/2016	M	1.28	26.63
3	Collinsville	22	lactofen	1.5	9	16.5	22	5/11/2016	M	0.79	4.83

3	Collinsville	26	lactofen	1.5	3	6	19.5	6/9/2016	M	1.39	18.29
3	Collinsville	28	lactofen	3	10	12	46.5	6/1/2016	F	1.53	16.75
3	Collinsville	35	lactofen	4	9.5	18	21	5/25/2016	M	1.03	11.56
3	Collinsville	45	lactofen	2.75	8.5	11	28	6/11/2016	M	1.32	17.25
3	Collinsville	48	lactofen	3	8	12	41	6/1/2016	F	1.4	21.14
3	Collinsville	52	fomesafen	2	6	8	31	6/10/2016	M	2.94	20.38
3	Collinsville	59	fomesafen	3	9	13	21	6/11/2016	F	4.62	22.64
3	Collinsville	60	fomesafen	1	8	16	32	6/12/2016	F	1.41	19.33
3	Collinsville	65	fomesafen	4.5	9	15	19	5/26/2016	F	2.02	11.23
3	Collinsville	71	fomesafen	2.5	11	16	37.5	6/11/2016	M	3.26	27.49
3	Collinsville	73	fomesafen	4.25	14	19	49.5	5/25/2016	F	1.87	20.48
3	Collinsville	79	fomesafen	1.25	10	16	21	5/25/2016	F	2.17	20.81
3	Collinsville	80	fomesafen	2	10	16	16	5/19/2016	M	2.07	13.8
3	Collinsville	81	fomesafen	1.75	9	17	18.5	5/24/2016	F	1.61	20.59
3	Collinsville	84	fomesafen	1	10	14	34.5	6/1/2016	F	1.97	20.34
3	Collinsville	101	control	6	12	18	25	5/5/2016	F	2.56	8.88

3	Collinsville	102	control	9	17.5	26.5	16	4/29/2016	M	2.27	7.54
3	Collinsville	103	control	5	10.5	13.5	19	5/31/2016	F	2.04	16.48
3	Collinsville	104	control	6.5	14	19	14	5/5/2016	M	1.62	7.65
3	Collinsville	105	control	8.75	14	19.5	12	4/29/2016	M	1.76	8.78
3	Collinsville	106	control	8.75	15	16	39.5	6/11/2016	M	2.9	26.62
3	Collinsville	107	control	6	15	17	15	5/5/2016	M	1.7	12.5
3	Collinsville	108	control	7.25	12	18	46.5	5/31/2016	F	1.55	21.18
3	Collinsville	109	control	6	13	16	6	4/20/2016	M	2.01	2.58
3	Collinsville	110	control	6.25	15.5	22	10	5/1/2016	M	1.24	8.19
1	Massac	15	lactofen	6.5	12.5	28	32	5/25/2016	F	1.54	10.47
1	Massac	19	lactofen	5	8	15	21	6/11/2016	F	1.02	16.62
1	Massac	28	lactofen	5.5	8	16	27	6/12/2016	F	3.73	12.54
1	Massac	31	lactofen	4	10	17	41	6/13/2016	M	0.94	25.25
1	Massac	35	lactofen	4	6	13	29	6/9/2016	F	1.69	19.77
1	Massac	39	lactofen	3.5	8	18	27	6/10/2016	M	1.63	14.76
1	Massac	42	lactofen	3.5	7	18	23	6/3/2016	F	1.71	11.51

1	Massac	44	lactofen	5	9	20	41.5	6/1/2016	F	1.42	15.98
1	Massac	49	lactofen	6	13	24	42.5	6/1/2016	F	2.28	15.58
1	Massac	50	lactofen	7	10	23	25	5/25/2016	F	2.09	13.81
1	Massac	55	fomesafen	4.5	9	24	34	6/12/2016	F	1.36	14.59
1	Massac	61	fomesafen	3	7	22	43	6/2/2016	M	1.08	7.84
1	Massac	64	fomesafen	3	3.5	7	32	6/12/2016	M	3.85	17.32
1	Massac	65	fomesafen	5	11	17	39	6/12/2016	M	3.85	17.32
1	Massac	73	fomesafen	6.5	12	29	29	5/31/2016	F	2.77	16.6
1	Massac	78	fomesafen	5	8	20	20	5/31/2016	M	2.58	8.65
1	Massac	82	fomesafen	5	13	27	27	5/31/2016	M	2.88	17.87
1	Massac	93	fomesafen	3	6.5	18	21	6/2/2016	M	2.14	12.81
1	Massac	94	fomesafen	3	8	27	39.5	6/2/2016	F	1.6	15.63
1	Massac	95	fomesafen	5	10.5	26	26	5/31/2016	F	1.71	14.8
1	Massac	101	control	14	21	37	45	6/3/2016	F	1.4	29.26
1	Massac	102	control	14	26	41	39	5/26/2016	F	4.11	24.89
1	Massac	103	control	19	29	35.5	33	5/16/2016	F	1.92	25.3

1	Massac	104	control	15	24.5	40.5	20	5/5/2016	M	10.48	1.89
1	Massac	105	control	9	12	32.5	32	5/30/2016	M	2.25	40.24
1	Massac	106	control	21	33	46	30	5/12/2016	F	2.17	23.23
1	Massac	107	control	16	26	42	16	5/5/2016	M	1.58	16.98
1	Massac	108	control	17.5	23	45	17	5/5/2016	M	1.77	14.23
1	Massac	109	control	17	29	47	27	5/12/2016	M	2.02	22.61
1	Massac	110	control	20	33	42.5	30.55	5/12/2016	F	1.59	22.21
2	Massac	6	lactofen	4.5	9	24	20.5	5/26/2016	F	0.46	7.59
2	Massac	7	lactofen	5	12	25	43.5	6/1/2016	F	1.01	16.34
2	Massac	10	lactofen	3	9	20	18.5	5/26/2016	F	1.32	12.7
2	Massac	11	lactofen	2	3	10	18.5	6/12/2016	M	1.85	13.07
2	Massac	19	lactofen	6	10	21	27	6/12/2016	M	2.67	12.43
2	Massac	20	lactofen	3	8	14.5	19.5	5/29/2016	M	1.21	7.8
2	Massac	36	lactofen	6.25	12	38	35.5	5/26/2016	M	0.86	8.2
2	Massac	38	lactofen	7	13	25	20.5	5/25/2016	F	1.01	10.97
2	Massac	45	lactofen	2.25	8	21	37	6/10/2016	F	5.78	29.35

2	Massac	48	lactofen	6	12	19	23	5/26/2016	M	3.63	12.8
2	Massac	54	fomesafen	3	11	24	36	6/3/2016	F	1.24	23.99
2	Massac	56	fomesafen	5	13	24	32.5	5/31/2016	F	1.1	23
2	Massac	57	fomesafen	7.5	15	38.5	42	5/30/2016	M	0.9	14.07
2	Massac	59	fomesafen	4	10	22	24.5	5/29/2016	F	1.58	11.73
2	Massac	60	fomesafen	4	12	22	47	5/31/2016	F	2.42	19.29
2	Massac	66	fomesafen	3	10	24	37.5	6/1/2016	M	2.15	15.13
2	Massac	67	fomesafen	3	9	22	25.5	5/26/2016	M	0.9	10.5
2	Massac	76	fomesafen	3	9	20	23.5	5/29/2016	M	1.04	5.54
2	Massac	85	fomesafen	4	9	21	42	6/10/2016	F	2.27	26.54
2	Massac	95	fomesafen	1	2	7	21.5	6/3/2016	F	0.56	10.4
2	Massac	101	control	15	25	39.5	42.5	5/16/2016	F	0.57	19.09
2	Massac	102	control	10.5	22		18.5	5/10/2016	M	3.33	6.91
2	Massac	103	control	15	25		23.5	5/16/2016	M	1.25	7.13
2	Massac	104	control	14.5	24.5		14.5	5/6/2016	M	8.26	1.93
2	Massac	105	control	9	13	38	12	5/16/2016	M	1.56	15.97

2	Massac	106	control	15.5	32		17	5/10/2016	M	2.06	11.63
2	Massac	107	control	15	29		25	5/12/2016	F	1.92	15.92
2	Massac	108	control	12.5	22	48.5	48.5	5/31/2016	F	1.37	26.03
2	Massac	109	control	12.5	31		29.5	5/16/2016	F	0.78	8.75
2	Massac	110	control	9.5	17		13	5/12/2016	F	0.69	11.29
3	Massac	2	lactofen	4	8	17	32.5	6/2/2016	F	2.22	24.5
3	Massac	9	lactofen	5	13	29	24.5	5/25/2016	F	1.95	8.59
3	Massac	19	lactofen	3	6	18	26	6/11/2016	F	3.63	11.51
3	Massac	22	lactofen	5	7	18	22.5	6/1/2016	M	2.91	12.88
3	Massac	25	lactofen	6	14	36	49.5	6/10/2016	F	2.49	35.98
3	Massac	31	lactofen	7	14	43	36.5	5/26/2016	M	1.19	12.56
3	Massac	32	lactofen	5	12	28	41	6/2/2016	F	1.2	24.78
3	Massac	33	lactofen	5.25	12	29	27.5	5/29/2016	F	1.53	11.08
3	Massac	39	lactofen	4	10	24	38	6/2/2016	M	2.4	20.19
3	Massac	42	lactofen	5	12.5	23	34	6/10/2016	M	3.24	16.25
3	Massac	56	fomesafen	8.5	19		18	5/16/2016	M	0.63	6.24

