Giant Ragweed (*Ambrosia trifida*) Seed Bank Dynamics and Management

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CHAPTER 1. A REVIEW OF LITERATURE

1.1 Giant Ragweed Biology

Giant ragweed (*Ambrosia trifida* L.) is a monoecious summer annual native to North America, traditionally inhabiting disturbed habitats with rich soils, such as floodplains and agricultural fields (Abul-Fatih and Bazzaz 1979a). Giant ragweed is one of the earliest emerging weeds in agricultural fields, with most seedlings emerging prior to the accumulation of 150 GDD (9^{o}C) (Werle et al. 2014). In Minnesota, Giant ragweed may begin emerging at the middle of March and continue through the middle of July (Abul-Fatih and Bazzaz 1979b) The majority of seedlings germinate at a depth of 2cm when soil temperature ranges between 10 and 24^{o}C and moisture ranges between 26 and 33% (Abul-Fatih and Bazzaz 1979b). Seedlings have two large cotyledons with true leaves being arranged oppositely on the stem. The first leaves are un-lobed while sequential leaves are typically lobed palmately with three or five lobes (Bassett and Crompton, 1982). Plant height tends to range from one to six meters, depending on the level of competition, and tend to grow taller than surrounding vegetation (Abul-Fatih et al. 1979). Giant Ragweed is photoperiod sensitive, and begins reproductive stages as daylight diminishes (<12 hrs) in late July with inflorescences emerging the beginning of August (Mann 1942). Giant ragweed produces female flowers at leaf axils and male inflorescences at meristems. At peak anthesis, giant ragweed plants produce over one million pollen grains per day, with the potential to disperse up to one kilometer (Raynor et al. 1970). Giant ragweed has been shown to produce up to 3,000 to 5,000 seeds m^{-2} in ideal environments, with agriculture fields typically producing 500 seeds m^{-2}, depending on life history (Abul-Fatih and Bazzaz 1979a; Harrison et al. 2001). Seeds (achenes) are surrounded by an involucre, have both covering structure enforced dormancy and physiological dormancy, and require cold stratification to alleviate dormancy (Bassett and Crompton, 1982; Schutte et al. 2012; Ballard et al. 1996). Schutte et al. (2008) determined agricultural populations from Ohio followed a biphasic emergence pattern, with an initial flush followed by a lag phase before a later flush emerged. It was determined that agricultural populations had greater levels of embryo dormancy but similar levels of covering structure-enforced dormancy, causing the agricultural populations to have an extended emergence period compared to successional populations (Schutte et al. 2012). Giant ragweed seed can be depleted relatively quickly, with up to 50% of seed being consumed by insects, rodents, and earthworms during one overwintering period (Harrison et al. 2003; Regnier et al. 2008). Giant ragweed produces a number of empty, non-viable seeds that deters seed predators by increasing foraging time, thereby increasing the survival rate of viable seeds. Giant ragweed seeds are large with little dispersal from the parent plant, and can remain viable in the soil seed bank for up to four years (Harrison et al. 2003).

1.2 Competitive ability of Giant Ragweed:

Giant ragweed is one of the most competitive agricultural weeds (Stoller et al. 1987; Webster et al. 1994). Webster et al. (1994) showed that a single giant ragweed plant m^{-2} can reduce soybean yields from 45-77%. Economic thresholds for giant ragweed in soybean have been determined to be 2 plants per $9m^{-1}$ of row, with the critical weed free period being 8-10 weeks (Baysinger and Sims 1991). With the development of herbicide resistance to ALS inhibitor and glyphosate herbicides, giant ragweed tends to be most problematic in soybean due to the few remaining herbicides that provide effective control.

Giant ragweed is highly competitive in corn as well, with up to 90% yield reduction with 13.8 plants per 10 m^{-2} when giant ragweed emerges with corn. Even one giant ragweed plant per 10 m^{-2} in corn can reduce corn yields by 13.6% (Harrison et al. 2001). However, if giant ragweed emergence is delayed by 4 weeks after corn emergence, the competitive ability of giant ragweed is reduced by 4 to 8 – fold (Harrison et al. 2001). When giant ragweed emerges with corn, the economic threshold is 0.4 plants per $10m^2$, but when giant ragweed emerges 4 weeks after corn, the economic threshold is increased to 4.2 plants per 10 m^2 (Harrison et al. 2001).

1.3 Herbicide Resistance

Throughout the world, agricultural weeds have historically been one of the most widespread and problematic factors influencing agriculture. In the United States alone, weeds annually cause a 12% overall reduction in yield, equating to approximately $33 billion in lost crop production (Pimentel et al. 2005). Weeds increase the cost of production through reduced crop yield, reduced commodity prices due to weed-seed contamination, increased necessity for mechanical and cultural controls, and the additional expense of herbicides, which cost an additional $4 billion annually (Pimentel et al. 2005). In addition to the current cost of weeds, the development of herbicide resistance adds additional concern (Asmus et al. 2013). Currently, there are 457 biotypes within 246 species of weeds known to have herbicide resistance worldwide (Heap, 2015).

This number continues to rise, increasing the density of resistant biotypes and thus increasing the chance of developing weed biotypes with resistance to multiple herbicide biochemical sites of action. Weeds with multiple resistances reduce the efficacy of existing and developing herbicide-resistant crop technologies, limit options for weed control, and decreases profitability.

Beginning with the release of glyphosate-resistant soybean in 1996, cultivars of glyphosate-resistant alfalfa, cotton, corn and sugarbeet have been developed, and have led to glyphosate's status as the most widely-used herbicide worldwide (Duke and Powles 2008). The availability of these glyphosate-resistant crops allowed producers to use glyphosate as an effective post-emergence, broad-spectrum herbicide with low cost, leading to glyphosate being used as a stand-alone herbicide on millions of hectares of cropland (Duke and Powles, 2008). Paired with the continual application to large weeds, the widespread and repeated use of glyphosate has caused tremendous selection pressure on weed populations and has resulted in the selection of glyphosate resistant weeds in at least 32 different weed species worldwide (Heap, 2015). In the Midwest alone, glyphosate resistant biotypes of common waterhemp, horseweed, kochia, common ragweed and giant ragweed have been identified and are becoming problematic (Heap, 2015). Several of these glyphosate resistant biotypes have previously been selected to be resistant to ALS herbicides. Since there is no fitness penalty associated with ALS resistance, the continual and widespread use of glyphosate has successfully stacked resistance to multiple modes of action into several weed populations, bringing much concern to growers.

Specialized management will increase the cost and complexity of crop production and will ultimately result in greater use of weed management strategies that potentially result in environmental damage, such as tillage. In the southern U.S., weeds with herbicide resistance are a major issue and are often managed reactively, costing farmers millions of dollars. For example, the occurrence of glyphosate resistant horseweed in the United States has resulted in a net increase of \$28.42 ha^{-1} in soybean production costs (Mueller et al. 2005). Moreover, effective management of glyphosate resistant Palmer amaranth in Georgia and Arkansas cotton production has increased production costs by an estimated \$48 ha^{-1} (Norsworthy et al. 2012). With such large economic consequences and the increasing prevalence of herbicide resistant weeds, new and integrated strategies are needed to improve the effectiveness of weed control. Despite the perceived cost and effort associated with preventing or delaying the development of herbicide resistant weeds, the cost of prevention can cost significantly less than dealing with herbicide-resistant weeds once established. One of the reasons for this perceived cost is the lack of ability for growers to assess economic risks associated with herbicide resistant weeds (Beckie 2006). An additional problem is the lack of information on how various crop rotations affect weed control and the economic returns associated with them. To sustain the efficacy of glyphosate and other herbicide technologies while providing acceptable economic return to the grower, it will be important to reduce the development of herbicide-resistant weeds through the use of integrated weed management strategies that control weeds using multiple approaches.

1.4 Crop Rotation for Weed Control.

Crop rotations have always been the foundation of good agronomic practice by controlling disease and insect pests. Rotation benefits to yield are well characterized, and tend to produce a yield-enhancing “rotation effect” that is related to factors such as increased nitrogen (N) availability, improved soil physical properties, and altered rhizosphere communities. While rotation benefits are provided by a soybean-corn rotation compared to continuous corn, the addition of alfalfa or wheat to the system amplifies crop rotation benefits. In particular, incorporating crop sequences that vary in patterns of resource competition, soil disturbance and mechanical damage create an unstable environment hostile to any particular weed species (Liebman and Dyck, 1993). However, crop rotation benefits to managing herbicide-resistant weed populations are poorly understood.

Alfalfa provides a two-fold approach to reducing the development and persistence of herbicide-resistant weed populations that plague row crop rotations. First, frequent harvests (3-4 times/year) reduce grass and eliminate broadleaf weed seed production for annual weeds adapted to corn-soybean systems (Olmstead and Brummer 2008). Second, since alfalfa is perennial it provides continuous year-round ground cover for multiple years, providing a favorable habitat for insects, rodents, and fungi that can prey on weed seeds within the seed bank (Meiss et al. 2010a; Meiss et al. 2010b). Alfalfa also reduces production costs for first-year corn in much of the Upper Midwest by providing adequate nitrogen without additional applications (Sheaffer et al. 2005) as well as reducing corn rootworm pressure, allowing growers to achieve maximum corn yields with hybrids lacking transgenic events for corn rootworm control. In addition, the year-round ground cover provided by alfalfa reduces soil exposure and limits soil erosion.

Wheat provides several additional mechanisms for weed control when included in a crop rotation. Since wheat differs in planting and harvest date, growth habitat, competitive ability, and production practices from either corn or soybean, incorporating wheat into a rotation favors different weed associations than in a traditional corn-soybean rotation since the wheat alters the cycle of adapted weeds (Buhler 2002). Breaking the cycle of adapted weeds allows enhanced control of dominant weeds, and leads to more diverse weed assemblages that are less problematic. For example, a single weed species comprised 71% of weeds present in continuous corn compared to no single species contributing more than 43% of total weeds present when corn was rotated with wheat (Liebman and Dyck 1993). Additionally, wheat is planted earlier than corn or soybean with high plant densities in narrow rows, allowing it to better compete with early emerging weeds like giant ragweed. Herbicides with alternative modes of action than corn or soybean herbicides can also be used in wheat. Alternative herbicides diversify the herbicide regimen and reduce the likelihood of weeds developing further resistance. Wheat is also harvested earlier than corn or soybean, providing the opportunity to harvest and cut off weeds prior to seed-set even when weed escapes do occur. Early harvest provides the opportunity for a multitude of weed control options to be employed following wheat harvest, including both chemical and mechanical control. Additionally, wheat stubble provides a favorable habitat for a variety of seed predators (Kaufman and Kaufman 1990; Hartzler et al. 2007).

Although crop rotation has been referred to as the most effective means of weed control since the 1930s, it has been in decline since the introduction of herbicides in the 1940s (Leighty 1938; Liebman and Dyck, 1993). Much of this is due to the overall ease and effectiveness of using herbicides for weed control, along with the general mechanization of agriculture (Liebman and Dyck, 1993). Developing herbicide resistant weeds, however, threatens the continued utility of herbicides for weed control. Despite the perceived cost and effort associated with preventing or delaying the development of herbicide resistant weeds, the cost of prevention can cost significantly less than dealing with herbicide-resistant weeds once established. One of the reasons for this perceived cost is the lack of ability for growers to assess economic risks associated with herbicide resistant weeds (Beckie, 2006). An additional problem is the lack of information on how crop rotations affect weed control and the economic returns associated with them.

