

SOYBEAN IRON DEFICIENCY CHLOROSIS AND *AMARANTHUS* SUPPRESSION BY AN  
OAT COMPANION CROP

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**Title**

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## ABSTRACT

Iron Deficiency Chlorosis (IDC) and weedy *Amaranthus* species are two production challenges affecting soybean production in North Dakota. Field experiments were conducted to evaluate the effect of an oat companion crop on soybean and to evaluate soybean preemergence herbicides effect on an oat companion crop and *Amaranthus*. An oat companion crop reduced IDC symptoms in one environment, but did not reduce IDC in others. An oat companion crop reduced *Amaranthus* biomass, but in many site years this suppression did not occur until soybean yield loss was realized. Flumioxazin and sulfentrazone consistently provided the greatest control of *Amaranthus*, but was also the most injurious to an oat companion crop. Greenhouse research evaluated competitiveness of two *Amaranthus* species, and factors tested did not influence competitiveness. Other forms of IDC and *Amaranthus* suppression may be more consistent with suppression and stable yields than that of an oat companion crop.

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## **CHAPTER 1. LITERATURE REVIEW**

### **1.1. Soybean**

Soybean [*Glycine max* (L.) Merr.] is an annual legume crop native to Southeast Asia with seeds found in China, Japan and Korea aged from 9,000 to 5,000 years before present. Evidence of soybean domestication tends to be concentrated about 3,000 years before present in the Huanghe-Yellow River basin in China (Franzen, 2017). Soybean was introduced to North America in 1765 by Samuel Bowen in Georgia (Hymowitz and Shurtleff 2005). Soybean has been traditionally grown in the eastern corn belt and the southern United States, where planting can occur earlier due to warmer weather conditions. Earlier maturing soybean varieties have allowed for the crop to be grown in North Dakota and Minnesota and has led to both states being in the top ten leading producers of the crop recently. The USDA National Agricultural Statistics Service reported 33.9 million hectares of soybean were grown in the United States in 2020 [USDA NASS 2020]. They also estimated 6 million hectares of soybean were harvested in North Dakota.

### **1.2. Iron Deficiency Chlorosis**

Iron Deficiency Chlorosis (IDC) is a soybean production challenge in the upper Midwest due to natural alkalinity of many soils in these areas, further aggravated by high soluble salts, which serve as an additional plant stress (Naeve 2006). The condition of IDC is an abiotic stress in soybean that can cause reduction of plant biomass and reduce seed yield of the plant (Helms et al. 2010). Soybean affected by IDC exhibit stunting, delayed canopy closure, and particularly yellowing and interveinal chlorosis, especially of early trifoliolate leaves (Bai et al. 2018) but almost never the unifoliolate leaves. Hansen et al. (2004) reported that IDC costs soybean growers 120 million U.S. dollars in North Dakota, South Dakota, and parts of north-western Minnesota

annually. IDC is directly related to a soybean plant not being able to uptake iron from the soil, because bicarbonates inhibit soybean protein exudate from changing insoluble iron into soluble iron (Liesch et al. 2012). Bicarbonates in high pH soils limit the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by neutralizing acidic exudates to lower the soil pH near the root (Lucena 2000). Soybean IDC severity has also been attributed to a combination of other factors, such as high soil moisture content, a high concentration of soluble salts and carbonates, pH, and temperature (Hansen et al. 2003; Inskip and Bloom 1986). Soybean IDC is not related to the absence of iron in the soil (Hansen et al. 2003), but results from low Fe solubility preventing transport of  $\text{Fe}^{3+}$  to the root surface where it is reduced to  $\text{Fe}^{2+}$  to be taken up and used by the plant (Kaiser et al. 2014). Without the necessary Fe in the plant, chlorophyll production for photosynthesis is hindered (Liesch et al. 2012). Many fields in the region only show IDC symptoms within a part of the field area where soils with free carbonates are present, though severe cases can show field-wide symptoms in the Red River Valley in broad areas of soils with high carbonates, or those that have been limed with sugar beet waste lime to reduce sugar beet *Aphanomyces* root rot (Helms et al. 2010).

Growers in areas affected by IDC have been trying to manage this production challenge with IDC-tolerant cultivars, but even those cultivars can exhibit IDC symptoms under certain soil/environmental conditions. Also, IDC tolerant cultivars historically have not produced as high of yield compared to other cultivars of soybean in the absence of IDC conditions (Helms et al. 2010). Using these tolerant cultivars can result in a similar yield that would be observed by using a higher-yielding cultivar that is not IDC tolerant, depending on the severity of the symptoms within the field (Helms et al. 2010). Still, use of IDC-tolerant cultivars is usually best method to reduce IDC symptoms in soybean (Helms et al 2010). The variation of IDC symptoms

within fields has led some growers to plant IDC tolerant cultivars in areas that regularly exhibit IDC symptoms throughout the season and plant the higher-yielding cultivars in the rest of the field to increase the field's overall yield (Helms et al. 2010). Other factors that have an influence on the severity of the IDC symptoms of soybean include soil nitrate, soil type, weed pressure and weather. Research has shown that soluble salts, soil pH, and soil Fe cannot be used to reliably predict where IDC will be present within a field (Franzen and Richardson 2000) but rather indicate how severe the condition might be once calcium carbonate is present. Foliar sprays of Fe compounds have been evaluated but have only been effective when used to alleviate mild IDC symptoms within a field, and the effect is short-lived (Naeve 2006). Certain Fe chelates, but not all, can reduce symptoms when applied to soil with carbonates. Ferreira et al. (2019) observed that applications of certain iron chelates to soil with chlorotic soybeans caused SPAD Logger readings and Fe concentration within the plants to increase. In-furrow applications of certain iron chelates can also reduce IDC symptoms in soybean and increase yields in IDC susceptible cultivars (Kaiser et al. 2014).

### **1.3. Companion Crop use for Iron Deficiency Chlorosis Symptoms**

Another method found to reduce IDC symptoms within soybean is to plant a companion crop soybean. A companion crop is a plant that is intercropped with the cash crop to try to gain benefits in the crop and for the environment. Utilizing a companion crop could help reduce the reliance on IDC tolerant soybean cultivars that are not as high yielding (Helms et. al. 2010) and enable use of higher yielding cultivars. A companion crop could alleviate IDC symptoms within soybean as it has been demonstrated that higher soybean seeding rates reduce chlorotic symptoms (Penas et al. 1990). Carbon dioxide produced through root respiration can become trapped in soil water and can dissolve to form bicarbonates (Bloom et al. 2011), so a companion

crop may reduce this occurrence by transpiring additional or excess water and reducing soil nitrate. Furthermore, a companion crop has the potential to increase overall health of the soybean within the field early in the season (Naeve 2006). This could also be beneficial through an economic standpoint, as purchasing a companion crop would cost less when compared to purchasing herbicide resistant soybean and increasing the overall rate of soybean within the field (Naeve 2006). Oat can also be conveniently terminated by using one of many different herbicide systems, such as glyphosate, if combined with glyphosate-resistant soybeans (Naeve 2006) that are already frequently planted throughout the Midwest.

IDC symptoms tend to appear earlier in the season and gradually disappear as the plants grow and enter the reproductive stages (Naeve 2006). This correlates with the timing at which the companion crop would need to be terminated to avoid yield loss due to competition. If the companion crop is only growing during the vegetative stages of soybean to mitigate IDC symptoms. Companion crops compete for light and nutrients, reducing the biomass and density of weeds, and reducing yield of the cash crop (Verret et al. 2017; Naeve 2006). The use of a companion crop and timing to terminate it in order to prevent yield loss would be determined by the critical period of weed control in soybean. The critical period of weed control is defined as an interval in the life cycle of the crop when it must be kept weed free to prevent yield loss (Van Acker et al. 1993). However, the critical period of weed control in soybean is variable depending on the farming practices (Green-Tracewicz et al. 2012). Determining a termination timing to capture the benefits of companion crop, while still being able to remove it before significant yield losses occur is important.

Research supports that a companion crop planted alongside soybean will reduce symptoms of IDC. The mechanism behind IDC symptom mitigation is largely unknown,

although the presence of greater total plant biomass has been shown to lessen the symptoms of IDC (Naeve 2006). It has been hypothesized that companion crops are able to absorb excess nitrates. Kaiser et al. (2014) reported that soybean planted with oat had reduced trifoliolate nitrate N and Fe within the soybean plants. Likewise, increased plant biomass can also come from increasing the soybean planting rate, which reduces IDC symptoms and increased final soybean yield (Naeve 2006). This reduction in IDC symptoms is thought to be due to the increase in the number of plants within a field that are reducing the water content in the soil (Goos and Johnson 2006), but this has only been demonstrated in certain environments (Naeve 2006). Under normal conditions however, increased soybean seeding rates and including a companion crop has not increased soybean yields (Naeve 2006). Increased seeding rates and using an oat companion crop have exhibited both a negative and a positive effect on yield, depending on the amount of time the companion crop is allowed to grow. Varying environmental effects were a determining factor in the success of this system in previous research (Naeve, 2006). Previous research about utilizing an oat companion crop in soybean was conducted in fields without glyphosate-resistant weeds. Glyphosate-resistant waterhemp (*Amaranthus tuberculatus*) has become a prevalent weed in many fields where IDC is also problematic. Waterhemp is becoming more difficult to manage in soybean fields in the Northern Great Plains, and utilizing a companion crop to manage waterhemp may prove beneficial, particularly in fields susceptible to IDC.

#### **1.4. Waterhemp Biology**

Waterhemp (*Amaranthus tuberculatus*) is a small seeded, summer annual broadleaf plant that is native to North America (Sauer 1956). Waterhemp is a dioecious member of the family *Amaranthaceae*. The main identifying characteristics that separate it from other members of the family are its smooth, waxy leaves, hairless stems and leaves, lance-shaped leaves, and the

potential to grow up to 3m in height (Costea et al. 2005). Waterhemp has a C4 photosynthetic pathway allowing it to be better adapted in warm seasons and to grow quickly once established.

Seedling emergence is important for plants as it is the moment the plant begins to compete for resources, along with determining if the plant will be able to compete with its neighbors (Forcella et al. 2000). Waterhemp has its peak germination at temperatures around 25 C, but is able to germinate from temperatures of 10 to 35+ C, with little to no germination observed at 10 and 35 C (Guo and Al-Khatib 2003; Leon et al. 2004). Waterhemp is also a prolific seed producer, with documentation of over a million seeds per plant (Heneghan and Johnson 2017) This trait allows the species to spread very quickly once a population is established. This large number of seed can be difficult to deplete from the seed bank, and requires waterhemp to be actively managed for years following establishment. Waterhemp is also able to emerge throughout the growing season with multiple flushes throughout the growing season (Nordby et al. 2007). Waterhemp's initial emergence is usually later in the growing season compared to other weed species (Hager et al. 1997). Its emergence patterns can make it difficult for growers to manage since it can emerge and compete with the cash crop all season long. Seedling emergence is also influenced by the tillage practices that are being used within a field. Seedling emergence can be four times greater in no-tillage systems compared to chisel or moldboard plow systems (Leon and Owen 2006) and waterhemp emergence can occur one month later in no-tillage systems compared to other tillage systems (Leon and Owen 2006).

Waterhemp can grow rapidly once it has emerged due to its C4 photosynthetic pathway (Steckel 2007). Waterhemp can grow 0.09 to 0.12 cm per growing degree day (Horak and Loughin 2000). Waterhemp grows and produces the most biomass at 25 C and is able to grow at temperatures between 10 and 35 degrees C, but it does not survive temperatures above 45 C



(Guo and Al-Khatib 2003). Waterhemp can adapt to different environments and climates, with different populations being more suited for the climate the parent plant was growing in (Wazelkov et al. 2020).

There are two different species of waterhemp, common waterhemp (*Amaranthus rudis*) and tall waterhemp (*Amaranthus tuberculatus*), the main difference between the two species being their place of origin (Waselkov and Olsen 2014). The two species spread across the US and eventually hybridized together due to both plants being compatible. This led to the progeny of the two plants being indistinguishable from one of the parent species. This led to some scientists proposing to combine them together under the name of *Amaranthus tuberculatus*, but *A. rudis* is still seen in some literature (Pratt and Clark 2001).

### **1.5. Management of Waterhemp**

Waterhemp control in soybean is important due to the impact that it can have on the yield. Waterhemp has caused yield losses in soybean of up to 73% (Vyn et al. 2007). Waterhemp is one of the most troublesome weeds to control in cropping systems in the Red River Valley of the north. This is due to its ability to emerge in flushes throughout the season, as well the abundance of seeds produced by the plant. Once established, the seed bank hard to deplete due to the number of seeds within the soil after one season alone. The seeds of waterhemp can also be viable for 3 years or more (Korres et al. 2018), making them a constant presence in a field once established.

Waterhemp has increased in prevalence following the introduction of glyphosate-resistant crops (Culpepper 2006). The over-reliance on glyphosate led to glyphosate-resistant waterhemp developing quickly. Onset of glyphosate-resistant populations has forced growers to seek other herbicides and cultural methods to control waterhemp (Young 2006). Waterhemp has developed

multiple resistances to different herbicide sites of action including ALS inhibitors, photosystem II inhibitors, EPSP synthase inhibitors, PPO inhibitors, HPPD inhibitors, auxin mimics, and very long-chain fatty acid synthesis inhibitors (Heap 2021). Resistance challenges and other characteristics including high seed production, dioecious, etc. has resulted in waterhemp being one of the most important weeds to control in the Midwest. Cultural practices to control waterhemp and reduce the seed bank includes cover crops, tillage, crop rotation, and cultivation (Korres et al. 2018; Benech-Arnold et al. 2000). The spread of waterhemp has led to many different cultural practices starting to be considered, including companion crops (Naeve 2006). Due to the multiple flushes throughout the growing season, multiple herbicide applications are required for effective season-long control of waterhemp in soybean (Vyn et al. 2017). A systems approach including both preemergence and postemergence herbicides have shown to provide better waterhemp within soybean compared to just applying one or the other (Schryver et al. 2017). Schuster and Smeda (2007) reported a preemergence herbicide suppressed waterhemp emergence and growth from 7 to 31 days and reported a single POST-herbicide application provided 69 to 100% control of waterhemp, while using multiple application strategies provided 77 to 100% control of waterhemp. This demonstrates the importance of multiple herbicide applications in order to provide better control of waterhemp throughout the growing season. Preemergence herbicides are also important to allow crops to become established with minimal competition for the crop to grow and be better able to compete with weeds once they finally emerge. Legleiter et al (2009) reported herbicide programs for glyphosate-resistant waterhemp utilizing a single preemergence herbicide reduced waterhemp densities to 5 plants m<sup>-2</sup>, as compared to densities at 38 to 70 plants m<sup>-2</sup> from glyphosate. The amount of precipitation needed to activate the preemergence herbicides is also important as this has an effect on the

herbicides ability to control weeds once applied (Splittoesser & Derscheid 1962). Recently, Hartzler has observed how the amount of precipitation had an effect on *S*-metolachlor and acetochlor abilities to control giant foxtail within a field (Hartzler 2017). The increased prevalence of multiple herbicide resistant waterhemp has led many herbicide programs to include multiple sites of action to control waterhemp instead of the overreliance of one site of action to avoid evolving resistance to additional sites of action. There have been no new herbicide sites of action introduced into row crops since the early 1990's, so many proactive farmers are looking for additional tools to help control waterhemp.