3	Massac	59	fomesafen	6.5	16.5	42	38.5	5/26/2016	M	0.92	8.11
3	Massac	61	fomesafen	5	9	16	29.5	6/5/2016	M	2.44	18.75
3	Massac	63	fomesafen	2.5	10	24	37.5	5/31/2016	M	1.25	15.45
3	Massac	68	fomesafen	3.5	6	11	35.5	6/1/2016	M	1.26	10.08
3	Massac	70	fomesafen	2	10	21	32.5	6/2/2016	F	2.93	12.99
3	Massac	71	fomesafen	4	9	30	39.5	6/1/2016	F	1.79	20.33
3	Massac	83	fomesafen	4.5	12	29	22.5	5/24/2016	M	1.6	9.35
3	Massac	89	fomesafen	2.5	8	20.5	14.5	5/24/2016	M	1.68	28.52
3	Massac	91	fomesafen	6	11	28	48.5	6/1/2016	F	1.77	20.88
3	Massac	101	control	13	25	41	23	5/16/2016	F	7.56	30.45
3	Massac	102	control	13	21	43	32	5/23/2016	F	5.76	27.21
3	Massac	103	control	15	27	47	23	5/16/2016	F	1.93	18.39
3	Massac	104	control	16	30	39	26	5/17/2016	F	3.44	32.21
3	Massac	105	control	19	34	59	29	5/18/2016	F	2.37	33.59
3	Massac	106	control	12	26	37	23.5	5/19/2016	F	1.01	12.13
3	Massac	107	control	13.5	20	41	51.5	6/1/2016	F	2.29	25.82

3	Massac	108	control	19.5	32	45	28.5	5/16/2016	F	5.75	25.27
3	Massac	109	control	14.5	22	43	27	5/21/2016	F	3.74	21.95
3	Massac	110	control	10	16	35	16	5/18/2016	F	4.96	32.01
1	Cahokia	1	lactofen	2	8	17	28.5	5/25/2016	M	2.58	10.55
1	Cahokia	8	lactofen	3	7	9	22.5	6/2/2016	F	1.19	13.88
1	Cahokia	17	lactofen	2.5	11	20	27	5/25/2016	F	1.39	16.39
1	Cahokia	18	lactofen	2	7	12	19.5	5/25/2016	M	1.69	10.92
1	Cahokia	26	lactofen	3	10.75	28.5	10	5/5/2016	M	0.77	4.34
1	Cahokia	35	lactofen	1.75	8	13	19.5	5/25/2016	M	3.28	19.47
1	Cahokia	38	lactofen	1.75	5	10	46.5	6/8/2016	F	2.79	42.54
1	Cahokia	42	lactofen	4.5	13	22.5	22.5	5/18/2016	M	1.96	13.49
1	Cahokia	45	lactofen	3.75	9	12	53	6/9/2016	M	4.71	45.14
1	Cahokia	46	lactofen	3	10	17	39	5/2/2016	F	3.21	34.6
1	Cahokia	59	fomesafen	9	13	19	39.5	6/1/2016	F	2.79	26.67
1	Cahokia	61	fomesafen	3.25	11	22	16.5	5/11/2016	M	1.99	11.6
1	Cahokia	62	fomesafen	3	10	24	27.5	5/25/2016	F	2.55	13.86

1	Cahokia	63	fomesafen	3.25	15	23.5	12	4/29/2016	M	4.91	1.93
1	Cahokia	72	fomesafen	3.25	13.3	20	41	6/11/2016	F	2.34	34.46
1	Cahokia	74	fomesafen	5.75	15	35	14.5	5/5/2016	M	1.04	9.03
1	Cahokia	79	fomesafen	2.75	12	20	26	5/25/2016	M	3	17.06
1	Cahokia	81	fomesafen	2.5	8	16	45	6/12/2016	M	4.62	32.33
1	Cahokia	97	fomesafen	3.5	8.5	15	39.5	6/1/2016	F	1.13	27.02
1	Cahokia	98	fomesafen	5.5	12	29	12	5/5/2016	M	1.4	9.07
1	Cahokia	101	control	6	16	29	13	4/29/2016	M	3.88	8.41
1	Cahokia	102	control	5.75	15	23	34.5	5/25/2016	F	2.31	20.13
1	Cahokia	103	control	8	17	30	17	5/6/2016	M	2.92	12.53
1	Cahokia	104	control	7	15	24	24	5/18/2016	F	2.42	15.45
1	Cahokia	105	control	7	11.5	20.5	11.5	5/6/2016	F	2.06	11.79
1	Cahokia	106	control	12	20	38	17.5	5/2/2016	M	1.21	12.91
1	Cahokia	107	control	7.5	14	22	43.5	5/19/2016	M	2.28	23.2
1	Cahokia	108	control	6.5	12	17	35	6/1/2016	F	2.36	19.82
1	Cahokia	109	control	11.5	18	26.5	14.5	4/19/2016	M	2.34	8.16

1	Cahokia	110	control	11	17	26	12.5	5/2/2016	F	0.91	8.12
2	Cahokia	3	lactofen	4.75	9	22	16.5	5/11/2016	F	0.53	6.81
2	Cahokia	11	lactofen	3.5	9	17	27	6/1/2016	F	5.77	16.26
2	Cahokia	15	lactofen	3	9	16	36	5/31/2016	F	3.04	36.02
2	Cahokia	18	lactofen	2	3	10	37	6/12/2016	M	5.49	22.37
2	Cahokia	26	lactofen	3.25	10	11	25	5/26/2016	F	2.61	16.45
2	Cahokia	29	lactofen	4	15	29.5	21.5	5/11/2016	F	0.48	8.22
2	Cahokia	30	lactofen	3.5	11.5	17.5	26	6/2/2016	F	1.26	10.08
2	Cahokia	31	lactofen	3	14	21	21	5/18/2016	M	2.54	17.71
2	Cahokia	35	lactofen	3	6	9	36	6/5/2016	F	4.51	21.72
2	Cahokia	36	lactofen	2	3	6	20.5	6/1/2016	M	3.53	14.12
2	Cahokia	65	fomesafen	5.5	10	16	39.5	5/25/2016	M	4.25	22.47
2	Cahokia	72	fomesafen	6.75	11.5	17	26.5	5/25/2016	F	2.36	16.79
2	Cahokia	78	fomesafen	3	4	5	23	6/10/2016	F	1.26	14.18
2	Cahokia	80	fomesafen	2	4	9	16	5/25/2016	M	1.72	6.22
2	Cahokia	81	fomesafen	2.75	10	19	19	5/19/2016	F	2.79	9.76

2	Cahokia	85	fomesafen	4.5	8.5	13	36	5/25/2016	F	2.49	20.93
2	Cahokia	89	fomesafen	6	9	20.5	14	5/11/2016	M	1.37	7.06
2	Cahokia	90	fomesafen	3	5.5	9	32	6/2/2016	M	1.52	21.17
2	Cahokia	94	fomesafen	5.75	11	18	42	6/11/2016	F	4.05	31.56
2	Cahokia	95	fomesafen	9			9.5	4/22/2016	F	2.91	1.51
2	Cahokia	101	control	11.5			12	4/22/2016	M	2.57	4.75
2	Cahokia	102	control	8.5	17.75	36	36	5/19/2016	M	2.48	21.68
2	Cahokia	103	control	9.5	17.75	36	36	5/19/2016	M	1.93	19.09
2	Cahokia	104	control	6.5	13.5	20.5	11	5/11/2016	M	2.56	13.63
2	Cahokia	105	control	5.75	14	28	12.5	5/2/2016	M	1.44	9.57
2	Cahokia	106	control	10.5	19	23.5	17	4/22/2016	M	2.51	6.67
2	Cahokia	107	control	12	20.5	27	18	4/29/2016	F	2.81	11
2	Cahokia	108	control	11			12	4/22/2016	M	1.63	4.44
2	Cahokia	109	control	11	18.5	25	16	4/29/2016	M	2.01	8.02
2	Cahokia	110	control	5.5	14.5	25	12	4/29/2016	M	1.53	8.37
3	Cahokia	2	lactofen	2	9	15	36.5	5/31/2016	F	2.94	18.42

3	Cahokia	8	lactofen	1.25	7	9	22.5	5/25/2016	M	2.69	15.2
3	Cahokia	9	lactofen	2.5	8	17	24	5/25/2016	F	1.45	11.96
3	Cahokia	10	lactofen	1.5	10	19	27	5/25/2016	M	2.1	16.71
3	Cahokia	15	lactofen	2	17	28	16.5	5/5/2016	F	2.13	6.54
3	Cahokia	18	lactofen	2	5	8	19	5/27/2016	F	2.15	8.66
3	Cahokia	23	lactofen	1.5	9.5	17	28	5/25/2016	F	1.72	18.02
3	Cahokia	26	lactofen	1	2	5	25.5	6/2/2016	M	1.18	10.72
3	Cahokia	31	lactofen	2	5	9	16.5	6/2/2016	F	1.26	12.17
3	Cahokia	45	lactofen	2	9.5	20.5	14	5/11/2016	M	2.39	12.86
3	Cahokia	51	fomesafen	1.75	8	13	11	5/16/2016	M	3.72	17.74
3	Cahokia	66	fomesafen	2.5	11	19	23	5/27/2016	F	1.86	10
3	Cahokia	67	fomesafen	2.5	10.5	18	24	5/25/2016	F	1.62	14.57
3	Cahokia	68	fomesafen	3	12	18.5	24.5	5/25/2016	M	2.47	19.29
3	Cahokia	74	fomesafen	2	7	9	29.5	5/31/2016	F	2.18	14.28
3	Cahokia	80	fomesafen	2.25	8	12	28.5	6/7/2016	F	2.77	32.98
3	Cahokia	85	fomesafen	1.75	5	9	30.5	6/5/2016	M	2.41	25.81

3	Cahokia	88	fomesafen	2.5	5	12.5	28	6/2/2016	M	5.46	30.01
3	Cahokia	92	fomesafen	2.5	9	34	54	6/7/2016	F	3.87	61.36
3	Cahokia	94	fomesafen	1.25	10.5	16.5	41	5/31/2016	M	2.74	32.67
3	Cahokia	101	control	8	13		13	5/6/2016	F	1.25	8.32
3	Cahokia	102	control	8.5	12	18	12	5/6/2016	M	0.84	7.29
3	Cahokia	103	control	9	16	20	13.5	4/29/2016	M	2.55	6.28
3	Cahokia	104	control	11	17	22.5	14	4/25/2016	M	1.78	6.08
3	Cahokia	105	control	9.25	14	23	14	5/6/2016	M	2.24	11.99
3	Cahokia	106	control	4.5	9	15	9	5/6/2016	F	1.78	7.58
3	Cahokia	107	control	6.5	15	20	13.5	4/29/2016	M	2.67	5.27
3	Cahokia	108	control	8	16	37	40.5	5/25/2016	F	2.27	22.43
3	Cahokia	109	control	5	10	15.5	12	5/11/2016	M	1.22	9.81
3	Cahokia	110	control	10	15	25.5	18.5	5/8/2016	F	1.66	14.56

APPENDIX D: Experimental design of field study both the year 2015 and 2016 (Randomized Complete Block Design with 12 pre and 2 post-emergence herbicides, 3 replications). Plots were 3 m wide by 10 m long.

a=	Non-treated	f=	Zidua	k=	Valor SX
b=	Anthem	g=	Sharpen	l=	Valor SX
c=	Dual-Magnum	h=	Spartan 4F	m=	Valor SX
d=	Warrent	i=	Spartan 4F	n=	Cobra
e=	Prowl H2O	j=	Spartan 4F	o=	Flexstar

APPENDIX E: Summary of 2015 field data. Final counts in 1 m^2 quadrats. Male weight = fresh weight of total male counts, Female weight = fresh weight of total female counts.