All weeds rely on the weed seed bank as a genetic resource to develop herbicide resistance. Additionally, annual weeds rely on the seed bank for species persistence. Therefore, the use of weed control strategies directly affecting weed emergence patterns and seed bank depletion represent ideal targets for integrated weed control. By using crop rotations that promote weed seed bank depletion via seed decay and predation, there is large potential to effectively manage herbicide resistant weeds over the long term. Seed predation has been shown to remove as much as 88% of giant ragweed seed over the course of one year in no-tillage corn (Harrison et al. 2003). It has also been found that the greatest seed predation occurs in small grain and alfalfa, since the rate of seed predation tends to increase as the crop canopy develops within a field (Westerman et al. 2005; Hartzler et al. 2007).

1.5 Emergence Timing

Applying herbicides when weeds are at the most vulnerable stages is critical for effective weed management (Menalled & Schonbeck, 2011). There are several models predicting the emergence timing of giant ragweed, which can be used to improve timing of field operations such as herbicide application, tillage, and date of crop planting (Archer et al. 2006; Schutte et al. 2008; Werle et al. 2014; Davis et al. 2013; Menalled and Schonbeck 2011). However, these models have not been analysed in the context of alternative crops and crop rotations, which may affect total and temporal patterns of giant ragweed emergence. Analysing soil environmental factors in respect to giant ragweed emergence in alternative crops allows the verification of existing giant ragweed emergence models in addition to providing information on how emergence differs in alternative crops. Different crops influence the soil environment in different ways, specifically in the amount of light reaching the surface, thus affecting soil temperature and moisture, which influence seedling emergence (Liebman and Dyck 1993). Analyzing soil environmental factors in respect to giant ragweed emergence will provide information on how emergence differs in alternative crops. A better understanding of giant ragweed emergence allows better strategies to be developed which proactively manage herbicide-resistant giant ragweed populations. Incorporating giant ragweed emergence models into weed management allows growers to optimize timing of cultivation schedules, planting dates, and herbicide application dates so practices affect weed populations when they are most vulnerable (Menalled & Schonbeck, 2011).

1.6 Harvest Weed Seed Control.

As weeds throughout the Midwest continue to develop resistance to herbicides, it will necessitate the development of alternative weed control strategies, including nonchemical approaches (Shaner and Beckie 2014). Weeds escaping herbicides and other in-season weed management practices are able to produce seed and replenish the weed seed bank. Weed seed banks allow weeds to persist through cropping phases and extend weed infestations (Fenner 1995). However, various late-season weed management practices are available to prevent weeds from depositing viable seed into the soil seed bank.

Uncontrolled weeds in crop fields will eventually mature and shatter seed onto the soil surface and repopulate the seed bank. However, weeds may retain seed until grain harvest. During normal grain harvest, the weed seed enters the harvester, is processed, separated from the grain, and spread over the field by the chaff-spreading system of the harvester (Barroso et al. 2006; Blanco-Moreno et al. 2004; Rew et al. 1996; Shirtliffe and Entz 2005; Walsh and Powles 2007). Mechanisms targeting escaped weed seed in the chaff fraction, such as harvest weed seed control (HWSC) systems, have been developed to destroy weed seed in the chaff fraction (Walsh and Powles 2007; Walsh and Newman 2007; Shirliffe and Entz 2005; Walsh et al. 2012). These systems have been reported to be from 60 to 99% effective in destroying seeds of various weed species (Walsh et al. 2013).

For HWSC systems to be effective, weeds need to retain seed until crop harvest, and there is good evidence that high levels of seed retention occur for weeds infesting some crops. For example, in wheat production fields in Western Australia, annual ryegrass (Lolium rigidum), wild radish (Raphanus raphanistrum), brome grass (Bromus spp. Roth), and wild oat (Avena fatua) retain 85%, 99%, 77% and 84% of seed until crop maturity, respectively (Walsh and Powles 2014). Seed retention of annual ryegrass in Spanish wheat fields was even greater, with 96% of seed being retained until crop maturity (Blanco-Moreno et al. 2004).

1.7 Summary and Research Objectives

Biotypes of giant ragweed resistant to both ALS inhibitors and glyphosate have developed, and are becoming increasingly problematic (Heap 2015). To prevent future infestations of giant ragweed, seed inputs into the soil seed bank must be limited. As herbicide control of herbicide resistant weeds becomes more difficult, it may require a zero weed threshold to prevent weed persistence (Norsworthy et al. 2014). To implement a zero weed threshold it may be necessary to implement nonchemical strategies such as hand weeding before seeds shatter or HWSC to prevent seed bank replenishment of resistant biotypes. However, these systems are reliant either on controlling weeds prior to seed production or that weeds retain seed until crop harvest, both of which are typically influenced by the growing environment (Shirtliffe et al. 2000, Taghizadeh et al. 2012). Information on giant ragweed seed production and retention as a factor in late season weed control strategies does not exist for Midwest growing conditions and cropping systems. The objectives of this research (Chapter 2) were to determine: (1) the rate of seed shattering of giant ragweed during the harvest season and (2) the level of seed retention at crop harvest in Midwestern soybean and field margins.

As an annual, giant ragweed relies on the weed seed bank to persist in agricultural fields (Fenner 1995). Therefore, the use of weed control strategies specifically targeting weed seed bank depletion and seedling emergence patterns appear to be ideal approaches for integrated weed control (Buhler et al. 1997). By using crop rotations that promote weed seed bank depletion via seed decay and predation, there is potential to effectively manage weeds over the long-term (Chee-Sanford et al. 2006). The objectives of this research (Chapter 3) were to determine how cropping systems common to the Midwest affect (1) the quantity of giant ragweed seed bank depletion, (2) total giant ragweed emergence and (3) emergence timing.

CHAPTER 2: GIANT RAGWEED (*AMBROSIA TRIFIDA*) SEED PRODUCTION AND RETENTION IN SOYBEAN AND FIELD MARGINS

2.1 Summary. As herbicide-resistant weed populations become increasingly problematic in crop production, alternative strategies of weed control are necessary. Giant ragweed (*Ambrosia trifida* L.), one of the most competitive agricultural weeds in row crops, has developed resistance to multiple herbicide biochemical sites of action within the plant, necessitating the development of new and integrated methods of weed control. This study assessed the quantity and duration of seed retention of giant ragweed grown in soybean [*Glycine max* (L.) Merr.] fields and adjacent field margins. Seed retention of giant ragweed was monitored weekly during the 2012 to 2014 harvest seasons using seed collection traps. Giant ragweed plants produced an average of 1818 seeds per plant with 66% being potentially viable. Giant ragweed on average began shattering hard (potentially viable) and soft (nonviable) seed September 12th and continued through October at an average rate of 0.75 and 0.44% of seeds per day during September and October, respectively. Seed remained on the plants well into the harvest season, with an average of 80% of seed being retained on October 11, when Minnesota soybean harvest was approximately 75% completed in the years of the study. These results suggest that there is ample time to remove escaped giant ragweed from production fields and field margins before the seed shatters by managing weed seed dispersal before or at crop harvest. This approach has potential to manage herbicide-resistant giant ragweed by limiting replenishment of the weed seed bank.

2.2 Introduction. As weeds throughout the midwestern United States continue to develop resistance to herbicides, there is becoming a greater need for alternative weed control strategies, including nonchemical approaches (Shaner and Beckie 2014). Weeds escaping herbicides and other in-season weed management practices are able to produce seed and replenish the weed seed bank. Weed seed banks allow weeds to persist through cropping phases and extend weed infestations (Fenner 1995). Various late-season weed management practices are available to prevent weeds from depositing additional seed into the soil seed bank.

Uncontrolled weeds in crop fields will eventually mature, shatter seed onto the soil surface, and repopulate the seed bank. Weeds also can retain seed until crop harvest. During normal harvest of grain crops, weed seed enters the harvester, is separated from the grain, and is distributed over the field by the chaff-spreading system of the harvester (Barroso et al. 2006; Blanco-Moreno et al. 2004; Rew et al. 1996; Shirtliffe and Entz 2005; Walsh and Powles 2007). Mechanisms targeting escaped weed seed in the chaff fraction, such as harvest weed seed control (HWSC) systems, have been developed to destroy weed seed at crop harvest (Walsh and Powles 2007; Walsh and Newman 2007; Shirliffe and Entz 2005; Walsh et al. 2012). These systems are reported to be from 60 to 99% effective in destroying seeds of various weed species (Walsh et al. 2013).

For HWSC systems to be effective, weeds need to retain seed until crop harvest, and there is evidence that high levels of seed retention occurs for many weeds. For example, in wheat production fields in western Australia, rigid ryegrass (*Lolium rigidum* Gaudin L.), wild radish (*Raphanus raphanistrum* L.), brome grass (*Bromus* spp. Roth L.), and wild oat (*Avena fatua* L.) retain 85, 99, 77 and 84% of seed until crop maturity, respectively (Walsh and Powles 2014). Seed retention of annual ryegrass in Spanish wheat fields was even greater, with 96% of seed retained until crop maturity (Blanco-Moreno et al. 2004).

Giant ragweed is one of the most competitive weeds infesting corn (*Zea mays* L.) and soybean across the midwestern United States (Stoller et al. 1987; Webster et al. 1994). Biotypes of giant ragweed resistant to both ALS inhibitors and glyphosate have developed, and are becoming increasingly problematic (Heap 2015). To prevent future infestations of giant ragweed, seed inputs into the soil seed bank must be limited. As herbicide control of herbicide resistant weeds becomes more difficult, a zero-weed threshold may be required to prevent weed persistence (Norsworthy et al. 2014). A zero-weed threshold may require nonchemical strategies such as hand weeding before seeds shatter or HWSC to prevent seed bank replenishment of resistant biotypes. However, these systems require that weeds are controlled prior to seed production or that weeds retain seed until crop harvest, both of which are influenced by the growing environment (Shirtliffe et al. 2000, Taghizadeh et al. 2012). There is no information on giant ragweed seed production and retention as a factor in late-season weed control strategies for the growing conditions and cropping systems of the midwestern United States. The objectives of this research were to determine: (1) the quantity of seed produced during the growing season and (2) the level of seed retention at crop harvest in midwestern soybean and field margins.