### **1.6. Palmer Amaranth Biology**

Palmer Amaranth (*Amaranthus palmeri*) is an erect, small seeded summer annual with hairless leaves and stems (Fernald 1950). The species is native to the Sonoran Desert and has been found in most of the southern half of the United States (Davis et al. 2015). Palmer amaranth is a dioecious plant having both male and female plants (Fernald 1950). The species can spread quickly with up to 600,000 seed being able to be produced by one plant (Keeley et al. 1987). The plant's appearance is very similar to that of waterhemp. The slight differences between the two can be observed in the leaves, with palmer amaranth having rhombic-ovate to rhombic-lanceolate leaves, with petioles as long or longer than the blades of the leaves (GPFA 1986). Some populations of waterhemp have been observed to have these characteristics as well, so it is not always the best way to distinguish the two from each other. The two species can also be differentiated at later growth stages, as the female flowers Palmer amaranth have stiff bracts (Fernald 1950). Palmer amaranth has the ability to exceed 2m in height (Horak and Loughlin 2000). Palmer amaranth also utilizes a C4 photosynthetic pathway, which can allow the plant to grow at a rapid pace and be very competitive in cropping systems (Ehleringer 1983). Palmer

amaranth can produce progeny that are better suited for the environment that the parent plant was growing in, for instance, producing bigger seeds that can be more successful than the parent plants in drought conditions (Matzrafi et al. 2020). Palmer amaranth has also been observed to grow larger than that of waterhemp and redroot pigweed when in warmer conditions. Guo and Al-Khatib observed Palmer amaranth growing to a larger biomass than waterhemp and redroot pigweed at day and night temperature cycles of 25/20 C and 35/25 C. However, Palmer produced less biomass than that of waterhemp and redroot pigweed when growing at a day and night temperature cycle of 15/10 C (Guo and Al-Khatib 2003).

Palmer amaranth has become a challenge for growers to control within fields over the last few decades. Palmer amaranth was listed as the #2 most troublesome weed in soybean in 2010, and the #7 most troublesome weed in corn in 2009 (Webster and Nichols 2012) as compared to #23 in 1995 in soybean and not being listed in corn. Palmer amaranth has also become one of the most challenging glyphosate-resistant weed species within the United States (Beckie 2006). The weed has also been confirmed in states with a cooler climate, such as North Dakota (Corn and Soybean Digest 2020). This shows the adaptability of the plant to be able to spread and grow within different environments and climates.

### **1.7. Companion Crop Effects on Weeds**

Companion crops can potentially reduce pigweed interference during the critical period of weed control. Companion crops compete with the weeds within the cropping system for light, nutrients, and water, but may also compete with the cash crop and reduce yield (Verret et al 2017). The species must be chosen carefully; they must be able to out-compete the weeds, while limiting competition with the cash crop to avoid or reduce yield loss. Verret et al (2017) tested multiple cropping systems and reported a 56% decrease in weed biomass compared to non-

weeded control treatments, with no significant change in yield. Companion crop biomass and shading has been studied in field trials, with differing results, but has been considered a main factor of weed suppression (Gfeller et al. 2018). Gfeller et al. (2018) also reported a greater competitiveness of the companion crop for a significant effect on weed control through shading. While companion crops may demonstrate an allelopathic effect towards weeds, more research is necessary to test potential allelopathic effects of different companion crop by weed combinations (Gfeller et. al 2018). Brassicaceae crops and oat have shown to effectively reduce pigweed biomass (Gfeller et. al. 2018). Other mechanisms to control pigweeds are root interactions and competition for light, water and nutrients. (Gfeller et. al. 2018). Buckwheat, black oat, and forage radish can suppress pigweed growth through indirect root interactions by 46%, 37%, and 49%, respectively, and they were able to reduce growth by 68%, 41% and 62% through direct root interactions, respectively.

### **1.8. Justification and Summary**

Waterhemp and other pigweeds are difficult to control in soybean due to the herbicide resistances, high seed production, and growth rate. This has caused many growers to search for a cultural practice to combine with the current herbicide strategies to control waterhemp in cropping systems and reduce yield loss from waterhemp. Cultural practices must be researched in order to determine if they can compliment control of pigweeds. Companion crops mixed with current herbicide programs show great promise to solve these challenges.

Iron Deficiency Chlorosis remains a prevalent production challenge within North Dakota and Minnesota due to the high pH levels in this soybean growing region. While tolerant cultivars are the best way to combat this production issue, IDC is still observed within these cultivars. Companion crops could be used to supplement the tolerant cultivars to reduce the

amount of IDC observed within soybean. The benefits that could be observed with the use of a companion crop for these issues is one that should be evaluated to help increase yields for growers.

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**CHAPTER 2. OAT (*AVENA SATIVA*) COMPANION CROP EFFECTS ON SOYBEAN (*GLYCINE MAX*), WATERHEMP (*AMARANTHUS TUBERCULATUS*), AND POWELL AMARANTH (*AMARANTHUS POWELLI*)**

**2.1. Introduction**

Iron deficiency chlorosis (IDC) is a persistent production challenge within soybean (*Glycine max*) in North Dakota and the upper Midwest of the USA due to the presence of alkaline soils (Naeve 2006). In-furrow applications of certain types of iron (Fe) chelate can reduce IDC symptoms in soybean and increase yields of IDC susceptible cultivars (Kaiser et al. 2014; Ferreira et al. 2019). The use of IDC tolerant soybean cultivars reduce the presence of IDC symptoms in soybean fields. Both of these IDC reduction methods may be expensive. Soybean IDC tolerant cultivars historically have produced less yield compared to other non-tolerant soybean cultivars in the absence of IDC conditions (Helms et al. 2010). This had led some growers to look for other options to reduce IDC symptoms within their soybean fields. The planting of a companion crop may contribute to reduced IDC symptoms within a field through increased soil drying and soil nitrate uptake. Greater soybean biomass with increased soybean seeding rate has been observed to reduce IDC symptoms within a field (Penas et al. 1990). A soybean grower could realize suppressed IDC symptoms by planting a companion crop that is easily controlled with herbicides, thus benefitting from increased soil water use and soil nitrate, while still being able to control and terminate the companion crop to ensure that it does not have an effect on yield. The companion crop may also suppress weeds within a field by competing with weeds for sunlight and other nutrients (Verret et al 2017). *Amaranthus* species have had an increased prevalence in North Dakota, especially since the emergence of herbicide-resistant waterhemp (*Amaranthus tuberculatus*(*Moq.*) *J.D. Sauer*) within the state. North Dakota is the

leading US state in oat grain production, producing a high quality oat crop for both the human food industry and for animal feed (Ransom et al. 2018). Growing oat as a companion crop would be easy for North Dakota growers to implement and would be expected to grow well in the state's multiple environments. The objectives of this experiment were: 1) determine the effects that an oat companion crop can have on soybean yield; 2) determine the effect of an oat companion crop on IDC suppression; and (3) measure any suppression of weeds from the oat companion crop.

## **2.2. Materials and Methods**

### **2.2.1. Field Design**

Field experiments were conducted at conventionally tilled locations near Fargo, ND (Fargo-Ryan series, Silty Clay, 5% OM, pH 7.4, 46°55'50.4"N 96°51'07.7"W) and near Prosper, ND (Kindred-Bearden, Silty Loam, 4.3% OM, pH 7, 47°00'01.9"N 97°07'16.7"W). The experiment was arranged 2x5x2 factorial in a RCBD split-block arrangement with four replications. Treatment factors were: a) presence of oat companion crop; b) timing of first POST herbicide application based on height of the companion crop; and c) number of POST herbicide applications. Factor A had two levels, oat and no oat. Factor B had 5 levels; 15cm, 30cm, 45cm, and 60cm oat height to determine termination timing, as well as a no POST timing/no oat termination. Factor C had two levels, one or two POST treatments of herbicide. A weed-free, oat-free treatment was included for comparison. The main pigweed species at the Fargo location was waterhemp, while the main pigweed species at the Prosper location was Powell amaranth (*Amaranthus powelli* S. Watson). The rainfall for both years and sites are listed below (Table 2.1 and 2.2).

Table 2.1. Weekly precipitation at Fargo and Prosper, ND locations for the experiments in 2020.

	Fargo	Prosper
<i>Weeks</i>	----- <i>cm</i> -----	
May 10- May 16	.51	.76
May 17 – May 23	0	0
May 24 – May 30	.28	.18
May 31 – June 06	.10	.05
June 07 – June 13	4.29	5.59
June 14 – June 20	.79	1.12
June 21 – June 27	1.37	1.23

Table 2.2. Weekly precipitation at Fargo and Prosper, ND locations for the experiments in 2021

	Fargo	Prosper
<i>Weeks</i>	----- <i>cm</i> -----	
May 02 – May 08	.03	.03
May 09 – May 15	0	.05
May 16 – May 22	.58	1.27
May 23 – May 29	.25	.58
May 30 – June 05	.03	.05
June 06 – June 12	5.84	2.11
June 13 – June 19	0	0
June 20 – June 26	2.76	2.54

### 2.2.2. Planting

A Roundup Ready Xtend® soybean cultivar, ‘AG06X8’(Bayer Crop Science, Creve Coeur, MO), was planted to a depth of 3 cm in 76-cm rows at a seeding rate of 385,320 seeds ha<sup>-1</sup> using a custom made Monosem vacuum-planter. The cultivar ‘ND Rockford’ oat (NDSU Foundation Seedstocks, Fargo, ND) was planted parallel to soybean rows to a depth of 3 cm in 19-cm rows at a seeding rate of 67 kg ha<sup>-1</sup> using a Great Plains 3P600 drill (Salina, KS). Fields were soil tested and fertilized according to the soil tests for nitrogen, phosphorus, and potassium. In 2020 the Fargo site was planted on May 19<sup>th</sup> and the Prosper site was planted on May 27<sup>th</sup>,

and in 2021 the Fargo site was planted on May 10<sup>th</sup> and the Prosper site was planted on May 17<sup>th</sup>. The size for each experimental unit within each experiment was 3m wide by 7.6m long.

### **2.2.3. Herbicide Application and Selection**

Herbicide POST treatments comprised of a tank-mixture of glyphosate (RoundUp PowerMax®, Bayer Crop Science, Creve Coeur, MO) at 1260 g ae ha<sup>-1</sup> and dicamba (Xtendimax®, Bayer Crop Science, Creve Coeur, MO) at 560 g ae ha<sup>-1</sup> with Class Act® Ridion® (Winfield United, LLC, St. Paul, MN), at 1% v/v. Experimental units with a secondary POST treatment received a repeat herbicide treatment 14 days after the initial termination. Glyphosate and dicamba were selected because the tank-mix would terminate the oat companion crop and to evaluate the efficacy of the herbicide mixture on the *Amaranthus* species present within the experimental unit. Treatments were applied using a CO<sub>2</sub>-pressurized backpack sprayer calibrated to deliver 140 L ha<sup>-1</sup> at a speed of 4.8 km h<sup>-1</sup> with TTI 11002 (Turbo TeeJet Induction, TeeJet® Technologies, Glendale Heights, IL) in 38 cm spacing with a 1.5 meter boom with a 2 meter spray width at 193 kPa of pressure.

### **2.2.4. Evaluation and Data Analysis**

The IDC symptom results were collected visibly and through the use of a Soil Plant Analysis Development (SPAD) logger (Spectrum Technologies, Inc., Aurora, IL) technology to collect chlorophyll readings on soybean. The newest trifoliolate leaf of 10 different plants were subject to chlorophyll readings using the SPAD logger and were recorded for the plot. A SPAD logger measures the “absorbance by the leaf of two different wavelengths in the spectral domain of red and near-infrared. As an output, they calculate index-values (SPAD-value) that specify leaf chlorophyll content.” (Süß et al. 2015). The SPAD value and the amount of chlorophyll in the plant are directly related. The visible IDC ratings were recorded using the system described

by Helms and Kandel (2020), whereby plots were rated on a scale of 1-5, with 1 representing no symptoms and with a 5 being a dead plant. Weed biomass and weed densities were collected at each of the four different termination timings. Oat biomass was also collected at termination timing. The termination timing is when the first postemergence herbicide application was applied. All plants were collected from a 1-m<sup>2</sup> quadrat, and were clipped at the soil surface and dried at 43 C° for 14 days using a force air dryer. Herbicide efficacy data and SPAD readings were collected every 14 days after the initial treatment until canopy closure. Soybean grain and moisture collected at the end of the season using a Hege 125C plot combine. The center 1.5 m of the 3 m wide experimental units were collected and were then weighed and measured for moisture content.

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). Data were subjected to ANOVA using PROC GLIMMIX to test for treatment effects and significant differences. Treatment means were separated using Tukey's HSD when data were found to be significantly different at  $P \leq 0.05$ . The presence of oat, termination timings, and the number of postemergence herbicide applications were considered to be fixed effects, while environment, year, and replicate were considered to be random effects. Fargo vs Prosper were analyzed separately due to primary pigweed species. Years were analyzed separately due to failing Levine's test at  $P \leq 0.05$ . Years were combined for analysis for aboveground oat biomass due to passing Levine's test at  $P \leq 0.05$ . Normality was verified using PROC UNIVARIATE within SAS. Pigweed control percentages were non-normal and data were arc sine square-root transformed ( $\arcsin((Y/100)^{1/2}))$ ) for mean separation, but non-transformed means are reported for clarity.

## 2.3. Results

### 2.3.1. SPAD Logger Results

IDC symptoms were not frequently observed at either site during either year. This may have been due the variety of soybean that was planted, as well as environment. Rainfall was not in excessive amount (Table 2.1 and 2.2) early in the season to increase severity of IDC within the soybean. Therefore, these data represent differences in SPAD logger readings within a field where IDC was not a production issue. The number of postemergence herbicide applications also did not have an effect on the SPAD readings, so data was pooled across that factor.

Table 2.3. Presence of oat companion crop and termination timing effect on SPAD logger values at Fargo, ND, in 2020 and 2021, 0, 14, and 28 days after oat termination.<sup>a,b</sup>

Main effects	Fargo 2020			Fargo 2021		
	0 DAT <sup>c</sup>	14 DAT	28 DAT	0 DAT	14 DAT	28 DAT
<i>Presence of oats</i>	-----SPAD value-----					
No oats	32.66 b	32.15	31.34	39.18 b	34.29	36.82
Oats	34.58 a	31.97	30.95	40.69 a	34.30	37.29
<i>Termination timing</i>						
15 cm	35.21 a	34.74 a	32.28	43.76 a	35.34 a	37.60 a
30 cm	34.39 ab	30.68 b	31.20	37.00 b	34.62 a	37.21 a
45 cm	33.63 b	31.63 b	29.86	38.14 b	33.58 ab	37.33 a
60 cm	31.88 c	30.54 b	31.51	37.49 b	32.34 b	35.44 b
<i>ANOVA</i>	-----p-value-----			-----p-value-----		
Presence of oats	<0.001	0.719	0.301	0.002	0.986	0.252
Termination timing	<0.001	<0.001	0.109	<0.001	0.007	0.005
Presence of oat *	0.897	0.213	0.125	0.779	0.794	0.102
<i>Termination Timing</i>						

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Newest trifoliolate was used to measure SPAD logger values

<sup>c</sup> Abbreviation: DAT = days after termination.