Treatment	Replication	Male weight	Female weight	Male count	Female count
Non treated	101	0.8	0.85	8	16
Non treated	208	0.2	3.1	3	47
Non treated	307	0.83	3.35	7	23
pyroxasulfone+fluthiacet-ethyl	102	1.35	0.5	2	2
pyroxasulfone+fluthiacet-ethyl	221	0.2	2.35	1	2
pyroxasulfone+fluthiacet-ethyl	316	0	0	0	0
s-metolacholr	103	2.95	2.1	8	5
s-metolacholr	218	0.85	3.9	3	7
s-metolacholr	315	1.65	3.55	3	4
acetochlor	104	0.37	1.95	9	15
acetochlor	219	1	1.05	7	6
acetochlor	309	2.55	2.3	12	7
pendimethalin	105	0.16	1.25	3	9

pendimethalin	209	2	2.05	3	5
pendimethalin	304	0.15	1.8	2	7
pyroxasulfone	106	0.03	0.13	4	1
pyroxasulfone	211	0	3.3	0	4
pyroxasulfone	301	0.1	1.85	1	3
saflufenacil	107	0.06	2.35	1	22
saflufenacil	206	1.2	2.1	7	16
saflufenacil	306	0.1	1.15	4	13
sulfentrazone 8 oz	108	0	2.15	0	2
sulfentrazone 8 oz	222	1.3	3.4	2	3
sulfentrazone 8 oz	313	0.55	0.95	3	3
sulfentrazone 10 oz	109	1.1	0	0	4
sulfentrazone 10 oz	212	0	2.15	0	4
sulfentrazone 10 oz	308	0.3	2.4	3	2
sulfentrazone 12 oz	110	0.4	2.65	1	2
sulfentrazone 12 oz	205	0	2.6	0	2

sulfentrazone 12 oz	317	0	0	0	0
flumioxazin 2 oz	111	1.55	1	5	4
flumioxazin 2 oz	201	0.45	1.2	2	5
flumioxazin 2 oz	312	1.1	0.75	3	2
flumioxazin 2.5 oz	112	0	0.7	0	3
flumioxazin 2.5 oz	214	3.6	0.6	5	2
flumioxazin 2.5 oz	318	0	0.7	0	1
flumioxazin 3 oz	113	1.65	0.55	3	3
flumioxazin 3 oz	202	0	0.15	0	2
flumioxazin 3 oz	322	0.3	0.3	1	2
lactofen	114	0.65	0.85	3	7
lactofen	213	0.25	1.6	3	6
lactofen	311	0.3	1.85	2	6
fomesafen	115	0.4	0.65	2	3
fomesafen	210	0.2	0.09	3	1
fomesafen	305	1.3	0.45	3	1

APPENDIX F: Summary of 2016 field data. Final counts in 1 m^2 quadrats. Male weight = fresh weight of total male counts, Female weight = fresh weight of total female counts.

Treatment	Plot NO.	Male weight	Female weight	Male count	Female count
Non treated	101	1.9	3.25	6	17
Non treated	209	2.5	0	7	10
Non treated	305	0	2.65	0	11
pyroxasulfone+fluthiacet-ethyl	102	0	0.45	0	1
pyroxasulfone+fluthiacet-ethyl	207	0	0.9	0	1
pyroxasulfone+fluthiacet-ethyl	313	1	0	1	0
s-metolacholr	103	0	1.45	0	6
s-metolacholr	206	0	0	0	0
s-metolacholr	309	0	4.4	0	6
acetochlor	104	2	0	2	0
acetochlor	201	0.8	0	1	0
acetochlor	317	0	1.35	0	3
pendimethalin	105	0	3.35	0	1

pendimethalin	212	0	2.2	0	3
pendimethalin	318	0	0.5	0	1
pyroxasulfone	106	0.2	0.9	3	3
pyroxasulfone	208	2.3	0	1	0
pyroxasulfone	311	0.95	1.2	1	2
saflufenacil	107	0	1.85	0	3
saflufenacil	211	1.45	0	1	0
saflufenacil	315	1.9	0	2	0
sulfentrazone 8 oz	108	0	0	0	0
sulfentrazone 8 oz	215	0	0	0	0
sulfentrazone 8 oz	312	0	1.4	0	1
sulfentrazone 10 oz	109	0	0	0	0
sulfentrazone 10 oz	213	0	0	0	0
sulfentrazone 10 oz	301	0	2.35	0	5
sulfentrazone 12 oz	110	1.05	0	1	0
sulfentrazone 12 oz	214	0	1.15	0	3

sulfentrazone 12 oz	308	0	0	0	0
flumioxazin 2 oz	111	0.5	2.9	1	3
flumioxazin 2 oz	218	0.9	2.1	2	4
flumioxazin 2 oz	306	0.22	1.8	1	3
flumioxazin 2.5 oz	112	0	0.95	0	1
flumioxazin 2.5 oz	210	0	2.3	0	4
flumioxazin 2.5 oz	307	0	1.45	0	1
flumioxazin 3 oz	113	2.65	0	6	0
flumioxazin 3 oz	217	0	0.6	0	1
flumioxazin 3 oz	302	0	0.8	0	2
lactofen	114	0.12	6.9	1	6
lactofen	216	0.16	2.59	1	2
lactofen	303	0.1	1.45	1	1
fomesafen	115	0.1	0.05	1	1
fomesafen	202	0.43	1.3	4	1
fomesafen	316	0.26	1.2	3	3

APPENDIX G: Summary of field data in 2016 for average density within two 0.5m^2 subplots per plot, showing the collection of data on 6 sampling dates.

Plot No.	Treatment	6/6/2016	6/20/2016	7/5/2016	7/18/2016	8/1/2016	8/15/2016
101	Non treated	148	62	44	25	26.5	18
102	pyroxasulfone+fluthiacet-ethyl	13.00	4	6.5	3.5	2.5	2.5
103	s-metolacholr	4.5	2	6	3.5	3.5	2
104	acetochlor	2	3.5	2.5	1	0.5	0
105	pendimethalin	16	4.5	6	3.5	3.5	2
106	pyroxasulfone	11	5.5	6	4	4	3
107	saflufenacil	14	4.5	2	2	1.5	1.5
108	sulfentrazone 8 oz	0.5	1	1	1	0.5	0.5
109	sulfentrazone 10 oz	0.5	1.5	0	0	0	0
110	sulfentrazone 12 oz	0.00	0	1	1	1	1
111	flumioxazin 2 oz	2	1.5	2.5	3.5	2.5	2
112	flumioxazin 2.5 oz	0.00	0.5	2	1.5	1.5	1.5
113	flumioxazin 3 oz	0.5	1	1.5	0.5	0.5	0.5

114	lactofen	0	0	70.5	18	9.50	5
115	fomesafen	0	0	65	17	12.5	8
209	Non treated	122.2	68	53.5	41.5	38.5	29
207	pyroxasulfone+fluthiacet-ethyl	1	1.00	2	1.5	0.5	0
206	s-metolacholr	2	0.00	0	0.5	0	0
201	acetochlor	8	4.5	3.5	4.5	2.5	2.5
212	pendimethalin	3.5	7	6	2.5	2.5	2
208	pyroxasulfone	8	3	2	1	0.5	0.5
211	saflufenacil	1	5.5	5	2.5	1.5	1.5
215	sulfentrazone 8 oz	0.5	0.5	1	1	1	1
213	sulfentrazone 10 oz	0.5	1.5	0.5	0.5	0.5	0.5
214	sulfentrazone 12 oz	0	0	0	0	0	0
218	flumioxazin 2 oz	2.5	0.5	1.5	0.5	0.5	0
210	flumioxazin 2.5 oz	0	1	1.5	0.5	0.5	0.5
217	flumioxazin 3 oz	0	4.5	3.5	0.5	0.5	0.5
216	lactofen	0	0	35.5	23.5	10	6.5

202	fomesafen	0	0	34	17	6.5	4.5
305	Non treated	86.5	62	40.5	22.5	18.5	17.5
313	pyroxasulfone+fluthiacet-ethyl	0	1	0.5	0.5	0.5	0.5
309	s-metolacholr	2	2	3.5	4	3.5	2.5
317	acetochlor	0.50	1	0.5	0.5	0	0
318	pendimethalin	0	3.5	1	1	1	0.5
311	pyroxasulfone	1	2.5	0.5	1.5	1	0.5
315	saflufenacil	1	8.5	4	2	0.5	0.5
312	sulfentrazone 8 oz	4.5	3.5	3	1.5	1	1
301	sulfentrazone 10 oz	1.5	2.5	2.5	2	2	2
308	sulfentrazone 12 oz	0.5	0	0	0	0	0
306	flumioxazin 2 oz	3.5	0.5	1	1	1	1
307	flumioxazin 2.5 oz	2	1	1	1	1	1
302	flumioxazin 3 oz	1	1	1	0.5	0	0
303	lactofen	0	0	16.5	10	8.5	7.5
316	fomesafen	0	0	19	15.5	7	4

APPENDIX H: Summary of field data 2016 (survivorship data, collection of data on 6 sampling dates). 6/6/2016 =1st, 6/20/2016 = 2nd, 7/5/2016 = 3rd, 7/18/2016 = 4th, 8/1/2016 = 5th, 8/15/2016 = 6th, DDA= Death day, X= death, A = Alive until last evaluation, F = female, M = Male.

