2021

Auxin-Based Herbicide Program and Rhizobia Application for Weed Control and Nodulation Potential in Auxin Tolerant Soybean

Joy Amajioyi

AUXIN-BASED HERBICIDE PROGRAM AND RHIZOBIA APPLICATION FOR WEED CONTROL AND NODULATION POTENTIAL IN AUXIN TOLERANT SOYBEAN

BY

JOY AMAJIOYI

A thesis submitted in partial fulfillment of the requirements for the degree

Master of Science

Major in Plant Science

South Dakota State University

2021

THESIS ACCEPTANCE PAGE

Joy Amajioyi

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Sharon Clay
Advisor Date

David Wright
Department Head Date

Nicole Lounsbery, PhD
Director, Graduate School Date

I would like to dedicate this work to my precious son, Dinobi Victor Amajioyi, parents, Victor and Grace Amajioyi, siblings, Stanley Amajioyi, Chibuzor Amajioyi, Samuel Amajioyi, Queen Amajioyi, and my amazing counselor, Amy Ward. This work would not have been possible without your love and encouragements. I am blessed to have you all in my life.

ACKNOWLEDGEMENTS

I would like to express the deepest gratitude to my major advisor, Dr. Sharon Clay. Thank you for giving me the opportunity to learn under your tutelage and for the great role you played during my moment of despair. Without your unwavering faith in my potentials, unending support, patience, encouragement, and occasional push out of my comfort zone, I would not have finished this great journey. You are the best advisor ever.

To my advisory committee, Dr David Clay, Dr Thandi Nyela. and Dr Jensen Amber, thank you for your relentless effort to impact knowledge and for your contributions towards the success of this program. You are the best! The significant contributions of faculty and students of Agronomy, Horticulture and Plant Science department did not go unnoticed.

To my parents, Victor and Grace Amajioyi, siblings, Stanley, Chibuzor, Samuel, and Queen, this work would not have been possible without your love, care, and blessings. Thank you for always having my back.

To Brookings multicultural center, and my dear friends Patra Akaya, Anuoluwa Sangotayo, Kim Chang, Cassandra Lamb, Katie Miller, Margaret Isedowo, Collete Nyuydze, Cindy, Richard, Damola, and Unius, thank you for making the journey far more interesting than it was trying. You all rock!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

AUXIN-BASED HERBICIDE PROGRAM AND RHIZOBIA APPLICATION FOR WEED CONTROL AND NODULATION POTENTIAL IN AUXIN TOLERANT SOYBEAN

JOY AMAJIOYI

2021

Foliar-applied Bradyrhizobia to V4 soybean has been reported to increase yield up to 5%. However, a stand-alone product application may not be practical. Applying with other treatments such as post-emergence herbicide application may be economical but herbicide and/or additives may be deleterious to rhizobial growth. A laboratory study investigated the impact of herbicides (glyphosate and dicamba), additives (an oil to improve absorption and spreading; and AMS used to overcome hard water impacts on glyphosate), and herbicide + additives on bacterial growth. Optical density (OD) measurements at the wavelength of 650 nm assessed solution turbidity, a surrogate measure of bacterial growth. Glyphosate, dicamba, and AMS, as stand-alone treatments, reduced OD values by 98, 64 and 100%, respectively, compared to control (deionized water + inoculant) after 72-hr. Herbicide + additives, however, had OD values 25 % greater than the control. Therefore, applying bradyrhizobia with post-emergence herbicide applications at labeled rates with typical mixtures of surfactants/additives should not be harmful to the bacteria.

Field experiments were conducted at three South Dakota locations for two years where Enlist E3 or Xtend soybean varieties were planted early, mid, or late season.

Treatments included preemergence (pre), pre + post emergence auxin herbicides (2,4-D or dicamba), or herbicide solutions mixed with bradyrhizobia to examine weed control, soybean nodulation and activity, yield, and seed protein. Pre-only herbicides resulted in poor weed control and reduced yields. Pre + post emergence treatments improved weed control and yield, with early and mid-planting having greater yields than late planting. Uncontrolled weeds in the pre and pre + auxin-based treatments were mostly grasses including barnyardgrass (*Echinochloa crus-galli*), volunteer wheat (*Triticum aestivum*), large crabgrass (*Digitaria sanguinalis*), green foxtail (*Setaria viridis*) and volunteer corn (*Zea mays*). Rhizobia application did not impact soybean nodulation, yield, or seed protein in 27 out of 30 treatments. The exception was dicamba + glyphosate + rhizobia that enhanced nodulation numbers (+30%) and activity (+54%) in one location in one year for all three planting dates compared to dicamba + glyphosate, although yield and seed protein content were similar among these treatments.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. Overview of soybean production and utilization

Soybean [*Glycine max* (L.) Merr.] is a member of the Leguminosae family and is the most widely grown oilseed and legume crop in the world (Lee et al. 2011). The Soyinfo Center has documented information on soybean history, use and bibliographies (www.soyinfocenter. com). Global production of soybean increased from 80.9 million metric tons in 1980 to 337 million metric tons in 2019. Farmers in the United States in 2019 produced 97 million metric tons of soybeans on 30 million hectares of land with a value of $31 billion (USDA-NASS, 2019). South Dakota's share of this production was about 4 million metric tons soybeans on 1.4 million hectares with a value of $1.2 billion (USDA-NASS, 2019). In May of 2020, Brazil became the leading soybean producing country with approximately 124 million metric tons production (https://www.worldatlas.com/articles/largest -soybean-producing-countries.html). When compared to 2019, soybean production in the United States and South Dakota in 2020 increased by 16% and 50% respectively (USDA-NASS, 2020a). The 2019 South Dakota growing season was a very wet year with rainfall amounts exceeding the 30-year average by 50% (https://mesonet.sdstate.edu/archive). The early rains in 2019 prevented planting of most crops as the fields were too wet thereby leaving most fields uncultivated. Also, the mid-summer rains in 2019 drowned out many areas and the few acres that were planted got flooded in July thus resulting in low crop yields.

The global demand for soy as food, vegetable oil, and animal feed has grown steadily over time. About 85 % of the world's soybean are processed into soybean meal and oil for livestock and aquaculture feed (Ali, 2010), whereas 2% are consumed directly by man as food (Goldsmith, 2008; Hartman et al. 2011). The soybean plant provides a complete protein as it contains all nine amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) essential for human health. Natto, soy milk, soy flour, tempeh, tofu, edamame, and miso are examples of food products made from soybeans (www.soyinfocenter.com). Soybean oil can be used industrially in the manufacture of products such as paints, fertilizers, adhesives, linoleum backing, as well as in biofuels (Liu, 2008).

1.2. Growing Soybean in South Dakota

Soybean is among the top five crops grown in South Dakota with most hectares in eastern South Dakota (USDA-NASS, 2020b) Unlike many other crops, soybean varieties are either determinate or indeterminate. In South Dakota, most varieties are indeterminate and continue to develop leaves on the main stem and branches throughout flowering. Determinate soybean varieties characteristically stop vegetative growth and do not produce nodes on the main stem soon after flowering begins and are typically grown in southern United States. The soybean plant thrives best on warm, fertile, moist but well-drained sandy loam soil (Martin, 1988). Timely planting of soybean is important to optimize yields in the northern Corn Belt regions. In South Dakota, soybean is planted no earlier than when soil temperature is at least 10 degrees Celsius, at a depth of 3 to 5 cm (Clay et al. 2013). When planted too early, soybean may be exposed to a spring killing frost, early season weeds, and insects and seedling diseases. These factors may result in suboptimal stands.

In recent years, scientists in the seed industry have focused on developing new soybean varieties with improved quality, including high yield, nematode resistance, reduced lodging and pod shattering traits, and herbicide tolerance (https://www.ilsoyadvisor.com/on-farm/ilsoyadviso r/soybean-breeding-today). The selection of an appropriate planting variety is the first step towards the successful production of soybean. However, during the selection process, care should be taken to balance the yield potentials with other management costs. There is a very short window available for growing soybean in South Dakota. Planting begins in early May/June, and it is harvested in September or early October (Clay et al. 2013). Planting dates may vary depending on location and maturity groups. Soybean varieties that maximize the entire growing season for a particular region have been reported to produce higher yield (Muller et al. 2013; Mourtzinis et al. 2015; Wu et al. 2017). Maturity ratings of soybean grown in the United States range from Group 00 (in the far north which includes northern Minnesota and North Dakota) to Group IX (in the far south which includes Florida, and the southern parts of the Gulf Coast states) (Mourtzinis and Conley, 2017). Group 00 matures in about 115 days or less while group IX have about 195 days to maturity. The number of days to reach maturity as group numbers increase is about 10 days (https://www.farmprogress.com/soybeans/soybean-maturity-group-planting-date-and-development-related). Relative maturity group I has a predicted maturity of around 127 days and is used in northeast South Dakota whereas group II, with 137 days to maturity, is better adapted to southeast South Dakota (Hall et al. 2012).

Row spacing and seeding rates have been reported to impact soybean yields in the Upper Midwest region of the United States. There are, however, different reports of increased yield or no difference in yield due to narrow row spacing. A marked increase in yield from 134 kg ha^{-1} to 604 kg ha^{-1} has been reported when soybean was planted in narrow rows (< 50 cm) than in wider rows (50 to 76 cm) (Lambert and Lowenberg-DeBoer, 2003; De Bruin and Pedersen, 2008a; Cox et al. 2012) while no yield difference was reported in another study (Pedersen and Lauer, 2003). Most soybean in South Dakota are grown in wider row (76 cm) spacing. About 69% of South Dakota soybean farmers grow the crop in a row spacing of 76 cm or more (USDA-NASS, 2015). Planting in wide rows delays canopy closure, reduces canopy density around the time of soybean flowering, and thus prevents favorable conditions for white mold development (https://extension.sdstate.edu/start-flowering-ideal-time-white-mold-management-soybeans). Previous research found that optimum seeding rate varied from 194 000 to 291 000 seeds ha^{-1} in narrow rows but from 157 000 to 212 000 seeds ha^{-1} in wide rows (De Bruin and Pedersen, 2008b).

The impact of delayed planting on the yield of soybean has been well studied (Staton, 2011; Roozeboom, 2012; Licht et al. 2013; Nleya et al. 2020). Research indicates that in the northern Corn Belt regions, high yielding soybean varieties would lose 17 to 67 kilograms of yield per hectare per day when planted after the optimum planting date, which is targeted for May 15 in South Dakota (https://www.sdsoybean.org/scoop-on-soybean-blog/early-does-it-optimal-soybean-yields-come-with-time-planting/). In Lincoln Nebraska, research has shown that soybean loses 17 to 42 kilograms per hectare for each day planting was delayed after the optimum planting timing of mid to late April (Bastidas et al. 2008). In 2014, delaying the planting of soybean by 42 days (from May 15 to June 27) resulted in a 29 % and 42 % decrease in the number of growing degree days during the reproductive phase (R1 – R8) for maturity groups 1.4 and 2.4, respectively (Nleya et al. 2020). Also, when soybean was planted late, 51 and 72 kilograms per hectare per day reduction in grain yield was reported for maturity groups 1.4 and 2.4, respectively. In addition, seed oil content for both maturity groups were reduced by 14% and 18% respectively, with late planting, whereas protein content was variable among planting dates (which ranged from May to early July) and between maturity groups (Nleya et al. 2020).

In another study conducted at Aberdeen (northern South Dakota) and Beresford (southeast South Dakota), Schutte and Nleya (2018) investigated the impact of row spacing and seeding rate on soybean performance. Results from the study showed that soybeans when planted in narrow rows (19 cm) yielded 0.8% to 10% more than those planted in wider rows (76 cm). The study also reported 3 to 7 % increase in soybean yield when the seeding rate was increased from 247,000 to 506,500 seeds per hectare. However, indeterminate varieties of soybean can compensate for space in the canopy as the plant adds branches when planted in wider rows.

1.3. Weeds: A major issue in soybean production

Weeds reduce soybean yields 37% worldwide (Oerke 2006, Vivian et al. 2013), and in the United States and Canada, weeds reduce yields up to 52% yield loss with an estimated monetary loss valued at $17 billion (Soltani et al. 2017). Weeds reduce harvest efficiency, decrease crop quality, produce seed that can impact future crops, and increase the cost of production. It has been found that crops have a critical weed free period which is defined as the interval in the life cycle of a crop when it must be kept weed-free to prevent yield loss (Zimdahl 1980; Zimdahl 1987). The impact of weed interference during the critical weed-free periods of many crops has been researched in many studies (Knezevic et al. 2002; Zimdahl, 2004; Clay et al. 2009; Moriles et al. 2012; Osipitan et al. 2013; Osipitan, 2016; Adigun et al. 2017; Horvath et al. 2018, Daramola et al. 2020). It is difficult to predict the exact time for the critical weed free period because it is dependent on many factors including soil moisture, weed emergence relative to crop emergence, weed density, and weed species.