Table 2.4. Presence of oats and termination timing effect on SPAD logger values at Prosper in 2020 and 2021, 0, 14, and 28 days after oat termination.<sup>a,b</sup>

Main effects	Prosper 2020			Prosper 2021		
	0 DAT <sup>c</sup>	14 DAT	28 DAT	0 DAT	14 DAT	28 DAT
<i>Presence of oats</i>						
-----SPAD value-----						
No Oats	32.02	29.73 b	31.88	36.48	35.67	38.07 a
Oats	31.49	30.82 a	32.51	37.36	35.38	37.37 b
<i>Termination timing</i>						
15 cm	31.13 b	30.51 a	31.22 b	43.75 a	36.82 a	40.17 a
30 cm	28.95 c	30.35 ab	31.53 b	35.31 b	35.59 ab	38.20 b
45 cm	35.42 a	29.56 ab	33.04 a	33.70 b	34.59 bc	36.40 c
60 cm	31.52 b	29.26 b	33.41 a	35.00 b	33.84 c	35.59 c
-----p-value-----						
ANOVA						
Presence of Oats	0.243	0.007	0.122	0.106	0.555	0.028
Termination Timing	<0.001	0.002	0.002	<0.001	<0.007	<0.001
Presence of Oats *	0.355	0.154	0.276	0.990	0.103	0.140
Termination Timing						

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Newest trifoliolate was used to measure SPAD logger values

<sup>c</sup> Abbreviation: DAT = days after termination.

The presence of oats gave a higher SPAD reading compared to when oats were absent at 0 days after termination in both years, indicating that the soybean plants in the plots with an oat companion crop had a higher amount of chlorophyll content (Table 2.1). Oats did not have an effect on the SPAD readings at Fargo at 14 and 28 days after termination. Earlier termination timings had higher SPAD readings than that of later termination timings at Fargo, decreasing with time.

The presence of oats did not have an effect on the SPAD readings at 0 and 28 days after termination in 2020 at Prosper (Table 2.2.). At 14 days after termination, plots with oats had higher SPAD readings than when oats were not present. In 2021 at Prosper the presence of oats did not have an effect on the SPAD readings at 0 and 14 days after termination. Treatments with

oats had lower SPAD readings than when oats were not present at 28 days after termination. It was not clear if the presence of an oat companion crop increased SPAD readings and reduced IDC ratings within the soybean. The presence of IDC symptoms within the soybean crop at the sites, as well as choosing a more IDC susceptible variety of soybean could yield more defining results. Terminating oat when it was shorter gave greater SPAD values with the exception of Prosper in 2020. IDC usually appears in younger soybeans, so if IDC symptoms were present, the 15 cm termination timing would be expected to have the lowest SPAD readings of all the termination timings. These results show the 15 cm termination timing had higher SPAD values, likely due to the soybeans being healthy and IDC symptoms not being present. The competition with the oats at the later timings may have also had a negative effect on the chlorophyll present within the plant, explaining why plants at later termination timings had lower SPAD values than that of the younger plants at earlier termination timings.

### **2.3.2. Iron Deficiency Chlorosis Visible Results**

IDC symptoms were not frequently observed at either site during either year. There were not any visible ratings assigned that were above a rating of 2. This lack of IDC symptomology could be contributed to the variety of soybeans that were used, as well as the environments of both the fields. The number of postemergence herbicide applications were not included during the analysis of visible IDC ratings due to number of post applications not having an effect on the IDC ratings.

Table 2.5. Presence of oats on iron deficiency chlorosis visible ratings at Fargo in 2020 and 2021, 0, 14, and 28 days after initial oat termination. <sup>a</sup>

Main effects	Fargo 2020			Fargo 2021		
	0 DAT <sup>b</sup>	14 DAT	28 DAT	0 DAT	14 DAT	28 DAT
<i>Presence of oats</i>	----- <i>IDC scale</i> -----					
No oats	1	1.05 a	1	1.11 a	1	1
Oats	1	1 b	1	1 b	1	1
<i>Termination timing</i>						
15 cm	1	1.13 a	1	1 b	1	1
30 cm	1	1 b	1	1.28 a	1	1
45 cm	1	1 b	1	1 b	1	1
60 cm	1	1 b	1	1 b	1	1
<i>Presence of oats * termination timing</i>						
Oats * 15 cm	1	1 b	1	1 b	1	1
Oats * 30 cm	1	1 b	1	1 b	1	1
Oats * 45 cm	1	1 b	1	1 b	1	1
Oats * 60 cm	1	1 b	1	1 b	1	1
No oats * 15 cm	1	1.25 a	1	1 b	1	1
No oats * 30 cm	1	1 b	1	1.56 a	1	1
No oats * 45 cm	1	1 b	1	1 b	1	1
No oats * 60 cm	1	1 b	1	1 b	1	1
<i>ANOVA</i>	----- <i>p-value</i> -----			----- <i>p-value</i> -----		
Presence of oats	1.000	0.015	1.000	<0.001	1.000	1.000
Termination timing	1.000	<0.001	1.000	<.0001	1.000	1.000
Presence of oats * termination timing	1.000	<0.001	1.000	<.0001	1.000	1.000

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Abbreviation: DAT = days after termination; IDC = Iron Deficiency Chlorosis

Table 2.6. Presence of oats on iron deficiency chlorosis visible ratings at Prosper in 2020 and 2021, 0, 14, and 28 days after initial oat termination. <sup>a</sup>

Main effects	Prosper 2020			Prosper 2021		
	0 DAT <sup>b</sup>	14 DAT	28 DAT	0 DAT	14 DAT	28 DAT
<i>Presence of oats</i>	----- <i>IDC scale</i> -----					
No oats	1.03 a	1	1	1 b	1	1
Oats	1.03 a	1	1	1.09 a	1	1
<i>Termination timing</i>						
15 cm	1 b	1	1	1 b	1	1
30 cm	1.13 a	1	1	1.22 a	1	1
45 cm	1 b	1	1	1 b	1	1
60 cm	1 b	1	1	1 b	1	1
<i>Presence of oats * termination timing</i>						
Oats * 15 cm	1	1	1	1 a	1	1
Oats * 30 cm	1.13	1	1	1.43 b	1	1
Oats * 45 cm	1	1	1	1 a	1	1
Oats * 60 cm	1	1	1	1 a	1	1
No oats * 15 cm	1	1	1	1 a	1	1
No oats * 30 cm	1.13	1	1	1 a	1	1
No oats * 45 cm	1	1	1	1 a	1	1
No oats * 60 cm	1	1	1	1 a	1	1
<i>ANOVA</i>	----- <i>p-value</i> -----			----- <i>p-value</i> -----		
Presence of oats	1.000	1.000	1.000	<0.001	1.000	1.000
Termination timing	0.002	1.000	1.000	<0.001	1.000	1.000
Presence of oats * termination timing	1.000	1.000	1.000	<0.001	1.000	1.000

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Abbreviation: DAT = days after termination; IDC = Iron Deficiency Chlorosis

At Fargo in 2020 the presence of oats did not affect visible IDC ratings 0 and 28 days after termination (Table 2.3.). The presence of oats resulted in less IDC symptoms 14 days after termination compared to oats not being present. At Fargo in 2021, plots with oats had greater IDC visible symptoms 0 days after termination compared to oats not being present. The presence of oat did not have an effects on IDC symptoms at 14 and 28 days after termination. Of note, IDC symptoms were less when oat was present at Fargo in 2021 at 0 days after termination and

the SPAD readings at that timing confirmed greater chlorophyll content in soybean when oat was present (Tables 2.1, 2.3). This was the only instance where visible rating of IDC symptoms aligned with SPAD readings to indicate oats alleviated IDC symptoms. These results reinforce the observation by Naeve (2006) that oats may have the ability to reduce IDC symptoms, but that the suppression effect was inconsistent.

The presence of oats did not have an effect on IDC symptoms at any timing At Prosper in 2020 (Table 2.4). In 2021, oats resulted in greater IDC symptoms at 0 days after termination. Oats did not affect IDC symptoms 14 and 28 days after termination. Greater IDC severity or a higher incidence of symptoms within the trial would be required for a more comprehensive evaluation of the effect of an oat companion crop on IDC symptoms in soybean. Naeve (2006) observed that an oat companion crop may reduce IDC symptoms within soybean, but the effect wasn't observed consistently, suggesting an oat companion crop may not be profitable due to the competition risk and inconsistency of the suppressed symptoms. Franzen and Richardson (2000) described the difficulty in predicting where IDC symptoms will appear. This prediction difficulty may make the decision to use oats as a method of reducing IDC difficult for growers due to input costs of oat seed. All visible IDC symptoms above 1 in these trials were observed at the 30 cm termination timing at 0 DAT or at the 15 cm termination timing at 14 DAT. These timings were similar and close to each other, at V2-V3 soybean, showing that this timeframe may have been when IDC symptoms were most present, and greater differences could have been detected if IDC symptoms had been more severe.

### **2.3.3. Pigweed Biomass**

There was an interaction between the presence of oat and termination timing on waterhemp biomass at Fargo in 2020 (Table 2.5). The presence of oats did not have an effect on

biomass until the 45 cm termination timing, when the oat treatment reduced biomass compared to the no oat treatment by 176.5 kg ha<sup>-1</sup> and reduced biomass at the 60 cm termination timing by 539.7 kg ha<sup>-1</sup>.

In 2021 the presence of oat had an effect on waterhemp biomass at the 30 cm termination timing; which was earlier in the season compared to 2020 (Table 2.5). The oat treatment reduced biomass at the 30 cm termination timing by 155.3 kg ha<sup>-1</sup>, by 221.4 kg ha<sup>-1</sup> at the 45 cm termination timing, and by 787.7 kg ha<sup>-1</sup> at the 60 cm termination timing. The waterhemp biomass in the oat treatment at the 30 cm termination timing was also not different from either 15 cm termination timing.

The presence of oat did not have an effect on Powell amaranth biomass until the 45 cm termination timing at Prosper in 2020 (Table 2.5). The oat treatment reduced biomass by 142.1 kg ha<sup>-1</sup> at the 45 cm termination timing, and reduced biomass by 220.4 kg ha<sup>-1</sup> at the 60 cm termination timing. The Powell amaranth biomass in the oat treatment at 45 cm termination timing was also not different from biomass in either of the 15 cm termination timing and 30 cm termination timing.

At Prosper in 2021, oats did not have an effect on the aboveground broadleaf weed biomass, but termination timing did have an effect (Table 2.5). The 60 cm termination timing had the greatest amount of aboveground Powell amaranth biomass (229.5 kg ha<sup>-1</sup>) and was different from every other termination timing. The next greatest amount of aboveground broadleaf weed biomass was at the 45 cm termination timing with 176.6 kg ha<sup>-1</sup>, which was also different from every other timing. The 15 cm and 30 cm termination timing had 1.7 kg ha<sup>-1</sup> and 43.6 kg ha<sup>-1</sup> of aboveground Powell amaranth biomass, respectively, which were the least amount and were different from every timing except for each other.

Table 2.7. Presence of oat and oat termination timing on pigweed aboveground biomass at Fargo and Prosper in 2020 and 2021.<sup>a,b</sup>

Main effects	Fargo 2020	Fargo 2021	Prosper 2020	Prosper 2021
<i>Presence of oats</i> -----kg ha <sup>-1</sup> -----				
No oats	312.0 a	446.4 a	174.0 a	119.3
Oats	134.4 b	98.7 b	81.5 b	106.4
<i>Termination timing</i>				
15 cm	37.3 c	12.1 d	14.6 c	1.7 c
30 cm	121.2 c	139.7 c	85.1 bc	43.6 c
45 cm	244.8 b	386.3 b	162.2 b	176.6 b
60 cm	489.7 a	552.2 a	249.2 a	229.5 a
<i>Presence of oats * Termination timing</i>				
No oats * 15 cm	36.8 d	14.5 e	13.8 d	1.4
No oats * 30 cm	118.8 cd	217.3 c	89.5 cd	30.8
No oats * 45 cm	333.0 b	607.8 b	233.2 b	191.4
No oats * 60 cm	759.5 a	946.0 a	359.4 a	253.6
Oats * 15 cm	37.8 d	9.7 e	15.3 d	2.0
Oats * 30 cm	123.6 cd	62.0 de	80.6 cd	56.5
Oats * 45 cm	156.5 c	164.9 c	91.1 cd	161.8
Oats * 60 cm	219.8 bc	158.3 cd	139.0 bc	205.5
<i>ANOVA</i> -----p-value-----				
Presence of oats	<0.001	<0.001	0.001	0.728
Termination timing	<0.001	<0.001	<0.001	<0.001
Presence of oats *	<0.001	<0.001	0.015	0.899
Termination timing				

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> The pigweed species at Prosper was Powell amaranth and the pigweed species at Fargo was waterhemp.

Oats had an impact on pigweed biomass between the 30 and 45 cm termination timings at 3 out of 4 site years. This biomass suppression does not occur until after recommended termination timings for oats used for suppression of IDC (Naeve 2006, Kandel et al. 2021). This means that oats that are terminated at recommended timings will not have reduced pigweed biomass, so they must be allowed to grow for longer in order to provide suppression. Allowing

oats to grow to 45 cm in order to suppress pigweed biomass could have other deleterious effects on soybean due to prolonged competition. However, a reduction in pigweed biomass can still be beneficial. Reduced pigweed biomass has been observed to assist in control of pigweeds using herbicides (Hay et al. 2019). The reduced biomass observed from oats could be combined with an herbicide program to better control pigweeds within soybean, and could be especially helpful when trying to control herbicide-resistant waterhemp.

#### **2.3.4. Pigweed Density**

None of the main effects had an influence on pigweed density at Fargo during either year (Table 2.6). The presence of oats resulted in a higher weed density compared to no oats at Prosper during both years. Termination timing did not have an effect on broadleaf weed density at Prosper during either year. The increase weed density that was observed from the oat treatment may be due to the planting of the oats with the grain drill creating more soil disturbance and a better seed bed for the weeds within the oats. Other explanations could be that better emergence conditions could have been present due to the increased plant biomass having an effect on the canopy microclimate such as increase humidity beneath the canopy, increased temperature, etc. (Sauer et al. 2007). In Fargo in 2021 the presence of oats and termination timing had a combined interaction. Comparing treatment factors, oats reduced waterhemp density at the 30 cm termination timing compared to no oats at the same timing. However, oats did not reduce density compared to no oats at any other termination timing. In general, fewer differences were found with waterhemp at Fargo compared to Powell amaranth at Prosper. This could be due to waterhemp's ability to remain competitive in high density environments (Steckel and Sprague 2004)



Table 2.8. Presence of oats and termination timing on pigweed density at Prosper and Fargo in 2020 and 2021. <sup>a,b</sup>

Main effects	Fargo 2020	Fargo 2021	Prosper 2020	Prosper 2021
<i>Presence of oats</i>	-----weeds m <sup>-1</sup> -----			
No oats	118	142	29 b	21 b
Oats	124	132	51 a	29 a
<i>Termination timing</i>				
15 cm	98	116	49	19
30 cm	120	142	52	26
45 cm	126	144	38	27
60 cm	140	145	21	29
<i>Presence of oats * termination timing</i>				
No oats * 15 cm	94	90 c	42	16
No oats * 30 cm	118	188 a	22	18
No oats * 45 cm	135	158 ab	26	24
No oats * 60 cm	122	131 bc	27	26
Oats * 15 cm	102	142 abc	57	22
Oats * 30 cm	122	96 c	83	33
Oats * 45 cm	117	129 bc	51	30
Oats * 60 cm	158	160 ab	15	32
<i>ANOVA</i>	-----p-value-----			
Presence of oats	0.129	0.480	0.030	0.050
Termination timing	0.365	0.420	0.126	0.302
Presence of oats * termination timing	0.546	0.004	0.090	0.832

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> The pigweed species at Prosper was Powell amaranth and the pigweed species at Fargo was waterhemp.