2.3 Materials and Methods

2.3.1 Site Details. Experiments were conducted at the Rosemount Research and Outreach Center near Rosemount, MN (44.71^{o}N, 93.12^{o}W) in 2012 to 2014 and near Rochester, MN (43.91^{o}N, 92.56^{o}W) in 2014 (Table 1). The soil at Rosemount was a Waukegan silt loam soil (fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls) and at Rochester was a Port Byron silt loam soil (fine-silty, mixed, superactive, mesic Typic Hapludolls). Weather data, including daily minimum and maximum air temperature, precipitation, wind speed, and frost dates were obtained from the National Weather Service station nearest each site. Growing degree days (GDD) were calculated using Equation 1, where T_{max} is the maximum daily temperature, T_{min} is the minimum daily temperature, b_0 is the base temperature (10°C), and S_1 and S_2 are months indicated in Table 1.

$$\text{GDD} = \sum_{S1}^{S2} \frac{(T_{max} - T_{min})}{2} - b_0 \qquad [1]$$

Giant ragweed seed retention was monitored weekly during September and October. Both research sites had known populations of glyphosate-resistant giant ragweed, and giant ragweed at the Rochester, MN site also had resistance to ALS inhibitors. At Rosemount, resident populations of giant ragweed plants were randomly selected each year in mid-July from both a conventionally managed soybean field and the adjacent field margin to monitor seed retention. Soybean were seeded at 345,947 seed/ha in 76cm rows with commercially available varieties. Field margins were not actively managed for weeds, and the primary vegetation providing competition was smooth bromegrass (*Bromus inermis* L.) and giant ragweed. Giant ragweed plants in both field locations were allowed to compete naturally with plants in the surrounding area. When traps were set up, the vegetation in the area surrounding the monitored giant ragweed plants was flattened to prevent interference with the seed trap and to prevent stray seed from falling into the seed trap.

2.3.2 Seed Trap Construction. Conical seed traps adapted from the gauze trap design outlined in Page et al. (2002) were installed around stems of individual giant ragweed plants to collect giant ragweed seed. Seed traps consisted of a plastic frame formed into a 0.9-m diameter circle supporting mesh fabric funnelling to a drained plastic collection bottle (1L) in the middle to capture seed and protect seed from predators. Traps were fastened and supported around giant ragweed plants just below the lowest seed-producing branch, and were sealed around the base of the plant using tape.

2.3.3 Seed Collection. Seed was collected weekly for eight weeks starting the first week of September through the last week of October, representing the typical time period from giant ragweed seed development to the end of soybean harvest. Traps were set up around 10 randomly selected giant ragweed plants in each field location at least 7 d prior to the monitoring period, which coincided with seed fill. Collected seed was dried at room temperature (18°C) for at least 7 d prior to analysis. Seed was separated from foreign material using an aspirator. Viability of giant ragweed seed was then determined by applying gentle pressure to each seed using a forceps and recorded as either hard (potentially viable) or soft (nonviable). For this test, all seed was probed with a similar amount of pressure; seed that was penetrated or crushed was considered nonviable, while hard seed was considered potentially viable (Ball and Miller 1989; Cardina and Sparrow

1996; Forcella 1992). Seed from each category was counted and weighed to determine total number and average mass of seed shattering each week.

To relate giant ragweed plant development with seed production and retention, plant development was monitored periodically through the growing season beginning in July in 2013 and 2014 to assess plant height, number of primary and secondary branches, number of nodes on the primary stem, leaf number, and stages of reproductive growth. At the end of the monitoring period (end of October), plants were clipped at ground level, bagged, and stripped of all seed to separate seed from the stems. Stems were dried in a forced-air oven at 60°C for 5 d to determine stem dry weight and moisture at the time of plant harvest. Since the majority of leaves were lost at the time of plant harvest, stem dry matter alone was used as a proxy for total plant dry matter. Seed was dried and processed as previously described.

2.3.4 Statistical Analysis. Plant development and seed production properties of giant ragweed by site-year and field location are summarized in Table 2. To determine site-year and field location effects of each plant biological factor, an ANOVA was performed treating site-year and field location as random effects, and Fisher's protected LSD ($P \leq 0.05$) were calculated for each biological factor. For total seed production, the average number of potentially viable and nonviable seed produced in each field location were calculated and ANOVA was performed treating site-year and field location as random effects. Total giant ragweed dry matter was determined to be a significant covariate using ANCOVA, and minimized differences in total seed production relative to field location.

To normalize seed retention data, the number of viable and nonviable seeds retained each week were converted to a percentage of the total viable and nonviable seed produced per plant, respectively. The relationship between percent seed remaining and day of year was linear, and a best fit linear regression equation (Equation 2) was fit to the normalized data (Walsh and Powles 2014), where Y is the proportion of seed retention, A is 100% seed retention, B is the rate of seed shed (% d^{-1}), and x is days after the start of seed shattering, which was predicted to be September 12 for hard (potentially viable) and soft (nonviable) seed.

$$Y = A + Bx \qquad [2]$$

To determine the effects of weather and plant development on seed retention, correlation analysis were performed to relate seed retention to all plant development and physical properties monitored as well as precipitation, wind speed, first frost date, and GDD (10°C). Multiple linear regression analysis was also performed to determine if a combination of weather factors affected seed retention. Although there were several weak correlations between seed retention and weather patterns, none were significant. All statistical analyses were performed using R version 3.1.3 (R Foundation for Statistical Computing, Wien, Austria).

2.4 Results and Discussion

2.4.1 Plant Development. Floral initiation of selected giant ragweed plants occurred near the end of July, but was delayed by several days in more competitive environments. Despite differences in floral initiation, pollination tended to be more uniform, and occurred in the third week of August, which was expected as giant ragweed is a short-day plant (Mann 1942). Although there were slight differences in floral development and plant structure, there were no associations among giant ragweed reproductive development, branching characteristics, and leaf number on seed production or retention characteristics.

There were significant effects of site-year and field location on giant ragweed development (Table 2). Seed production varied by site-year, but was largely dependent on plant size. Across all site-years and field locations, giant ragweed plants in soybean produced more biomass and seed, while plants in field margins grew taller and had lower reproductive ratios (Table 2). These differences are typical of competition effects and are likely due to increased densities of neighboring giant ragweed and smooth brome grass in field margins. Consequently, giant ragweed plants in this environment may be competing for light which causes plants to be etiolated with fewer branches, less stem biomass, and fewer leaves per plant (Jurik 1991). The result is an altered allocation of resources for seed development as reflected by the lower reproductive ratios observed in field margins (Table 2). The reproductive ratio, calculated as the percentage of hard seed biomass relative to the total stem and nonviable seed biomass, was 22% for giant ragweed plants in field margins and 29% for plants in soybean, indicating that a larger proportion of plant biomass goes into seed production in a soybean field (Table 2). If giant ragweed leaf biomass was accounted for in this study, which typically comprises 10 to 20% of plant biomass, these results would likely be similar to those reported previously, in that reproductive ratios are typically less than 20% for giant ragweed grown in a similar soybean monoculture setting (Jurik 1991; Brabham et al. 2011).

Seed Production. Although total seed production varied by site-year, plants produced substantial amounts of seed, demonstrating the high potential of weed seed contribution to the seed bank. Giant ragweed plants produced an average of 1796 seeds per plant with 64% being potentially viable in 2012, 1115 seeds per plant with 77% being potentially viable in 2013, and 2302 seeds per plant with 59% being potentially viable in 2014 (Figure 1; Table 2). These results are similar to those previously reported, in that giant ragweed typically produce 500 to 5000 seeds per plant (Brabham et al. 2011; Baysinger and Sims 1992). The percentage of potentially viable seeds was also similar to that reported by Vitolo and Stiles (1987), who found 65% of seed being viable from giant ragweed grown in a soybean field. This is in contrast with Harrison et al. (2001), who reported giant ragweed seed viability of only 50% in a corn field. In addition to variation by site-year, seed production also varied by field location; plants in soybean produced 72% more seeds than plants in field margins (Figure 1). This increase in seed production was a result of an increase in both hard (potentially viable) and soft (nonviable) seed. However, field location effects on seed production were eliminated if total plant dry matter was accounted for as a covariate. Over all site-years and field locations, seed production was correlated with aboveground plant biomass ($r^2 = 0.31$, $p < 0.001$). Giant ragweed plants in field margins typically weighed less and were at higher densities than plants in soybean, resulting in fewer seeds being produced, which is in line with what Jurik (1991) reported. Similarly, Harrison et al. (2001) found that giant ragweed emerging 4 wk after corn and therefore subjected to greater competition, had lower overall fecundity than giant ragweed emerging simultaneously with corn.

Rate of Seed Shatter. In each site-year, giant ragweed began shattering seed the first week of September and continued through October. Since giant ragweed is a short day plant, (Mann 1942), it was not surprising that giant ragweed began shattering seed at relatively the same date each year despite weather differences (Table 1). In this study, seed shattering began slightly earlier than reported from Ohio by Harrison et al. (2001), where seed did not begin to shatter until September 20. Differences in the start of seed shattering could be due to differences in biotype, weather, or latitude (Shirtliffe et al. 2000).

Giant ragweed seed shattering occurred at a linear rate over time, with a considerable amount of plant-to-plant variation. On average, potentially viable and nonviable seed shattered from plants at a rate of 0.75 and 0.44% of seeds per day, respectively, beginning on September 12 (Figure 2). Harrison et al. (2001) also observed a linear rate of seed shatter over time for giant ragweed in corn, despite it being delayed in plants with delayed emergence. Similar results have been observed for other weed species in Australia, including annual ryegrass (*Lolium rigidum*), wild radish (*Raphanus raphanistrum*), brome grass (*Bromus* spp. Roth), and wild oat (*Avena fatua*) (Walsh and Powles 2014).

The primary focus of this study was to monitor giant ragweed seed shattering through the harvest period. Consequently, seed retention was only monitored through the end of October, since soybean is typically harvested by this time in the midwestern

United States. When comparing giant ragweed seed retention with typical soybean harvest dates in 2012-2014, potentially viable giant ragweed seed retention was on average 75.3% on the date when 75% of soybean were harvested in Minnesota each year as inferred from crop progress reports (Table 1) (USDA-NASS 2014). Despite a large percentage of the seed being retained until soybean harvest, there was large variation in the seed retention characteristics of individual plants in various site-years and field locations (Figure 2), potentially due to variation in genetic background, rate of plant development, and specific environmental conditions.

Weather and Pest Effects. Although individual weather events likely did affect seed retention, no correlations were observed between weather data, plant developmental properties, and seed retention. Growing degree day accumulation beginning in April each year was not associated with seed retention patterns. However, there was a linear association between GDD accumulation after September 1 and seed retention ($r^2 = 0.49$, $p < 0.001$), indicating that increased GDD (10°C) accumulation in September and October increases seed shattering. Specifically, years with greater GDD accumulation after September 1st accounted for plants with less seed retention, indicating that a warmer harvest season may increase seed shattering (Figure 2). Although it was expected that seed shatter would increase following the first frost date, we found no associations between first frost dates or the number of accumulated freezing days with rates of seed shatter. This lack of association may have occurred because plants reached physiological maturity prior to the first frost in each year. It was expected that wind and precipitation events would increase seed shattering due to an increase in self-threshing among branches on a given plant. Wind and precipitation events, however, when determined alone or in combination via multiple linear regression, did not appear to play a significant role in influencing seed shatter ($r^2 \leq 0.05$, $P \leq 0.001$).

Aside from weather, other factors also likely influenced the rate of giant ragweed seed shatter. For example, there was evidence of birds, rodents, insects, and plant pathogens interacting with giant ragweed plants, and likely influencing rates of seed shattering. Several studies have found that 2 to 19% of giant ragweed seeds are infested by various insects that consume at least some portion of the embryo (Amatangelo 1974; Harrison et al. 2001; Vitolo and Stiles 1987). Abul-Fatih et al. (1979c) proposed that taller, isolated giant ragweed plants attract and experience the most granivory from seed-feeding insects, which may explain why rates of seed shatter were greater in taller plants in field margins than the typically shorter plants in soybean. The incidence of stem boring insect infestation was assessed at plant harvest in 2013 and 2014, which determined that nearly all plants had stem boring damage and that there was no correlation with rate of seed shatter. Complex combinations of weather, biological, and other environmental factors appear to ultimately influence giant ragweed seed retention.