Plot No.	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	Plot No.	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	
101	1	x				14		1		x	28	102	1				A	56	F	1	x		14
	2				A	56	F	2	x		14		2	x				14		2	x		14
	3		x			28		3	x		14		3				A	56	F	3	x		14
	4				A	56	F	4	x		14		4			x		42		4	x		14
	5				A	56	F	5	x		14		5	x				14		5	x		14
	6				A	56	F	6		x	28		6		x			28		6	x		14
	7	x				14		7	x		14		7		x			28		7	x		14
	8	x				14		8	x		14		8				A	56	M	8		x	28

	9	x				14		9		x	28		9	x				14		9	x		14
	10	x				14		10	x		14		10		x			28		10		x	28
	11				A	56	M	11	x		14		11				A	56	M	11	x		14
	12				x	56		12	x		14		12	x				14		12		x	28
	13	x			x	56		13	x		14		13		x			28		13	x		14
	14				x	56		14		x	28		14			x		42		14		x	28
	15				x	56		15	x		14		15		x			28		15		x	28
	16	x				14		16	x		14		16							16			
	17		x			28		17	x		14		17							17			
	18		x			28		18		x	28		18							18			
	19		x			28		19	x		14		19							19			
	20	x				14		20	x		14		20							20			
	2			A		56	F	2	x		14		2	x				14		2	x		14
	3			A		56	F	3	x		14		3		x			28		3	x		14

Plot No.	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Plot No.	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA
101	1	x				14		1	x		14	102	1		x			28		1	x		14
	5	x				14		5	x		14		5	x				14		5			
	6	x				14		6	x		14		6	x				14		6			
	7		x			28		7	x		14		7			A		56	F	7			
	8	x				14		8	x		14		8	x				14		8			
	9	x				14		9	x		14		9		x			28		9			
	10		x			28		10	x		14		10	x				14		10			
	11		x			28		11					11	x				14		11			
	12	x				14		12					12	x				14		12			
	13	x				14		13					13	x				14		13			
	14		x			28		14					14		x			28		14			

	15	x				14		15					15		x			28		15			
	16	x				14		16					16							16			
	17	x				14		17					17							17			
	18			A		56	M	18					18							18			
	19	x				14		19					19							19			
	20		x			28		20					20							20			
20 9	1st	2n d	3r d	4t h	5t h	DD A	se x	3rd visi t	4t h	5t h	DD A	20 7	1st	2n d	3r d	4t h	5th	DD A	se x	3rd visi t	4t h	5t h	6th
	1				A	56	F	1	x		14		1				A	56	F	1			
	2				A	56	F	2	x		14		2				A	56	F	2			
	3				A	56	F	3	x		14		3	x				14		3			
	4	x				14		4		x	28		4				A	56	M	4			
	5	x				14		5	x		14		5				A	56	M	5			
	6				A	56	F	6		x	28		6				A	56	M	6			

	7				A	56	M	7	x		14		7				A	56	F	7			
209	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	207	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	6th
	8				A	56	M	8	x		14		8	x				14		8			
	9		x			28		9		x	28		9	x				14		9			
	10		x			28		10	x		14		10				A	56	F	10			
	11		x			28		11	x		14		11				A	56	F	11			
	12		x			28		12	x		14		12		x			28		12			
	13	x				14		13		x	28		13		x			28		13			
	14	x				14		14	x		14		14		x			28		14			
	15	x				14		15	x		14		15		x			28		15			
	16	x				14		16	x		14		16		x			28		16			
	17		x			28		17	x		14		17		x			28		17			
	18			x		42		18	x		14		18		x			28		18			
	19		x			28		19	x		14		19				A	56	M	19			

	20			x		42		20	x		14		20				A	56	F	20			
	2n d	3r d	4t h	5t h	6t h	DD A	se x	4th visi t	5t h	6t h	DD A	Se x	2n d	3r d	4t h	5t h	6th	DD A	se x	4th visi t	5t h	6t h	DD A
	1	x				14		1					1	x				14		1			
	2	x				14		2					2	x				14		2			
	3	x				14		3					3	x				14		3			
	4	x				14		4					4	x				14		4			
	5	x				14		5					5	x				14		5			
	6	x				14		6					6			A		56	F	6			
	7	x				14		7					7	x				14		7			
	8	x				14		8					8	x				14		8			
	9	x				14		9					9	x				14		9			
	10	x				14		10					10			A		56	F	10			
	11	x				14		11					11		x			28		11			

	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Sex	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA
	12	x				14		12					12	x				14		12			
	13	x				14		13					13	x				14		13			
	14	x				14		14					14	x				14		14			
	15	x				14		15					15	x				14		15			
	16	x				14		16					16	x				14		16			
	17	x				14		17					17	x				14		17			
	18	x				14		18					18	x				14		18			
	19	x				14		19					19	x				14		19			
	20	x				14		20					20	x				14		20			
305	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	313	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA

	1	x				14		1	x		14		1		x			28		1	x		14
	2	x				14		2	x		14		2		x			28		2	x		14
	3	x				14		3	x		14		3		x			28		3	x		14
	4	x				14		4	x		14		4		x			28		4	x		14
	5	x				14		5	x		14		5		x			28		5	x		14
	6	x				14		6	x		14		6			x		42		6	x		14
	7	x				14		7	x		14		7			x		42		7			
	8				A	56	F	8	x		14		8	x				14		8			
	9				A	56	F	9	x		14		9	x				14		9			
	10				A	56	M	10	x		14		10	x				14		10			
	11				A	56	M	11	x		14		11							11			
	12				A	56	F	12	x		14		12							12			
	13				A	56	F	13	x		14		13							13			
	14				A	56	F	14	x		14		14							14			
	15				A	56	M	15	x		14		15							15			
	16				A	56	M	16	x		14		16							16			

	17				A	56	F	17	x		14		17							17			
	18		x			28		18	x		14		18							18			
	19		x			28		19	x		14		19							19			
	20		x			28		20	x		14		20							20			
	2n d	3r d	4t h	5t h	6t h	DD A	se x	4th visi t	5t h	6t h	DD A	Se x	2n d	3r d	4t h	5t h	6th	DD A	se x	4th visi t	5t h	6t h	DD A
	1	x				14		1					1	x				14		1			
	2	x				14		2					2	x				14		2			
	3	x				14		3					3	x				14		3			
	4	x				14		4					4	x				14		4			
	5	x				14		5					5	x				14		5			
	6	x				14		6					6	x				14		6			
	7	x				14		7					7	x				14		7			
	8	x				14		8					8	x				14		8			

	9	x				14		9					9	x				14		9			
	10	x				14		10					10	x				14		10			
	11	x				14		11					11	x				14		11			
	12	x				14		12					12	x				14		12			
	13	x				14		13					13	x				14		13			
	14	x				14		14					14	x				14		14			
	15	x				14		15					15	x				14		15			
	16	x				14		16					16	x				14		16			
	17	x				14		17					17	x				14		17			
	18	x				14		18					18	x				14		18			
	19	x				14		19					19	x				14		19			
	20	x				14		20					20	x				14		20			

103	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	1	x				14		1		x	28

	2	x				14		2		x	28
	3				A	56	F	3		x	28
	4				A	56	F	4	x		14
	5				A	56	M	5		x	28
	6				A	56	M	6		x	28
	7	x				14		7		x	28
	8	x				14		8		x	28
	9				A	56	F	9		x	28
	10		x			28		10		x	28
	11		x			28		11			
	12		x			28		12			
	13				A	56	F	13			
	14		x			28		14			
	15							15			
	16							16			
	17							17			

	18							18			
	19							19			
	20							20			
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA
	1			A		56	F	1			
	2			A		56	F	2			
	3		x			28		3			
	4		x			28		4			
	5		x			28		5			
	6		x			28		6			
	7		x			28		7			
	8		x			28		8			
	9		x			28		9			
2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Sex
	10		x			28		10			

	11							11			
	12							12			
	13							13			
	14							14			
	15							15			
	16							16			
	17							17			
	18							18			
	19							19			
	20							20			
206	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	6th
	1				A	56	F	1			
	2				A	56	F	2			
	3	x				14		3			
	4	x				14		4			
	5	x				14		5			

	6	x				14		6			
	7				A	56	F	7			
	8				A	56	F	8			
	9				A	56	M	9			
	10				A	56	M	10			
	11				A	56	F	11			
	12				A	56	F	12			
	13		x			28		13			
	14		x			28		14			
	15		x			28		15			
	16		x			28		16			
	17				A	56	F	17			
206	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	6th
	18				A	56	M	18			
	19		x			28		19			
	20				A	14	F	20			

	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	Sex
	1	x				14		1			
	2	x				14		2			
	3	x				14		3			
	4	x				14		4			
	5	x				14		5			
	6	x				14		6			
	7	x				14		7			
	8	x				14		8			
	9	x				14		9			
	10	x				14		10			
	11	x				14		11			
	12	x				14		12			
	13	x				14		13			
	14	x				14		14			

	15	x				14		15			
	16	x				14		16			
	17	x				14		17			
	18	x				14		18			
	19	x				14		19			
	20	x				14		20			
309	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	6th
	1	x				14		1			
	2				A	56	F	2			
	3				A	56	F	3			
	4				A	56	M	4			
309	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	6th
	5				A	56	F	5			
	6				A	56	M	6			
	7				A	56	F	7			
	8				A	56	F	8			

	9				A	56	M	9			
	10				A	56	M	10			
	11				A	56	F	11			
	12				A	56	M	12			
	13				A	56	M	13			
	14				A	56	F	14			
	15				A	56	M	15			
	16				A	56	F	16			
	17		x			28		17			
	18		x			28		18			
	19		x			28		19			
	20		x			28		20			
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA
	1	x				14		1			
	2	x				14		2			
	3	x				14		3			