Soybeans can sense weeds before they emerge and may change their morphology in response (https://www.syngenta.ca/smw/articles/critical-weed-free-period-soybeans) (Horvath et al. 2015) although this may not reduce yield. The start of the critical weed-free period in soybean is typically between the first to the third trifoliate growth stage (14 to 43 days after emergence), and it continues to the beginning bloom growth stage (R1) (Eaton et al. 1976; Harris and Ritter, 1987; Stoller et al. 1987; Zimdahl, 1987; Van Acker et al. 1993). To avoid yield losses, weeds should be removed before the onset of the reproductive growth stages R1 (Halford et al. 2001). Changes in gene expression, decreased photosynthetic pigment contents, nitrogen content in roots and leaves, reduced nodulation as well as increased oxidative stress levels, among other effects, have been detected due to early weed presence in soybeans (Afifi and Swanton, 2012; Horvath et al. 2015; Mckenzie-Gopsill et al. 2016). However, these changes may be subtle and not directly reduce soybean yield.

Weeds can have a mixed impact on soybean yields. Weeds that emerge and grow with soybean during the first three weeks, but are then removed, may or may not negatively impact yield. However, when weeds remain in the crop from three to eight weeks after soybean emergence (VE), they have the greatest potential to reduce yield. Late emerging weeds in soybean fields that have been kept weed-free up to eight weeks after crop emergence are unlikely to result in yield reductions or have a negative economic impact relative to the cost of in-season treatment. However, these late emerging weeds can cause harvest problems, decrease crop quality, or produce more weed seeds (Clay et al. 2005; Uscanga-Mortera et al. 2007; Moechnig et al. 2013). Smooth pigweed (*Amaranthus hybridus*) interference with soybeans has been reported to cause an average yield loss of 55% (Moolani et al. 1964). Also, 25 to 30% soybean yield reduction was reported by Nave and Wax (1971) with one smooth pigweed 0.3 m^{-1} in 76 cm^{-1} spaced soybean rows.

Common waterhemp [*Amaranthus tuberculatus* - a common weed in eastern South Dakota] interference up to 10 weeks after soybean unifoliate leaf expansion has been reported to cause an average yield loss of 43% during a study in Illinois (Hager et al. 2002). Another study in Nebraska reported 40-76% yield losses with two common ragweed (*Ambrosia artemisiifolia*) plants per 0.9 m of soybean row (Barnes et al. 2018). In South Dakota, common ragweed interference with soybean at low density (2 or fewer plants m^{-2}) has been reported to cause a 10% yield reduction (Clay et al. 2006; Clay 2013). Also, 51% yield loss was reported when volunteer corn competed with soybean at an average density of 4 plants m^{-2} (Alms et al. 2016). A recent study on soybean weed management indicates high yield losses with Palmer amaranth (*Amaranthus palmeri*) emergence even after an early weed control effort. According to the findings of Van De Stroet and Clay (2019), 35% to 45% yield losses occurred with moderate Palmer amaranth densities (6 to 10 plants m^{-2}) most likely due to intraspecific Palmer amaranth competition, whereas Bensch et al. (2003) reported up to 91 % yield loss with a single Palmer amaranth per 0.13 m of row in soybean in Kansas.

Herbicides provide a convenient, economical, and effective method to control weeds in row crops, where they are applied as pre- or post-emergence to maximize crop productivity by minimizing other vegetation. In the United States, herbicides make up 60% of the volume and 65% of the expenditures for all pesticides used by growers (Donaldson et al. 2002). Although found to be an important weed control tool, the use of herbicides has been implicated in several health and environmental issues, non-selective vegetation removal, crop injury concerns and herbicide drift injury to neighboring fields. Common soybean herbicides used in the United States include the WSSA group 1 ACCase inhibitors (e.g. fluazifop, quizalofop) for grass control, WSSA group 2 ALS inhibitors (e.g. imazamox, cloransulam), WSSA group 4 synthetic auxins (e.g. 2,4-D, dicamba; mainly applied PRE until the recent development of the auxin resistant (GMO) soybean which allows for POST application), WSSA group 5 photosynthesis inhibitors (e.g. metribuzin, bentazon), WSSA group 9 amino acid inhibitors (e.g. glyphosate), WSSA group 10 Glutamine synthetase inhibitors (e.g. glufosinate), WSSA group 14 Cell membrane disrupter or PPO inhibitors (e.g. acifluorfen, flumioxazin, sulfentrazone), and the WSSA group 15 Seedling shoot inhibitors (e.g. acetochlor, metolachlor) (Shaner, 2014).

1.4. Glyphosate and glyphosate tolerant crop technology

Glyphosate [N-(phosphonomethyl) glycine), formulated and marketed as Roundup by Monsanto in 1974 (Duke and Powles, 2008) is widely used to control dicotyledonous and monocot weeds (Anonymous, 2014b) in both cropping and non-cropping situations. This non-selective WSSA group 9 herbicide can be applied in a variety of forms including isopropylamine salt, ammonium salt, diammonium salt, dimethylammonium salt, and potassium salt (Dill et al. 2010). After its introduction in the mid-1970s, glyphosate was used primarily for burndown of emerged weeds and for perennial weed control in corn and soybeans. Prior to GMO crop introduction, the non-selectivity of glyphosate limited the number of applications and acres sprayed. Glyphosate has no soil activity and therefore allows for flexible crop rotations. The herbicide has been considered to have low environment and human health risks until recent reports challenged these perceptions. Glyphosate kills plants by inhibiting the EPSPS (5-enolpyruvyl-shikimate-3-phosphate synthase) enzymes, the shikimic acid pathway vital for synthesizing the three essential aromatic amino acids phenylalanine, tyrosine, and tryptophan (Schonbrunn et al. 2001; Reddy, 2001). The EPSPS enzyme is present only in plants and microorganisms but absent in humans (Bentley 1990; Richards et al. 2006).

Glyphosate tolerant crops (commonly referred to as Roundup Ready crops) were first approved for planting in the United States in 1996. This cutting-edge technology simplified weed management and no-till practices in agronomic cropping systems. Roundup Ready crops like soybean, corn, and cotton are tolerant to the herbicide glyphosate, and have enabled growers to spray glyphosate postemergence during the crop season to achieve excellent, broad spectrum weed control. Additional crops with the tolerance trait include canola and sugar beet. Weed management in glyphosate tolerant crops had been excellent. The technology gave farmers a simpler, inexpensive means of weed control using glyphosate-based herbicides. However, with several glyphosate applications over space (millions of hectares) and time (5 to 7 years) came the widespread selection for weed populations that are resistant to glyphosate. As resistant weed biotypes increased, growers in response, also increased their rate of glyphosate application, as well as the number of applications (Benbrook, 2012; Mortensen et al. 2012; Duke, 2014; Heap, 2014; USDA-NASS, 2014) while many other growers integrated additional herbicides into their spray programs (Christoffoleti et al. 2008; Mortensen et al. 2012; Owen et al. 2014; Heap, 2014). In 2021, thirty-eight weed species worldwide have been reported in both crop and non-crop situations to have resistance to glyphosate, out of which seventeen cases are recorded in the United States (Heap, 2021). In South Dakota, glyphosate resistance has been confirmed in four weed species including common ragweed (*Ambrosia artemisiifolia*), kochia (*Bassia scoparia*), tall or common waterhemp (*Amaranthus tuberculatus*) and marestail or horseweed (*Conyza canadensis*) (Moechnig et al. 2013; Heap, 2021).

The economic losses from glyphosate-resistant weeds pose a serious threat to crop production and have highlighted the need to consider alternatives for weed management in field crop production. To control resistant weeds in soybean fields, glyphosate may be tank-mixed with other postemergence (POST) herbicides. In order to achieve a more consistent and effective control of most resistant weeds, applying preemergence (PRE) followed by postemergence (POST) herbicides, tank-mixing herbicides with multiple modes of action, and herbicide rotation are some herbicide programs that have been adopted by soybean growers. However, additional herbicide applications, and combining herbicide chemistries increase weed management costs.

1.5. Auxin herbicides (2,4-D and dicamba) and tolerant crop traits.

WSSA group 4 herbicides 2,4-D (2,4-Dichlorophenoxyacetic acid) and dicamba (3,6-dichloro-2-methoxybenzoic acid) are growth regulators commonly used as postemergence and inexpensive herbicide treatments to selectively control broadleaf weeds in corn, pastures, small grains such as wheat, and turf. They can also be used to kill existing broadleaf weeds prior to planting of other agronomic crops. Although in use for over 50 years, 2,4-D and dicamba herbicide chemistries have shown excellent resilience with few herbicide-resistant weeds occurring. Both herbicides also provide excellent control of glyphosate-resistant broadleaf weeds like marestail, common and giant ragweed, common waterhemp, and other dicot weeds.

Auxin tolerant soybean was introduced in the United States in 2016 (dicamba tolerant) and 2019 (2,4-D tolerant). Prior to the introduction of the genetically modified soybean varieties, growers planted their fields to conventional soybean varieties (USDA-ERS, 2014; USDA-ERS, 2019a and 2019b). However, drift from adjacent fields or tank contamination was a major challenge as conventional soybean are very sensitive to auxin herbicides which causes cosmetic damage to total loss of crop depending on the amount and timing of exposure (Andersen et al. 2004). Also, the selection for glyphosate resistant weeds made broadleaf weed control in conventional soybean challenging. Growers dealt with this challenge by increasing their application rate or making double, and in some cases, triple applications of glyphosate to get the weeds under control. This promoted the buildup of more resistant weed biotypes in the population and increased production cost for growers.

Agricultural chemical companies [Bayer and Corteva], in response to the rapid evolution of glyphosate-resistant weeds, introduced the Roundup Ready 2 Xtend® and Enlist™ tolerant soybean varieties in 2016 and 2019, respectively. These traits have provided new ways to maximize broadleaf weed control flexibility using the approved auxin-based herbicides. The Enlist™ soybeans possesses gene modifications that make them tolerant to 2,4-D, glyphosate and glufosinate herbicides, whereas the Xtend® soybean type is resistant to dicamba and glyphosate herbicides. Tolerance to glyphosate in Enlist E3 soybean is conferred by the 2mepspsgene (Lepping et al., 2013), whereas in RR soybean, it is conferred by the cp4epspsgene (Padgette et al., 1995). The Enlist™ weed control systems allows for an over-the-top application of herbicides including 2,4-D, glyphosate and glufosinate to Enlist™ soybean, whereas the Xtend weed control system allows for the POST applications of dicamba and glyphosate to Xtend® soybean for more efficacious management of problem dicot or broadleaf weeds in soybean fields (https://weedscience.missouri.edu/publications/Dicamba_24D_Factsheet.pdf).

Auxin herbicides are a large family of herbicides that began with 2,4-D in the 1940's and have expanded since then to include the sub-families of phenoxy [2,4-D, MCPP, MCPA - (Circa 1945)], benzoic acid [dicamba (Circa 1965)] and pyridines [triclopyr, fluroxypyr, picloram, aminopyralid, clopyralid, aminocyclopyrachlor – (Circa 1970)]. While each sub-family has a different chemical structure, they all act as auxins and are growth regulators. They regulate cell division and elongation, and they impact plant processes such as vascular tissue, meristem differentiation and leaf initiation. The Phenoxy herbicide, 2,4-D, works by mimicking a naturally occurring plant chemical called indole acetic acid (IAA). When applied to a target (susceptible) plant, 2,4-D causes unregulated IAA production which results in uncontrolled growth, twisting and elongation of the stem, thickening of the leaves and the eventual death of the plant. Dicamba also works by stimulating abnormal cell growth in meristematic cells of susceptible plants, thus blocking the vascular tissue of the phloem. This destroys the cambium and phloem cells near the meristems and plants are killed by starvation resulting from an inability to translocate photosynthates through the phloem to other parts of the plant (Tu et al. 2001).

2,4-D and dicamba use are on the increase since the introduction of tolerant soybean varieties (Ganie and Jhala, 2017; Osipitan and Dille, 2017; Underwood et al. 2017). In 2019 and 2020, the acres planted with genetically engineered soybeans in South Dakota was 93 and 95%, respectively (USDA-ERS, 2019b). An ever-present concern for the use of 2,4-D and dicamba herbicides for the control of dicotyledonous weeds in the newly developed Enlist™ and Xtend® weed control technology is the off-location movement of herbicide through particle drift or volatility to sensitive crops that do not carry the tolerance trait. Herbicide drift occurs when windy conditions are combined with poor application techniques whereas volatility involves a phase change and is the movement of the gaseous form of the herbicide after it has been deposited on its intended target as a liquid due to high temperatures evaporating the herbicide from the leaf. Regardless of efforts to reduce vapor drift though improved formulations, training of spray operators, and label restrictions, many cases of off-target movement of dicamba have been reported over the past years, even before the introduction of auxin tolerant soybeans. Dicamba drift has been reported in Missouri (Bradley 2017a and b), Illinois (Illinois DOA), Indiana (Office of Indiana State Chemist 2019), and South Dakota (Andersen et al. 2004). The effect of auxin drift to conventional soybean growth and yield performance has been well studied (Wax et al. 1969; Auch and Arnold, 1978; Andersen et al. 2004; Robinson et al. 2013a and b; Solomon and Bradley, 2014; Osipitan et al. 2019; Costa et al. 2020). The injury caused by dicamba drift rates to non-tolerant soybeans may be slight to highly damaging with high yield losses (Andersen et al. 2004).