Conclusions. Giant ragweed plants escaping early-season weed control strategies produce substantial amounts of seed, providing the opportunity for escaped weeds to proliferate. Results from this study indicate that when adhering to a zero-weed threshold, there is a substantial window of time before seed development to remove giant ragweed plants from production fields and adjacent field margins to prevent seed bank replenishment. Once plants have developed seed, giant ragweed plants did show high seed retention rates through the harvest months. Hard (potentially viable) seed tended to shatter from plants at a higher rate than soft (nonviable) seed, which both began shattering September 12 on average and continued through October. This indicates that earlier harvest dates for soybean would provide increased potential to capture giant ragweed seed if implementing HWSC mechanisms at harvest. Even with an average harvest date in Minnesota of October 8 (USDA-NASS 2010), 80% of the hard (potentially viable) seed is retained on giant ragweed, indicating there is potential to capture giant ragweed seed at crop harvest.

These results indicate that there is potential for HWSC methods to be effective against giant ragweed. Due to the nature of harvesting equipment for crops common to the midwestern United States, HWSC mechanisms may only be effective in soybean, as the harvesting equipment has greater potential to feed weed biomass into the harvester than corn harvesting equipment. The overall variation in seed retention of giant ragweed (Figure 2) suggests that it is highly likely that implementing HWSC would select for giant ragweed plants that shatter seed earlier. However, if HWSC is used as part of an integrated weed management plan, these strategies have potential to control herbicide resistant giant ragweed. Overall, these results indicate that harvesting equipment is likely a primary mechanism of giant ragweed seed spread, since the majority (>63%) of giant ragweed seed is retained on plants through the end of October. To proactively manage giant ragweed, it will be important to consider the role that harvesting equipment has on mechanically spreading giant ragweed seed both within fields and across agricultural regions, especially when dealing with herbicide-resistant biotypes.

Table 2-1. Growing season details for each year and field location that seed retention of giant ragweed was assessed for approximately 60 d surrounding crop harvest. Weather information was obtained from National Weather Service station nearest each site.

	Rosemount						Rochester	
	2012		2013		2014		2014	
	Apr-Oct	Sept-Oct	Apr-Oct	Sept-Oct	Apr-Oct	Sept-Oct	Apr-Oct	Sept-Oct
Growing degree days (10°C)	1822	277	1719	350	1521	248	1318	189
Precipitation (mm)	606	41	675	111	763	68	717	150
Average wind speed (m/s)		3.6		3.9		4.1		4.4
First frost date		7-Oct		21-Oct		11-Oct		5-Oct
MN: 75% soybean harvest date		30-Sept		18-Oct		16-Oct		16-Oct

a. Growing degree days calculated using Equation 1

Table 2-2. Summary of seed production and plant development properties in each site-year from 2012 to 2014 in Rosemount and Rochester, MN. Least significant differences are shown ($P \leq 0.05$) for each site-year and field location combination (n=10). Average columns are the average for field location across all site-years.

	Rosemount						Rochester			
	2012		2013		2014		2014		Average	
	Soybean Field	Field Margin	Soybean Field	Field Margin	Soybean Field	Field Margin	Soybean Field	LSD (0.05)	Soybean Field	Field Margin
Hard (potentially viable) seed	852	1541	1299	409	2093	529	1434	595	1420	826
Soft (nonviable) seed	488	712	404	119	1465	284	1100	375	864	372
Total seed	1340	2253	1703	528	3557	814	2534	865	2284	1198
Hard (potentially viable) seed mass (mg/seed)	26	23	21	22	22	24	28	4	24	23
Soft (nonviable) seed mass (mg/seed)	8	6	10	9	5	7	7	2	7	7
Plant height (m)	1.6	2.0	1.9	2.7	2.1	2.3	2.2	0.3	1.9	2.3
Stem dry matter (g)	53	77	116	44	81	44	75	40	81	55
Reproductive ratio (% hard seed by weight)	27	30	19	17	35	19	38	7	29	22

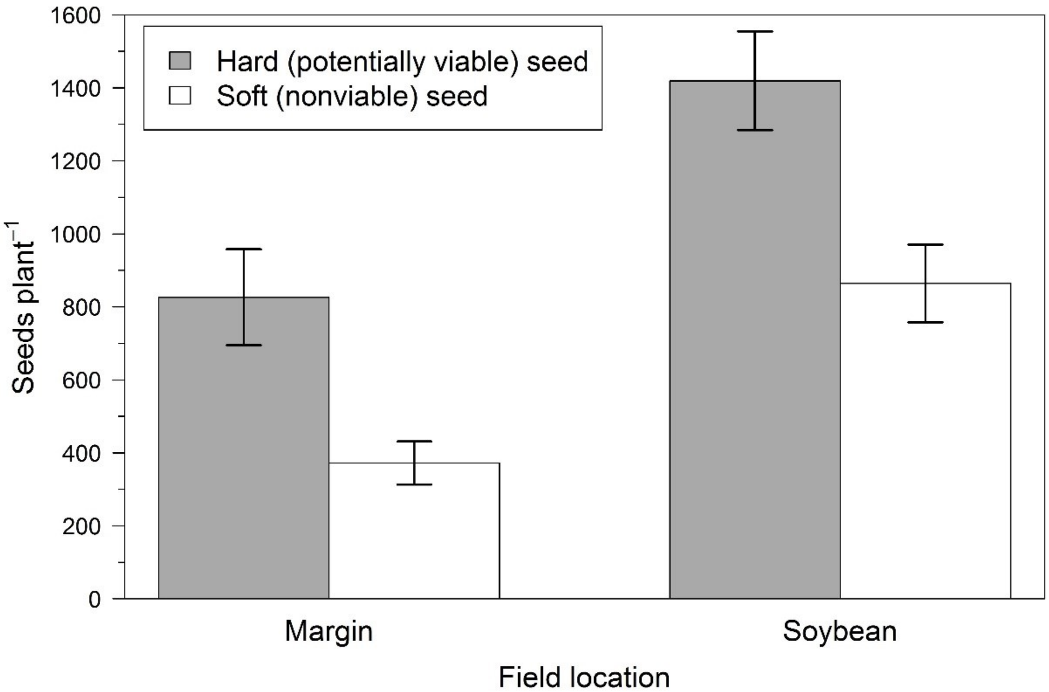

Figure 2-1. Average total giant ragweed potentially viable (hard) and nonviable (soft) seed production by field location averaged across 2012 to 2014. Mean seed production shown with standard error bars.

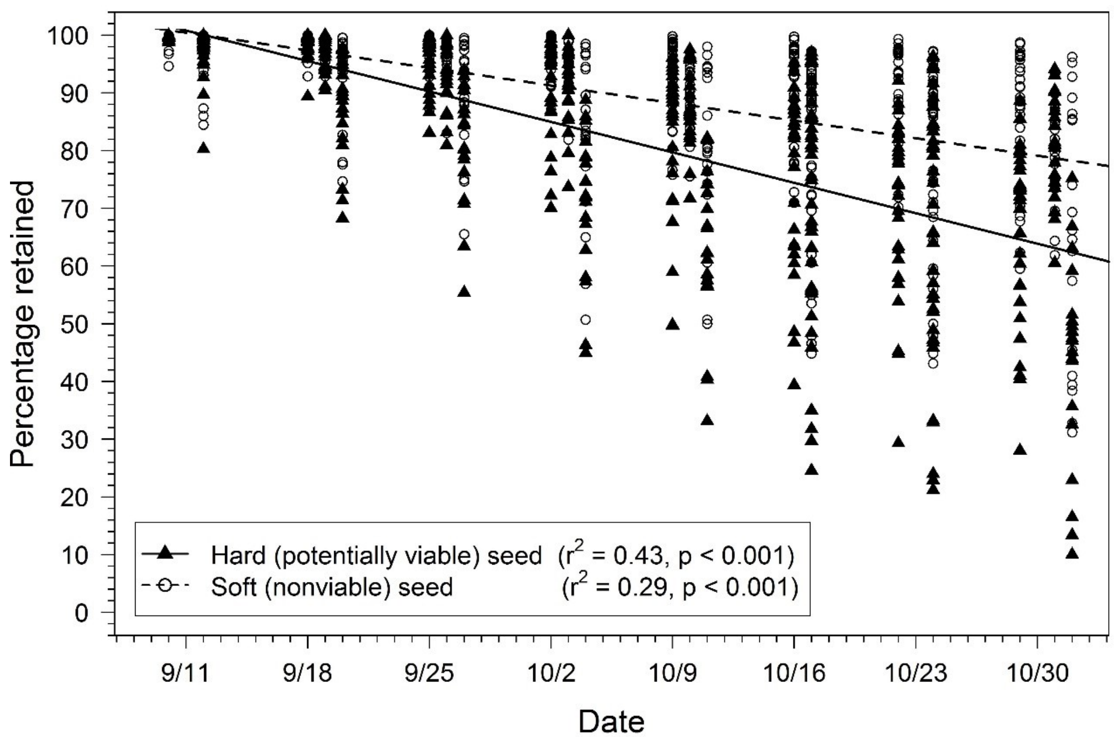

Figure 2-2. Hard (potentially viable) and soft (nonviable) seed retention of giant ragweed across all site-years and field locations in 2012 to 2014. Seed began shattering on September 12 on average. Lines represent a best fit linear model for hard ($y = 100 - 0.754x$) and soft seed ($Y = 100 - 0.435x$) retention.

CHAPTER 3: SEED BANK DEPLETION AND EMERGENCE PATTERNS OF GIANT RAGWEED (*AMBROSIA TRIFIDA*) IN SEVERAL MIDWESTERN CROPPING SYSTEMS.

3.1 Summary. In the Midwest, biotypes of giant ragweed (*Ambrosia trifida*) resistant to multiple herbicide biochemical sites of action have been identified. Weeds with resistance to multiple herbicides reduce the utility of existing herbicides and necessitates the development of alternative weed control strategies. From 2012-2014 in southern Minnesota, we determined the effect of six three year crop rotations containing corn (*Zea mays*) (C), soybean (*Glycine max*) (S), alfalfa (*Medicago sativa*) (A), and wheat (*Triticum aestivum*) (W): (CCC, SCC, CSC, SWC, SAC, AAC) on giant ragweed seed bank depletion and emergence patterns. Crop rotation had no effect on the amount of seed bank depletion when a zero weed threshold was maintained, with 97% of the giant ragweed seed bank being depleted in two years. However, this quantity of seed bank depletion was primarily through seedling emergence in annual crop rotation treatments, while similar seed bank depletion totals were observed alongside low levels of seedling recruitment after two years of alfalfa, possibly indicating an increase in seed predation or fatal germination. Giant ragweed emerged early across all treatments, with 90% emergence occurring by June 4th. In comparison to corn or soybean, total emergence was reduced when wheat or alfalfa were planted, indicating that seedling recruitment is affected by crop rotation. These results indicate that various crop rotations are more conducive to giant ragweed emergence than others, and that long term giant ragweed management can be accomplished by implementing a zero weed threshold to deplete the weed seed bank.