### 2.3.5. Aboveground Oat Biomass Results

Termination timing of oats affected aboveground oat biomass at all sites and years (Table 2.7). The 60 cm termination timing had the highest amount of oat biomass followed by 45 cm, then 30 cm, and 15 cm having the least amount of aboveground oat biomass at all sites and years. Each termination timing was different from each other. Higher oat biomass may be able to reduce additional waterhemp flushes later in the season. Increased biomass could also delay the

number of days it would take for waterhemp to emerge and grow to 10 cm. For example, Wiggins et al (2017) found that 1540 kg ha<sup>-1</sup> of cereal rye can delay Palmer amaranth germination and growth to 10 cm by 16.5 days. In another study, increasing the amount of biomass from a *Brassica* cover crop has been observed to be able to reduce the amount of weeds that emerge, as well as delay emergence by two days (Haramoto and Gallandt 2005). The increased amount of oat biomass at later timings may be able to reduce and delay emergence of waterhemp. This could be helpful to growers later in the season, due to waterhemp emerging throughout the season in later flushes giving better control of these late waterhemp flushes.

Table 2.9. Termination timing on aboveground oat biomass at Prosper and Fargo. <sup>a</sup>

Termination Timing	Fargo	Prosper
	-----kg ha <sup>-1</sup> -----	
15 cm	108.3 d	100.2 d
30 cm	398.6 c	420.7 c
45 cm	913.8 b	839.4 b
60 cm	1510.2 a	1277.0 a
<i>ANOVA</i>	-----p-value-----	
Termination Timing	<.001	<.001

<sup>a</sup> Means within a column that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

### 2.3.6. Pigweed Efficacy Results

The presence of oat did not have an effect on pigweed efficacy so the data were combined across that factor. Termination timing was the only main effect to have an influence on waterhemp efficacy at 14 days after termination at Fargo in 2020 (Table 2.8). The 15 cm termination timing had the greatest efficacy (94%) and was different from every other treatment. The 30 cm and 45 cm termination timings had 74% and 73% waterhemp control, respectively, and were different from every other treatment except for each other. The 60 cm termination timing resulted in 58% waterhemp control and was less than every other termination timing.

There was a combined interaction between the main effects of termination timing and number of postemergence herbicide applications at 28 days after treatment (14 days after second POST). The 15 cm termination timing with two postemergence herbicide applications had the greatest waterhemp control (97%) and was different from every other treatment. The treatments that had the next highest waterhemp control was the 30 cm termination timing with two postemergence herbicide applications (91%) and the 45 cm termination timing with two postemergence herbicide applications (92%), which were different from every other treatment except for each other. Every other treatment combination provided less than 90% waterhemp control.

At Fargo in 2021, termination timing was the only main effect to have an influence on waterhemp efficacy at 14 days after termination (Table 2.8). The treatments were all different from each other with 15, 30, 45, and 60 cm termination timings providing 96, 83, 67, and 51% waterhemp control, respectively. There was a combined interaction between the main effects of termination timing and the number of postemergence applications at 28 days after termination at Fargo in 2021. The 15 cm termination timing with two postemergence herbicide applications had the highest waterhemp efficacy (94%) and was different from every other treatment. The 30 cm termination timing with two postemergence herbicide applications provided the next best control at 88% waterhemp control. The 45 cm termination timing with two postemergence herbicide applications provided 76% control, and all other treatments resulted in less than 70% control. Overall, An early herbicide application followed up by a second application had the best control. A second application was required on the earliest terminated plots due to additional waterhemp flushes after application (*data not shown*), while the herbicide mix of dicamba and glyphosate provided less control of larger waterhemp with later termination timings. Dicamba is labeled to be used on weeds that are less than 10.16 cm (Xtendimax® with VaporGrip® Technology,

Bayer CropScience 2021). All of the waterhemp that was treated within this experiment was above this labeled limit, which could also explain the low control of waterhemp observed, especially at the later termination timings when waterhemp was much taller than the labeled limit which has been observed in other studies (Spaunhorst and Bradley 2013. The second application is especially important, as while the first application may control the initial waterhemp as seen at the 15 cm termination timing, the newly emerging waterhemp is capable of growing at a fast rate and still potentially have an effect on soybean later in the season. Sequential applications of dicamba when initially applied to waterhemp below 10 cm has resulted in the best control of waterhemp (Spaunhorst and Bradley 2017). A preemergence herbicide may also need to be used in combination with an oat companion crop and postemergence herbicides in order to control waterhemp within soybean to prevent yield loss due to the presence of waterhemp.

Table 2.10. Termination timing and number of postemergence herbicide applications on waterhemp efficacy at Fargo in 2020 and 2021. <sup>a</sup>

Main effects	Fargo 2020		Fargo 2021	
	14 DAT <sup>b</sup>	28 DAT <sup>c</sup>	14 DAT	28 DAT
<i>Termination timing</i>	-----%-----			
15 cm	94 a	66 b	96 a	58 b
30 cm	74 b	72 ab	83 b	68 a
45 cm	73 b	81 a	67 c	67 a
60 cm	58 c	66 b	51 d	61 b
<i>No. of post applications</i>				
1 post application	75	57 b	74	46 b
2 post applications	74	85 a	75	81 a
<i>Termination timing * No. of post applications</i>				
15 cm * 1 post	93	35 f	96	23 g
30 cm * 1 post	71	53 e	82	48 f
45 cm * 1 post	72	70 c	68	59 e
60 cm * 1 post	65	71 c	50	57 e
15 cm * 2 post	94	97 a	96	94 a
30 cm * 2 post	76	91 b	85	88 b
45 cm * 2 post	74	92 b	66	76 c
60 cm * 2 post	52	61 d	52	64 d
<i>ANOVA</i>				
	-----p-value-----			
Termination timing	<0.001	<0.001	<0.001	<0.001
no. of post applications	0.630	<0.001	0.561	<0.001
Termination timing * no. of post applications	0.243	<0.001	0.758	<0.001

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Abbreviation: DAT = days after initial treatment; No. = number

<sup>c</sup> 28 DAT was 14 days after 14 DAT (28 days after initial treatment, and 14 days after sequential treatment)

Table 2.11. Termination timing and number of postemergence herbicide applications on Powell amaranth efficacy at Prosper in 2020 and 2021. <sup>a</sup>

Main effects	Prosper 2020		Prosper 2021	
	14 DAT <sup>b</sup>	28 DAT <sup>c</sup>	14 DAT	28 DAT
<i>Termination timing</i>	-----%-----			
15 cm	99	91 b	97	77 b
30 cm	97	96 a	97	98 a
45 cm	97	97 a	96	99 a
60 cm	98	98 a	95	98 a
<i>No. of post applications</i>				
1 post application	98	93 b	96	88 b
2 post applications	98	98 a	97	98 a
<i>Termination timing * no. of post applications</i>				
15 cm * 1 post	99	83 b	97	60 b
30 cm * 1 post	96	95 a	97	97 a
45 cm * 1 post	97	97 a	97	98 a
60 cm * 1 post	99	99 a	94	98 a
15 cm * 2 post	98	99 a	98	94 a
30 cm * 2 post	98	98 a	97	99 a
45 cm * 2 post	97	98 a	96	99 a
60 cm * 2 post	98	98 a	96	99 a
<i>ANOVA</i>	----- <i>p-value</i> -----			
Termination timing	0.368	<0.001	0.399	<0.001
no. of post applications	1.000	<0.001	0.354	<0.001
Termination timing * no. of post applications	0.528	<0.001	0.567	<0.001

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Abbreviation: DAT = days after initial treatment; no. = number

<sup>c</sup> 28 DAT was 14 days after 14 DAT

Glyphosate + dicamba applied at Prosper in both years provided 94% to 99% control of Powell amaranth at 14 days after treatment and none of the main effects had an influence on the control (Table 2.9). There was an interaction between termination timing and the number of post

applications at 28 days after treatment. One herbicide application at the 15 cm termination timing was different from every other treatment and resulted in least control of Powell amaranth. This is due to initial flush of Powell amaranth being controlled, but later emerging cohorts of Powell amaranth that emerged after the POST application (*data not shown*). A second application of herbicides or the addition of an herbicide with residual control is necessary in order to control *Amaranthus* species due to the ability of the species to emerge throughout the growing season. This is especially important with *Amaranthus* species that are glyphosate-resistant, as the glyphosate and dicamba tank mix was able to control the glyphosate-susceptible Powell amaranth at later termination timings, while reduced control was seen with glyphosate-resistant waterhemp at the Fargo location.

### **2.3.7. Soybean Yield Results**

At Fargo in 2020 and 2021, the presence of oats reduced soybean yield compared to oats not being present (Table 2.10). Termination timing also had an effect on soybean yield in both years with 45 cm and 60 cm being lower in 2020 and 60 cm being lower in 2021. The number of post applications had an effect on soybean yield at Fargo in 2020, with two post applications having a higher yield than that of one post application. This suggests that multiple herbicide applications may be needed in order to control waterhemp and reduce its effect on the yield of soybean. This could be due to waterhemp having multiple flushes throughout the season (Nordby et al. 2007). There was a combined interaction between the presence of oats and termination timing at Fargo in 2020. Plots with no oats had greater yield than corresponding plots with oats at every termination timing. There was also a combined interaction between termination timing and the number of post applications at Fargo in 2020. Two post applications increased yield by 781 kg ha<sup>-1</sup> compared to one post application at the 15 cm termination timing. All other timings

were not different when comparing the number of post applications. This further suggests the need for two post applications in order to control subsequent waterhemp flushes and prevent yield loss. The lack of differences at other timings when comparing the number of post applications could be due to later flushes having to compete with waterhemp already present in the plot, or emerging too late in the season to have much of an effect on the yield. Termination timing had an effect on yield in both years at Prosper with later termination timings having lower yields than that of earlier termination timings (Table 2.10). The 45 cm and 60 cm termination timing were lower in 2020, with 60 cm having the least yield. The lowest termination timing in 2021 was also 60 cm termination timing, followed by the 45 cm termination timing and then the 30 cm termination timing. The presence of oats and termination timing had a combined interaction at Prosper in 2021. Oat increased yields at the 15 cm termination timing by 772 kg ha<sup>-1</sup>. This could potentially be due to a positive effect that the oat companion crop had early on in the growing season. This isn't supported by the other data recorded, however, as oat did not suppress IDC or Powell amaranth at this timing, so other factors may have had an effect. Oat didn't have an effect at any other termination timing.

All treatments were analyzed to compare with the competition-free. In Fargo, treatments that had no oats and had earlier termination timings tended to yield similarly to the competition free (Table 2.11). At Fargo in 2020, No oat at the 15 cm termination timing with 2 post applications and no oat at the 30 cm termination timing with 2 post were the only treatments not different from the competition free treatment. At Fargo in 2021, no oat at all timings had treatments that were not different from the competition free treatment. Oat with post applications at the 15 cm and 30 cm termination timing were also not different from the competition free treatment. Prosper in 2020 had treatments at 15 and 30 cm that were not different from the



competition free. At Prosper in 2021 both the oat and no oat treatments at the 15 cm termination timing with 2 post applications were the only treatments similar to the competition free treatment. Each year and site had different treatments that were similar to the competition-free treatment, but earlier termination timings tended to be similar to the competition-free treatment. Treatments with two post applications also tended to be similar to the competition-free treatment compared to treatments with one application. If the oat companion crop is allowed to grow for too long, it will start to have adverse effects on the soybean yield due to competition for light, water, and nutrients. The longer that oat is allowed to grow within the field, the more the competition of the oat will have an effect on the soybean yield. Multiple herbicide applications are also needed to control herbicide-resistant waterhemp, as multiple flushes throughout the season give the weed the chance to affect yields later in the season if a second treatments is not applied.

Table 2.12. Presence of oats, termination timing, and number of postemergence herbicide applications on soybean yield at Prosper and Fargo in 2020 and 2021.<sup>a</sup>

Main effects	Fargo 2020	Fargo 2021	Prosper 2020	Prosper 2021
<i>Presence of oats</i>	-----kg ha <sup>-1</sup> -----			
No oats	1039 a	1064 a	2982	2383
Oats	630 b	632 b	3011	2140
<i>Termination timing</i>				
No termination	132 d	500 b	703 d	436 e
15 cm	1406 a	1040 a	4027 a	3802 a
30 cm	1555 a	1027 a	3833 a	3215 b
45 cm	761 b	921 a	3449 b	2279 c
60 cm	320 c	749 b	2970 c	1576 d
<i>No. of post applications</i>				
1 post application	729 b	722	2956	2134
2 post applications	940 a	972	3036	2389
<i>Presence of Oats *</i>				
<i>Termination Timing</i>				
No oats * no termination	185 e	694	791	469 d
No oats * 15 cm	1654 ab	1188	3945	3416 b
No oats * 30 cm	1718 a	1072	3875	3242 b
No oats * 45 cm	1160 c	1255	3452	2770 b
No oats * 60 cm	480 d	1111	2864	2019 c
Oats * no termination	78 e	305	616	404 d
Oats * 15 cm	1157 c	891	4109	4188 a
Oats * 30 cm	1393 bc	982	3792	3187 b
Oats * 45 cm	362 d	585	3446	2770 b
Oats * 60 cm	160 e	386	3095	2019 c
<i>Termination timing *</i>				
<i>no. of post applications</i>				
15 cm * 1 post	1015 c	548	3947	3335
30 cm * 1 post	1442 b	1038	3785	3022
45 cm * 1 post	651 de	808	3310	2373
60 cm * 1 post	411 ef	1033	3039	1455
15 cm * 2 post	1796 a	1531	4106	4269
30 cm * 2 post	1668 ab	1016	3881	3407
45 cm * 2 post	870 cd	1033	3588	2184
60 cm * 2 post	229 f	848	2902	1696

Table 2.12. Presence of oats, termination timing, and number of postemergence herbicide applications on soybean yield at Prosper and Fargo in 2020 and 2021(continued)

Main effects	Fargo 2020	Fargo 2021	Prosper 2020	Prosper 2021
<i>ANOVA</i>	<i>p-value</i>			
Presence of oats	<0.001	0.005	0.742	0.099
Termination timing	<0.001	0.046	<0.001	<0.001
No. of post applications	<0.001	0.097	0.378	0.084
Presence of oats *	0.007	0.643	0.563	0.001
Termination timing				
Presence of oats * no. of post applications	0.576	0.987	0.756	0.754
Termination timing * no. of post applications	<0.001	0.167	0.659	0.126
Presence of oats * termination timing * no. of post applications	0.699	0.312	0.837	0.653

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> no. = number

Table 2.13. Treatment differences on soybean yield at Prosper and Fargo in 2020 and 2021. <sup>a</sup>