2,4-D herbicide is less injurious to soybean than dicamba. Dicamba doses as low as 1/100 of the label rate (5.6 g a.e. ha^{-1}) when applied to conventional soybean at V3 stage of growth have been reported to reduce yield by up to 34%, whereas about 1/10 of 2,4-D label rate (112 g a.e. ha^{-1}) was necessary to reduce soybean productivity within a range of 25 to 32% (Andersen et al. 2004). In another study (Osipitan et al. 2019), dicamba drift to sensitive soybean, regardless of the dicamba product technology or formulation used, caused substantial crop injury (80%), plant height reduction (65%), delay in maturity (22 days) and yield loss (96%) when 1/10 of the dicamba label rate (56 g a.e. ha^{-1}) was applied at V7/R1 soybean growth stage. Similarly, crop injuries up to 41% and 70%, plant height reduction of about 61%, and yield losses up to 29 % and 76 % was reported when low doses (1/10, 1/100, and 1/1000) of dicamba label rate (28 g a.e. ha^{-1}) were applied to soybean at V4 and R2 growth stages, whereas injuries caused by the same low doses of 2,4-D were neither enough to damage the crop, nor affect yield (Andersen et al. 2004; Costa et al. 2020).

1.6. Soybean nitrogen demand

Nitrogen (N) is a primary essential nutrient required by plants in comparatively large amounts for proper growth and development. Among all 16 essential nutrients, nitrogen is a fertilizer component required by plants in the highest quantity. Nitrogen plays a vital role in photosynthesis and the manufacturing of protein. When deficient in plants, poor growth and yellowing of leaves occurs (Fageria and Baligar, 2005). Excess nitrogen can result in excessive vegetative growth at the expense of flowering and fruiting in plants. Environmental problems can also arise when excess nitrogen from fertilizers is carried by runoff into groundwater or surface water. In surface waters, nitrogen pollutants can stimulate excessive algae growth (https://www.epa.gov/nutrientpollution/issue) and if found in aquifers used for drinking water, can cause methemoglobinemia in infants ("blue baby syndrome") (Brender, 2020). Soybean, unlike other row crops, have a high demand for nitrogen due to the high protein content (which is about 40 % or more on a dry weight basis) in the grain. The higher the soybean yield, the higher the nitrogen requirement (Salvagiotti et al. 2008). Demand for nitrogen in soybean peaks during pod development. An application of 22 to 44 kg N ha^{-1} at the R3 stage of soybean could alleviate the effect of nitrogen deficiency that occurred during the time of pod set/seed fill on yield (Wortmann et al. 2018).

Typically, nitrogen fertilizer is rarely applied in soybean fields. The atmosphere and the soil are the two major sources of nitrogen supply to soybean plants. Through nitrogen fixation process, soybean obtains nitrogen from the atmosphere. The Gram-negative, rod-shaped diazotrophic bacteria (rhizobia) fixes atmospheric nitrogen after becoming established inside the root nodules of legumes. The specific rhizobia responsible for biological nitrogen fixation (BNF) in soybean is the *Bradyrhizobium japonicum*. The bacteria colonize soybean roots forming nodules. Within the nodule, *B. japonicum* convert nitrogen gas (N_2) into ammonium (NH_4^+) which is an available form of nitrogen for plant use. The process of root nodule initiation and development is complex and is regulated by several phytohormones like auxins, cytokinins, gibberellins, and brassinosteroids as positive regulators of nodule formation (Ferguson et al. 2005; Maekawa et al. 2009), while ethylene, jasmonic acid and abscisic acid are negative regulators (Ding et al. 2008; Nakagawa and Kawaguchi, 2006; Penmetsa et al. 2008). Root nodulation has been reported to be inhibited by excess natural auxin levels in soybean roots (Turner et al. 2013), and consequently influences the amount of nitrogen fixed by crop and yield. At a yield level of 4035 kg ha^{-1}, N fixation provides 65% to 70% of the total nitrogen required by soybean. Between 50 to 100 kg N ha^{-1} has also been found to be provided typically by the mineralization process (Thies et al. 1995; Schmidt et al. 2000; Salvagiotti et al. 2009). Research also suggests that growers are unlikely to see yield increases when additional N fertilizer is applied to soybean, either as preplant or after the crop is up, except in the case of high-yielding, irrigated soybeans (Salvagiotti et al. 2008; Taylor, 2012; Cafaro La Menza et al. 2017). At increased soil nitrate (NO_2^-) levels, or when nitrogen fertilizer is applied, N fixation by soybean is inhibited. However, when nitrogen fertilizers are applied at levels less than the required amounts (<34 kg ha^{-1}), N fixation compensates for the remaining N nutrient required to obtain maximum soybean yield (Schmidt et al. 2000). Some recommendations found in literature for obtaining maximum benefits from nitrogen applications to soybean, include keeping N rates low (<34 kg ha^{-1}), applying fertilizer in season between growth stages R2 to R4 when uptake is most rapid, and seed inoculation with rhizobia or foliar application of *B. japonicum* at a rate of 0.6 kg ha^{-1} in addition to N fertilization at < 34 kg ha^{-1} (https://extension.sdstate.edu/late-season-nitrogen-soybean; Wesley et al. 1998; Ulzen et al. 2016; Leggett et al. 2017; Wortmann et al. 2018). It is known that broadleaf weed management in soybean has been revised by the recent development of auxin tolerant soybean varieties in the United States. About 44 % of soybean acres in South Dakota are planted to genetically modified varieties that permit a postemergence application of auxin herbicide to soybean foliage for the purpose of controlling mostly broadleaf weeds resistant to ALS and glyphosate.

These studies investigated the efficacy of 2,4-D and dicamba-based herbicide program for broadleaf weed control, and its impact on soybean greenness, nodule number, nodule activity, yield, 100-seed weight, seed oil, and seed protein contents of Roundup Ready 2 Xtend and Enlist soybean varieties planted at three timings across three eastern South Dakota locations in 2019 and 2020 crop seasons. The goal of the study was to confirm the efficacy of auxin herbicide applications, and to determine planting date yield responses for auxin tolerant soybean varieties grown in South Dakota. The result of this study will provide South Dakota soybean growers the information required to effectively manage weeds and improve soybean productivity.

In the laboratory, the study examined how herbicide (glyphosate, dicamba), surfactant (Duce HSOC), adjuvant (AMS), and a mixture of herbicide + surfactant + adjuvant influenced the growth of *Bradyrhizobium japonicum* – the nitrogen fixing bacteria in soybean, when cultured in yeast extract media (YEM) broth and deionized water, respectively.

CHAPTER 2: HERBICIDE IMPACT ON GROWTH OF BRADYRHIZOBIUM JAPONICUM (USDA 110)

2.1. Introduction

Rhizobia are important group of rhizobacteria that live in the soil or in root nodules of legumes. In the root nodules, rhizobia form symbiotic association with the legume, capturing atmospheric nitrogen and making it available to the plant through a process called biological nitrogen fixation (Willey et al. 2011). Based on their growth in yeast extract media (YEM), they can be classified as either fast growing rhizobium or slow growing bradyrhizobium. The compatible rhizobacteria specie that nodulates the soybean crop is the *Bradyrhizobium japonicum,* and *approximately* 50 to 60 percent of soybean nitrogen requirement can be supplied by *B. japonicum* and *B. elkanii*. (Salvagiotti et al. 2008). It has been reported that nitrogen obtained through the biological nitrogen fixation process was more effective at promoting plant growth compared to chemical fertilizers (Esmailpour et al. 2012). The amount of nitrogen (N) supplied by the fixation process depends on the ability of rhizobia to effectively fix nitrogen, and on the ability of the plant to provide rhizobia with the energy required to drive the process. Several other factors like temperature, light, soil moisture, and soil pH have been reported to influence the growth of rhizobia (Dart 1977; Gibson 1977; Munns 1977; Gibson and Jordan 1983). The use of herbicide for weed control is essential for yield and profit maximization in large scale conventional cropping systems. In the United States, the most heavily used herbicides in soybean production include pendimethalin, metolachlor, imazethapyr, trifluralin, thifensulfuron, glyphosate (https://www.epa.gov/caddis-vol2/caddis-volume-2-sources-stressors-responses-herbicides), and recently, the auxin herbicides dicamba and 2,4-D (Heap I, 2021). Since the introduction of herbicide tolerant trait technology, crop acres sown to herbicide-resistant varieties globally has increased significantly, thus causing a corresponding rise in the use of their approved herbicides for weed control by crop producers. It is known that auxin herbicides (for example 2,4-D and dicamba), which mimics natural plant hormones, provide another mode of action to kill weeds in tolerant soybean varieties. Research suggests that natural plant hormones, including auxins, influence root nodule formation. In a laboratory study, soybean root was found to be sensitive to auxin, and showed reduced nodule development when a set of repressor auxin response factor (ARF) was silenced by overexpressing microRNA160 (Turner et al. 2013).

There are diverging reports on the effect of herbicides on soil microbial activities. While some studies found no adverse effect of herbicides on the growth of rhizobia [Cardina et al. 1986; Moorman, 1986; Mårtensson and Nilsson, 1989; Sprout et al. 1992; Yueh and Hensley, 1993; Gonzalez et al. 1996; Drouin et al. 2010], others reported rhizobial growth inhibition due to herbicide application (Clark and Mahanty, 1991; Mårtensson, 1992). The deleterious effect of 2,4-D herbicide on the growth of rhizobia (measured as changes in the optical density) is found in literature (Fabra de Peretti et al. 1987; Arias and Peretti, 1993). Decreased turbidity was observed when 2,4-D was applied to rhizobium sp. at a concentration of 1 mM at the beginning of the incubation. The application of glyphosate herbicide in glyphosate-resistant soybean have also been reported to have negative impacts on rhizobial activities (Zablotwicz and Reddy, 2004; Bohm et al. 2009). In midwestern U.S., up to 623 kg ha^{-1} yield increase was reported when new fields were planted to seeds inoculated with *Bradyrhizobium japonicum*

(Abendroth et al. 2006). A supplementary application of 1L/ha foliar rhizobia at critical growth stages (V3 and R2) of soybean is reported to increase yields up to 5% (https://onfarmresearch.sdsoybean.org/archives/ reports/primo-foliar-inoculant-high-yield-zone-location-1-55-bu-ac). However, a sole application of foliar rhizobia may not be economical to soybean growers, as yield increases have been modest. On the other hand, if rhizobia are foliar applied in combination with other management operations, for example a herbicide application, the net return may be profitable.

Since beneficial soil organisms like rhizobia are well known to help in legume nodulation, and excess auxin in soybean roots has been shown to inhibit nodulation, and not much is known on the effect of auxin herbicides, surfactants, and adjuvants on the activities of rhizobia, the question remains: can rhizobia still be viable if mixed with herbicide solutions? There is the need to further investigate the direct effect of herbicides and surfactants on the viability of rhizobia. This study investigated the effects of herbicides (glyphosate and dicamba), and surfactants/adjuvants [Duce HSOC (designed for use with herbicides that require an oil or surfactant to improve absorption and spreading); Ammonium sulfate (AMS; 21-0-0 spray grade) – a fertilizer additive added at 4 kg/379 L spray to overcome spray water antagonism of glyphosate] and herbicide mixtures containing dicamba + glyphosate + duce + AMS on the growth of *Bradyrhizobium japonicum* – USDA 110 strain when cultured in yeast extract mannitol (YEM) broth under laboratory conditions. The specific objectives of our study were to determine over a 3-day exposure:

1. the effect of herbicides (glyphosate and dicamba) on the growth of *Bradyrhizobium japonicum*, strain USDA 110.

2. the effect of surfactants/adjuvants (Duce and ammonium sulfate) on the growth of *Bradyrhizobium japonicum,* strain USDA 110.
3. herbicides combined with surfactant/adjuvant effects on the growth of *Bradyrhizobium japonicum,* strain USDA 110.

Research hypothesis

Null hypothesis (H_O): herbicides alone, surfactant/adjuvant alone, and a combination of herbicides with surfactant/adjuvant will not inhibit *Bradyrhizobium japonicum* growth.