3.2 Introduction. Giant ragweed is one of the most competitive agricultural weeds plaguing crops in the Midwest (Webster et al. 1994), and its control has become complicated due to the development of resistance to multiple herbicide mechanisms of action (Heap 2015). As an annual, giant ragweed relies on the weed seed bank to persist in agricultural fields (Fenner 1995). Therefore, the use of weed control strategies specifically targeting weed seed bank depletion and seedling emergence patterns appear to be ideal approaches for integrated weed control (Buhler et al. 1997). By using crop rotations that promote weed seed bank depletion via seed decay and predation, there is potential to effectively manage weeds over the long-term (Chee-Sanford et al. 2006).

Seed predation by rodents and invertebrates has been shown to remove as much as 88% of giant ragweed seed in one year in no-tillage corn (Harrison et al. 2003). Seed predation increases in wheat (*Triticum aestivum*) and alfalfa (*Medicago sativa*) compared to annual row crops due to increases in crop canopy (Westerman et al. 2005; Hartzler et al. 2007). In addition to promoting seed predation, alfalfa is harvested frequently throughout the growing season (3-4 times/year), eliminating the ability for giant ragweed to produce seed to replenish the weed seed bank. Wheat increases early season competition by being planted earlier than corn or soybean in narrow rows, therefore increasing early season competition with emerging giant ragweed. Additionally, wheat allows the incorporation of herbicides with alternative mechanisms of action that are more effective against herbicide resistant populations of giant ragweed. In the event of weed escapes, wheat is harvested prior to giant ragweed seed production, preventing replenishment of the seed bank and offering multiple mechanical and chemical weed control options following wheat harvest.

Different crops influence weed seedling emergence due to differences in the amount of light reaching the soil surface, soil temperature and moisture (Liebman and Dyck 1993). Applying herbicides when weeds are most vulnerable is critical for effective weed management. There are several models predicting the emergence timing of giant ragweed, which can be used to improve timing of field operations such as herbicide application, tillage, and date of crop planting (Archer et al. 2006; Schutte et al. 2008; Werle et al. 2014; Davis et al. 2013; Menalled and Schonbeck 2011). However, these models have not been analysed in the context of alternative crops and crop rotations, which may affect total and temporal patterns of giant ragweed emergence. Analysing soil environmental factors in respect to giant ragweed emergence in alternative crops provides information on how emergence differs in alternative crops.

The objectives of this research are to determine how cropping systems common to the Midwest affect (1) the quantity of giant ragweed seed bank depletion, (2) total giant ragweed emergence and (3) emergence timing. This research allows the determination of which crop rotations have soil conditions most conducive to minimizing giant ragweed emergence and maximizing seed bank degradation to allow growers to determine the most effective ways to proactively manage herbicide resistant giant ragweed infestations.

3.3 Materials and Methods

3.3.1 Site Details. Experiments were initiated in 2012 near Rochester, MN (43.91^{o}N, 92.56^{o}W) on a Port Byron silt loam (Fine-silty, mixed, superactive, mesic Typic Hapludoll) with a pH of 7.0 and 4.0% organic matter with a history of corn and soybean rotation. The research site had known populations of naturally occurring giant ragweed resistant to glyphosate and ALS inhibitor herbicide chemistries.

3.3.2 Crop Management. The experiment had a foundation of six crop rotations applied in a randomized complete block design with four replications. Crop rotation treatments consisted of: continuous corn (CCC); soybean-corn-corn (SCC); corn-soybean-corn (CSC); soybean-wheat-corn (SWC); soybean-alfalfa-corn (SAC); and alfalfa-alfalfa-corn (AAC) (Table 1). Plots were 10 by 15 m. Corn and alfalfa varieties had resistance to glyphosate (Roundup Ready®), while corn and soybean cultivars were glufosinate-resistant (LibertyLink®). Corn was seeded with DeKalb DKC 53-78RIB at 86,486 seed/ha in 76cm rows (John Deere model 7000 planter). Soybean plots were seeded with Stine 19LD08 LibertyLink at 345,947 seed/ha in 76cm rows (John Deere model 7000 planter). Inoculated alfalfa (DeKalb DKA41-18RR) was direct seeded (Great Plains model 3P606NT no-till drill) at 16.8kg/ha in 19cm rows. Wheat was seeded (Great Plains model 3P606NT no-till drill) with MN RB07 at 135 kg/ha in 19cm rows. Fertilizer applications were made according to University of Minnesota recommendations. Phosphorus, K, and S were uniformly applied across the entire study area the fall of each year to maintain adequate levels of these nutrients for all crops grown. All N was applied in the form of ammonium nitrate (34-0-0) at time of planting. Corn following corn or wheat received 191 kg N/ha, corn following soybean or a single year of alfalfa received 135kg N/ha, corn following two years of alfalfa received no additional N, and wheat following soybean received 129kg N/ha.

Corn plots were chisel plowed in the fall following corn harvest and stover chopping, and field cultivated twice in the spring prior to planting. Soybean plots were field cultivated in the spring with two passes prior to planting. Soybean stubble following harvest was chisel plowed when corn was to be planted the following year, and left fallow when wheat or alfalfa were to be seeded the following year. Wheat was no-tilled into standing soybean stubble and chisel plowed in the fall after harvest. Alfalfa plots that were seeded in the first year of the rotation received a single pass with a field cultivator prior to planting, while alfalfa plots seeded in the second year of the rotation were no-till seeded into standing soybean stubble.

Fields were scouted for insects and diseases using University of Minnesota recommendations. However, no insects or diseases reached levels warranting treatment throughout the study. Wheat plots were sprayed prophylactically with Folicure® (Tebuconazole, alpha-[2-(4-chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol at 126g ai/ha) at the flowering stage to prevent the development of Fusariam head blight in wheat plots.

A zero weed threshold was maintained throughout the study to allow the accurate study of seed bank depletion. Due to the presence of glyphosate- and ALS- resistant weed populations, herbicides specifically targeting resistant weeds were used. When herbicides with residual activity on giant ragweed were used, quadrats where emergence was monitored were covered at time of application to prevent herbicide coverage. Corn and soybean plots had a single PRE application of Dual II Magnum (S-metolachlor at 2.14kg ai/ha) on the date of planting each year. Corn and soybean plots received two POST applications of Liberty (glufosinate-ammonium at 450g ai/ha) targeted at approximately 3 and 6 weeks post-planting. Alfalfa plots received a single application of Butyrac 200 (4-(2,4-Dichlorophenoxy)butyric acid, dimethylamine salt at 1.12kg ai/ha) 2 weeks following planting in the seeding year, while second year alfalfa received no herbicide application. Wheat plots received a tank-mixed application of Widematch (clopyralid MEA salt: 3,6-dichloro-2-pyridinecarboxylic acid, monoethanolamine salt at 105g ai/ha, fluroxypyr 1-methylheptyl ester: (((4-amino-3,5-dichloro-6fluoro-2-pyridinyl)oxy)acetic acid, 1-methylheptyl ester at 105g ai/ha) and MCPE (2-methyl-4-chlorophenoxyacetic acid isooctyl (2-ethylhexyl) ester at 389g ai/ha) at approximately 2 weeks post-planting, when wheat was beyond the 2-leaf stage. Weeds escaping herbicide control were hand weeded to ensure seed inputs into the seed bank were eliminated.

Corn yields were determined in 2012 by hand harvesting and shelling three 1.5m by 6m areas per plot. In 2013 and 2014, corn yield was determined by harvesting two 1.5m by 9m areas per plot with a plot combine. In 2012 to 2014, grain subsamples (~1000g) were taken from shelled samples and dried at 60C for 5 days to determine grain moisture and kernel weight. Average kernel weight was determined by weighing 300 dried kernels and grain yield was adjusted to 155 g kg^{-1} moisture content. Each year plots were cleared following corn harvest using a combine with a chopping corn head. Soybean was harvested from three 1.5m by 6m areas per plot. Total soybean grain mass was determined for each sampling location and subsamples (~1000g) were placed in 60C dryer for 5 days to determine grain moisture. Grain yield was adjusted to 130 g kg^{-1} moisture content. Seed weight was determined by weighing 300 dried seeds. Alfalfa plots were harvested from three 0.9m by 6.4m areas per plot to determine total wet biomass of each sample area. A subsample (~1000g) was clipped adjacent to the sampling area, weighed and dried for 3d at 60C to determine total dry matter of each of the subsample areas. In SAC treatments, where only a single year of alfalfa was maintained, plots were harvested 3 times at about 30 day intervals beginning in July. In AAC treatments, alfalfa was cut 2 times in the seedling year at approximately 30 day intervals beginning in July, and 4 times in the subsequent year. Harvests were targeted to occur when alfalfa was at the early flower stage. Wheat grain was harvested from three 1.5m by 9.1m areas with a plot combine to determine grain mass. Subsamples (~1000g) were weighed and dried for 3d at 60C to determine grain moisture. Grain yield was adjusted to 135 g kg^{-1} moisture content. Wheat protein content was determined using NIR analysis. To determine wheat straw yield, 4 samples of whole wheat plants were clipped at 10cm above the soil surface in an area of 0.76 x 0.91m in each plot. Whole samples were dried at 60C for 3d and threshed using a stationary thresher to separate grain from straw, and straw biomass was determined. Following yield sampling for all crops, the remaining unharvested crop was cleared using a combine harvester.

3.3.3 Seed Bank Monitoring. Giant ragweed was managed by maintaining a zero weed threshold, which prevented giant ragweed seed production. This was done utilizing both herbicides as well as hand weeding any escapes to ensure no seed inputs entered back into the seed bank. Giant ragweed seed bank densities were determined in the initial and final year of each crop rotation. Seed-bank samples in the first year were taken from three quadrat locations in each plot (25cm x 40cm x 15cm). Due to the amount of time necessary to extract each sample, an alternative sampling method was used to determine seed-bank densities in the final year of the rotation. Soil seed bank samples in the final year were taken from the same quadrat locations as samples taken in the first year of the rotation. In the final year, a hole cutter was used, where 10 10cm diameter holes were taken in a systematic pattern from the same sampling area and compiled together to compose a large, single sample, to obtain more reliable seedbank prediction levels (Forcella, 1992). Weed seed was separated from compiled samples using a modified version of physical extraction procedures adapted from Ball and Miller (1989), Standifer (1980), and Cardina and Sparrow (1996), where compiled samples were wet-sieved to separate seeds. Samples were soaked with water and mixed several times over 20min using a paint stirring attachment on an electric drill. Once the soil was in suspension, samples were poured through a 0.16cm sieve to extract seed. Remaining soil was soaked again and mixed until the entire sample passed through sieve. A low pressure shower of water was also sprayed on the sample to speed the passing of soil through the sieve. Once the organic material larger than 0.16cm was separated from soil, the seed bank extract was placed in 60C drier for 2d to dry the sample before seeds and seed fragments were hand-picked from the samples. Seeds were then determined to be viable or nonviable by dissecting seed to determine the presence of an intact embryo and counted. Seeds with embryos intact were weighed and all seeds without an embryo were weighed with seed fragments to determine weight of nonviable giant ragweed seed and seed fragments.