	4	x				14		4			
	5	x				14		5			
	6	x				14		6			
	7	x				14		7			
	8	x				14		8			
	9	x				14		9			
	10	x				14		10			
	11	x				14		11			
	12	x				14		12			
	13	x				14		13			
2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	
	14	x				14		14			
	15	x				14		15			
	16	x				14		16			
	17	x				14		17			

	18	x				14		18			
	19	x				14		19			
	20	x				14		20			

104	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	sex	DDA
	1				A	56	F	1	x			14
	2				A	56	F	2	x			14
	3				A	56	F	3		A	M	56
	4	x				14		4	x			14
	5	x				14		5	x			14
	6	x				14		6	x			14
	7	x				14		7	x			14
	8	x				14		8				
	9	x				14		9				

	10	x				14		10				
	11							11				
	12							12				
	13							13				
	14							14				
	15							15				
	16							16				
	17							17				
	18							18				
	19							19				
	20							20				
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Sex
	1			A		56	F	1				
	2			A		56	F	2				

	3			A		56	M	3				
	4		x			28		4				
	5		x			28		5				
	6	x				14		6				
2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Sex	
	7	x				14		7				
	8	x				14		8				
	9	x				14		9				
	10	x				14		10				
	11							11				
	12							12				
	13							13				
	14							14				
	15							15				
	16							16				

	17							17				
	18							18				
	19							19				
	20							20				
201	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	6th	DDA
	1				A	56	F	1	x			14
	2	x				14		2	x			14
	3				A	56	F	3	x			14
	4		x			28		4	x			14
	5	x				14		5	x			14
	6	x				14		6	x			14
	7		x			28		7				
	8		x			28		8				
	9				A	56	M	9				

	10				A	56	M	10				
	11				A	56	F	11				
	12				A	56	M	12				
201	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	6th	DDA
	13		x			28		13				
	14		x			28		14				
	15		x			28		15				
	16		x			28		16				
	17		x			28		17				
	18		x			28		18				
	19		x			28		19				
	20		x			28		20				
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Sex

	1	x				14		1				
	2	x				14		2				
	3		x			28		3				
	4	x				14		4				
	5	x				14		5				
	6	x				14		6				
	7	x				14		7				
	8		x			28		8				
	9	x				14		9				
	10	x				14		10				
	11	x				14		11				
	12	x				14		12				
	13	x				14		13				
	14	x				14		14				
	15	x				14		15				
	16	x				14		16				

	17	x				14		17				
	18	x				14		18				
2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Sex	
	19	x				14		19				
	20	x				14		20				
317	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	6th	Sex
	1				A	56	F	1				
	2				A	56	M	2				
	3				A	56	F	3				
	4				A	56	F	4				
	5		x			28		5				
	6		x			28		6				
	7		x			28		7				

	8		x			28		8				
	9							9				
	10							10				
	11							11				
	12							12				
	13							13				
	14							14				
	15							15				
	16							16				
	17							17				
	18							18				
	19							19				
	20							20				
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Sex

	1	x				14		1				
	2	x				14		2				
	3	x				14		3				
	4	x				14		4				
	5	x				14		5				
	6	x				14		6				
	7	x				14		7				
	8	x				14		8				
	9	x				14		9				
	10							10				
	11							11				
	12							12				
	13							13				
	14							14				
	15							15				
	16							16				

	17							17				
	18							18				
	19							19				
	20							20				

105	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	106	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	1				A	56	F	1	x		14		1				A	56	F	1		x	28
	2		x			28		2		x	28		2				A	56	F	2	x		14
	3				A	56	F	3	x		14		3	x				14		3	x		14
	4				A	56	F	4	x		14		4				A	56	M	4	x		14
	5	x				14		5	x		14		5	x				14		5	x		14
	6	x				14		6		x	28		6	x				14		6			
	7				A	56	F	7	x		14		7	x				14		7			
	8				A	56	M	8	x		14		8				A	56	M	8			
	9	x				14		9		x	28		9				A	56	M	9			
	10		x			28		10		x	28		10	x				14		10			
	11		x			28		11		x	28		11				A	56	M	11			
	12		x			28		12	x		14		12	x				14		12			
	13			x		42		13	x		14		13		x			28		13			

	14			x		42		14	x		14		14	x				14		14			
	15		x			28		15	x		14		15		x			28		15			
	16			x		42		16	x		14		16		x			28		16			
	17	x				14		17	x		14		17		x			28		17			
	18	x				14		18	x		14		18		x			28		18			
	19		x			28		19	x		14		19		x			28		19			
	20		x			28		20	x		14		20		x			28		20			
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Sex	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA
	1		x			28		1					1		x			28		1	x		14
	2		x			28		2					2	x				14		2	x		14
	3		x			28		3					3	x				14		3			
	4			A	M	56		4					4	x				14		4			

2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	Sex	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA	
	5			A	F	56		5					5	x				14		5			
	6			A	F	56		6					6		x			28		6			
	7		x			28		7					7		x			28		7			
	8		x			28		8					8		x			28		8			
	9	x				14		9					9		x			28		9			
	10	x				14		10					10		x			28		10			
	11	x				14		11					11							11			
	12	x				14		12					12							12			
	13	x				14		13					13							13			
	14	x				14		14					14							14			
	15	x				14		15					15							15			
	16	x				14		16					16							16			
	17	x				14		17					17							17			

	18	x				14		18					18							18			
	19		x			28		19					19							19			
	20		x			28		20					20							20			
212	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	208	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	
	1		x			28		1	x		14		1	x				14		1			
	2		x			28		2	x		14		2	x				14		2			
	3		x			28		3	x		14		3	x				14		3			
	4	x				14		4	x		14		4				A	56	F	4			
	5	x				14		5	x		14		5				A	56	F	5			
	6	x				14		6	x		14		6				A	56	F	6			
	7	x				14		7	x		14		7				A	56	M	7			
	8	x				14		8	x		14		8	x				14		8			

212	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	208	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	
	9	x				14		9	x		14		9	x				14		9			
	10	x				14		10	x		14		10				A	56	M	10			
	11	x				14		11	x		14		11				A	56	M	11			
	12	x				14		12	x		14		12				A	56	M	12			
	13	x				14		13	x		14		13				A	56	F	13			
	14	x				14		14	x		14		14				A	56	F	14			
	15	x				14		15	x		14		15				A	56	F	15			
	16	x				14		16	x		14		16				A	56	M	16			
	17	x				14		17	x		14		17				A	56	F	17			
	18	x				14		18	x		14		18	x				14		18			
	19	x				14		19	x		14		19				A	56	M	19			
	20	x				14		20	x		14		20				A	56	F	20			

	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA		2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	
	1	x				14		1					1	x				14		1			
	2	x				14		2					2	x				14		2			
	3	x				14		3					3			A		56	F	3			
	4	x				14		4					4	x				14		4			
	5	x				14		5					5			A		56	M	5			
	6	x				14		6					6	x						6			
	7	x				14		7					7	x						7			
	8		x			28		8					8	x						8			
	9	x				14		9					9	x						9			
	10	x				14		10					10	x						10			
	11		x			28		11					11	x						11			
	12		x			28		12					12	x						12			

2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA		2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th		
	13	x				14		13					13	x						13			
	14	x				14		14					14	x						14			
	15	x				14		15					15	x						15			
	16	x				14		16					16	x						16			
	17	x				14		17					17	x						17			
	18	x				14		18					18	x						18			
	19	x				14		19					19	x						19			
	20	x				14		20					20	x						20			
318	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	311	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	1		x			28		1	x		14		1	x				14		1	x		14

	2		x			28		2	x		14		2	x				14		2	x		14
	3		x			28		3	x		14		3	x				14		3	x		14
	4		x			28		4	x		14		4	x				14		4	x		14
	5	x				14		5	x		14		5		x			28		5	x		14
	6	x				14		6	x		14		6		x			28		6	x		14
	7	x				14		7	x		14		7		x			28		7	x		14
	8	x				14		8	x		14		8	x				14		8	x		14
	9	x				14		9	x		14		9	x				14		9	x		14
	10	x				14		10	x		14		10	x				14		10	x		14
	11	x				14		11					11							11			
	12	x				14		12					12							12			
	13	x				14		13					13							13			
	14	x				14		14					14							14			
	15	x				14		15					15							15			
	16							16					16							16			

318	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	311	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	17							17					17							17			
	18							18					18							18			
	19							19					19							19			
	20							20					20							20			
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA		2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	
	1		x			28		1					1		x			28		1			
	2	x				14		2					2	x				14		2			
	3	x				14		3					3	x				14		3			
	4	x				14		4					4	x				14		4			
	5	x				14		5					5	x				14		5			

	6	x				14		6					6	x				14		6			
	7	x				14		7					7	x				14		7			
	8	x				14		8					8	x				14		8			
	9	x				14		9					9	x				14		9			
	10	x				14		10					10	x				14		10			
	11	x				14		11					11	x				14		11			
	12	x				14		12					12	x				14		12			
	13	x				14		13					13	x				14		13			
	14	x				14		14					14	x				14		14			
	15	x				14		15					15	x				14		15			
	16	x				14		16					16	x				14		16			
	17	x				14		17					17	x				14		17			
	18	x				14		18					18	x				14		18			
	19	x				14		19					19	x				14		19			
	20	x				14		20					20	x				14		20			

107	1st	2n d	3r d	4th	5th		se x	3r d vis it	4th	5t h	D D A	108	1st	2n d	3r d	4th	5th		se x	3r d vis it	4th	5t h	D D A
	1	x				14		1	x		14		1				A	56	M	1	x		14
	2	x				14		2	x		14		2				A	56	F	2	x		14
	3	x				14		3	x		14		3				A	56	M	3	x		14
	4	x				14		4	x		14		4				A	56	F	4	x		14
	5	x				14		5	x		14		5				A	56	F	5	x		14
	6				A	56	F	6	x		14		6		x			28		6	x		14
	7				A	56	M	7	x		14		7		x			28		7	x		14
	8				A	56	F	8	x		14		8		x			28		8	x		14
	9				A	56	F	9	x		14		9							9	x		14
	10				A	56	M	10	x		14		10							10	x		14
	11				A	56	F	11	x		14		11							11			
	12		x			28		12	x		14		12							12			