Alternative Hypothesis (H_A): herbicides alone, surfactant/adjuvant alone, and a mixture of herbicides with surfactant/adjuvant will inhibit the growth of *Bradyrhizobium japonicum.*

2.2. Materials and methods

Location, treatments, and experimental design

The experiment was conducted in the laboratories of Agronomy, Horticulture and Plant Science Department (South Dakota State University, Brookings, SD). The experiment, which was performed under aseptic conditions at 30 ^{0}C, investigated how herbicides, surfactant and adjuvant influenced the growth of *Bradyrhizobium japonicum*, strain USDA 110 when treated cultures were inoculated in yeast extract mannitol (YEM) broth and deionized water. YEM broth is widely used for the cultivation of several agrobacterium species (Gram-negative bacteria), as well as the symbiotic nitrogen fixing microorganisms like Rhizobium species to make it suitable to produce legume inoculants. The broth contains mannitol as a carbon source and yeast extract as a source of both nitrogen and growth factor, and balances oxidation - reduction potential of medium in the range favorable for rhizobia and serves as hydrogen donor in respiratory process (Allen and Allen, 1950).

Treatments used in the study include the potassium salt formulation of 49 % acid equivalent glyphosate [Roundup PowerMAX®], diglycolamine salt formulation of 43 % acid equivalent dicamba [Xtendimax®], ammonium sulfate (AMS 21-0-0) fertilizer additive, Duce HSOC manufactured by Helena Agri Enterprises, and a combination of herbicide and surfactant/adjuvant [glyphosate + dicamba + AMS + Duce + Strike zone (a drift reduction and deposition aid)], each cultured in yeast extract mannitol (YEM) and deionized water media. The experimental design for the study was a 5 x 2 factorial, with three replicates and repeated in time. Positive and negative control groups were included with the treatments. The positive control which had 9 ml of yeast extract mannitol (YEM) broth and 1 ml of *B. japonicum* inoculant was used to compare the growth rate of *B. japonicum* in the treated cultures. The negative control contained 10 ml of sterile YEM broth and was used as a check to ensure there was no contamination in the setup. Optical density values obtained were analyzed using R-statistical software program. The runs across replicates were combined (n=6), and the treatment by time interaction was examined. Least significant difference values were calculated when the F value was found to be significant.

Glassware/equipment and reagents

All glassware was thoroughly washed with liquid detergent, rinsed, dried, and autoclaved (model SV120; manufacturer Steris; serial number 0114005-27) using the liquid 25 cycle for one hour. Sterile glassware was carefully set apart until needed. Work areas including benches and the fume hood was sterilized/disinfected with 70 % ethanol before starting the experiment. The materials used in this study are grouped into two categories: the glassware and equipment, and reagents. Glassware and equipment included the Erlenmeyer flask, conical flask, measuring cylinder, 500 ml glass jars/bottles, 55 ml test-tube with plastic stopper, micropipettes and micropipette tips, aluminum foil, masking tape, sterile toothpicks, spatula, stericup and steritop filtration system (0.22µm pore size rating), fume hood, autoclave, electronic weighing balance [Mettler Toledo, OH], pH meter [Mettler Toledo, OH], 28-degree orbit shaker [New Brunswick Scientific Excella E24 incubator shaker series], spectrophotometer cuvettes, and the ultrospec 10 cell density meter or spectrophotometer [Amersham Biosciences]. Reagents included glycerol stock of *B. japonicum,* strain USDA 110; chloramphenicol (20 mg/ml); yeast extract mannitol broth containing the following ingredients: yeast extract agar (0.4 g), D-mannitol (10 g), potassium phosphate (0.65 g), sodium chloride (0.1 g), and magnesium sulphate (0.2 g); and herbicide stock solution.

Ammonium sulfate fertilizer (AMS 21-0-0) is used in post-emergent glyphosate application at 4 kg / 379 L spray to enhance herbicide performance by increasing uptake. AMS helps to overcome hard water antagonism during glyphosate application (that is, it conditions hard water to help glyphosate work better). By preventing glyphosate from binding to calcium, magnesium or iron in the water, or dirt on the surface of the leaf, AMS forms an $NH4^{+}$ glyphosate complex that enters the waxy layer on the leaf surface of target plants and kills it (http://www.ianrpubs. unl.edu/sendIt/ec130.pdf). Duce HSOC (Helena Agri-enterprises) is a blend of non-ionic surfactant and methylated seed oil, a kind of fatty acid from seed oil esterified with methyl alcohol (Miller and Westra, 1996; Young et al. 2016). Duce meets herbicide label requirement for high surfactant oil concentrate; it is compatible with multiple tank-mix partners and also improves herbicide absorption and spreading (https:// helenaagri.com/products/nonionic-surfact ants/duce/). Strike Zone® LC (Helena Agri-enterprises) is a drift reduction and deposition aid containing 95 % polyethoxylated hydroxyl aliphatics and carbohydrate polymers. This adjuvant mixes easily with tank-mix partners, improves drift control and deposition regardless of the nozzle chosen for the application, and also reduces spray droplet bounce and evaporation to enhance the absorption of active ingredients on target weeds (https://helenaagri.com/products/ drift-reduction-and-deposition-aids/strike-zone-lc/).

Pre-bacterial culturing

The liquid media (YEM broth) was prepared prior to the culturing of *B. japonicum*. In preparing the YEM broth, 1L of MilliQ water that had been purified using resin filters and deionized to a high degree by a water purification system was measured into 1L Erlenmeyer flask, after which 0.65 g of potassium phosphate [Amresco biochemicals, Solon, Ohio], 0.1 g sodium chloride [Sigma Aldrich, St. Louis, MO], 0.2 g magnesium sulfate [VWR chemicals, Radnor, PA], 0.4 g yeast extract agar [Sigma Aldrich, St. Louis, MO], and 10.0 g D-mannitol [Sigma Aldrich, St. Louis, MO] were measured and added into the flask. The broth was mixed thoroughly using the Fisher Scientific Isotemp equipment and the pH adjusted to 6.8. To adjust the pH of the YEM broth solution, a pH probe (Mettler Toledo, OH) was inserted into the media, and one to two drops of concentrated HCL or NaOH was pipetted into the solution to either reduce or increase the pH of the YEM broth until a stable pH of 6.8 was attained as *Bradyrhizobium japonicum* prefer a neutral to slightly basic environment for optimal growth (Vincent 1970; Somasegaran and Hoben 1994). The broth was thereafter filter sterilized using the vacuum stericup and steritop system and stored in the refrigerator until needed.

Culturing of Bradyrhizobium japonicum

B. japonicum, strain USDA 110 was cultured under a sterile hood. A 50-ml aliquot of yeast extract mannitol (YEM) broth was measured into a conical flask and 50 µl of chloramphenicol was added to the broth in the flask. Using a sterile toothpick, a fraction of glycerol stock of *B. japonicum* USDA 110 obtained from Subramanian laboratory at South Dakota State University was added to the broth. The bacteria culture was then incubated in a 28-degree rotary shaker at 30 ^{o}C for 3 days. Optical density readings at 650 nm wavelength (OD_{650}) were taken using the spectrophotometer at 24-, 48-, and 72-hours incubation period. Once the bacteria culture attained an OD_{650} value in the range 0.20 to 0.30 (this was the starting OD range used for the entire experiment), and this occurred after 72 hours incubation, the culture was removed from the incubator and kept in the refrigerator until needed.

Herbicide stock preparation

Herbicide stock solutions were prepared in the CLAY herbicide degradation laboratory, McFadden Biostress Laboratory, South Dakota state University. The herbicide laboratory concentrations were derived using the method given by Fletcher (1956). Herbicide formulations containing potassium salt of glyphosate [2.5 ml of formulation containing 1.4 g a.e. of glyphosate (Bayer)] and diglycolamine salt of dicamba [1.4 ml of formulation containing 0.4 g a.e. of dicamba (Bayer)] were each measured into a 100 ml volumetric flask after which milliQ water was added into the flask to make a 100 ml of individual herbicide stock solution. To make the adjuvant stock solution, 2 g of ammonium sulfate salt was measured into a 100 ml volumetric flask and milliQ water added to make 100 ml stock solution. For the surfactant stock solution, 7.5 ml of Duce HSOC was measured into a 100 ml volumetric flask and milliQ water added into the flask to make 100 ml stock. The stock solution that contained a mixture of herbicide, adjuvant and surfactants was made by weighing 2.5 ml glyphosate + 1.4 ml dicamba + 2.0 g dry ammonium sulfate + 7.5 ml Duce HSOC + 0.125 ml Strike zone, into a 100 ml volumetric flask and the solution made up to 100 ml by adding milliQ water (Table 2.1). All herbicide stock solutions were kept in a refrigerator maintained at 4.4 ^{0}C temperature until needed.

Culturing of samples

Bradyrhizobium japonicum (USDA 110) cultures were treated with glyphosate alone, dicamba alone, adjuvant (AMS) alone, surfactant (Duce HSOC) alone, and a herbicide mixture that contained glyphosate + dicamba + AMS + Duce HSOC+ Strike Zone). A 1-ml aliquot of *Bradyrhizobium japonicum* (USDA 110) was added into a sterile test tube containing 7 ml of yeast extract media (YEM) broth or DI water, and 2 ml of herbicide alone, or adjuvant alone, or surfactant alone, and herbicide mixture containing glyphosate, AMS, Duce HSOC, and Strike Zone. The treated cultures were then incubated in a 28-degree orbit shaker at 30 ^{0}C for 3 days (72- hours).

Measurement of optical density in treated culture samples

After 0, 24-, 48-, and 72-hours incubation period, respectively, A 1-ml aliquot of the treated bacteria culture was pipetted into a spectrophotometer cuvette for optical density measurement. The growth of *Bradyrhizobium japonicum* (USDA 110 strain) was measured as light absorbance at 650 nm wavelength (OD_{650}) using a spectrophotometer [Ultrospec 10, Amershan Biosciences] calibrated with an uninoculated untreated blank that contained sterile YEM broth alone (Carpenter 1977; Cardina et al. 1986; Gonzalez et al. 1996).

Figure 2.1. Some equipment used in the laboratory for the in-vitro culturing and determination of the growth rate of B. japonicum (USDA 110) by optical density measurements at 650 nm wavelength.

(A) fumehood; (B) spectrophotometer; (C) filter sterilization of water using steritop and stericup system; (D) culture incubation in 28-degree rotatory shaker at 30 ^{0}C to stimulate growth of B. japonicum (USDA 110 strain).

2.3. Results

Table 2.1. shows the laboratory concentration of herbicide stock, electrical conductivity, and pH of treated culture at day 0 to 3. The optical densities of treatment [measured at 650 nm wavelength (OD_{650})] at the start of the experiment (0-hour incubation) was in the range of 0.20 to 0.30. The positive control group which consisted of an aliquot of *B. japonicum*, and yeast extract media (YEM) had an optical density value that increased over 72-hours incubation time. An interaction was observed between culture media and incubation time ($p \leq 0.01$) for all treatments [herbicide (glyphosate, dicamba), adjuvant (AMS), surfactant (Duce HSOC), and herbicide mixture (glyphosate + dicamba + AMS + Duce HSOC + Strike Zone)] (Table 2.2).

A reduction in the growth rate of *Bradyrhizobium japonicum* was observed over 72-hours incubation time when bacteria cultures in yeast extract media (YEM) and deionized (DI) water were treated with glyphosate and dicamba herbicides (Figure 2.2). Comparing the effect of the herbicides with the positive control group that had no herbicide, and over a 3-day incubation period, glyphosate in YEM and DI water reduced the optical density value of bacteria culture by 65 % and 98 %, respectively, whereas dicamba reduced optical density of *B. japonicum* culture by 64 % and 43 % in YEM and DI water media, respectively (Figure 2.2).

Also, when compared to the positive control group, ammonium sulfate (AMS), both at low (0.2 mg/ml) and high (20 mg/ml) concentrations had a negative impact on the growth of *B. japonicum* in YEM and DI water media over the 72-hours incubation time (Figure 2.3). At a lower concentration of AMS (0.2 mg/ml), bacteria cultures in DI water showed no growth and had an optical density reading of zero throughout the incubation time (Figure 2.3).

The surfactant Duce HSOC, depending on the concentration and growth media (YEM or DI water), positively influenced the growth of *B. japonicum*. Bacteria cultures treated with low concentration (0.75 ml) of Duce HSOC and grown in YEM media had optical densities that were 117%, 115%, and 48% more than the positive control at 24-, 48-, and 72-hours incubation time, respectively (Figure 2.4), whereas bacteria cultures treated with a higher Duce concentration (7.5 ml) and grown in deionized water (DI) media increased optical density value by 91% at the third day (72-hours) of incubation (Figure 2.4).

Herbmix containing 2.5 ml glyphosate + 1.4 ml dicamba + 2.0 g AMS + 7.5 ml Duce HSOC + 0.13 ml Strike Zone LLC enhanced *B. japonicum's* growth in deionized (DI) water only. At 24-, 48-, and 72-hours of incubation, the optical density value from bacteria (*B. japonicum*) culture was 22%, 29%, and 25% higher than what was found in the positive control, respectively, at $p \leq 0.01$ (Figure 2.5).

Table 2.1. Herbicide, adjuvant, surfactants and herbicide mixture concentrations, electrical conductivity (EC), and pH of cultures after 3 days incubation period.