	Fargo 2020	Fargo 2021	Prosper 2020	Prosper 2021
<i>Treatment</i>	----- <i>kg ha<sup>-1</sup></i> -----			
Oat * no termination * 1 post	65 f	383 de	669 f	323 i
Oat * no termination * 2 post	92 f	228 e	563 f	484 i
Oat * 15 cm * 1 post	795 d	695 cde	3995 ab	3581 bc
Oat * 15 cm * 2 post	1519 b	1086 abcd	4222 a	4795 a
Oat * 30 cm * 1 post	1348 bc	835 cde	3807 abc	2945 bcde
Oat * 30 cm * 2 post	1437 b	1129 abcd	3777 abcd	3430 bc
Oat * 45 cm * 1 post	297 e	479 cde	3239 de	1917 efg
Oat * 45 cm * 2 post	426 ef	692 cde	3652 bcd	1658 fgh
Oat * 60 cm * 1 post	198 f	127 e	3217 de	1180 ghi
Oat * 60 cm * 2 post	122 f	646 cde	2972 e	1085 ghi
No oat * no termination * 1 post	186 f	752 cde	733 f	644 hi
No oat * no termination * 2 post	184 f	636 cde	848 f	295 i
No oat * 15 cm * 1 post	1239 bc	400 de	3899 ab	3090 bcd
No oat * 15 cm * 2 post	2073 a	1977 ab	3990 ab	3743 abc
No oat * 30 cm * 1 post	1536 b	1238 abcd	3764 abcd	3099 bcd
No oat * 30 cm * 2 post	1900 a	903 cde	3985 ab	3385 bcd
No oat * 45 cm * 1 post	1003 cd	1136 abcd	3380 cde	2829 cde
No oat * 45 cm * 2 post	1313 bc	1374 abc	3524 bcde	2710 cde
No oat * 60 cm * 1 post	624 de	1171 abcd	2861 e	1731 efg
No oat * 60 cm * 2 post	336 ef	1050 bcd	2831 e	2308 def
Competition free	2009 a	2057 a	3676 abcd	3971 ab
<i>ANOVA</i>	----- <i>p-value</i> -----			
Treatment	<.0001	.014	<.0001	<.0001

<sup>a</sup> Means within a column that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

## 2.4. Conclusion

We did not observe severe or frequent IDC symptoms throughout the experiment, and the only differences that occurred multiple times was SPAD readings being affected from the presence of oats at 0 days after termination (Table 2.1; Table 2.2), but this was only observed at the Fargo site. The lack of IDC symptoms could be due to the amount of precipitation, the variety of soybeans used, or other combination of environmental effects. Kaiser et al. (2014) hypothesized that the reducing the amount of nitrates within the soil may also help to reduce IDC

symptoms within a field. One other reason why IDC symptoms may not have been as common within the trials are due to pigweeds and oats being nitrate accumulating plants (USDA 2018). Many fields as well only show IDC symptoms in specific parts of the field, which can make it difficult to utilize areas with constant IDC issues for research (Helms et al. 2010). Naeve (2006) reported IDC symptoms were reduced by the presence of oats and in some years they were not. He also observed the potential yield loss that oats can cause similar to what was observed during this experiment. This would be due to the oat competing with the soybean for water, sunlight, and other nutrients. The use of IDC tolerant cultivars and the use of iron chelate may be a more profitable and less risky option to control and suppress IDC symptoms within a soybean field rather than the use of an oat companion crop (Ferreira et al. 2019; Kaiser et al. 2014; Helms et al. 2010). Evaluation of other potential companion crops could prove useful to understand the effects of different companion crops, as well as study potential benefits of allelopathic effects that some crops have (Gfeller et. al 2018).

An oat companion crop is able to suppress pigweed biomass within the field, but the suppression may occur too late in the soybean growth stages to the point that soybean yield has been compromised. A companion crop that has allelopathic effects could be more suitable if the goal of the companion crop is to reduce weed biomass (Rehman et al. 2018), as it seems the early competition of the oat companion crop is not enough to reduce weed biomass and density within soybean. In order for the companion crop to be able to reduce a highly competitive weed such as waterhemp, it must also be highly competitive (Gfeller et al. 2018). Introducing another highly competitive plant into a field may not be profitable, as while it may suppress the weeds present, this suppression effect would also occur on the main crop as well and cause the plants to be stunted and reduce yield (Verret et al 2017). A rye cover crop terminated at planting has been

observed to reduce weed biomass within soybean (Bish et al. 2021) and may prove to be a better option at reducing weeds within soybean without the risk of losing yield.

IDC has been observed to reduce yields even when the chlorotic symptoms are slight (Niebur and Fehr 1981). The competition from the oat companion crop was observed in this study to reduce yield at later termination timings. In addition, weed biomass was not reduced by oats until later termination timings. If an oat companion could have the ability to reduce IDC early within soybean and be terminated by the 30 cm termination timing, then it may be an acceptable and profitable way to control IDC within soybean as long as the weeds present are glyphosate susceptible. If the weeds present are not glyphosate susceptible, such as waterhemp, other combinations with this method may be needed, such as a preemergence herbicide that can control the waterhemp while not being injurious to oats.

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## **CHAPTER 3. SOYBEAN (*GLYCINE MAX*) PREEMERGENCE HERBICIDE EFFECTS ON AN OAT (*AVENA SATIVA*) COMPANION CROP**

### **3.1. Introduction**

An oat companion crop has been previously evaluated as a solution to controlling Iron Deficiency Chlorosis in soybean (Naeve 2006). An additional benefit of the oat companion crop could be weed suppression within the soybean crop. However, preemergence herbicides are often used to control early season weeds in a soybean crop, and some of these soybean preemergence herbicides may injure the oat companion crop. Companion crop biomass and shading have been considered main factors of weed suppression in previous research (Gfeller et al. 2018) but have also can compete with the cash crop and reduce yield (Verret et al 2017). Increased plant biomass can also suppress IDC symptoms in soybean (Penas et al. 1990). A preemergence herbicide may have an effect on the growth and biomass accumulation of these companion crops, which could also alter the ability to suppress both the weeds and IDC symptoms. It is important to understand the effects preemergence herbicides may have on a companion crop, since the injury or stand loss of a companion crop may compromise weed control or other desirable benefits. If an herbicide that is used to primarily control weeds, also controls the companion crop, then the producer would have lost time and inputs on the companion crop seed. Conversely, a preemergence herbicide can be used that controls the target weed species, without compromising the companion crop, then overall weed control should improve and other benefits of planting the companion crop could be realized. The objectives of this experiment were to: (1) understand the effects that different preemergence herbicides have on an oat companion crop's growth and biomass; and (2) evaluate the effects of those herbicides on *Amaranthus* spp. control.

## **3.2. Materials and Methods**

### **3.2.1. Field Design**

Field experiments were conducted at conventionally tilled locations near Fargo, ND (Silty Clay, 5% OM, pH 7.4, 46°55'50.4"N 96°51'07.7"W) and near Prosper, ND (Silty Loam, 4.3% OM, pH 7, 47°00'01.9"N 97°07'16.7"W). These sites were selected for their differences in soil type and weed spectrum. The experiment was arranged in a random complete block design with four replications. The treatment factor in this experiment was preemergence herbicide treatments.

### **3.2.2. Planting**

A Roundup Ready Xtend® soybean cultivar, 'AG06X8' (Bayer Crop Science, Creve Coeur, MO), was planted to a depth of 3 cm in 76-cm rows at a seeding rate of 385,320 seeds ha<sup>-1</sup> using a custom made Monosem vacuum-planter. 'ND Rockford' oat (NDSU Foundation Seedstocks, Fargo, ND) was planted parallel to soybean rows to a depth of 3 cm in 19 cm rows at a seeding rate of 67 kg ha<sup>-1</sup> using a Great Plains 3P600 drill. The fields were soil tested and fertilized according to the soil tests for nitrogen, phosphorus, and potassium. In 2020 the Fargo site was planted on May 19<sup>th</sup> and the Prosper site was planted on May 27<sup>th</sup>, and in 2021 the Fargo site was planted on May 10<sup>th</sup> and the Prosper site was planted on May 17<sup>th</sup>. The size for each plot was 3m by 7.6m.

It is important to observe the rainfall that occurred during the weeks preceding and following the initial application dates of the preemergence herbicides due to the influence that precipitation can have on the preemergence herbicide activity. (Tables 3.1 and 3.2).

Table 3.1. Weekly precipitation at Fargo and Prosper, ND locations for the experiments in 2020.

	Fargo	Prosper
<i>Weeks</i>	----- <i>cm</i> -----	
May 10- May 16	.51	.76
May 17 – May 23	0	0
May 24 – May 30	.28	.18
May 31 – June 06	.10	.05
June 07 – June 13	4.29	5.59
June 14 – June 20	.79	1.12

Table 3.2. Weekly precipitation at Fargo and Prosper, ND locations for the experiments in 2021

	Fargo	Prosper
<i>Weeks</i>	----- <i>cm</i> -----	
May 02 – May 08	.03	.03
May 09 – May 15	0	.05
May 16 – May 22	.58	1.27
May 23 – May 29	.25	.58
May 30 – June 05	.03	.05
June 06 – June 12	5.84	2.11
June 13 – June 19	0	0

### 3.2.3. Herbicide Selection and Application

Treatments were applied with a CO<sup>2</sup>-pressurized backpack sprayer calibrated to deliver 140 L ha<sup>-1</sup> at a speed of 4.8 km h<sup>-1</sup> with TTI 11002 (Turbo TeeJet Induction, TeeJet Technologies, Glendale Heights, IL) nozzles on 38-cm spacing with a 1.5 m boom with a 2 m spray width at 193 kPa of pressure. The treatments were applied after planting on the day each site was planted. The herbicides used for this experiment are listed in table (Table 3.3).

These preemergence herbicides were chosen as they are common preemergence herbicides used and labeled for use in soybean in North Dakota to control *Amaranthus* spp. One exception is that Prowl H<sub>2</sub>O (pendimethalin) is not labeled for preemergence use in areas North of Interstate 80 (except in the states of Indiana, Michigan, and Ohio), but it was included to

evaluate another mode of action and its effects on weeds, oats, and soybean (Prowl H<sub>2</sub>O, BASF Ag Products 2021). Some of the herbicides chosen are also labelled in other small grain crops in North Dakota, but are not labeled in oat. The herbicides chosen represent four different sites of action and are effective against *Amaranthus* species, which was the main species within the fields.

Table 3.3. Herbicide product information for treatments applied

Herbicide	Trade name	kg ai ha <sup>-1</sup>	Herbicide manufacturer	Site of Action
1	Untreated	-	-	-
2	Dual II Magnum ( <i>S-metolachlor</i> )	1.783	Syngenta <sup>1</sup>	VLCFA Synthesis Inhibitor <sup>a</sup>
3	Outlook (dimethenamid-P)	0.946	BASF <sup>2</sup>	VLCFA Synthesis Inhibitor
4	Zidua SC (pyroxasulfone)	0.183	BASF <sup>2</sup>	VLCFA Synthesis Inhibitor
5	Warrant (acetochlor)	1.597	Bayer <sup>3</sup>	VLCFA Synthesis Inhibitor
6	Valor SX (flumioxazin)	0.107	Valent <sup>4</sup>	PPO Inhibitor
7	Spartan (sulfentrazone)	0.420	FMC <sup>5</sup>	PPO Inhibitor
8	Flexstar (fomesafen)	0.013	Syngenta <sup>1</sup>	PPO Inhibitor
9	Tricor 75 DF(metribuzin)	0.420	UPL <sup>6</sup>	PSII Inhibitor
10	Prowl H20 (pendimethalin)	1.597	BASF <sup>2</sup>	Microtubule Assembly Inhibitor

<sup>a</sup> Abbreviations: VLCFA = very long-chain fatty acid; PPO = protoporphyrinogen oxidase; PSII = Photosystem II.

<sup>1</sup> Syngenta Crop Protection AG, Basel, Switzerland

<sup>2</sup> BASF, Ludwigshafen, Germany

<sup>3</sup> Bayer AG, Leverkusen, Germany

<sup>4</sup> Valent U.S.A. LLC, Walnut Creek, CA

<sup>5</sup> FMC, Philadelphia, PA

<sup>6</sup> UPL, Mumbai, Maharashtra, India

### 3.2.4. Data Collection and Analysis

*Amaranthus spp.* control and oat injury were visibly rated on a scale from 0 to 100% with 0 being no weed control or injury to the oats, and 100% being complete control or plant death. Plots were evaluated 14, 28, and 42 days after herbicide treatment for pigweed efficacy. Plots were evaluated 7, 14, and 28 days after herbicide treatment for oat injury. Oat above ground biomass was collected at 28 DAT using a 1-m<sup>2</sup> quadrat to determine the area from which the oats were harvested. Oat biomass was dried at 43 degrees C for 14 days using a forced air dryer and

weighed. Waterhemp (*Amaranthus tuberculatus*) was the main species of pigweed at Fargo, while Powell amaranth (*Amaranthus powelli*) was the main species of pigweed at Prosper.

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). Data were subjected to ANOVA using PROC GLIMMIX to test for treatment effects and significant differences. Treatment means were separated using Tukey's HSD with  $P \leq 0.05$ . Herbicide treatments were considered to be fixed effects, while environment and replicate were considered to be random effects. All environments were analyzed separately due to primary pigweed species and precipitation. Normality was verified using the UNIVARIATE procedure within SAS. Oat injury and pigweed control percentages were non-normal and data were arc sine square-root transformed ( $\arcsin((Y/100)^{1/2}))$  for mean separation, but non-transformed means are reported.

### **3.3. Results**

#### **3.3.1. Pigweed Control**

Pigweed control was greater at both locations in 2021 compared to 2020 (Tables 3.4 and 3.5). In 2020, both locations received reduced rainfall after planting compared to 2021. This reduced precipitation is likely a primary reason for reduced weed control and oat injury due to less moisture being available to move the herbicides into the soil. Prosper in 2020 received 2 cm of precipitation on June 7<sup>th</sup>, 11 days after planting. Prosper in 2021 received 1.3 cm of precipitation May 20<sup>th</sup>, 3 days after planting. In 2020, Fargo received 0.3 cm of precipitation on May 26<sup>th</sup>, 7 days after planting. This was likely not enough rain to solubilize enough herbicide into the soil solution to be available for plant uptake. The next precipitation event after this date was on June 7<sup>th</sup>, 19 days after planting (1.5 cm). Fargo in 2021 received 0.6cm of precipitation on May 20<sup>th</sup>, 10 days after planting. Overall moisture conditions in 2020 and 2021 were dry. Of

note, pigweed emergence was observed at the sites in 2020 prior to trial initiation, but was not observed at sites in 2021 prior to trial initiation.

Table 3.4. Effect of herbicide on control of Powell amaranth Prosper in 2020 and 2021.<sup>a</sup>

<i>Herbicide</i>	2020			2021		
	14 DAT <sup>b</sup>	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT
	-----%-----					
Nontreated	0 d	0 d	0 c	0 d	0 e	0 e
S-metolachlor	11 cd	13 cd	0 c	38 bc	28 bcde	15 cde
dimethenamid-P	15 c	16 cd	5 c	73 ab	58 ab	33 bc
Pyroxasulfone	16 c	16 cd	1 c	60 ab	48 bc	26 bc
Acetochlor	23 bc	16 cd	4 c	50 bc	45 bcd	28 bc
Flumioxazin	89 a	55 b	21 b	65 ab	50 abc	24 bcd
Sulfentrazone	85 a	81 a	38 a	92 a	84 a	55 a
Fomesafen	35 b	20 c	8 c	70 ab	58 ab	36 ab
Metribuzin	15 c	19 cd	0 c	35 bc	20 cde	15 cde
Pendimethalin	18 c	9 cd	0 c	18 cd	13 de	6 de
<i>ANOVA</i>	----- <i>p-value</i> -----					
Herbicide	<.001	<.001	<.001	<.001	<.001	<.001

<sup>a</sup> Means within a column that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Abbreviation: DAT = days after treatment.