3.3.4 Emergence Monitoring. Giant ragweed emergence counts were made on a weekly basis starting at the onset of emergence and continued for 10 weeks, or until emergence ceased each year. Giant ragweed emergence was monitored in six 30 by 76cm quadrats within each plot. Three quadrats were placed between rows while the alternate three quadrats were placed over the crop row. Each week, seedlings were counted and pulled from the quadrat by clipping seedlings at the soil surface without disturbing the soil.

3.3.5 Environmental data. In addition to emergence data, various environmental data were monitored to determine their effects on giant ragweed emergence. Daily precipitation and minimum and maximum temperature were obtained from a nearby weather station. Weather data were then input into the soil temperature and moisture model software (STM^2) (Spokas and Forcella 2009) to predict daily soil moisture (kPa) at a 5-cm depth. Predictions were based on daily maximum and minimum soil temperature, daily precipitation, and soil properties (sand, silt, clay, and organic matter content), latitude, longitude, and elevation. To verify the STM^2 model predictions, soil moisture was determined on a weekly basis by taking three soil samples of 2.5cm x 10cm from each plot, combining samples for each sampling date, and stored in a plastic bag before being weighed and placed in a 60C dryer for 5d to determine dry weight. Soil temperature was monitored using temperature sensors (Hobo Water Temp Pro v2) at a 5cm depth logging temperature at hourly intervals. Growing degree days (GDD) were calculated using Equation 1, where T_{max} is the maximum daily temperature,

$$GDD = \sum_{S1}^{S2} \frac{(T_{max} - T_{min})}{2} - b_0 \quad [1]$$

T_{min} is the minimum daily temperature, b_0 is the base temperature (10C), and S_1 and S_2 are April 1 and July 31, respectively (Table 2).

3.3.6 Statistical analysis. Seed bank depletion data were analyzed using the MIXED procedure of SAS (SAS Institute, 2012). Crop rotation treatment was considered as a fixed effect, while block and interactions between block and crop rotation treatment were considered random. Mean comparisons were made using Fisher's protected LSD test (α = 0.05) when appropriate.

Total giant ragweed emergence in each year of each crop rotation were analyzed using the MIXED procedure of SAS (SAS Institute, 2012). Crop rotation treatment was considered as a fixed effect, while location, block (nested within location), the starting seed bank densities as a covariate, interactions, and subsampling effects were considered random. Mean comparisons were made using Fisher's protected LSD test (α = 0.05) when appropriate.

To evaluate emergence timing of giant ragweed, weekly emergence counts were converted to a cumulative emergence (%) based on total seedling emergence each year. The cumulative percent emergence of giant ragweed in each year of each crop rotation was pooled and modelled with a best fit logistic function over day of year (DOY), to calculate the date when 50 and 90% giant ragweed emergence occurred in the second year of each crop rotation system.

3.4 Results and Discussion

3.4.1 Seed Bank Depletion. There were no differences in seed bank depletion in the six crop rotation systems when weed seed bank replenishment was eliminated via a zero weed threshold. On average, 97% of the giant ragweed seed bank was depleted in 2 years in any crop rotation system, indicating that the giant ragweed seed bank is short lived regardless of cropping system (Figure 1). These results support previous findings that the giant ragweed seed bank is short lived. Nordby et al. (2005) found greater than 95% of giant ragweed seed is lost within 2yr in both conventional and no-tillage crop fields, while Harrison et al. (2007) found seed depletion levels were dependent on burial depth, and that seeds closer to the soil surface were degraded more quickly than seeds deeper than 10cm. However, a small percentage of giant ragweed seed remaining in the seed bank has been shown to persist for up to 15 years (Loux and Berry 1991; Hartnett et al. 1987), exemplifying the importance of long term weed management.

There are multiple ways weed seeds can be depleted from the weed seed bank. Weed seeds may germinate and emerge or die, fungi and other soil microorganisms may decay the seed, or seed predators such as birds and rodents may consume seeds (Buhler et al. 1997; Kremer 1993; Chee-Sanford et al. 2006). Each of these mechanisms of seed bank degradation have potential to cause significant seed bank losses. Harrison et al. (2003) found that up to 90% of giant ragweed seeds deposited on the soil surface of a no-tillage cornfield can be eliminated by predation in a single year. Additionally, the rate of seed predation increases as the crop canopy develops within a field, with wheat and alfalfa typically having higher seed predation than corn (Westerman et al. 2005; Hartzler et al. 2007). Interestingly, we found the same amount of seed bank depletion regardless of crop rotation system in this study, highlighting the importance of eliminating seed inputs. This study did not determine the fate of seeds degraded in the seed bank. However, emergence was accounted for to document the quantity of the seed bank depletion that was due to emergence. Cropping systems that included wheat or alfalfa had lowest total giant ragweed emergence, which only accounted for 81, 79, and 42% of seed bank depletion in SWC, SAC, and AAC treatments, respectfully. In contrast, approximately 100% of seed bank depletion was accounted for due to emergence in CCC, SCC, and CSC treatments. These results indicate that there was increased depletion due to factors other than seedling emergence in treatments containing wheat or alfalfa. Previous studies support these results, and have found that increased seed bank depletion due to seed predators and soil microorganisms typically occurs in more diverse cropping systems due to the increased habitat (Brust and House 1988).

3.4.2 Total Emergence. Giant ragweed emergence was highly variable among years, with the overall depletion of the weed seed bank over time corresponding to fewer giant ragweed seedlings emerging each year. Across the entire experiment, 125, 34, and 4 seedlings m^{-2} yr^{-1} emerged in year 1, 2 and 3 of the crop rotation system, respectively. These high amounts of emergence observed in the first several years represent the major threat for crop yield and control costs (Buhler et al. 1997), and indicate the importance of adhering to a zero weed threshold in the first two years. Due to the spatial variation of the weed seed bank observed across the experiment, estimates of the starting seed bank density were included as a covariate in the analysis of total emergence in each year of each cropping system. Accounting for the spatial variation of the seed bank via ANCOVA allowed more accurate comparisons of total giant ragweed emergence in each crop rotation system. In the first year, there were no differences in giant ragweed emergence in any of the crop rotation systems (Figure 2), which was expected since all crops were planted into a site with the same management history. Additionally, the weather in the first year of the cropping systems at site year one in 2013 had average temperatures and above normal precipitation, which resulted in a large percentage of giant ragweed seedlings emerging prior to crop planting (Table 2). There were differences in total giant ragweed emergence in the second year of the crop rotation system, with corn planted into soybean stubble having the greatest amount of giant ragweed emergence and second year alfalfa having the least (Figure 3). This trend was similar in the third year of the cropping system, where once again corn planted into soybean stubble had the greatest giant ragweed emergence, despite much lower densities (Figure 4). Interestingly, these results were observed despite different tillage strategies for corn following soybean in the second and third years of the rotational systems. In the second year of the SCC system, corn was planted into no tilled soybean stubble which was left untilled the previous fall and had two passes with a field cultivator prior to crop planting. In the CSC treatment, soybean stubble was chisel plowed the fall of year two and field cultivated twice in the spring of year three. Despite these differences in tillage, similar emergence results were observed in both years. These crop rotation systems had no-tilled soybean stubble and thus similar overwinter and early spring soil environments, and only differed at the time of crop planting, where SWC and SAC were planted to wheat and alfalfa, respectively, and SCC was planted to corn following two passes of spring tillage. However, even slight adjustments in planting date, cultivation timing, harvest methods and residue management can influence seed dormancy dynamics and thus emergence (Dyer 1995; Buhler et al. 1997). Therefore, it is possible that the spring tillage subjected to the SCC treatment in year two affected emergence. Tillage causes vertical seed movement in the soil and influences weed emergence (Buhler 1995; Cousens and Moss 1990; Staricka et al. 1990), which could explain the differences in emergence observed in the different cropping systems.

Spring tillage does not explain why the SCC treatment had greater emergence in year three of the crop rotation system, since spring tillage in year three was uniform across all treatments. The increased emergence could be due to alternative soil environmental differences, as there were slight soil temperature and residue differences among treatments (Figure 5). The second year of the AAC system had the least giant ragweed emergence, which was expected since giant ragweed is least adapted to the perennial environment of alfalfa. In the fall of year one of the system, there was significant alfalfa canopy coverage, likely buffering the soil environment throughout the winter. In the spring of year two, this canopy coverage caused less extreme temperature fluctuations, typically keeping the soil temperature cooler in the established alfalfa than in the exposed soil of the other treatments (Figure 5). Additionally, there was less tillage that occurred in the AAC system. Previous work has shown that mean seedling emergence depth is smallest with no-tillage, while chisel plow and moldboard plowing gradually increase mean emergence depth of most weed species (Buhler and Mester 1991; citation). The seed of large-seeded weeds like giant ragweed tend to remain near the soil surface with less intensive tillage, which has been shown to inhibit establishment of these species (Lueschen and Anderson 1980). Therefore, the lower soil temperatures along with less intensive tillage likely provided a soil environment less conducive to giant ragweed emergence. This environment was also likely most conducive to seed bank degradation through fatal germination or seed degradation, which ultimately resulted in lower emergence with similar seed bank depletion amounts.

3.4.3 Emergence Timing. Giant Ragweed exhibited similar emergence patterns in each of the six crop rotation systems, following a logistic growth curve relative to date in each site year. Giant ragweed began emerging slowly in the early weeks of each growing season before having a period of rapid emergence throughout May. Emergence then tapered off and nearly terminated mid-June. On average, 50 and 90% emergence of giant ragweed occurred on May 21 and June 4, respectively, which is indicative of the early emergence pattern of giant ragweed (Table 3). There was slight variation in emergence timing between 2013 and 2014, which was expected due to environmental differences. The accumulation of growing degree days was slightly less in the spring months of 2014 than 2013, which was associated with less rapid emergence in all crop rotation systems. All crop rotation treatments exhibited a similar emergence pattern except for the second year of the AAC system. The second year of the AAC system had a similar early season emergence pattern, reaching 50% emergence at the same time as the other crop rotation systems. However, the AAC system had an extended period of emergence than the other treatments, and did not reach 90% emergence until June 18, several weeks after the other crop rotation systems (Table 3). Previous work modelling giant ragweed emergence has indicated that giant ragweed emergence is associated with the accumulation of thermal time (Werle et al. 2014; Schutte et al. 2008; Davis et al. 2013). Therefore, the prolonged pattern of emergence of giant ragweed in the second year of the AAC treatment is likely due to the increased crop canopy of alfalfa early in the growing season. The crop canopy diminishes the amount of sunlight reaching the soil surface, which decreases soil temperature and thus slows the accumulation of thermal time at the soil level. Aside from the slightly slower emergence pattern of giant ragweed in established alfalfa, these results indicate that giant ragweed emerges early in the growing season regardless of the cropping system.

3.4.4 Conclusion. Results from this study indicate that the seed bank of giant ragweed is short lived, and that nearly all of the giant ragweed seed bank is depleted within two years in any crop rotation system where a zero weed threshold is implemented. More specifically, these results indicated that weed seed inputs only need to be eliminated for two years to nearly eliminate the weed seed bank. Implementing a zero weed threshold, however, may be easier in some cropping systems due to lower total emergence. Corn planted into soybean stubble resulted in the greatest total emergence regardless of tillage practices. Conversely, emergence in the AAC system was lower than other cropping systems, indicating that the inclusion of alfalfa in the cropping system has large potential to improve giant ragweed control despite the extended emergence period observed in this system. Although the emergence period is slightly extended in the AAC system, the harvesting schedule of established alfalfa will still prevent seed bank inputs without the reliance on herbicides for control.