Treatment	Stock solution (ml/50ml H_2O)	laboratory concentration of solution (a.e./g/ml)	Electrical conductivity (mS/cm)	pH at start of experiment (Day 0)	pH at end of experiment (Day 3)
Glyphosate [K salt formulation (Bayer)]	2.50	1.40	5.80	4.27	4.00
Dicamba [DGA salt formulation (Bayer)][a]	1.40	0.40	2.20	5.65	5.16
Ammonium sulfate [Winfield United]	2.00	2.0	28.00	5.38	3.13
Ammonium sulfate [Winfield United]	0.20	0.20	18.10	5.12	3.07
Duce HSOC [Helena Agri-sciences]	7.50	7.50	0.55	7.48	6.75
Duce HSOC [Helena Agri-sciences]	0.75	0.75	0.55	7.48	5.39
Strike zone [Helena Agri-sciences][b]	0.125	0.125	0.61	7.96	ND[c]
Herbicide + adjuvant + surfactant		12.38	32.50	6.98	6.51

[a]Dicamba [DGA salt formulation (Bayer) – DGA is the diglycolamide formulation used.

[b]Strike zone (manufactured by Helena Agri-enterprises is a drift reduction and deposition aid that contains 95% polyethoxylated hydroxyl aliphatics and carbohydrate polymers) was added to the herbicide mixture, but not tested alone.

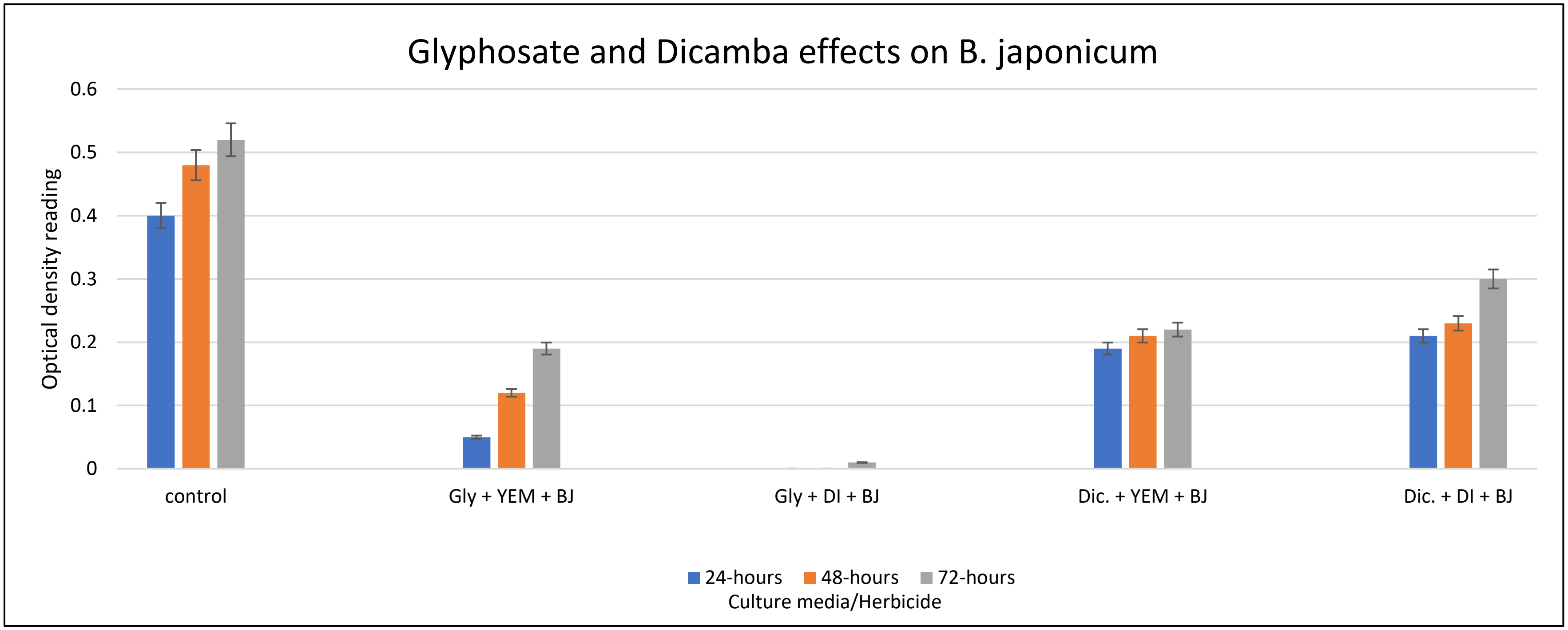
[c]ND: not determined

Table 2.2. Analysis of variance showing mean of treatment effect on Bradyrhizobium japonicum's (USDA 110) growth in yeast extract media (YEM) and deionized (DI) water over 3-day incubation period.

Source of variation	DF	glyphosate	dicamba	AMS 0.2	AMS 2.0	Duce 0.75	Duce 7.5	Herbmix[a]
factor A (culture media)[b]	2	0.85	0.30	0.18	0.14	0.24	0.34	0.04
factor B (incubation time)[c]	2	0.03	0.02	0.02	0.00	0.02	0.01	0.01
factor A x factor B	4	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Residuals	36							
standard error A (culture media)		0.01	0.01	0.00	0.00	0.01	0.01	0.01
standard error B (incubation time)		0.01	0.01	0.00	0.00	0.01	0.01	0.01
standard error A x B		0.01	0.01	0.01	0.01	0.01	0.02	0.01
R square		0.99	0.98	1.00	1.00	0.99	0.99	0.91
LSD (0.05)		0.02	0.03	0.02	0.01	0.03	0.05	0.02

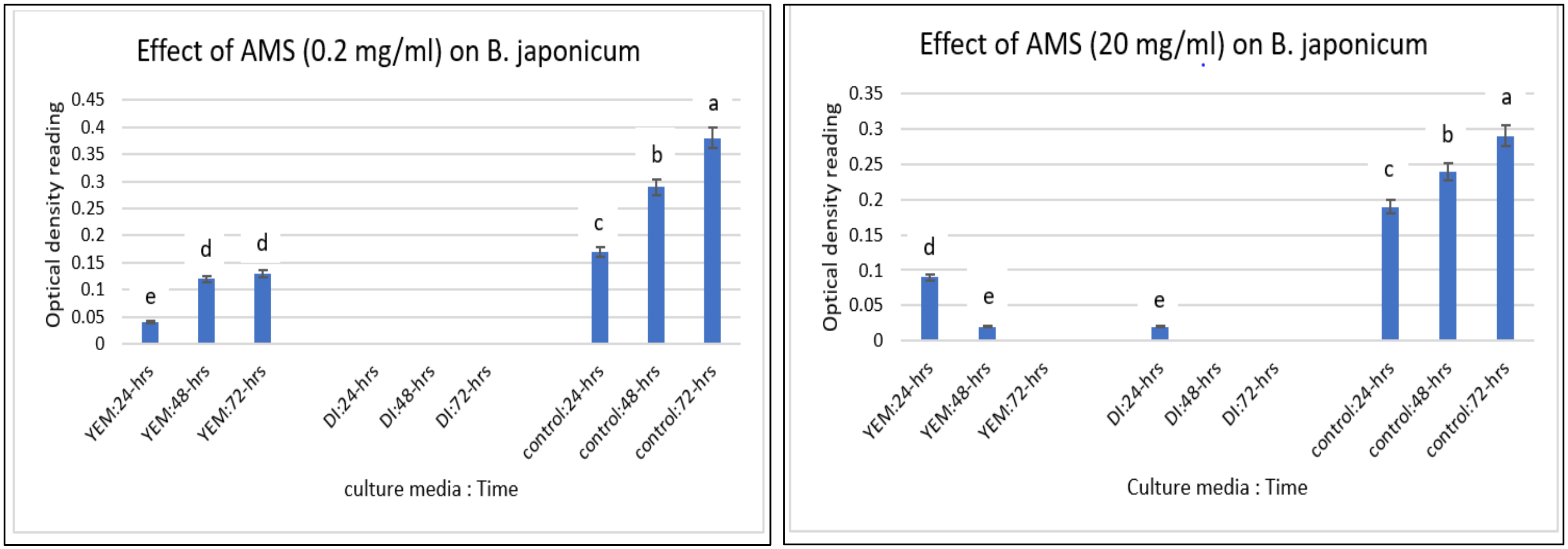
[*]Significant at probability level ($\alpha \leq 0.05$).
[a]Herbmix contained a mixture of glyphosate (2.5 ml), dicamba (1.4 ml), ammonium sulfate (2.0 g), Duce HSOC (7.5 ml), and Strike Zone (0.13 ml).
[b]Culture media 1: yeast extract mannitol (YEM) broth + herbicide or adjuvant or surfactant or herbmix + *Bradyrhizobium japonicum* (USDA 110).
[b]Culture media 2: deionized water + herbicide or adjuvant or surfactant or herbmix + *Bradyrhizobium japonicum* (USDA 110).
[b]Control: yeast extract mannitol (YEM) broth + *Bradyrhizobium japonicum* (USDA 110).
[c]Incubation time – 24-hours, 48-hours, and 72-hours.

Figure 2.2. Effect of glyphosate and dicamba herbicides on the growth of Bradyrhizobium japonicum (USDA 110) in yeast extract media (YEM) and deionized (DI) water media over 3-day incubation period.



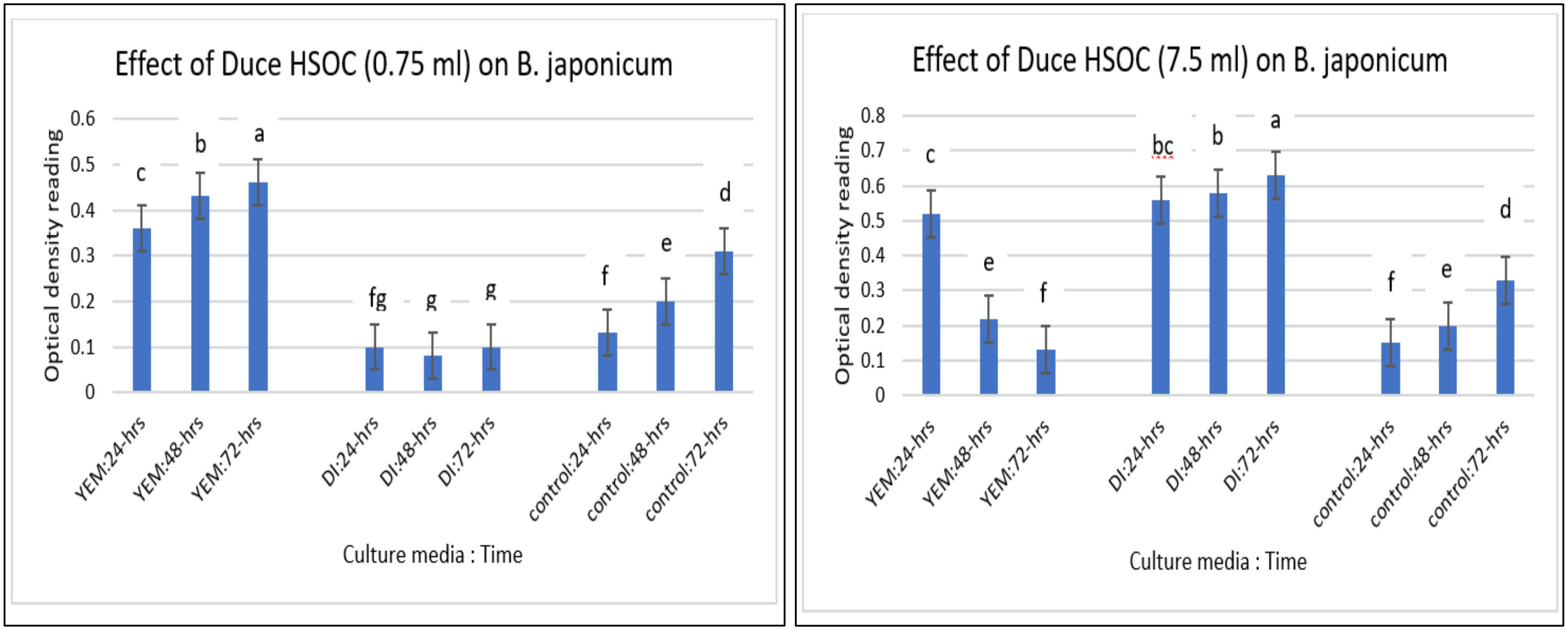
* Optical density (OD) was measured at 650 nm wavelength in a spectrophotometer.
* Control media had yeast extract mannitol broth (9 ml) and glycerol stock (1 ml) of *Bradyrhizobium japonicum* (USDA 110).
* Each treatment had 2 ml glyphosate or dicamba + 7 ml yeast extract mannitol broth or deionized water + 1 ml Bradyrhizobium (USDA 110).
* Incubation time: 24-,48-, and 72-hours.
* Bars indicate standard deviation from treatment mean.