	13		x			28		13	x		14		13							13			
	14		x			28		14	x		14		14							14			
	15			x		42		15	x		14		15							15			
	16		x			28		16	x		14		16							16			
	17			x		42		17	x		14		17							17			
	18		x			28		18	x		14		18							18			
	19			x		42		19	x		14		19							19			
	20		x			28		20	x		14		20							20			
	2nd	3rd	4th	5th	6th	DDA	sex	4th	5th	6th			2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA
	1		x			28		1					1	x				14		1	x		14
	2	x				14		2					2		x			28		2	x		14
	3	x				14		3					3	x				14		3	x		14
	4	x				14		4					4	x				14		4	x		14

	2n d	3r d	4t h	5th	6th	D DA	se x	4th	5th	6t h			2n d	3r d	4t h	5th	6th	D DA	se x	4th vis it	5th	6t h	D D A
	5	x				14		5					5	x				14		5			
	6	x				14		6					6	x				14		6			
	7	x				14		7					7	x				14		7			
	8	x				14		8					8		x			28		8			
	9	x				14		9					9		x			28		9			
	10			A		56	F	10					10		x			28		10			
	11			A		56	F	11					11		x			28		11			
	12		x			28		12					12							12			
	13	x				14		13					13							13			
	14	x				14		14					14							14			
	15		x			28		15					15							15			
	16	x				14		16					16							16			
	17	x				14		17					17							17			

	18		x			28		18					18							18			
	19	x				14		19					19							19			
	20	x				14		20					20							20			
211	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	215	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	1				A	56	F	1	x		14		1	x				14		1	x		14
	2				A	56	F	2	x		14		2		x			28		2	x		14
	3				A	56	M	3	x		14		3	x				14		3	x		14
	4				A	56	M	4	x		14		4		x			28		4	x		14
	5	x				14		5	x		14		5	x				14		5			
	6	x				14		6	x		14		6	x				14		6			
	7				A	56	F	7	x		14		7							7			
	8		x			28		8	x		14		8							8			

211	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	215	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	9		x			28		9	x		14		9							9			
	10							10	x		14		10							10			
	11							11	x		14		11							11			
	12							12	x		14		12							12			
	13							13	x		14		13							13			
	14							14	x		14		14							14			
	15							15	x		14		15							15			
	16							16	x		14		16							16			
	17							17	x		14		17							17			
	18							18	x		14		18							18			
	19							19	x		14		19							19			
	20							20	x		14		20							20			

	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	
	1	x				14		1					1	x				14		1			
	2	x				14		2					2	x				14		2			
	3	x				14		3					3	x				14		3			
	4	x				14		4					4	x				14		4			
	5	x				14		5					5	x				14		5			
	6		x			28		6					6	x				14		6			
	7		x			28		7					7		x			28		7			
	8		x			28		8					8	x				14		8			
	9		x			28		9					9	x				14		9			
	10	x				14		10					10	x				14		10			
	11	x				14		11					11							11			
	12	x				14		12					12							12			

2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th		
	13	x				14		13					13							13			
	14	x				14		14					14							14			
	15		x			28		15					15							15			
	16		x			28		16					16							16			
	17		x			28		17					17							17			
	18		x			28		18					18							18			
	19	x				14		19					19							19			
	20	x				14		20					20							20			
315	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	312	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA

	1		x			28		1	x		14		1	x				14		1	x		14
	2		x			28		2	x		14		2	x				14		2	x		14
	3				A	56	F	3	x		14		3		x			28		3	x		14
	4				A	56	F	4	x		14		4		x			28		4			
	5				A	56	F	5	x		14		5		x			28		5			
	6				A	56	F	6	x		14		6			x		42		6			
	7		x			28		7	x		14		7			x		42		7			
	8	x				14		8	x		14		8			x		42		8			
	9	x				14		9	x		14		9			x		42		9			
	10	x				14		10	x		14		10		x			28		10			
	11	x				14		11	x		14		11		x			28		11			
	12							12	x		14		12							12			
	13							13	x		14		13							13			
	14							14	x		14		14							14			
	15							15					15							15			
	16							16					16							16			

315	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	312	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	17							17					17							17			
	18							18					18							18			
	19							19					19							19			
	20							20					20							20			
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th		DDA	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	
	1		x			28		1					1	x				14		1			
	2		x			28		2					2	x				14		2			
	3	x				14		3					3	x				14		3			
	4	x				14		4					4	x				14		4			

	5	x				14		5					5	x				14		5			
	6	x				14		6					6	x				14		6			
	7	x				14		7					7	x				14		7			
	8	x				14		8					8	x				14		8			
	9	x				14		9					9	x				14		9			
	10	x				14		10					10	x				14		10			
	11							11					11	x				14		11			
	12							12					12	x				14		12			
	13							13					13	x				14		13			
	14							14					14	x				14		14			
	15							15					15	x				14		15			
	16							16					16							16			
	17							17					17							17			
	18							18					18							18			
	19							19					19							19			
	20							20					20							20			

109	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	110	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	1				A	56	F	1	x		14		1				A	56	F	1	x		14
	2		x			28		2		x	28		2				A	56	M	2	x		14
	3		x			28		3	x		14		3				A	56	M	3	x		14
	4	x				14		4		x	28		4				A	56	F	4		x	28
	5	x				14		5	x		14		5				A	56	F	5	x		14
	6	x				14		6	x		14		6							6	x		14
	7				A	56	F	7	x		14		7							7			
	8				A	56	F	8	x		14		8							8			
	9				A	56	M	9	x		14		9							9			
	10		x			28		10		x	28		10							10			
	11							11					11							11			
	12							12					12							12			
	13							13					13							13			

	14							14					14							14			
	15							15					15							15			
	16							16					16							16			
	17							17					17							17			
	18							18					18							18			
	19							19					19							19			
	20							20					20							20			
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA		2nd	3rd	4th	5th	6th		sex	4th visit	5th	6th	
	1		x			28		1	x		14		1			A		56	F	1			
	2	x				14		2	x		14		2	x				14		2			
	3	x				14		3	x		14		3	x				14		3			
	4	x				14		4	x		14		4	x				14		4			

2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA		2nd	3rd	4th	5th	6th		sex	4th visit	5th	6th		
	5	x				14		5	x		14		5	x				14		5			
	6	x				14		6					6							6			
	7	x				14		7					7							7			
	8		x			28		8					8							8			
	9	x				14		9					9							9			
	10		x			28		10					10							10			
	11		x			28		11					11							11			
	12	x				14		12					12							12			
	13							13					13							13			
	14							14					14							14			
	15							15					15							15			
	16							16					16							16			
	17							17					17							17			

	18							18					18							18			
	19							19					19							19			
	20							20					20							20			
21 3	1st	2n d	3r d	4t h	5th	DD A	sex	3rd vis it	4t h	5th		21 4	1st	2n d	3r d	4t h	5t h	DD A	sex	3rd vis it	4t h	5t h	DD A
	1	x				14		1					1		x			28		1	x		14
	2	x				14		2					2		x			28		2	x		14
	3	x				14		3					3	x				14		3	x		14
	4		x			28		4					4	x				14		4	x		14
	5		x			28		5					5	x				14		5	x		14
	6	x				14		6					6	x				14		6	x		14
	7	x				14		7					7	x				14		7	x		14
	8							8					8	x				14		8	x		14

21 3	1st	2n d	3r d	4t h	5th	DD A	sex	3rd vis it	4t h	5th		21 4	1st	2n d	3r d	4t h	5t h	DD A	sex	3rd vis it	4t h	5t h	DD A
	9							9					9	x				14		9	x		14
	10							10					10	x				14		10	x		14
	11							11					11							11	x		14
	12							12					12							12			
	13							13					13							13			
	14							14					14							14			
	15							15					15							15			
	16							16					16							16			
	17							17					17							17			
	18							18					18							18			
	19							19					19							19			
	20							20					20							20			

	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	
	1	x				14		1					1		x			28		1			
	2	x				14		2					2		x			28		2			
	3	x				14		3					3	x				14		3			
	4	x				14		4					4	x				14		4			
	5	x				14		5					5	x				14		5			
	6		x			28		6					6	x				14		6			
	7		x			28		7					7	x				14		7			
	8							8					8	x				14		8			
	9							9					9	x				14		9			
	10							10					10	x				14		10			
	11							11					11							11			
	12							12					12							12			

	2n d	3r d	4t h	5t h	6th	DD A	sex	4th vis it	5t h	6th			2n d	3r d	4t h	5t h	6t h	DD A	sex	4th vis it	5t h	6t h	
	13							13					13							13			
	14							14					14							14			
	15							15					15							15			
	16							16					16							16			
	17							17					17							17			
	18							18					18							18			
	19							19					19							19			
	20							20					20							20			
30 1	1st	2n d	3r d	4t h	5th	DD A	sex	3rd vis it	4t h	5th		30 8	1st	2n d	3r d	4t h	5t h	DD A	sex	3rd vis it	4t h	5t h	
	1	x				14		1					1			x		42		1			

	2				A	56	M	2					2			A		56	F	2			
	3				A	56	F	3					3			x		42		3			
	4				A	56	F	4					4							4			
	5				A	56	M	5					5							5			
	6				A	56	F	6					6							6			
	7		x			28		7					7							7			
	8		x			28		8					8							8			
	9		x			28		9					9							9			
	10		x			28		10					10							10			
	11		x			28		11					11							11			
	12							12					12							12			
	13							13					13							13			
	14							14					14							14			
	15							15					15							15			
	16							16					16							16			

30 1	1st	2n d	3r d	4t h	5th	DD A	sex	3rd vis it	4t h	5th		30 8	1st	2n d	3r d	4t h	5t h	DD A	sex	3rd vis it	4t h	5t h	
	17							17					17							17			
	18							18					18							18			
	19							19					19							19			
	20							20					20							20			
	2n d	3r d	4t h	5t h	6th	DD A	sex	4th vis it	5t h	6th			2n d	3r d	4t h	5t h	6t h	DD A	sex	4th vis it	5t h	6t h	
	1	x				14		1					1	x				14		1			
	2	x				14		2					2	x				14		2			
	3	x				14		3					3	x				14		3			
	4	x				14		4					4	x				14		4			
	5	x				14		5					5							5			