Table 3.5. Effect of herbicide on control of waterhemp at Fargo in 2020 and 2021.<sup>a</sup>

<i>Herbicide</i>	2020			2021		
	14 DAT <sup>b</sup>	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT
	-----%-----					
No herbicide	0 d	0 c	0 d	0 d	0 e	0 d
S-metolachlor	10 cd	9 c	8 cd	96 a	90 a	60 ab
dimethenamid-P	8 cd	6 c	8 cd	80 ab	80 ab	50 bc
Pyroxasulfone	10 cd	10 c	8 cd	85 ab	74 abc	40 bc
Acetochlor	40 bc	29 b	23 b	93 a	84 ab	54 ab
Flumioxazin	55 b	35 b	20 bc	86 ab	78 ab	53 ab
Sulfentrazone	80 a	69 a	50 a	98 a	95 a	70 a
Fomesafen	20 c	13 bc	10 bcd	79 ab	66 bc	43 bc
Metribuzin	5 d	3 c	3 d	70 b	53 c	33 c
Pendimethalin	13 cd	8 c	8 cd	30 c	23 d	11 d
<i>ANOVA</i>	----- <i>p-value</i> -----					
Herbicide	<.001	<.001	<.001	<.001	<.001	<.001

<sup>a</sup> Means within a column that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Abbreviation: DAT = days after treatment.

There were differences between treatments for control of Powell amaranth at Prosper in 2020 (Table 3.4). At 14 days after treatment (DAT), all herbicide except *S*-metolachlor provided greater control of Powell amaranth than the nontreated check. Flumioxazin and sulfentrazone (89 and 85%) provided similar control, and greater control than all other treatments. Fomesafen (35%) provided less control than flumioxazin and sulfentrazone, but provided greater control than the remaining treatments, except acetochlor. In summary, the PPO-inhibiting herbicides provided the greatest control of Powell amaranth at 14 DAT at Prosper in 2020. At 28 DAT, sulfentrazone provided the greatest control (81%), while flumioxazin provided 55% control. These two treatments provided more control than all other herbicides evaluated.. At the same rating timing, fomesafen provided greater control than the nontreated check. All other treatments were equivalent to the control. By 42 DAT, only sulfentrazone (38%) and flumioxazin (21%) were different from the control treatment, with sulfentrazone providing the best control.

Control of Powell amaranth at Prosper in 2021 was generally greater than in 2020 (Table 3.4). *S*-metolachlor, metribuzin, and pendimethalin were the only treatments not different from the control treatment at 14 DAT. Sulfentrazone provided the greatest control (92%), but was not different from dimethenamid-P (73%), fomesafen (70%), flumioxazin (65%), and pyroxasulfone (60%). At 28 DAT, pendimethalin, metribuzin, and *S*-metolachlor were not different from the control. Sulfentrazone again provided the greatest control at 84%, but was not different from fomesafen, dimethenamid-P, and flumioxazin. Sulfentrazone did provided greater control than pyroxasulfone at 28 DAT. By 42 DAT, *S*-metolachlor, pendimethalin, and metribuzin were not different from the control treatment. Sulfentrazone provided 55% control and was greater than every treatment except for fomesafen (36%). Sulfentrazone was observed to provide the greatest control of Powell amaranth throughout the experiment in both years. Flumioxazin and fomesafen

also provided greater control early in the experiment, similar to that of sulfentrazone, but lost effectiveness later into the evaluation timings.

Most herbicide treatments provided poor control of waterhemp at Fargo in 2020 (Table 3.5). The lack of control is likely due to low rainfall totals for the first 20 days after planting (Table 3.1). At 14 DAT, *S*-metolachlor, dimethenamid-P, pyroxasulfone, metribuzin, and pendimethalin were not different from the nontreated control. Sulfentrazone provided 80% waterhemp control, which was more than every other treatment. Flumioxazin and acetochlor provided 55% and 40% control, respectively. Fomesafen (20%) was the only other treatment to provide more waterhemp control than the nontreated check. At 28 DAT, Sulfentrazone again provided the greatest control of waterhemp (69%). Acetochlor and flumioxazin, with 29% and 35%, respectively, were the only other treatments that provided more waterhemp control than the check. This trend remained the same at 42 DAT, where only sulfentrazone, acetochlor, and flumioxazin provided greater waterhemp control than the check (50, 23, and 20%, respectively). In summary, sulfentrazone provided the best waterhemp control throughout the experiment at Fargo in 2020

There was more precipitation within the first 7 days after planting at Fargo in 2021 than in 2020 (Table 3.2), and subsequently visible weed control was greater for all treatments (Table 3.5). Every herbicide provided greater waterhemp control than nontreated check at 14 DAT. Sulfentrazone (98%), *S*-metolachlor (96%), and acetochlor (93%) provided the greatest control, but were only different from metribuzin (70%), pendimethalin (30%) and the control treatment. By 28 DAT, every herbicide still provided more waterhemp control compared to the nontreated control. Sulfentrazone and *S*-metolachlor provided the greatest control, but were not different from dimethenamid-P, pyroxasulfone, acetochlor, and flumioxazin. By 42 DAT, only



pendimethalin was not different from the control treatment. Sulfentrazone once again provided the greatest control 42 DAT (70%), but was not different from *S*-metolachlor, acetochlor, and flumioxazin. Despite the differences between 2020 and 2021, sulfentrazone had the best waterhemp control at Fargo in both years throughout the entirety of the experiment. Compared to other literature, glyphosate resistant waterhemp was better controlled by flumioxazin, metribuzin, or pyroxasulfone when compared to the control due to sulfentrazone (Schryver et al. 2017).

### **3.3.2. Oats**

Similar to *Amaranthus* control, the results for herbicide injury to oats was different amongst the environments. However, there were instances when all herbicides were similar to the nontreated check. The amount of precipitation to activate the herbicides, as well as the initial moisture available for germination at planting, are some of the reasons for the differences observed at the different environments.

Table 3.6. Effect of herbicide on oat injury at Prosper in 2020 and 2021, 7, 14, and 28 days after treatment. <sup>a</sup>

<i>Herbicide</i>	2020			2021		
	7DAT <sup>b</sup>	14DAT	28DAT	7DAT	14DAT	28DAT
	-----%-----					
No herbicide	0 d	0 d	0 b	0 c	0 d	0
<i>S</i> -metolachlor	13 c	5 cd	3 b	11 bc	10 bc	1
dimethenamid-P	11 c	9 bcd	4 b	10 bc	13 ab	5
Pyroxasulfone	16 c	18 b	18 a	13 ab	9 bc	3
Acetochlor	11 c	8 bcd	1 b	13 ab	11 bc	6
Flumioxazin	60 a	44 a	23 a	18 ab	20 a	4
Sulfentrazone	63 a	38 a	19 a	23 a	20 a	5
Fomesafen	29 b	14 bc	3 b	18 ab	10 bc	6
Metribuzin	13 c	15 bc	15 a	10 bc	13 ab	6
Pendimethalin	9 cd	8 bcd	4 b	20 ab	6 b	4
<i>ANOVA</i>	----- <i>p</i> -value-----					
Herbicide	<.001	<.001	<.001	<.001	<.001	.062

<sup>a</sup> Means within a column that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Abbreviation: DAT = days after treatment.

Table 3.7. Effect of herbicide on oat injury at Fargo in 2020 and 2021, 7, 14, and 28 days after treatment. <sup>a</sup>

<i>Herbicide</i>	2020			2021		
	7 DAT <sup>b</sup>	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
	-----%-----					
No herbicide	0 c	0 c	0	0 c	0 e	0 d
<i>S</i> -metolachlor	0 c	0 c	0	3 c	8 ed	3 cd
dimethenamid-P	0 c	1 bc	0	5 bc	9 d	5 bcd
Pyroxasulfone	0 c	1 bc	0	15 abc	20 bc	9 b
Acetochlor	0 c	1 bc	1	20 ab	11 d	6 bc
Flumioxazin	4 b	4 ab	1	23 a	15 cd	8 bc
Sulfentrazone	9 a	6 a	4	25 a	35 a	15 a
Fomesafen	0 c	4 ab	4	0 a	11 d	6 bc
Metribuzin	0 c	3 bc	0	13 abc	25 b	9 b
Pendimethalin	0 c	0 c	0	3 a	8 de	4 bcd
<i>ANOVA</i>	----- <i>p</i> -value-----					
Herbicide	<.0001	.0005	.082	.0004	<.0001	<.0001

<sup>a</sup> Means within a column that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

<sup>b</sup> Abbreviation: DAT = days after treatment.

At Prosper in 2020 pendimethalin was the only herbicide not different from the control treatment 7 DAT (Table 3.6.). Sulfentrazone and flumioxazin caused 63% and 60% injury, respectively, which was the greatest amount of oat injury. Fomesafen caused 29% injury, which was the next greatest amount, and it was different from every other treatment. *S*-metolachlor, dimethenamid-P, pyroxasulfone, acetochlor, and metribuzin all provided 11% to 16% injury, which was only greater than the nontreated control. By 14 DAT, *S*-metolachlor, dimethenamid-P, acetochlor, and pendimethalin all were similar to the no herbicide treatment. Flumioxazin and sulfentrazone still caused more oat injury than any other herbicide at 44% and 38%, respectively. Pyroxasulfone, dimethenamid-P, acetochlor, pendimethalin, fomesafen, and metribuzin all caused similar amounts of injury ranging from 9% to 18% injury. At 28 DAT, *S*-metolachlor, dimethenamid-P, acetochlor, fomesafen, and pendimethalin were all similar to the control treatment. Pyroxasulfone, flumioxazin, sulfentrazone, and metribuzin all caused the greatest amount of injury to the oats, ranging from 15% to 23% injury.

At the Prosper site in 2021 *S*-metolachlor, dimethenamid-P, and metribuzin were not different from the control 7 DAT (Table 3.6.). Sulfentrazone caused the greatest injury to the oat (23%), but was not different from pyroxasulfone (13%), acetochlor (13%), flumioxazin (18%), fomesafen (18%), and pendimethalin (20%), which all injured the oats more than the control. By 14 DAT, pendimethalin was the only herbicide not different from the control treatment. Sulfentrazone and flumioxazin both caused 20% injury to the oats, and they caused more injury than every other treatment. *S*-metolachlor, dimethenamid-P, pyroxasulfone, acetochlor, fomesafen, and metribuzin all caused between 9% and 13% injury. By 28 DAT, herbicide treatment no longer had an effect on oat injury.

Sulfentrazone and flumioxazin caused the most visible oat damage throughout the experiment at both years at Prosper. Fomesafen was also another injurious herbicide to the oat companion crop in both years, but not at the same level as sulfentrazone and flumioxazin. *S*-metolachlor, dimethenamid-P, pendimethalin, and acetochlor caused damage early in the experiment, but were not as damaging to the oat companion crop later into the experiment and were not different the non-treated treatment at the later evaluation timings.

At Fargo in 2020, no herbicide ever caused more than 9% injury to oats (Table 3.7.). At 7 DAT, sulfentrazone caused the greatest injury to the oats (9%), followed by flumioxazin (4%), and were the only treatments different from the nontreated control. By 14 DAT, sulfentrazone caused 6% injury, but was not different from flumioxazin and fomesafen, which both caused 4% injury. These PPO-inhibiting herbicides were the only treatments different from the control treatment at that rating. By the final rating at 28 DAT, herbicide did not have an effect on oat injury.

There was more injury observed at Fargo in the 2021 season (Table 3.7). At 7 DAT, sulfentrazone, flumioxazin, and acetochlor were the only treatments different than the control, and provided 20% to 25% injury to oats. By 14 DAT, *S*-metolachlor and pendimethalin were the only treatments not different from the control treatment. Sulfentrazone caused 35% injury, which was greater than every other treatment. Metribuzin caused the next greatest amount of injury (25%) and was different from every other treatment except for pyroxasulfone, which caused 20% injury. At 28 DAT, flumioxazin, pyroxasulfone, *S*-metolachlor, dimethenamid-P, acetochlor, fomesafen, and pendimethalin all caused 4% to 11% injury. *S*-metolachlor, dimethenamid-P, and pendimethalin were the only treatments at 28 days after treatment to not be different from the

control treatment. Sulfentrazone caused the greatest amount of injury to the oats at 15%, which was more than every other treatment.

Sulfentrazone caused the greatest damage to the oat companion crop throughout the experiments at Fargo in both years. Flumioxazin also was more injurious to the oat companion crop early in the experiment, but was not as damaging as sulfentrazone at later evaluation timings. . S-metolachlor, dimethenamid-P, and pendimethalin caused the least amount of damage and were not different later into the experiment when compared to the non-treated control treatment.

### 3.3.3. Final Oat Biomass

The data for final oat biomass were analyzed separately for each environment. These differing effects between the environments is likely due to the differing amounts of precipitation which would have had an effect on the activation on the herbicides.

Table 3.8. Effect of herbicide on oat above ground biomass at Prosper in 2020 and 2021. <sup>a</sup>

<i>Herbicide</i>	2020	2021
	----- <i>kg ha<sup>-1</sup></i> -----	
No herbicide	165 ab	323
S-metolachlor	194 a	332
dimethenamid-P	137 bc	311
Pyroxasulfone	94 cd	259
Acetochlor	155 ab	279
Flumioxazin	65 d	293
Sulfentrazone	128 bc	261
Fomesafen	150 abc	270
Metribuzin	68 d	263
Pendimethalin	117 bcd	235
<i>ANOVA</i>	----- <i>p-value</i> -----	
Herbicide	.003	.694

<sup>a</sup> Means within a column that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

Table 3.9. Effect of herbicide on oat above ground biomass at Fargo in 2020 and 2021.<sup>a</sup>

<i>Herbicide</i>	2020	2021
	----- <i>kg ha<sup>-1</sup></i> -----	
No herbicide	164	259 a
<i>S</i> -metolachlor	206	120 cd
dimethenamid-P	188	184 abc
Pyroxasulfone	199	106 cd
Acetochlor	220	157 bcd
Flumioxazin	133	120 cd
Sulfentrazone	135	98 cd
Fomesafen	144	213 ab
Metribuzin	180	87 d
Pendimethalin	228	103 cd
<i>ANOVA</i>	----- <i>p-value</i> -----	
Herbicide	.176	.007

<sup>a</sup> Means within a column that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

At Prosper in 2020 pyroxasulfone, flumioxazin, and metribuzin were the only treatments that reduced oat biomass compared to the control treatment (Table 3.8.). Herbicide did not have an effect on the biomass of the oat in 2021 at Prosper.

At Fargo in 2020 herbicide did not have an effect the above ground biomass of the oats (Table 3.9). In 2021 dimethenamid-P and fomesafen were the only treatments that did not reduce biomass compared to the control treatment. Metribuzin caused the least amount of oat biomass and was not different from pendimethalin, sulfentrazone, flumioxazin, acetochlor, pyroxasulfone, and *S*-metachlor.

Flumioxazin was one of the more injurious herbicides while also causing one of the lowest biomasses when comparing Prosper's final oat biomass and oat visible injury in 2020. Sulfentrazone did not reduce oat biomass as expected when compared to the visible injury at Prosper, but had one of the lowest final oat biomasses at Fargo. Metribuzin also had a greater

effect on final oat biomass than it did on the injury of the oats at both sites. Fomesafen was the only herbicide to not affect oat biomass across all years.