Figure 2.3. Effect of adjuvant [ammonium sulfate fertilizer (AMS 20-0-0)] on the growth of Bradyrhizobium japonicum (USDA 110) in yeast extract media (YEM) and deionized (DI) water media over 3-day incubation period.



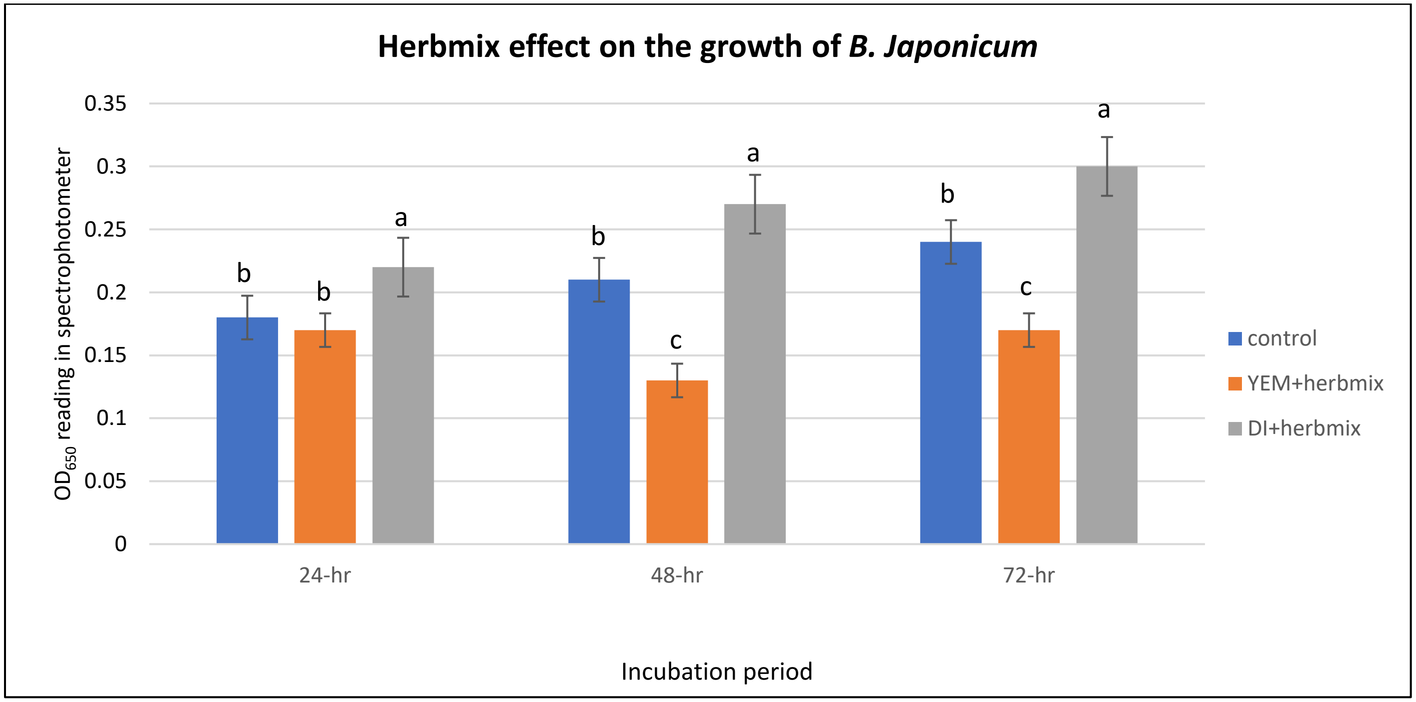
* Optical density (OD) reading in spectrophotometer was at 650 nm wavelength.
* Control media had yeast extract mannitol broth (9 ml) and glycerol stock (1 ml) of *Bradyrhizobium japonicum* (USDA 110).
* YEM: yeast extract mannitol broth
* DI: deionized water.
* Incubation time: 24-,48-, and 72-hours.
* Bars indicate standard error of treatment mean. Different letters above the error bars are significantly different from each other at 0.05 probability level.

Figure 2.4. Impact of surfactant [Duce HSOC] on growth of Bradyrhizobium japonicum (USDA 110) in yeast extract media (YEM) and deionized (DI) water media over 3-day incubation period.



* Optical density (OD) reading in spectrophotometer was at 650 nm wavelength.
* Control media had yeast extract mannitol broth (9 ml) and glycerol stock (1 ml) of *Bradyrhizobium japonicum* (USDA 110).
* YEM: yeast extract mannitol broth
* DI: deionized water.
* Incubation time: 24-,48-, and 72-hours.
* Bars indicate standard error of treatment mean. Different letters above the error bars are significantly different from each other at 0.05 probability level.

Figure 2.5. Effect of Herbicide mixture on the growth of Bradyrhizobium japonicum (USDA 110) in yeast extract media (YEM) and deionized (DI) water media over 3-day incubation period.



* Herbmix contained a mixture of glyphosate (2.5 ml), dicamba (1.4 ml), ammonium sulfate (2.0 g), Duce HSOC (7.5 ml), and Strike Zone (0.125 ml).
* OD_{650} - optical density reading in spectrophotometer at 650 nm wavelength.
* Control contained 9 ml of yeast extract mannitol (YEM) broth and 1ml glycerol stock of *Bradyrhizobium japonicum* (USDA 110).
* YEM: yeast extract mannitol broth; DI: deionized water.
* Incubation time: 24-,48-, and 72-hours.
* Bars indicate standard error of treatment mean. Different letters above the error bars are significantly different from each other at 0.05 probability level.

2.4. Discussion

Bradyrhizobium japonicum's growth over an incubation period of 72-hours was influenced by the additions to the culture media. In deionized water, all the additions except Duce HSOC at a higher concentration (7.5 mg/ml) and herbmix reduced *Bradyrhizobium japonicum* growth compared to the untreated control. Changes in the pH of the media environment for the duration of the study may be responsible for the increase or decrease in the growth rate of *Bradyrhizobium japonicum*. The optimum pH suitable for the culturing of *B. japonicum* is 6.8 (Vincent 1970; Somasegaran and Hoben, 1994). Our data supports previous literature that herbicide, adjuvant, and surfactant influenced rhizobial growth in agitated liquid media (Mallik and Tesfai, 1985; Schuls et al. 1985; Eberbach and Douglas, 1989; Moorman et al. 1992; Singh and Wright 2002; Santos et al. 2005).

Past studies that investigated the effect of herbicides on the growth of rhizobia species obtained different results. Bentazon was found to have an inhibitory effect on the growth of *R. trifolii* when applied at the recommended rate (2.50 $\mu g\ g^{-1}$ soil), and ten times above the recommended rate (25.0 $\mu g\ g^{-1}$ soil), as well as when grown on agar plates, but not in broth cultures (Clark and Mahanty, 1991). Results presented in Figure 2.2. showed a growth retarding effect of glyphosate on soybean N- fixing bacteria (*Bradyrhizobium japonicum* – USDA 110 strain) cultured in yeast extract mannitol broth and deionized water, when compared to the control.

Glyphosate inhibits the synthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) in microorganisms (Jaworski, 1972; Fisher et al. 1986). Specifically, the herbicide inhibits 5-enolpyruvylshikimic acid-3-phosphate synthase

(EPSPS) enzyme which catalyzes the condensation of shikimic acid and phosphoenolpyruvate (Steinrucken and Amrhein, 1980). Inhibition of the shikimic acid pathway by glyphosate results in the accumulation of shikimic acid and/or certain hydroxybenzoic acids such as protocatechuic acid (PCA) and gallic acid (GA) in *B. japonicum* (Moorman et al. 1992; Hernandez et al. 1999). The PCA and GA are phenolic acids with antibacterial properties (www.ncbi.nlm.nih.gov/pmc/articles/PMC8216263/). Hence, the likely reasons for the growth inhibition of *B. japonicum* by glyphosate may be attributed to (i) the inability of the organism to synthesize aromatic amino acids; (ii) an energy drain on the organism resulting from adenosine triphosphate and phosphoenolpyruvate (PEP) spent in the accumulation of shikimate, 3-deoxy-D-arabino-heptulose-7-phosphate (DAHP), and hydroxybenzoic acids; and (iii) toxicity of accumulated intermediates of the shikimic acid pathway (Fisher et al., 1986). Also, it is known that *B. japonicum* requires an optimum pH of 6.8 to maintain its metabolic activities (growth, respiration, and reproduction) (Somasegaran and Hoben, 1994). Therefore, the low pH (4.0) obtained in cultures treated with glyphosate at the end of the experiment may be responsible for the reduced growth observed.

2,4-D, although not tested in the current study, has been reported to have an inhibitory effect on soil bacteria activity (Arias and Fabra, 1993; Balagué et al. 2001; Fabra et al. 1997; Fanous et al. 2007; and Jofré et al. 1996). However, soil bacteria like Pseudomonas, Alcaligenes, Ralstonia, Delftia, Arthrobacter, and Burkholderia were found to breakdown 2,4-D molecules. These organisms used the herbicide molecules as an energy source for their growth (Marron et al. 2006; Baelum et al. 2010; Sandoval-Carrasco et al. 2013; Singh and Singh, 2014). Furthermore, microbial strains, including

Pseudomonas fluorescens bacteria, have been reported to be able to mineralize dicamba under aerobic conditions (Smith, 1973; Smith and Cullimore, 1975; Krueger et al. 1989; and Krueger et al. 1991) and at an optimal temperature of 30 ^{0}C (Speed, 1990). In our study, the optical density value of bacteria culture treated with dicamba in YEM and deionized (DI) water was lower than that of the positive control at 30 ^{0}C. A possible reason for the reduced growth rate of *B. japonicum* compared to the untreated control may be because of the inability of the bacteria to breakdown the dicamba molecules present in the media solution. Over time, nutrient sources present in YEM, and DI water media got depleted and bacterial metabolic activities declined as indicated by the low optical densities (OD_{650}) measured in the dicamba-treated culture compared to the positive control.

The pH of the media solution at the end of the experiment (72-hours) may be another reason for the reduced growth rate of *B. japonicum*. The dicamba-treated bacteria culture, with a pH of 5.7 at the start of the experiment, had a pH of 5.2 at the end of 72-hours incubation period and this was below the optimum pH (6.8) needed to maintain the bacteria's metabolic activities (Somasegaran and Hoben, 1994). This implies that the growth of *Bradyrhizobium japonicum* will be inhibited in a strongly acidic medium. The biodegradation of dicamba herbicide has been found to reduce at pH less than 6 (Krueger,1989). In our study, the low pH or acidic environment obtained at the end of the incubation period (pH = 5.2) may be the most likely cause of the low optical density values found in bacteria cultures treated with dicamba herbicide. Therefore, it is possible that the acidic growth media caused a reduction in the metabolic activities (respiration, growth, and reproduction) of *Bradyrhizobium japonicum*.

Past studies have reported the negative effects of adjuvant and surfactants on the development of soil microorganisms (Katan and Eshel, 1973; Johal and Rahe, 1984; Sawada et al. 1988; Berner et al. 1991). Some special class of adjuvants/surfactants have the tendency to increase herbicide effects by decreasing or removing crop wax layer (Kissmann, 1997). By reducing surface tension, the penetration of herbicide is facilitated, and bacteria become more sensitive to their action (Malkones, 2000). It is hard to come by documented studies that report on the impact of ammonium sulfate (AMS) on *B. japonicum* growth in liquid media. Our study found that ammonium sulfate fertilizer [AMS; 28-0-0] typically used to enhance herbicide efficacy by preventing cations in hard water from binding to glyphosate, suppressed the growth of *Bradyrhizobium japonicum* in YEM and deionized water throughout the incubation time (Table 2.3.1). A likely explanation for the inhibitory effect of AMS on the growth of *B. japonicum* could be because of the low pH of the culture solution. At the end of the experiment, AMS-treated cultures at high (20 mg/ml) and low (0.2 mg/ml) levels had pH values of 3.07 and 3.13, respectively, thus indicating a strongly acidic environment that inhibited bacterial growth since *B. japonicum* is known to thrive in environments with an optimum pH of 6.8 (Somasegaran and Hoben, 1994).

According to Santos et al. 2005, the surfactant ethylamine used in commercial herbicide formulations (for example Roundup Transorb®) can affect beneficial microorganisms including some strains of Bradyrhizobium. Our study which examined surfactant impact on the growth of *B. japonicum* saw some measure of growth increase when bacterial cultures were treated with Duce high surfactant oil concentrate at low concentration (0.75 mg/ml) and grown in yeast extract mannitol broth, and at high concentration (7.5 mg/ml) and cultured in deionized water. Duce HSOC contains a blend of methylated seed oil (a kind of fatty acid esterified with methyl alcohol) and non-ionic surfactants. Fatty acids or lipid molecules are reported to serve as a source of nutrient, storage form of carbon, energy storage molecules, or structural components of membranes and hormones (OpenStax, 2021). Also, according to Bergey et al. (1923), the rhizobia group of bacteria can metabolize glyoxylate, which is a degradation product of fatty acids. Therefore, it is possible that the bacteria (*B. japonicum*) in our study expended its energy degrading the surfactant Duce as it may have used the fatty acid present in Duce as a nutrient and energy source to sustain its metabolic activities throughout the incubation period (24-, 48-, and 72-hours).