	6	x				14		6					6							6			
	7	x				14		7					7							7			
	8	x				14		8					8							8			
	9	x				14		9					9							9			
	10	x				14		10					10							10			
	11	x				14		11					11							11			
	12	x				14		12					12							12			
	13	x				14		13					13							13			
	14	x				14		14					14							14			
	15	x				14		15					15							15			
	16	x				14		16					16							16			
	17	x				14		17					17							17			
	18	x				14		18					18							18			
	19	x				14		19					19							19			
	20	x				14		20					20							20			

111	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	112	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	1	x				14		1	x		14		1	x				14		1	x		14
	2	x				14		2	x		14		2	x				14		2	x		14
	3				A	56	F	3	x		14		3	x				14		3	x		14
	4				A	56	M	4	x		14		4	x				14		4	x		14
	5				A	56	F	5	x		14		5	x				14		5	x		14
	6				A	56	M	6	x		14		6	x				14		6	x		14
	7				A	56	F	7	x		14		7	x				14		7		x	28
	8				A	56	F	8	x		14		8	x				14		8		x	28
	9	x				14		9	x		14		9	x				14		9	x		14
	10				A	56	M	10	x		14		10	x				14		10		x	28
	11	x				14		11	x		14		11				A	56	F	11		x	28
	12				A	56	F	12	x		14		12				A	56	F	12	x		14
	13				A	56	M	13	x		14		13				A	56	F	13	x		14

	14				A	56	M	14	x		14		14				A	56	M	14	x		14
	15	x				14		15	x		14		15				A	56	F	15	x		14
	16	x				14		16			14		16				A	56	F	16	x		14
	17	x				14		17			14		17				A	56	M	17		x	28
	18	x				14		18			14		18				A	56	M	18		x	28
	19	x				14		19			14		19	x				14		19		x	28
	20	x				14		20			14		20	x				14		20	x		14
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA		2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA
	1			A		56	F	1	x		14		1		x			28		1	x		14
	2			A		56	F	2	x		14		2		x			28		2	x		14
	3			A		56	F	3					3	x				14		3	x		14
	4	x				14		4					4	x				14		4	x		14

	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA		2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	DDA
	5	x				14		5					5		x			28		5	x		14
	6	x				14		6					6	x				14		6	x		14
	7	x				14		7					7		x			28		7	x		14
	8	x				14		8					8	x				14		8	x		14
	9	x				14		9					9	x				14		9	x		14
	10	x				14		10					10		x			28		10	x		14
	11	x				14		11					11		x			28		11			
	12	x				14		12					12		x			28		12			
	13	x				14		13					13		x			28		13			
	14			A		42	M	14					14		x			28		14			
	15			A		42	M	15					15		x			28		15			
	16	x				14		16					16		x			28		16			
	17		x			28		17					17	x				14		17			

	18		x			28		18					18		x			28		18			
	19		x			28		19					19		x			28		19			
	20		x			28		20					20			x		42		20			
218	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	210	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	1		x			28		1	x		14		1	x				14		1	x		14
	2		x			28		2	x		14		2				A	56	F	2	x		14
	3	x				14		3	x		14		3				A	56	F	3	x		14
	4	x				14		4		x	28		4				A	56	F	4	x		14
	5	x				14		5		x	28		5				A	56	M	5	x		14
	6	x				14		6		x	28		6				A	56	M	6	x		14
	7	x				14		7		x	28		7				A	56	M	7	x		14
	8	x				14		8					8				A	56	F	8	x		14
	9	x				14		9					9				A	56	F	9	x		14

	10		x			28		10					10				A	56	F	10	x		14
	11		x			28		11					11				A	56	M	11	x		14
	12		x			28		12					12				A	56	M	12	x		14
	13		x			28		13					13				A	56	F	13	x		14
	14			x		42		14					14				A	56	F	14	x		14
	15			x		42		15					15				A	56		15	x		14
	16							16					16		x			28		16	x		14
	17							17					17		x			28		17	x		14
	18							18					18							18	x		14
	19							19					19							19	x		14
	20							20					20							20	x		14
	2nd	3rd	4th	5th	6th	DDDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	
	1		x			28		1					1	x				14		1			

	2		x			28		2					2		x			28		2			
	3	x				14		3					3			A		56	F	3			
	4	x				14		4					4		x			28		4			
	5	x				14		5					5		x			28		5			
	6	x				14		6					6		x			28		6			
	7	x				14		7					7		x			28		7			
	8	x				14		8					8	x				14		8			
	9	x				14		9					9	x				14		9			
	10	x				14		10					10	x				14		10			
	11	x				14		11					11		x			28		11			
	12	x				14		12					12	x				14		12			
	13	x				14		13					13		x			28		13			
	14	x				14		14					14	x				14		14			
	15	x				14		15					15	x				14		15			

	2nd	3rd	4th	5th	6th	DDDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	
	16	x				14		16					16	x				14		16			
	17	x				14		17					17	x				14		17			
	18	x				14		18					18	x				14		18			
	19	x				14		19					19		x			28		19			
	20	x				14		20					20		x			28		20			
306	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	307	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA
	1	x				14		1		x	28		1		x			28		1	x		14
	2	x				14		2		x	28		2		x			28		2	x		14
	3	x				14		3	x		14		3			x		42		3	x		14
	4	x				14		4	x		14		4				A	56	M	4	x		14

	5				A	56	F	5	x		14		5				A	56	M	5	x		14
	6				A	56	F	6	x		14		6				A	56	M	6	x		14
	7				A	56	F	7	x		14		7				A	56	F	7	x		14
	8				A	56	F	8	x		14		8				A	56	F	8	x		14
	9		x			28		9	x		14		9				A	56	F	9	x		14
	10				A	56	M	10	x		14		10				A	56	F	10	x		14
	11				A	56	F	11					11				A	56	M	11	x		14
	12				A	56	F	12					12		x			28		12	x		14
	13				A	56	F	13					13	x				14		13	x		14
	14				A	56	M	14					14	x				14		14	x		14
	15				A	56	M	15					15	x				14		15	x		14
	16							16					16							16			
	17							17					17							17			
	18							18					18							18			
	19							19					19							19			
	20							20					20							20			

	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th	
	1		x			28		1					1	x				14		1			
	2		x			28		2					2	x				14		2			
	3	x				14		3					3	x				14		3			
	4	x				14		4					4	x				14		4			
	5	x				14		5					5	x				14		5			
	6	x				14		6					6	x				14		6			
	7	x				14		7					7	x				14		7			
	8	x				14		8					8	x				14		8			
	9	x				14		9					9	x				14		9			
	10	x				14		10					10	x				14		10			
	11	x				14		11					11	x				14		11			
	12	x				14		12					12	x				14		12			
	13	x				14		13					13	x				14		13			

	14	x				14		14					14	x				14		14			
	15	x				14		15					15	x				14		15			
	16	x				14		16					16							16			
	17	x				14		17					17							17			
	18	x				14		18					18							18			
	19	x				14		19					19							19			
	20	x				14		20					20							20			

113	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	114	1st	2nd	3rd	4th	5th	sex	3rd visit	4th	5th	sex	DDA

	1	x				14		1	x		14		1						1	x			14
	2				A	56	F	2	x		14		2						2	x			14
	3				A	56	F	3	x		14		3						3	x			14
	4	x				14		4	x		14		4						4		A	F	56
	5	x				14		5		x	28		5						5	x			14
	6				A	56	F	6	x		14		6						6	x			14
	7	x				14		7	x		14		7						7	x			14
	8	x				14		8	x		14		8						8		A	F	56
	9				A	56	M	9		x	28		9						9	x			14
	10	x				14		10	x		14		10						10	x			14
	11				A	56	F	11	x		14		11						11	x			14
	12				A	56	F	12	x		14		12						12	x			14
	13				A	56	M	13	x		14		13						13	x			14
	14	x				14		14	x		14		14						14	x			14
	15				A	56	M	15	x		14		15						15	x			14
	16		x			28		16	x		14		16						16		A	F	56

	17	x				14		17	x		14		17						17	x			14
	18		x			28		18	x		14		18						18	x			14
	19		x			28		19	x		14		19						19		x		28
	20		x			28		20	x		14		20						20		x		28
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	sex	4th visit	5th	6th		
	1		x			28		1					1						1				
	2		x			28		2					2						2				
	3	x				14		3					3						3				
	4			A		56	M	4					4						4				
	5			A		56	M	5					5						5				
	6		x			28		6					6						6				
	7		x			28		7					7						7				
	8		x			28		8					8						8				

	9		x			28		9					9						9				
	10		x			28		10					10						10				
	11		x			28		11					11						11				
	12		x			28		12					12						12				
	13		x			28		13					13						13				
	14	x				14		14					14						14				
	15	x				14		15					15						15				
	16	x				14		16					16						16				
	17	x				14		17					17						17				
	18		x			28		18					18						18				
	19	x				14		19					19						19				
	20	x				14		20					20						20				
21 7	1st	2n d	3r d	4t h	5t h	DD A	se x	3rd visi t	4t h	5t h	DD A	21 6	1st	2n d	3r d	4t h	5t h	se x	3rd visi t	4t h	5t h	6th	DD A

	1		x			28		1	x		14		1						1	x			14
	2		x			28		2	x		14		2						2	x			14
	3		x			28		3	x		14		3						3	x			14
	4	x				14		4	x		14		4						4	x			14
	5	x				14		5	x		14		5						5	x			14
	6	x				14		6	x		14		6						6	x			14
	7	x				14		7	x		14		7						7	x			14
	8	x				14		8	x		14		8						8	x			14
	9	x				14		9	x		14		9						9	x			14
	10	x				14		10	x		14		10						10	x			14
	11	x				14		11					11						11	x			14
217	1st	2nd	3rd	4th	5th	DDA	sex	3rd visit	4th	5th	DDA	216	1st	2nd	3rd	4th	5th	sex	3rd visit	4th	5th	6th	DDA
	12	x				14		12					12						12	x			14
	13	x				14		13					13						13	x			14