### **3.4. Conclusion**

Flumioxazin and sulfentrazone consistently provided the greatest control of pigweeds between all the sites. These two herbicides, however, were also the most harmful when it came to oat injury. It may more profitable to use flumioxazin and sulfentrazone, in conjunction with post-emergence herbicides to control the waterhemp if the oat companion crop is being planted in order to try and suppress pigweeds within the field. This would allow the grower more choices in herbicides and be able to use more preemergence and post-emergence herbicide combinations that would allow greater control of waterhemp (Schryver et al. 2017). If the companion crop is being planted to reduce IDC symptoms within the soybean crop, there are a few options that could be available. Fomesafen, dimethenamid-P, acetochlor, and pyroxasulfone provided some pigweed control in 2021, when a significant precipitation event occurred within 14 days after planting. These herbicides also caused less injury to the oat companion crop when compared to other herbicides. These herbicides, except for acetochlor, also were not different from the control treatment when looking at the amount of above ground oat biomass at the end of the season. The ability to be able to use soybean preemergence herbicides in conjunction with using an oat companion crop could be useful due to the prevalence that waterhemp has within the Red River Valley of the North, as well as IDC being a common issue within the area (Naeve 2006). Herbicide options that tolerate an oat companion crop could allow growers more options to control pigweeds while increasing plant biomass early in the growing season. This is important due to greater plant biomass within a field has been observed to reduce IDC symptoms (Penas et al. 1990; Goos and Johnson 2006). Controlling herbicide resistant waterhemp within a field

becomes more difficult when less control options are available, which would be the case if a grower did not utilize herbicides in order to avoid injury to an oat companion crop (Vyn et al. 2017). The option to use preemergence herbicides in soybean without causing significant injury to an oat companion crop could provide new options to growers looking to use oat as an option to control IDC within their fields. Increased biomass from a terminated winter cover crop like cereal rye has been observed to suppress waterhemp emergence while not having adverse effects on soybean (Bish et al. 2021). Similar results could be observed with an oat companion crop at reducing pigweed emergence within a field (Wiggins et al. 2017). An oat companion crop in combination with an herbicide that is able to control waterhemp while not reducing the biomass of the oat within the field could prove to provide another weed control option as control of waterhemp becomes more difficult due to the multiple herbicide resistances observed in waterhemp in North Dakota and Minnesota (Heap 2021). Other companion crops, such as brassica, have not been as effective at reducing pigweed suppression, so continued research of the effects of cereal companion crops on pigweed suppression is necessary to better understand if this is a viable and profitable option for growers (Haramoto and Gallandt 2005).

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## **CHAPTER 4. INFLUENCE OF SOIL TEXTURE AND MOISTURE ON THE GROWTH OF WATERHEMP (*AMARANTHUS TUBERCULATUS*) AND PALMER AMARANTH (*AMARANTHUS PALMERI*)**

### **4.1. Introduction**

Palmer amaranth (*Amaranthus palmeri* S. Watson) has recently been introduced into North Dakota and is quickly becoming an agronomic issue within the state. Palmer amaranth exhibits a rapid growth of 0.2 cm per growing degree day and is able to survive in drought conditions (Davis et al. 2015; Horak and Loughlin 2000; Matzrafi et al. 2020). There are questions about the competitive ability of Palmer amaranth within North Dakota cropping systems. Waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer) is another *Amaranthus* species that is native to the Midwest region of the United States. Due to Palmer amaranth being native to the Sonoran Desert region (Davis et al. 2015) and the plant being an agronomic issue in warmer climates within the United States, there are some that hypothesize it will not be as competitive in a cool, wet climate such as North Dakota. Some observations are that Palmer amaranth can thrive and grow faster than waterhemp (Baker 2021, Sellers et al. 2003, Bensch et al. 2003). Baker observed that Palmer amaranth produced twice as much biomass as waterhemp within the first 6 weeks of growth. The concern for Palmer amaranth becoming an agronomic issue in North Dakota stems from the issues that are present due to waterhemp. Waterhemp was present in weed surveys in North Dakota in 2000, but was listed as a footnote due to the rarity of finding it (Zollinger et al. 2000). Waterhemp has since spread quickly throughout eastern North Dakota and has become a production challenge due to the herbicide resistance trait observed within waterhemp populations, and abundant seed production and yield interference that it can have on crops. Waterhemp has been observed to have resistance towards seven different

herbicide sites of action (Heap 2021) and has been documented of producing over a million seeds per plant (Heneghan and Johnson 2017). Palmer amaranth is capable of producing 1,800,000 seed per plant (Bryson and DeFelice 2009; Smith et al. 2012). Resistance towards 9 different herbicide sites of action have been observed in Palmer amaranth as well (Heap 2021). Much is still unknown on Palmer amaranth's ability to grow in a cooler climate, like that of North Dakota. The objectives of this study are: 1.) evaluate the ability of Palmer amaranth and waterhemp to grow in different soil types and moisture conditions observed in North Dakota; 2.) evaluate the competitive effect of the two weeds when grown together; 3.) compare growth of Palmer amaranth to waterhemp in order to better understand how Palmer amaranth may become a production challenge for growers in North Dakota.

## **4.2. Materials and Methods**

### **4.2.1. Experiment Design**

The greenhouse experiment was arranged in a 6x2x3 factorial in a RCBD with four replications. The treatments for this experiment were: a) the ratio of waterhemp:Palmer amaranth of the five plants within the pots; b) the soil used within the greenhouse pots; c) the saturation level of the pot. Soils were a Towner soil series (sandy loam, pH 6.5, OM 3.4%) and Hegne series (clay, 7.5 pH, OM 7.4%). The two species of *Amaranthus* were Palmer amaranth and waterhemp. Watering regimes were 25%, 50%, and 75% field capacity. Pots were 16.5 cm diameter by 17.8 cm height cylindrical pots. Greenhouse was set to a 30/25 C day/night cycle for temperature and a 16 hour photoperiod from 5 am to 9 pm Central Standard Time using 600 watt high pressure sodium lights. This experiment had two runs, with both runs having different methods of evaluating the water contents of each pot. For the first run, water contents were determined using weight of the pots. The weights for each of the water levels were determined

using similar calculations as Sarangi et al. (2016). The weight of the pots for both types of soil were measured when they were filled with dry soil. The pots were saturated with water and then allowed to drain for 24 hours and reweighed. The water content for the percentages were calculated using the equation  $WC = [(W_w - W_d) / d]$  with WC for water content,  $W_w$  for the wet weight of the soil,  $W_d$  for the dry weight of the soil, and  $d$  for the density of water. For the second run of this experiment, the water contents were monitored using a Fieldscout soil sensor reader (Spectrum Technologies, Inc., Aurora, IL). This soil sensor reader was used to monitor the water contents of the soil within the pots to help keep the pots at the desired percentages of field capacity. The soil sensor reader water content levels were determined by weighing out the pots for water content as was done in the first experiment and then measured the soil sensor levels at the different weight levels. This was done for the second run for ease of use, as well as to account for the weight of the plants within the pot that added to the overall weight of the pot.

#### **4.2.2. Planting**

Waterhemp and Palmer amaranth were seeded into CN-PLG-084ST plug flats (Greenhouse Megastore, Danville, IL). The plugs were transplanted into the pots full of soil once the plants had emerged and were at one true leaf. Five plants were planted into each pot with the ratio of the plants being 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5 (Waterhemp : Palmer amaranth). The plants were planted with one plant in the middle of the pot, with the other four around the edge of the pot. The plant that had the least amount within the ratio always had at one of the plants within the middle of the pot (1 waterhemp : 4 Palmer amaranth, the 1 waterhemp would be in the middle. After transplanting, the pots were watered every day to their respective weights for 8 weeks. The waterhemp population collected from a research site near Fargo, North Dakota, while the Palmer amaranth that was used was from Mississippi (Azlin Seed Services, Leland, MS).

### **4.2.3. Evaluation and Data Analysis**

Heights of the plants were measured weekly beginning at the week five. Above ground biomass of the plants was harvested at the end of the 8-week period by clipping the plants at soil level. Final height measurements were also taken at this time. Harvested above ground biomass was dried at 43 degrees C for 14 days using a force air dryer and weighed.

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). Data were subjected to ANOVA using PROC GLIMMIX to test for treatment effects and significant differences. Treatment means were separated using Tukey's HSD when data were found to be significantly different at  $P \leq 0.05$ . Pigweed ratio, soil type, and % field capacity were considered to be fixed effects, while environment and replicate were considered to be random effects. Both runs of data were combined as they were found to not be different using the Levine's test at  $P \leq 0.05$ . Normality was verified using PROC UNIVARIATE within SAS.

## **4.3. Results**

### **4.3.1. Palmer Amaranth Growth**

Palmer amaranth growth were compared amongst the three factors in the experiment. Soil type, percent field capacity of water, and waterhemp:Palmer amaranth ratio had no effect on the biomass (Table 4.1) or height (Table 4.2) of Palmer amaranth. These results are different from other studies that have observed a reduction in Palmer amaranth biomass and height in soils below 50% field capacity (Chahal et al. 2018). Baker (2021) also found a reduction in Palmer amaranth biomass and height in poorly-drained soils compared to well-drained soils. This difference in this study compared to previous research may be due to the competition with the other plants in pot, the point at which we harvested Palmer amaranth (8 weeks), and other environmental factors that differ from the greenhouse compared to a field study. Palmer

amaranth may also be a plant that exhibits a high plasticity, being able to easily adapt and grow similarly in different environments (Beckie 2006; Corn and Soybean Digest 2020; Matzrafi et al. 2020; Webster and Nichols 2012). The pigweeds may also not exhibit competitive effects at the numbers that were used in this experiment. Increasing the pigweed density may cause the pigweed ratio to have an effect. At the density used within this experiment, it seems as if Palmer amaranth is not affected by having another pigweed species within the area that it is growing.

Table 4.1. Effects of pigweed ratio, soil type, and % field capacity on Palmer amaranth aboveground biomass. <sup>a</sup>

Main effects	
<i>Waterhemp:Palmer amaranth ratio</i>	-----grams per plant-----
0:5	2.39
1:4	2.33
2:3	2.06
3:2	1.98
4:1	2.35
<i>Soil type</i>	
Hegne	2.17
Towner	2.27
<i>% field capacity</i>	
25%	2.01
50%	2.36
75%	2.29
<i>ANOVA</i>	-----p value-----
Waterhemp:Palmer amaranth ratio	0.766
Soil type	0.708
% field capacity	0.682
Waterhemp:Palmer amaranth ratio * soil type	0.443
Waterhemp:Palmer amaranth ratio * % field capacity	0.782
Soil type * % field capacity	0.862
Waterhemp:Palmer amaranth ratio * soil type * % field capacity	0.893

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

Table 4.2. Effects of pigweed ratio, soil type, and % field capacity on Palmer amaranth final height. <sup>a</sup>

Main effects	
<i>Waterhemp:Palmer amaranth ratio</i>	-----cm per plant-----
0:5	34.56
1:4	34.24
2:3	33.89
3:2	34.63
4:1	35.00
<i>Soil type</i>	
Fargo	33.38
Town	35.55
<i>% field capacity</i>	
25%	30.70
50%	37.39
75%	35.30
<i>ANOVA</i>	-----p value-----
Waterhemp:Palmer amaranth ratio	0.994
Soil type	0.456
% field capacity	0.202
Waterhemp:Palmer amaranth ratio * soil type	0.345
Waterhemp:Palmer amaranth ratio * % field capacity	0.234
Soil type * % field capacity	0.946
Waterhemp:Palmer amaranth ratio * soil type * % field capacity	0.647

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

### 4.3.2. Waterhemp Growth

Soil type, percent field capacity of soil, and waterhemp:Palmer amaranth ratio had no effect on the height or biomass of waterhemp. These results differ from research reporting water stress influencing waterhemp height and biomass at 75%, 50% and 25% field capacity (Sarangi et al. 2017). Waterhemp has also been reported to have higher biomass in well-drained soils compared to poorly-drained soils (Becker 2021). The results in this experiment could be due to the competition between the plants within the pots, the size of the pots themselves, and other

greenhouse environmental factors. Sarangi et al. (2017) deployed a 2-day watering interval, compared to this study in which the pots were watered every day to their respective moisture. Plants at 25% capacity after the 5-week mark would wilt dailt (data not shown). The 25% capacity wou;d have had a greater effect if watering occurred every two days instead of every day. None of the factors had an effect on waterhemp growth, suggesting that waterhemp has a high plasticity, which reflects previous reports that waterhemp grows in many different climates around the United States and has shown the ability to adapt to those different environments (Waselkov et al. 2020). These results also suggest that Palmer amaranth does not reduce height or biomass of waterhemp, indicating there is no competitive effect on waterhemp.



Table 4.3. Main effects of pigweed ratio, soil type, and % field capacity on waterhemp aboveground biomass. <sup>a</sup>

Main effects	
<i>Waterhemp:Palmer amaranth ratio</i>	-----grams per plant-----
1:4	3.77
2:3	4.31
3:2	3.98
4:1	3.78
5:0	3.87
<i>Soil type</i>	
Hegne	4.02
Town	3.86
<i>% field capacity</i>	
25%	3.65
50%	4.03
75%	4.14
<i>ANOVA</i>	-----p value-----
Waterhemp:Palmer amaranth ratio	0.922
Soil type	0.671
% field capacity	0.364
Waterhemp:Palmer amaranth ratio * soil type	0.554
Waterhemp:Palmer amaranth ratio * % field capacity	0.234
soil type * % field capacity	0.465
Waterhemp:Palmer amaranth ratio * soil type * % field capacity	0.354

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

Table 4.4. Main effects of pigweed ratio, soil type, and % field capacity on waterhemp final height. <sup>a</sup>

Main effects	
<i>Waterhemp:Palmer amaranth ratio</i>	----- <i>cm per plant</i> -----
1:4	71.88
2:3	71.33
3:2	70.07
4:1	68.09
5:0	70.27
<i>Soil type</i>	
Hegne	70.38
Town	70.27
<i>% field capacity</i>	
25%	64.96
50%	72.83
75%	73.19
<i>ANOVA</i>	
	----- <i>p value</i> -----
Waterhemp:Palmer amaranth ratio	0.823
Soil type	0.987
% field capacity	0.137
Waterhemp:Palmer amaranth ratio * soil type	0.873
Waterhemp:Palmer amaranth ratio * % field capacity	0.191
soil type * % field capacity	0.536
Waterhemp:Palmer amaranth ratio * soil type * % field capacity	0.454

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

#### 4.3.3. Palmer Amaranth Monoculture Growth

The effects of soil type and moisture on Palmer amaranth growth and biomass accumulation were evaluated using the pots containing a monoculture of only Palmer amaranth (0:5 waterhemp:Palmer amaranth). Soil type and percent field capacity did not effect the height and biomass of Palmer amaranth in the monoculture pots. There was no effect of percent field capacity on height (Figure 1a). These results confirm previous reports that Palmer amaranth can grow and propagate in many different environments (Beckie 2006; Corn and Soybean Digest

2020; Matzrafi et al. 2020; Webster and Nichols 2012). The fact that we saw no differences in soil type or moisture indicates that Palmer amaranth growth would be similar across many areas of North Dakota. Similar to the competition study, these results differ from other research that observed Palmer amaranth grows better in well-drained soils (Baker 2021) and in soils where water is more available to the plants (Chahal et al. 2018). These differences we observed compared to the literature could be due to different populations of Palmer amaranth utilized and different greenhouse environmental factors.