In the present study, higher optical density (OD_{650}) values obtained in culture solutions treated with Duce HSOC surfactant, compared to the positive control, is an indication of continued bacterial growth after 24-, 48-, and 72-hours of incubation. In a recent study, peanut Bradyrhizobium was found to have metabolized Tween 40 and Tween 80, both surfactants with fatty acid structures (Li et al. 2019). Their findings suggest that *Bradyrhizobium japonicum* may have metabolized Duce HSOC (a surfactant with fatty acid structure) and used the fatty acid molecules as energy source for growth when cultures were treated at lower concentration (0.75 mg/ml) and grown in yeast extract media or at higher concentration (7.5 mg/ml) and grown in deionized water. Since the pH of the Duce-treated culture solutions were stable and within the optimum value of 6.8, the metabolic activities of *Bradyrhizobium japonicum*, including its respiration, growth, and reproduction, could have been enhanced. This may explain the high OD_{650} readings observed for the Duce-treated bacteria cultures.

Some herbicide mixtures have been reported to have no inhibitory effect on the growth of bacteria (Flores and Barbachano, 1992). In the present study, *B. japonicum* (USDA 110 strain) cultures treated with a mixture of herbicide and adjuvant and surfactants (glyphosate + dicamba + AMS + duce + strike zone) increased the growth rate of Bradyrhizobia in deionized water but not in yeast extract media when compared to the untreated control in our study (Figure 2.5). Strike zone LC (manufactured by Helena Agri-enterprises) contain 95% polyethoxylated hydroxyl aliphatics and carbohydrate polymers, whereas Duce HSOC (manufactured by Helena Agri-enterprises), contain a blend of methylated seed oil (a kind of fatty acid esterified with methyl alcohol). The increase in the growth of *B. japonicum* observed in this study may be due to the bacteria metabolizing Strike Zone and/or Duce HSOC in the herbicide mixture as a nutrient and energy source for sustained metabolic activities. However, since the surfactant Strike Zone was not tested alone, it is unknown if the growth of Bradyrhizobium in cultures treated with the herbicide mixture was caused by either Strike Zone or Duce HSOC or both. The pH of the herbicide mixture after 72-hours incubation was 6.51 and is suitable for the growth of *Bradyrhizobium japonicum*.

In summary, the present study saw a reduction in the growth rate of *B. japonicum* when the bacteria cultures were treated with herbicide (glyphosate, dicamba), and adjuvant (AMS), and grown in YEM and DI water. The concentration of Duce HSOC (0.75 ml in YEM or 7.5 ml in DI water) enhanced the growth of bacteria, whereas higher optical densities were obtained for the bacteria cultures grown in deionized water and treated with herbicide mixtures containing 2.5 ml glyphosate + 1.4 ml dicamba + 2.0 g AMS + 7.5 ml Duce HSOC + 0.13 ml Strike Zone LLC compared to the positive control.

Throughout the incubation time of 72-hours, the pH of the non-treated control remained at a range of 6.8 ± 0.2. Therefore, the low optical density values found (which indicates reduced bacterial growth) in cultures treated with glyphosate, dicamba, and AMS were most likely due to low pH of these solutions.

Any factor that affects the cell division process of rhizobia bacteria will also impact nodulation, nitrogen fixation and ultimately yield in legume crops. From the current research findings, the surfactant type, herbicide type, and the concentration used for herbicide application can either positively or negatively impact the growth of *Bradyrhizobium japonicum* in liquid (YEM or DI) media culture. Therefore, there is the need for further studies into the effect of other groups of adjuvant, surfactant, herbicide, and herbicide mixtures on nitrogen fixing bacteria as little is known about their effects on *Bradyrhizobium japonicum*.

2.5. Conclusion

The adjuvant (AMS; 20-0-0 grade) greatly reduced the growth of *B. japonicum* in agitated liquid (YEM or DI) media at low (0.2 mg/ml) and high (20 mg/ml) concentrations throughout the incubation time (24-, 48- and 72-hours). Compared to the control, *B. japonicum's* growth rate increased when the bacteria were cultured in YEM media and treated with 0.75 ml Duce HSOC surfactant, whereas an increase in growth was observed when bacteria was grown in deionized water media and treated with high levels of Duce HSOC (7.5 ml). Glyphosate and dicamba herbicides reduced the growth rate of bacteria in agitated liquid media, but when mixed with adjuvant/surfactants, the herbicide mixture in deionized water did not inhibit *B. japonicum's* growth as the optical density value of bacteria were similar to the positive control at 72-hours incubation time.

Nitrification or fermentation that occurred in the treated culture solutions over 72-hours incubation period may reduce pH of the solutions, and the reduction in the growth of *B. japonicum* may be attributed to this low pH.

Based on the results of our findings, we can conclude that rhizobia inoculant may be mixed with some herbicide combinations and not be adversely impacted. However, if rhizobia are to be mixed with herbicide for foliar application on the field, those mixtures that have no impact or a positive impact would be preferable, rather than those that inhibit the growth of bacteria.

2.6. Recommendation

An ideal condition for the survival and growth of the Bradyrhizobium species must be ensured, as well as the use of aseptic techniques throughout the experiment period to ascertain that the changes in the growth pattern of the bacteria was due to the effect of the formulated herbicides, surfactants and adjuvant and not as a result of external contamination or protocol error. Since little is known about the effect of surfactant like Strike Zone on rhizobia, it is recommended that additional testing be carried out to validate the results obtained in this study.

CHAPTER 3: EVALUATING 2,4-D AND DICAMBA BASED HERBICIDE PROGRAM FOR WEED CONTROL IN AUXIN TOLERANT SOYBEAN

3.1. Introduction

Weeds pose a serious threat to successful soybean production worldwide as they can reduce crop yield if left uncontrolled, particularly during the critical weed free period of soybean. Tillage program, crop rotation practices and management inputs can influence weed species and densities present (Kegode et al. 1999). In large scale conventional farming, herbicides are relied upon for weed control. The repeated use of herbicides with the same mode of action increases weed selection pressure and contributes to the development of herbicide-resistant weed biotypes (Norsworthy et al. 2012; William et al. 2012). About 522 unique cases of herbicide-resistant weeds (species x location of action) have been reported globally (Heap, 2021), with several weed species found to be resistant to 23 of the 26 known herbicide locations of action and to 167 different herbicides (Heap, 2021).

According to the international survey of herbicide resistant weeds, the most widespread herbicide resistant weed of South Dakota is kochia (*Bassia scoparia*) (Heap, 2021). The ALSresistant kochia which infested wheat and soybean was first reported in South Dakota in 1988 (Wolf et al. 2000, Heap, 2021). A glyphosate-resistant kochia biotype was later reported in corn and soybean fields in 2009 (Heap, 2021). The herbicide resistant weeds found in soybean fields and their reported year in South Dakota include glyphosate resistant kochia (*Bassia scoparia* –2009), common ragweed (*Ambrosia artemisiifolia* –2007), tall or common waterhemp (*Amaranthus tuberculatus* (=*A. rudis*) –

2010), and horseweed or marestail (*Conyza canadensis* –2010) (Heap, 2021). The presence of herbicide resistant weeds in soybean fields has increased grower's production costs as several herbicide applications or an investment in herbicide tank-mix partners are needed to prevent weed stress to crops as this can lead to yield reductions if left uncontrolled.

Biotechnology companies continue to seek new ways to combat the rise of "super weeds" by providing innovative technology to manage these resistant weeds in fields. The recent development and launching of auxin-tolerant crop traits by Monsanto [now Bayer (Roundup Ready 2 Xtend)] and Dow AgroSciences (Enlist E3) have provided another mode of action to control broadleaf weeds, including the glyphosate-resistant weeds found in soybean fields. Auxin-tolerant soybean varieties were developed using high-tech methods (genetic engineering) to insert desirable genes (herbicide-resistance) from one species (plant, animal, or micro-organism) into the genome of soybean. It is the inserted genes that confer the herbicide resistant trait to the crop (https://www.ecofarmingdaily.com/grow-crops/grow-soybeans/ choosing-soybean-seeds/are-gmo-soybeans-the-way-to go/#:~:text=The%20major%20development%20in%20soybean%20agriculture%20over%20the,now%20planted%20on%2090%25%20of%20U.S.%20soybean%20acres)(https://www.croplife.com/crop-inputs/herbicides/dow-agrosciences-anno unces-new-enlist-e3-soybean-brand/).

3.1.1. 2,4-D based herbicide program

Dow Agrosciences, now Corteva, launched the Enlist E3™ soybean with multiple herbicide tolerant crop traits. This technology was developed to maximize weed control flexi-bility by providing tolerance to 2,4-D choline, glyphosate and glufosinate herbicides

(Simpson et al. 2014). By using the Enlist weed control system, soybean growers can make applications of approved Enlist herbicides (Enlist One and Enlist Duo) in-season to soybean for an effective control of both monocot and dicot weeds, including glyphosate-resistant waterhemp, horseweed, and kochia. The Enlist Duo® herbicide with Colex-D™ technology combines 2,4-D choline and glyphosate to control weeds, whereas the Enlist One™ herbicide offers an additional tank-mix flexibility with glufosinate to provide weed control in Enlist E3 soybeans.

The Enlist weed control program has some merits which include a near-zero volatility, reduced physical drift potential, better handling characteristics, and a longer application window through R2 or the full flowering growth stage. The three herbicide tolerances of Enlist E3 soybeans, 2,4-D, glyphosate, and glufosinate, are used in a variety of cropping situations (Anonymous 2014a, 2014b). 2,4-D (WSSA group 4) herbicide is effective against a wide range of broadleaf weeds. The herbicide imitates the natural hormone indole 3-acetic acid present in plants (Grossman 2010; Shaner 2014). In multiple environments, 2,4-D herbicides have been found to effectively control most dicot weeds including the Amaranthus species (Shaner 2014).

2,4-D choline herbicides are currently labelled for pre and postemergence applications in Enlist E3™ (2,4-D tolerant) soybeans (Anonymous 2017a and b). The 2,4-D choline formulation has a reduced drift potential from intended target during applications than 2,4-D ester and salt formulations. Susceptible plants show symptoms similar to those of other auxin herbicides when exposed to 2,4-D applications and symptoms include leaf cupping and curling, stem elongation and epinasty within hours after application. However, susceptible plants completely die in 3 to 5 weeks of exposure to the herbicide (Shaner 2014). Therefore, the use of auxin (2,4-D) tolerant soybean provides growers more flexibility for controlling glyphosate-resistant broadleaf weeds common in fields (Johnson et al. 2012).

Glyphosate, another herbicide used with the Enlist technology is a broad-spectrum, non-selective herbicide developed in the 1970s (Davies, 2011; Heap, 2021). Glyphosate herbicide is commonly used in the control of many dicotyledonous and monocotyledonous weeds in both cropping and non-cropping situations (Anonymous 2014b). In recent years, glyphosate applications over expansive croplands resulted in the selection of glyphosate-resistant weed biotypes (Franz et al. 1997; Heap and Duke, 2018). However, glyphosate, applied post emergent, continues to control many species and is relatively inexpensive and therefore is a go-to-product for many producers.

Miller and Norsworthy (2016) controlled Palmer amaranth up to 95 % with the sequential application of 2,4-D choline plus glyphosate at early-POST followed by mid-POST timings. Another research study reports that an application of the herbicides 2,4-D choline plus glyphosate, followed by glufosinate, had 99 % control of Palmer amaranth when trifluralin was applied as a pre-plant incorporate (Manuchehri et al. 2017). In addition, herbicide applications that contained a mixture of glufosinate and 2,4-D has been reported to result in more than 96 % control of glyphosate-resistant horseweed, giant ragweed (*Ambrosia trifida*), and common lambsquarters (*Chenopodium album*) (Barnett et al. 2013; Chahal and Johnson 2012).

Glufosinate (WSSA group 10) herbicide is in the phosphinic acid herbicide family. The herbicide is a non-selective, contact herbicide with limited translocation within the plant and can be applied to the Enlist E3 soybean. Glufosinate provides control of several annual grass and broadleaf weeds, including Palmer amaranth and volunteer corn (Coetzer et al. 2002; Chahal and Johnson, 2012; Chahal et al. 2014, Chahal and Jhala, 2015). However, crop injury occurs with spray contact with non-tolerant crops. Common glufosinate trade names include Liberty® (BASF), Interline® (United Phosphorus, Inc.), and Ignite®(Bayer).