	14	x				14		14					14						14	x			14
	15	x				14		15					15						15		A	F	56
	16	x				14		16					16						16		A	F	56
	17	x				14		17					17						17		A	M	56
	18	x				14		18					18						18	x			14
	19	x				14		19					19						19	x			14
	20	x				14		20					20						20	x			14
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	sex	4th visit	5th	6th		
	1	x				14		1					1						1				
	2		x			28		2					2						2				
	3	x				14		3					3						3				
	4	x				14		4					4						4				
	5	x				14		5					5						5				
	6	x				14		6					6						6				

	7	x				14		7					7						7				
	8	x				14		8					8						8				
	9	x				14		9					9						9				
	10	x				14		10					10						10				
	11	x				14		11					11						11				
	12		x			28		12					12						12				
	13		x			28		13					13						13				
	14	x				14		14					14						14				
	15	x				14		15					15						15				
	16	x				14		16					16						16				
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	sex	4th visit	5th	6th		
	17	x				14		17					17						17				
	18	x				14		18					18						18				
	19	x				14		19					19						19				

	20	x				14		20					20						20				
30 2	1st	2n d	3r d	4t h	5t h	DD A	se x	3rd visi t	4t h	5t h	DD A	30 3	1st	2n d	3r d	4t h	5t h	se x	3rd visi t	4t h	5t h	Se x	DD A
	1				A	56	M	1	x		14		1						1		A	F	56
	2				A	56	F	2	x		14		2						2		A	M	56
	3				A	56	F	3	x		14		3						3		A	F	56
	4				A	56	F	4	x		14		4						4		A	M	56
	5				A	56	F	5	x		14		5						5		A	F	56
	6				A	56	F	6	x		14		6						6		A	M	56
	7				A	56	M	7	x		14		7						7	x			14
	8							8	x		14		8						8	x			14
	9							9	x		14		9						9	x			14
	10							10	x		14		10						10	x			14
	11							11	x		14		11						11	x			14

	12							12	x		14		12						12	x			14
	13							13	x		14		13						13	x			14
	14							14	x		14		14						14	x			14
	15							15	x		14		15						15	x			14
	16							16	x		14		16						16	x			14
	17							17	x		14		17						17	x			14
	18							18	x		14		18						18	x			14
	19							19	x		14		19						19	x			14
	20							20	x		14		20						20	x			14
	2nd	3rd	4th	5th	6th	DDA	sex	4th visit	5th	6th			2nd	3rd	4th	5th	6th	sex	4th visit	5th	6th		
	1	x				14		1					1						1				
	2	x				14		2					2						2				
	3	x				14		3					3						3				
	4	x				14		4					4						4				

	5	x				14		5					5						5				
	6	x				14		6					6						6				
	7	x				14		7					7						7				
	8	x				14		8					8						8				
	9	x				14		9					9						9				
	10	x				14		10					10						10				
	11	x				14		11					11						11				
	12	x				14		12					12						12				
	13	x				14		13					13						13				
	14	x				14		14					14						14				
	15	x				14		15					15						15				
	16	x				14		16					16						16				
	17	x				14		17					17						17				
	18	x				14		18					18						18				
	19	x				14		19					19						19				
	20	x				14		20					20						20				

115	1st	2nd	3rd	4th	5th	sex	3rd visit	4th	5th	sex	DDA
	1						1	x			14
	2						2	x			14
	3						3	x			14
	4						4	x			14
	5						5		A	F	56
	6						6	x			14
	7						7	x			14
	8						8	x			14
	9						9		x		28
	10						10	x			14
	11						11	x			14
	12						12	x			14
	13						13	x			14

	14						14		x		28
	15						15	x			14
	16						16	x			14
	17						17	x			14
	18						18	x			14
	19						19	x			14
	20						20	x			14
	2nd	3rd	4th	5th	6th	sex	4th visit	5th	6th	Sex	DDA
	1						1				
	2						2				
	3						3				
	4						4				
	5						5				
	6						6				
	7						7				
	8						8				

	9						9				
	2nd	3rd	4th	5th	6th	sex	4th visit	5th	6th	Sex	DDA
	10						10				
	11						11				
	12						12				
	13						13				
	14						14				
	15						15				
	16						16				
	17						17				
	18						18				
	19						19				
	20						20				
202	1st	2nd	3rd	4th	5th	sex	3rd visit	4th	5th	Sex	DDA
	1						1	x			14
	2						2	x			14

	3						3	x			14
	4						4	x			14
	5						5		A	F	56
	6						6		x		28
	7						7		x		28
	8						8	x			14
	9						9	x			14
	10						10	x			14
	11						11	x			14
	12						12	x			14
	13						13	x			14
	14						14	x			14
	15						15	x			14
	16						16	x			14
	17						17	x			14
	18						18	x			14

202	1st	2nd	3rd	4th	5th	sex	3rd visit	4th	5th	Sex	DDA
	19						19	x			14
	20						20	x			14
	2nd	3rd	4th	5th	6th	sex	4th visit	5th	6th	Sex	DDA
	1						1				
	2						2				
	3						3				
	4						4				
	5						5				
	6						6				
	7						7				
	8						8				
	9						9				
	10						10				
	11						11				

	12						12				
	13						13				
	14						14				
	15						15				
	16						16				
	17						17				
	18						18				
	19						19				
	20						20				
316	1st	2nd	3rd	4th	5th	sex	3rd visit	4th	5th	Sex	DDA
	1						1		x		28
	2						2	x			14
	3						3	x			14
	4						4	x			14
316	1st	2nd	3rd	4th	5th	sex	3rd visit	4th	5th	Sex	DDA

	5						5		A	F	56
	6						6	x			14
	7						7	x			14
	8						8	x			14
	9						9	x			14
	10						10		A	M	56
	11						11	x			14
	12						12	x			14
	13						13	x			14
	14						14		A	M	56
	15						15	x			14
	16						16	x			14
	17						17	x			14
	18						18	x			14
	19						19	x			14
	20						20	x			14

	2nd	3rd	4th	5th	6th	sex	4th visit	5th	6th	Sex	DDA
	1						1				
	2						2				
	3						3				
	4						4				
	5						5				
	6						6				
	7						7				
	8						8				
	9						9				
	10						10				
	11						11				
	12						12				
	13						13				
	14						14				

	15						15					
	16						16					
	17						17					
	18						18					
	19						19					
	20						20					

APPENDIX I: Rainfall data 2016.

Date- 2016	Precipitation(cm)
4-1	0
4-2	0
4-3	0
4-4	0
4-5	0
4-6	1.24
4-7	0.02
4-8	0
4-9	0
4-10	0.76
4-11	1.7
4-12	0
4-13	0
4-14	0
4-15	0
4-16	0
4-17	0
4-18	0
4-19	0
4-20	1.04

4-21	0.07
4-22	0.02
4-23	0
4-24	0
4-25	0
4-26	1.34
4-27	3.07
4-28	0
4-29	0.27
4-30	4.64
5-1	0
5-2	0
5-3	0
5-4	0.07
5-5	0
5-6	0
5-7	0.17
5-8	0
5-9	1.54
5-10	0.17
5-11	2.2
5-12	1.16
5-13	0.27

5-14	0
5-15	0
5-16	0.71
5-17	1.98
5-18	0
5-19	0
5-20	0.15
5-21	0
5-22	0
5-23	0
5-24	0
5-25	0.127
5-26	0.66
5-27	0.93
5-28	0
5-29	0
5-30	0
5-31	0.05
6-1	0.02
6-2	0
6-3	0.02
6-4	1.82
6-5	0

6-6	0
6-7	0
6-8	0
6-9	0
6-10	0
6-11	0
6-12	0
6-13	0.07
6-14	0
6-15	0
6-16	0
6-17	0
6-18	0
6-19	0
6-20	0
6-21	0.02
6-22	0
6-23	0
6-24	0
6-25	0.05
6-26	0
6-27	0
6-28	0

6-29	0
6-30	0.76
7-1	1.52
7-2	0.48
7-3	2.87
7-4	0.86
7-5	0
7-6	0.33
7-7	0.3
7-8	0.17
7-9	0
7-10	0
7-11	0
7-12	0
7-13	0.43
7-14	0.83
7-15	0
7-16	0
7-17	0.05
7-18	0
7-19	0.71
7-20	3.02
7-21	0

7-22	0
7-23	0
7-24	0
7-25	0.25
7-26	0
7-27	0
7-28	0
7-29	1.19
7-30	0
7-31	0.02
8-1	1.24
8-2	0.05
8-3	1.9
8-4	0
8-5	0.15
8-6	0
8-7	0
8-8	0
8-9	0
8-10	0
8-11	0
8-12	0.66
8-13	0.45

8-14	3.88
8-15	7.06
8-16	0.25
8-17	0
8-18	0
8-19	0
8-20	0
8-21	0
8-22	0
8-23	0.05
8-24	0
8-25	0
8-26	0
8-27	0
8-28	0
8-29	0
8-30	0.07
8-31	0
9-1	0
9-2	0
9-3	0
9-4	0
9-5	0

9-6	0
9-7	0
9-8	0.76
9-9	8.99
9-10	3.32
9-11	0
9-12	0
9-13	0
9-14	0.07
9-15	0
9-16	3.81
9-17	0.07
9-18	0
9-19	0
9-20	0
9-21	0
9-22	0
9-23	0
9-24	0
9-25	0.38
9-26	0.17
9-27	0
9-28	0

9-29	0.07
9-30	0.5
10-1	0.02
10-2	0
10-3	0
10-4	0
10-5	0
10-6	0
10-7	0
10-8	0
10-9	0
10-10	0
10-11	0
10-12	0.17
10-13	0
10-14	0
10-15	0
10-16	0
10-17	0
10-18	0
10-19	2.38
10-20	3.3
10-21	0

10-22	0
10-23	0
10-24	0
10-25	0
10-26	0.25
10-27	0
10-28	0
10-29	0
10-30	0
10-31	0

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Effect of PPO-inhibiting herbicides on male-to-female sex ratio of *Amaranthus palmeri*

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