Overall, our results align with previous research in that giant ragweed is an early emerging weed with a short duration of emergence. This early season emergence pattern indicates that there is potential to enhance giant ragweed control through improving the timeliness of field operations. For example, delayed planting allows a greater percentage of seedlings to emerge prior to planting, when tillage and nonselective herbicides can be used to control early emerging weeds (Gill 1996; Walsh and Powles 2007). If weed seed inputs are eliminated over the course of two years, 97% of the giant ragweed seed bank is depleted, which significantly reduces the weed pressure from giant ragweed. These results indicate that there is potential to manage fields infested with giant ragweed in the long term by eliminating seed bank inputs and degrading the weed seed bank, to ultimately improve control of these herbicide-resistant weeds.

Table 3-1. Sequence of crops in each crop rotation system. Note differences in tillage in each of the crop rotation systems.

Year	Crop Rotation System					
	CCC	SCC	CSC	SWC	SAC	AAC
1	Corn	Soybean	Corn	Soybean	Soybean	Alfalfa
2	Corn	Corn	Soybean	Wheat	Alfalfa	Alfalfa
3	Corn	Corn	Corn	Corn	Corn	Corn

Table 3-2. Growing season details over the study period. Weather information was obtained from nearby weather stations.

	2012		2013		2014	
	Apr-Jul	Apr-Oct	Apr-Jul	Apr-Oct	Apr-Jul	Apr-Oct
Average Temperature (C)	18.4	17.2	14.8	15.2	15.1	15.1
Growing Degree Days (10C)	1083	1674	812	1443	777	1318
Precipitation (mm)	356	486	712	865	430	717

Table 3-3. Date when 50% and 90% of giant ragweed was emerged in the second year of the crop rotation systems. Dates were calculated from the best fit logistic function fit to each crop rotation system.

Treatment	% Emerged	
	50%	90%
Corn - Corn	May 22	Jun 3
Soybean - Corn	May 22	Jun 2
Corn - Soybean	May 21	May 31
Soybean - Wheat	May 22	Jun 5
Soybean - Alfalfa	May 21	Jun 5
Alfalfa - Alfalfa	May 21	Jun 18
Average	May 21	Jun 4

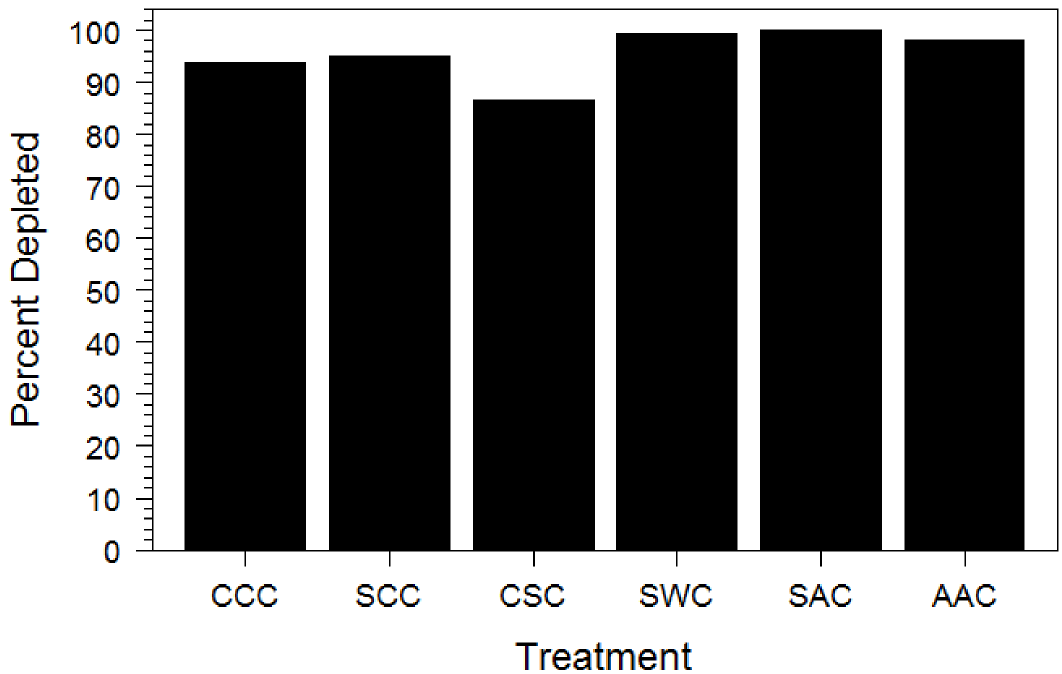

Figure 3-1. Percentage of seed bank depleted in each crop rotation treatment between year one and three of each crop rotation system. Treatments were not significantly different.

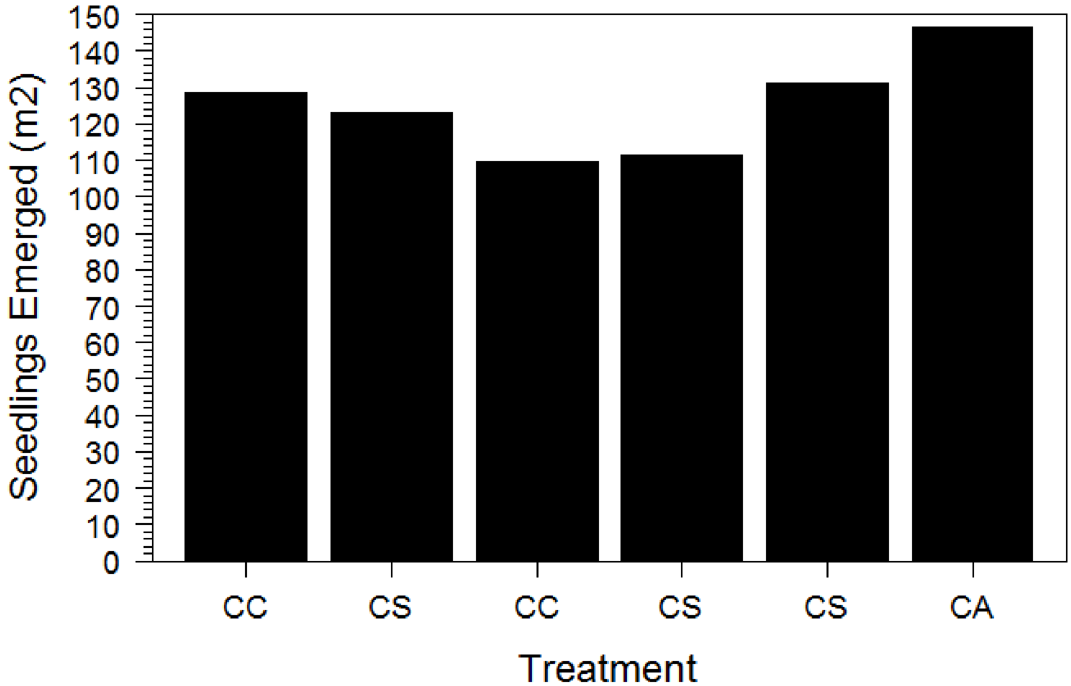

Figure 3-2. Total seedling emergence in the first year of each crop rotation system. Corn was the previous crop for each crop rotation system followed by the crop planted in year one of the crop rotation system. Treatments were not significantly different.

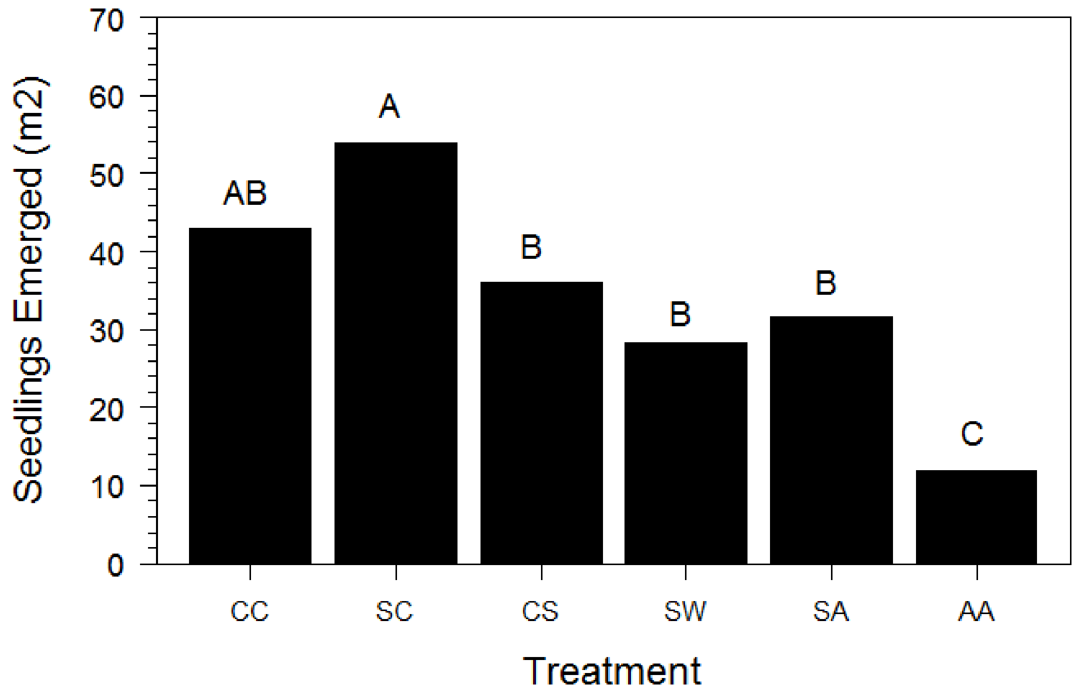

Figure 3-3. Total seedling emergence in the second year of each crop rotation. Cropping sequence is shown with year 1 crop designator followed by year 2 crop designator. Letters represent means are significantly different at the 0.05 level.