Table 4.5. Main effects of soil type and % field capacity on palmer amaranth monoculture final height. <sup>a</sup>

Main effects	
<i>Soil type</i>	----- <i>cm per plant</i> -----
Hegne	34.99
Towner	34.12
<i>% field capacity</i>	
25%	34.92
50%	35.54
75%	33.22
<i>ANOVA</i>	----- <i>p value</i> -----
soil type	0.840
% field capacity	0.686
soil type * % field capacity	0.410

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

Table 4.6. Main effects of soil type and % field capacity on palmer amaranth monoculture aboveground biomass. <sup>a</sup>

Main effects	
<i>Soil type</i>	<i>grams per plant</i>
Hegne	2.49
Town	2.29
<i>% field capacity</i>	
25%	2.53
50%	2.53
75%	2.10
<i>ANOVA</i>	<i>p value</i>
Soil type	0.646
% field capacity	0.352
Soil type * % field capacity	0.682

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

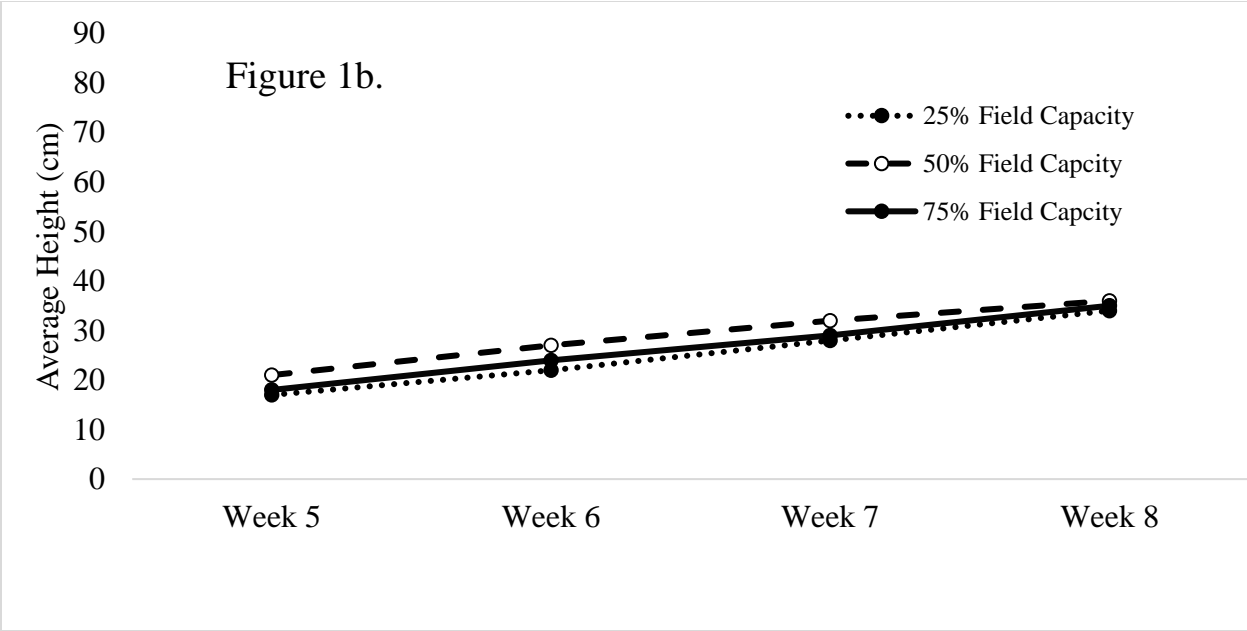
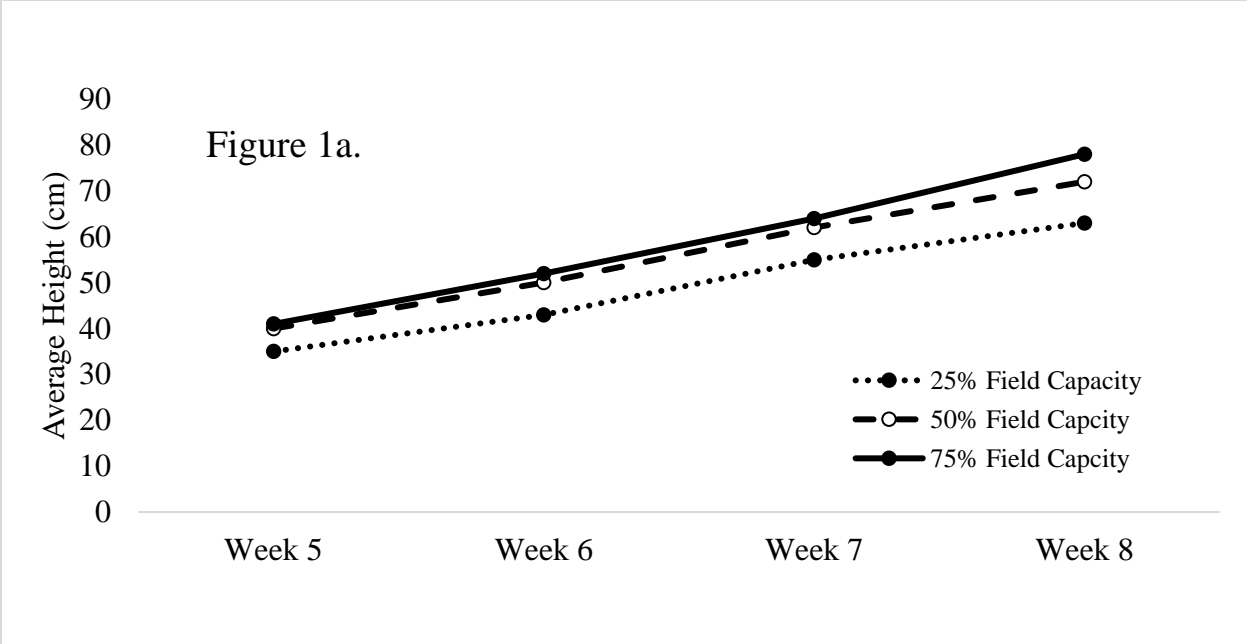


Figure 4.1. **a)** Effect of percent field capacity of soil on average waterhemp height over week 5 through week 8. Growth per day was 1.76 cm at 75% field capacity, 1.51 cm at 50% field capacity, and 1.33 cm at 25% field capacity. **b)** Effect of percent field capacity of soil on average Palmer amaranth height over week 5 through week 8. Growth per day was 0.81 cm at 75% field capacity, .71 cm at 50% field capacity, and 0.81 cm at 25% field capacity.

#### 4.3.4. Waterhemp Monoculture Growth

Biomass and height from pots containing only a monoculture of waterhemp (5:0 waterhemp:Palmer amaranth) were analyzed to determine the effects without competition with Palmer amaranth. The data were compared against the factors of soil type and percent field capacity of soil. The factor of waterhemp:Palmer amaranth was not compared due to the only ratio being analyzed was 5:0. Soil type did not effect waterhemp height or biomass in monoculture pots. Percent field capacity had  $P = .074$  for waterhemp height and  $P = .088$  for waterhemp biomass but did not have an effect on either at our established  $\alpha=0.05$ . The growth rate of waterhemp over time at the different field capacity are reported in Figure 1a. Similar to the waterhemp growth in competition with Palmer amaranth analysis, waterhemp in monoculture is able to grow in many different environments and adapt to those environments (Waselkov et al. 2020). These results differ again from other studies observing waterhemp growing faster in well-drained soils with higher field capacities (Baker 2021; Sarangi et al. 2017).

Table 4.7. Main effects of soil type and % field capacity on waterhemp final height. <sup>a</sup>

Main effects	
<i>Soil type</i>	<i>-----cm per plant-----</i>
Hegne	70.09
Town	70.50
<i>% field capacity</i>	
25%	61.58
50%	71.11
75%	78.19
<i>ANOVA</i>	<i>-----p value-----</i>
Soil type	0.958
% field capacity	0.074
Soil type * % field capacity	0.254

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

Table 4.8. Main effects of soil type and % field capacity on waterhemp aboveground biomass. <sup>a</sup>

Main effects	
<i>Soil type</i>	<i>grams per plant</i>
Hegne	3.81
Town	3.94
<i>% field capacity</i>	
25%	3.20
50%	3.83
75%	4.60
ANOVA	
	<i>p value</i>
Soil type	0.683
% field capacity	0.088
Soil type * % field capacity	0.375

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

#### 4.3.5. Comparative Pigweed Growth

Waterhemp grew taller and produced more biomass than Palmer amaranth (Tables 4.9 and 4.10). The species of pigweed did not have any interaction with any other main effect. These results differ from other experiments reporting Palmer amaranth grew at a faster rate than that of waterhemp (Baker 2021; Sellers et al. 2003). Waterhemp was observed to be more competitive in all conditions, whether it was a poorly-drained or well-drained soil, or at different saturation levels of the soil. Other studies have evaluated the growth rate within garden studies and field sites, while our experiment was conducted within a greenhouse environment. This experiment was also initiated with both of these species at the one true leaf stage. This could have an effect as Palmer amaranth has been reported to have a faster emergence rate than that of waterhemp (Sellers et al. 2003). Other reasons for differences observed in this study compared to the literature could be the amount of sunlight that the plants were subjected to compared to other studies, as this experiment was conducted in winter in a greenhouse in North Dakota, compared to other studies conducted outdoors in summer (Baker 2021; Sellers et al. 2003). However,

Sarangi et al. (2017) reported a range of waterhemp heights and biomass accumulation at different field capacities within a greenhouse environment. Another reason why our results may be different from Sarangi et al. (2017) could be due to the waterhemp population in North Dakota not being affected by different soil environments.

Table 4.9. Main effects of pigweed species, soil type, and % field capacity on monoculture pigweed aboveground biomass. <sup>a</sup>

Main effects	
<i>Pigweed species</i>	-----grams per plant-----
Palmer amaranth	2.39 b
Waterhemp	3.87 a
<i>Soil type</i>	
Hegne	3.15
Town	3.12
<i>% field capacity</i>	
25%	2.86
50%	3.18
75%	3.94
ANOVA	
	-----p value-----
Pigweed species	0.048
Soil type	0.930
% field capacity	0.213
Pigweed species* soil type	0.420
Pigweed species * % field capacity	0.071
Soil type * % field capacity	0.365
Pigweed species * soil type * % field capacity	0.798

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.



Table 4.10. Main effects of pigweed species, soil type, and % field capacity on monoculture pigweed height. <sup>a</sup>

Main effects	
<i>Pigweed species</i>	----- <i>cm per plant</i> -----
Palmer amaranth	34.56 b
Waterhemp	70.29 a
<i>Soil type</i>	
Hegne	52.54
Town	52.31
<i>% field capacity</i>	
25%	48.22
50%	53.33
75%	55.73
<i>ANOVA</i>	----- <i>p value</i> -----
Pigweed species	0.033
Soil type	0.971
% field capacity	0.149
Pigweed species * soil type	0.785
Pigweed species * % field capacity	0.108
Soil type * % field capacity	0.214
Pigweed species * soil type * % field capacity	0.719

<sup>a</sup> Means within a column and under the same factor that do not share the same letters are significantly different using Tukey's HSD at the 5% level of significance.

#### 4.4. Conclusion

Based on the data analysis Palmer amaranth will be able to grow and spread within North Dakota. There were not any significant effects in growth between the different factors tested in the experiment. Even though Palmer amaranth is native to environments with well-drained soils (Davis et al. 2015), the population used within this experiment was able to grow without significant negative effect in a poorly drained clay soil, such as what is found in Fargo, ND. It would be expected that Palmer amaranth would be able to grow in the varying soils found throughout North Dakota and in the more arid climates in western North Dakota. Another factor that would need to be tested would be the temperatures typically observed in North Dakota to see

how the weed would be able to grow in the colder regions of North Dakota. Palmer amaranth has been observed to produce less biomass compared to waterhemp when grown in colder temperatures (Guo and Al-Khatib 2003). Guo and Al-Khatib (2003) observed Palmer amaranth produces less biomass than waterhemp when the temperature for a day and night cycle was 15/10 C. At higher temperature day/night cycles they found Palmer amaranth grew at a faster rate than waterhemp. Palmer amaranth produced more biomass at every temperature cycle higher than that. Waterhemp was also observed to have higher germination rates than that Palmer amaranth at day/night cycles lower than 35/30 C. The greenhouse for this study was set at a temperature day/night cycle of 30/25 C, and waterhemp produced more biomass and height than that of Palmer amaranth. Waterhemp was observed to grow faster and larger than Palmer amaranth at colder temperatures, but in our study waterhemp was observed to grow faster and larger than Palmer amaranth in warm temperatures. Based off of the results of this greenhouse research, coupled with how fast Palmer amaranth is able to produce seed and the high abundance of seed produced (Keeley et al. 1987), Palmer amaranth should be able to spread throughout North Dakota; though it may not spread and grow at the same rapid rate that has been observed with waterhemp. Palmer amaranth could become a larger agronomic issue for growers in North Dakota compared to waterhemp if Palmer amaranth has the potential to grow at rates observed in other studies (Sellers 2003; Baker 2021), but based off the results of this study, it does not seem likely that Palmer amaranth will grow faster than waterhemp. Due to the amount of seed that Palmer amaranth is able to produce and the herbicide resistances that have been observed within Palmer amaranth populations, it is still important for growers to control the weed, even if the plant may not become as problematic as waterhemp is within North Dakota.

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## CHAPTER 5. SUMMARY

Iron Deficiency Chlorosis (IDC) and glyphosate-resistant *Amaranthus* species have been challenges for soybean growers in North Dakota. The yield loss caused by both of these issues has led to growers looking for other options to manage them. Small-grain companion crops have been observed to alleviate IDC symptoms within soybean, but little information is available about the capability of weed suppression capability of small grains planted simultaneously with soybean.

Two field trials were conducted in 2020 and 2021 in Cass County, North Dakota to evaluate the effects of oat companion crop terminated at different timings, and if they would influence IDC symptoms in soybean, *Amaranthus* species populations, and end of season yield (Chapter 2). IDC symptoms were not frequent at either site, but SPAD ratings were greater at Fargo when an oat companion crop was present at termination timing, indicating greater chlorophyll content and less chlorosis in soybean plants in those treatments. An oat companion crop reduced pigweed biomass, but this effect did not occur until the 30 to 45 cm termination timings. Yield losses were observed as early as the 30 cm termination timing. Pigweed biomass reduction may not be observed until after yield losses occurred. Oat also reduced yield in Fargo. An oat companion crop may not be a viable option to reduce both *Amaranthus* and IDC symptoms without the risk of yield loss.

Two field trials were conducted in 2020 and 2021 in Cass County, North Dakota to evaluate the effects of soybean preemergence herbicides on an oat companion crop and *Amaranthus* species (Chapter 3). Flumioxazin and sulfentrazone consistently provided the greatest control of pigweeds between all the sites, but also caused the greatest injury to the oat companion crop. Fomesafen, dimethenamid-P, acetochlor, and pyroxasulfone provided some

pigweed control in 2021. These herbicides also caused less oat injury when compared to other herbicides and could potentially be used in conjunction with an oat companion crop. Using sulfentrazone or flumioxazin in a herbicide system may be a better option if *Amaranthus* control is the main concern.

Palmer amaranth (*Amaranthus palmeri*) is a recently introduced weed to North Dakota. Palmer amaranth has been a major problem in growing systems in other states, and there is concern of the same issue occurring in North Dakota. Waterhemp (*Amaranthus tuberculatus*) has been researched extensively in North Dakota and is a major issue in cropping systems due to the quick spread of waterhemp and the difficulty to control the weed due to herbicide resistances and competitiveness of the weed. A greenhouse experiment was conducted to compare the growth of waterhemp and Palmer amaranth in two soil types and three water content levels (Chapter 4). There were no differences observed in growth of the two weeds amongst the different environmental factors. Waterhemp grew twice as tall as Palmer amaranth. Waterhemp also produced twice as much biomass of Palmer amaranth. Palmer amaranth may be able to grow within North Dakota environments, but may not grow as well compared to what is observed in waterhemp currently in the state.

Other options may be more viable for controlling IDC and *Amaranthus* species than that of an oat companion crop in order to reduce the risk of yield loss. Preemergence herbicides options could be available if controlling *Amaranthus* species early in a soybean crop using oat as a companion crop. While Palmer amaranth is definitely a concern for North Dakota growers to control, waterhemp may still be a more problematic weed within the state at this time.