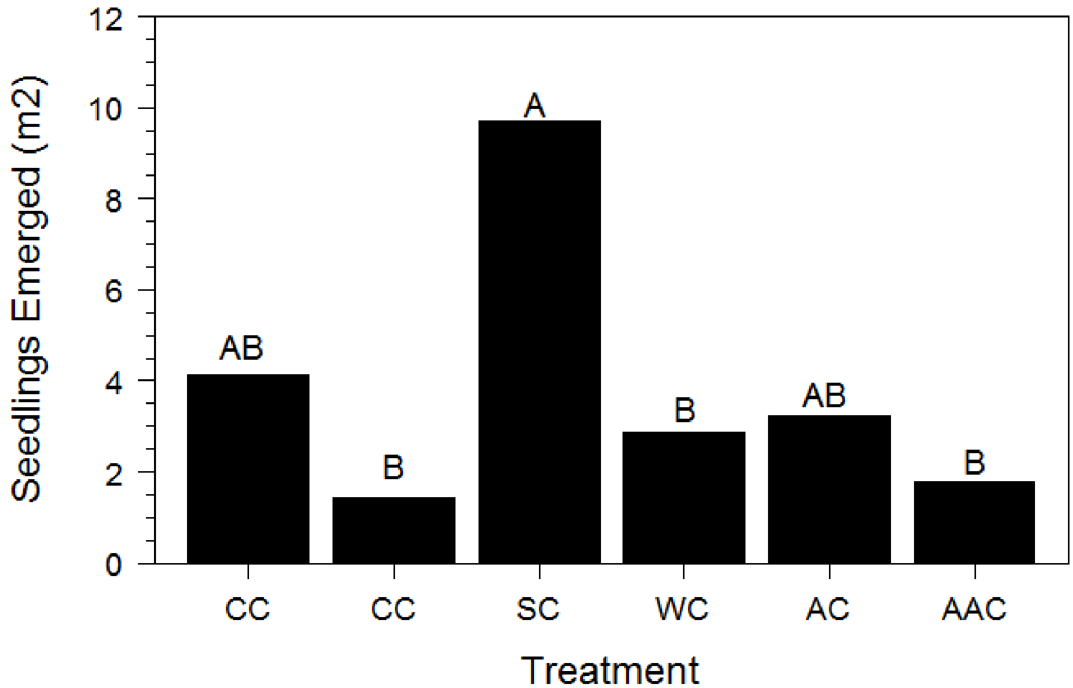

Figure 3-4. Total seedling emergence in the third year of each crop rotation. Cropping sequence is shown with year 2 crop designator followed by year 3 crop designator. Letters represent means are significantly different at the 0.05 level.

Figure 3-5. Average daily soil temperature at 5cm throughout the emergence period in the six different cropping systems in 2014. The crop planted in 2014 is bolded and shown with the crop grown in 2013 preceding.

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APPENDIX

Table A-1. Average crop yields over in each year of each crop rotation system. Corn yields are expressed as Mg ha^{-1} adjusted to 155 g kg^{-1} moisture content. Soybean yields expressed as Mg ha^{-1} adjusted to 13 g kg^{-1} moisture content. Wheat yields expressed as Mg ha^{-1} adjusted to 13.5 g kg^{-1} moisture content. Alfalfa yields expressed as DM (Mg) ha^{-1}. Data from year 1 and 2 is from both site years while data from year 3 is only from site year 1.

Year	Cropping rotation system					
	CCC	SCC	CSC	SWC	SAC	AAC
1	12.8	2.9	12.8	2.8	2.6	2.4
2	11.5	12.4	3	3.4	4.6	8.9
3	13.5	13.4	14	14.4	14.4	12.7

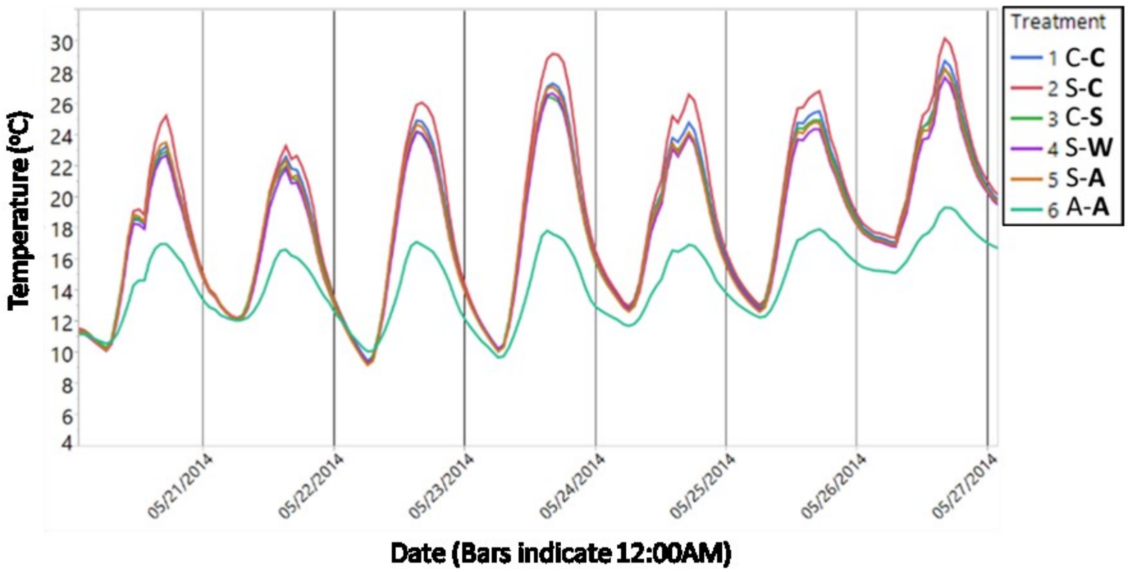

Figure A-1. Daily temperature fluctuations observed in each of the cropping system treatments from May 20 to May 27 in 2014.

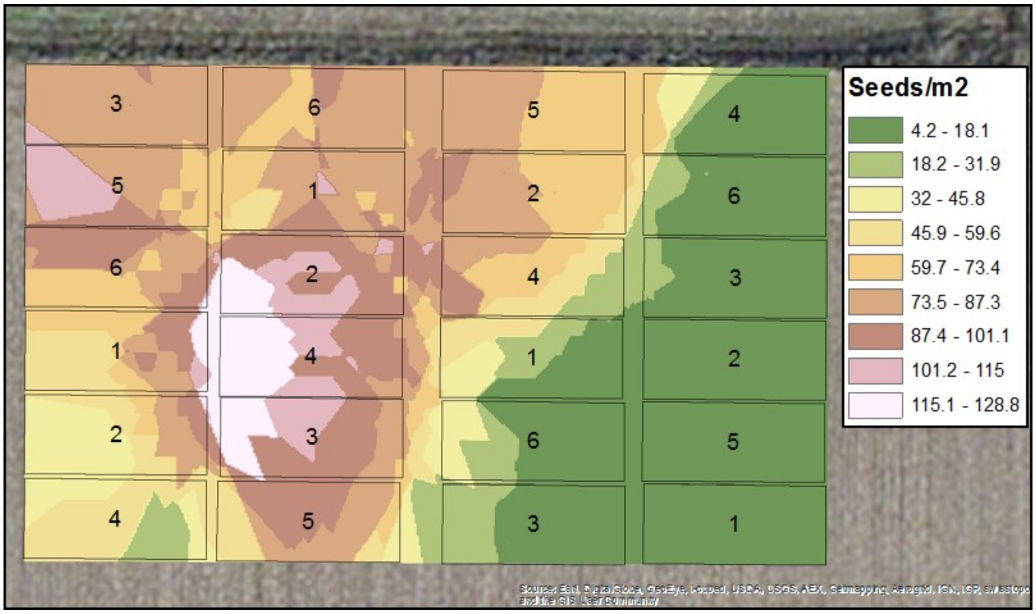

Figure A-2. Spatial distribution of starting seed bank density in experiment 1 at Rochester, MN taken in 2012. The krigging method of spatial interpolation was used to interpolate data and produce the seed density map.

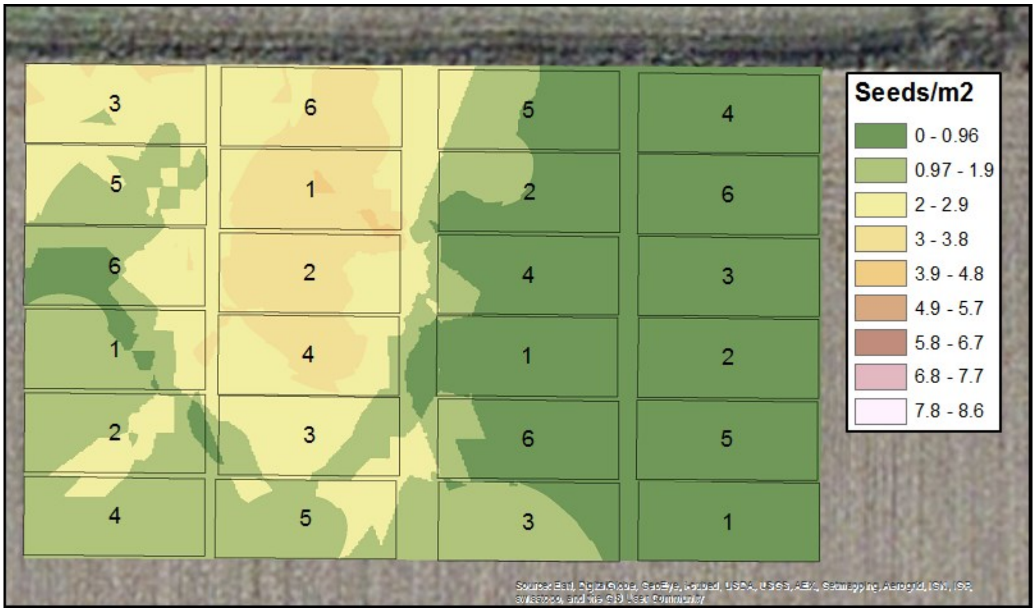

Figure A-3. Spatial distribution of final seed bank density in experiment 1 at Rochester, MN taken in 2014. The krigging method of spatial interpolation was used to interpolate data and produce the seed density map.

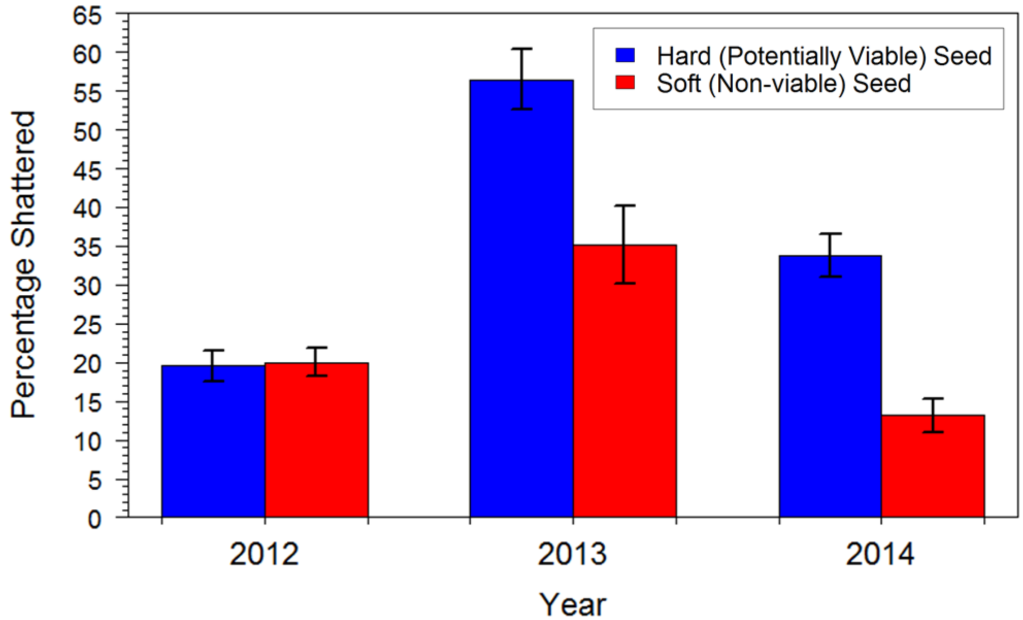

Figure A-4. Percentage of seed shattered at the end of October in 2012-2014 from both field locations in Rosemount, MN and Rochester, MN. Mean percentage shattered is shown with standard error bars.