Glufosinate inhibits the glutamine synthetase pathway by binding to the active location of glutamine synthetase and competing for glutamate (Manderscheid and Wild 1986). The glutamine synthetase pathway is an efficient pathway for ammonia detoxification, which inhibits photosynthesis (Sauer et al. 1987; Wild and Manderscheid 1984). Inhibition of this enzyme causes a buildup of phytotoxic ammonia in plants which disrupts cell membranes. Weed control with glufosinate is best when weeds are actively growing and not under stress.

3.1.2. Dicamba based herbicide program

Dicamba (WSSA group 4 herbicide) is a highly volatile auxin-mimic chemical with vapor pressure of 4.5 x 10^{-3} Pa at 25 ^{0}C (https://wssa.net/wp-content/uploads/Dicamba-Report_6_30_2018.pdf), and can damage non-target plant species through spray drift and/or volatilization (vapor drift). Volatility is influenced by several factors including temperature, relative humidity, and application rates. The misuse of dicamba may cause serious damage to broadleaf plants including non-dicamba-tolerant soybeans, grapes, peas, flax, canola, and non-crop plants (Strachan et al. 2010). Because of the problem with volatility, cutoff dates for dicamba application have been established. The cutoff date for use of dicamba products like XtendiMax and Engenia in

South Dakota is June 30 (https://www.sdaba.org/dicamba-information). Applicators can use dicamba products until soybeans reach the R1 growth stage, or 45 days after planting, or June 30, whichever comes first.

Bayer's Roundup Ready 2 Xtend soybean variety was developed in 2006. The Xtend soybean is the company's first biotech-stacked soybean trait having both dicamba and glyphosate tolerance. During the process of development, herbicide resistant genes were incorporated into the soybean genome which enabled the crop to metabolize the herbicide dicamba. The Xtend weed control system allows for the control of glyphosate resistant weeds in soybean fields by using another herbicide mode of action to kill weeds, while increasing yields. Glyphosate-resistant broadleaf weeds controlled by dicamba applications in fields planted to Xtend soybean include Palmer amaranth, waterhemp, and horseweed.

Because glyphosate (for both Enlist™ and Xtend® soybeans) and glufosinate (Enlist™ only) are non-selective herbicides, their applications will help control grass weeds and provide another mode of action for controlling weeds present in fields. As the numbers of herbicide resistant weeds increase globally, using multiple modes of action at different application timings for weed control are critical as this will help slow down or prevent the selection of resistant weed biotypes. An understanding of POST control of glyphosate-resistant weeds (particularly those common in eastern South Dakota such as waterhemp, kochia, and horseweed) with multiple herbicide modes of action including auxinic herbicides is crucial to minimize the interference of these weeds within the broadleaf crop.

However, high auxin levels in soybean roots (Turner et al. 2013), as well as weed stress (Gal et al. 2015), other herbicide stress (Tortosa et al. 2021), and high soil nitrogen (Gresshoff, 1990) have all been reported to inhibit nodulation. An on-farm study suggested up to 5% yield increase with Primo foliar rhizobia inoculant (manufactured by Verdesian, Cary, NC) when applied to soybean at V3 and R2 stages of growth (https://onfarmresearch.sdsoybean.org/archives/ reports/primo-foliar-inoculant-high-yield-zone-location-1-55-bu-ac). Research investigating the impact of auxin-based herbicides (2,4-D and dicamba) on auxin tolerant soybeans (Enlist E3 and Xtend) and on the nodulation potential of these varieties has not been done. Also, the application of foliar rhizobia with herbicide has not been studied. The objective of this study was to evaluate the efficacy of auxin-based herbicide programs (2,4-D and dicamba) and rhizobia application with herbicide in auxin tolerant soybean for weed control and nodulation potential in South Dakota soybean. This study specifically examined:

1) the performance of 2,4-D alone for broadleaf weed control and with mixtures of glyphosate and glufosinate for annual grass and broadleaf weed control in Enlist E3 soybeans;
2) the performance of dicamba for broadleaf weed control, and mixed with glyphosate for grasses and broadleaf weed control in Roundup Ready 2 Xtend soybean;
3) planting date influence on auxin-tolerant soybean performance and weed control;
4) if auxin-tolerant soybean nodulation was impacted by auxin herbicide; and

5) if foliar application of Bradyrhizobium japonicum (USDA 110) at V3 stage of soybean when combined with auxin herbicide would improve soybean nodulation, nodule activity, yield, 100-seed weight, seed protein and oil contents.

3.2. Materials and methods

Location description

Field experiments were conducted over five location years at three eastern South Dakota locations [northeast - South Shore (elevation:1831 ft, latitude:44 45N, longitude:096 41W, season: 2019); southeast - Beresford (elevation:1257 ft, latitude:43 03N, longitude:096 54W, season: 2019 & 2020); east-central - Brookings (elevation:1637 ft, latitude:44 18N, longitude:096 49W, season: 2019 & 2020). The Koppen climate classification subtype for the study locations is the "Dfa" (hot summer continental climate) for southeast location, and "Dfb" (warm humid continental climate) for northeast and east-central locations (https://www.weather-base.com/search/search.php3?query=south+dakota). The soil type at the experimental locations (northeast, southeast, and east-central) were Brookings clay loam, 0-2% slope (fine-silty, super- active, frigid Cumulic Hapludoll), Egan silty clay, 0-2% slope (fine-silty, mixed, superactive, mesic Udic Hapludoll), and Brandt silty clay loam, 0-2% (fine-silty, mixed, superactive, frigid Calcic Haplodolls), respectively.

Land preparation and planting

Study locations at northeast and east-central South Dakota were tilled with a field cultivator to a depth of about 10 cm before planting, whereas southeast was under no-till system. The previous crop at all locations was corn. Auxin tolerant soybeans (Enlist E3 and Xtend) were planted at three timings (early-PD1, mid-PD2, and late-PD3), with approximately two weeks between planting dates, using a seeding rate of 350,000 seeds ha^{-1} at a planting depth of 2.5 cm. Planting dates and soybean genotypes differed based on climate. The relative maturity group (MG) of soybean planted at northeast was 1.0 – 1.1

MG (short maturity). The mid-maturity varieties (1.3 – 1.7 MG) were planted at east-central, whereas southeast was planted to a longed maturity varieties (2.0 MG). Information on soybean variety planted with their relative maturity group is presented in Table 3.1. The planting dates, harvest dates and growing degree days (GDD) across locations are presented in Table 3.2.

The Enlist seed was not treated whereas the Roundup Ready 2 Xtend seeds were treated with fungicide/insecticide combination that contained the active ingredients metalaxyl, fluxapyroxad, and pyraclostrobin (Acceleron, Monsanto, St. Louis Mo). Preemergence herbicide tank mix containing flumioxazin, metribuzin, glyphosate, S-metolachlor, pendimethalin, ammonium sulfate and surfactants (Table 3.3) was applied over all plots at each planting date to burndown emerged weeds (mid-PD2 and late-PD3) and to provide early season residual weed control (all planting dates), especially for grass weeds. At northeast location (2019), the preemergence (PRE) treatment for early planting date had no glyphosate as tillage prior to planting left few emerged weeds. However, glyphosate and adjuvant (AMS) were added to the preemergence treatments for mid-PD2 and late-PD3 planting dates. Also, the PRE herbicide was applied to all planting dates at southeast on May 11 in 2020 growing season, whereas at east central location, the PRE herbicide was applied to individual planting date in 2019 and 2020 (Table 3.3).

Table 3.1. Soybean varieties and relative maturity groups planted at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing seasons.

Experiment location	Soybean variety	Maturity group	Days to maturity
Northeast[a]	Enlist E3 (Stine 11EC20)	1.1	≤120
	Xtend (Asgrow 10X9)	1.0	
East-central[b]	Enlist E3 (Stine 13EA12)	1.3	≤127
	Xtend (Asgrow 17X8)	1.7	
Southeast[c]	Enlist E3 (Stine 22EB23)	2.0	≤137
	Xtend (Asgrow 20X7)	2.0	

[a]South Dakota State University northeast research farm (SDSU - NERF) located at South Shore, South Dakota. Elevation: 558 m, latitude: 44 45N, longitude: 096 41W. Study was conducted in 2019 growing season only.

[b]South Dakota State University Aurora experiment research station, South Dakota. Elevation: 499 m, latitude: 44 18N, longitude: 096 49W. Study was conducted at the location in 2019 and 2020 growing seasons.

[c]South Dakota State University southeast research farm (SDSU – SERF) located at Beresford, South Dakota. Elevation: 383 m, latitude: 43 03N, longitude: 096 54W. Study was conducted at the location in 2019 and 2020 growing seasons.

Table 3.2. Planting date, growing degree days, and harvest date of Enlist and Xtend soybean varieties evaluated for weed control and nodulation potential at three eastern South Dakota.

Planting date	Growing degree days (GDD)[a]	Harvest date
Northeast 2019		
PD1 - May 15	1178	October 30
PD2 - May 30	1136	
PD3 - June 15	1008	
East-central 2019		
PD1 - May 15	1279	October 29
PD2 - June 2	1198	
PD3 - June 19	1047	
Southeast 2019		
PD1 - May 7	1535	October 19
PD2 - June 5	1378	
PD3 - June 19	1236	
East-central 2020		
PD1 - May 20	1420	October 9
PD2 - June 3	1314	
PD3 - June 16	1166	
Southeast 2020		
PD1 - May 15	1553	October 15
PD2 - May 29	1458	
PD3 - June 12	1292	

[a]GDD: growing degree days from planting to harvest. A base temperature of 10 ^{0}C was used to calculate GDD.

Table 3.3. Preemergence herbicide applications to auxin-tolerant soybeans at northeast, east-central, and southeastern South Dakota in 2019 and 2020.

Pre-herbicide	Rate [a.i. or a.e. ha^{-1}]	*Northeast location*	
Flumioxazin[1]	0.42	2019 application date	2020 application date
Metribuzin[2]	0.56	PD1 – May 6	N/A
S-metolachlor[3]	0.12	PD2 – May 30	N/A
Glyphosate[4]	0.34	PD3 – June 14	N/A
Pendimethalin[5]	0.29	*East-central location*	
		2019 application date	2020 application date
		PD1 – May 15	PD1 – May 15
		PD2 – June 5	PD2 – June 2
		PD3 – June 19	PD3 – June 19
		Southeast location	
		2019 application date	2020 application date
		PD1 – May 7	PD1 – May 11
		PD2 – June 2	PD2 – May 11
		PD3 – June 19	PD3 – May 11

[1]Flumioxazin 51% [Valor SX (Valent BioSciences LLC USA, Walnut Creek, Ca) or Panther SC (Nufarm Americas Inc. Alsip, IL.)]. Flumioxazin is a light-dependent peroxidizing herbicide (LDPH), which acts by blocking heme and chlorophyll biosynthesis resulting in an endogenous accumulation of phototoxic porphyrins.

[2]Metribuzin 75% [Glory (ADAMA USA. Raleigh, NC) or Dimetric (WinField United, Shoreview, MN)]. The mode of action of metribuzin is that it acts by inhibiting photosystem II of photosynthesis by disrupting electron transfer.

[3] S-metolachlor 82% [Me-Too-Lachlor™ (Drexel chemical company. Memphis, TN) or Medal II EC (Syngenta, Greensboro, NC, USA)]. S-metolachlor act by inhibiting the biosynthesis of several plant components such as fatty acids, lipids, proteins, isoprenoids, and flavonoids.

[4]Glyphosate 49% [Roundup PowerMax® (Bayer, Whippany,NJ) or Tomahawk (WinField United, Shoreview, MN)]. Glyphosate interferes with the shikimate pathway, which produces the aromatic amino acids phenylalanine, tyrosine and tryptophan in plants and microorganisms.

[8]Prowl® [39% pendimethalin] manufactured by BASF. Pendimethalin acts both pre-emergence, that is before weed seedlings have emerged, and early post-emergence. Pendimethalin inhibits root and shoot growth.

*Adjuvant [AMS at 3 kg ha^{-1}], and surfactants [Duce HSOC at 1.67 Lha^{-1}) or Destiny HSOC (1.38 Lha^{-1}), NIS (0.01 Lha^{-}1), UAN (0.01 Lha^{-}1) and StrikeZone (0.21 Lha^{-1})] were added into the preemergence spray tank. N/A – not applicable.

Treatments

Postemergence herbicide treatments used in the study are reported in Tables 3.4, 3.5, and 3.6. The postemergence herbicides applied to Enlist E3 soybean include 2,4-D (as choline salt) + clethodim or 2,4-D + glufosinate. A no POST herbicide treatment was included to determine weed problems after preemergence treatment application. Herbicide treatments to Xtend soybean varieties were diglycolamine salt of dicamba + glyphosate, acifluorfen + clethodim, and a no POST herbicide treatment. Rhizobia (*Bradyrhizobium japonicum*-USDA-110) inoculant cultured in yeast extract mannitol (YEM) media at 1 L/ha and delivering 2.7 ml inoculant per plot was foliar applied with each POST treatment to determine if inoculant addition with the herbicide improved nodulation.

Postemergence auxin herbicide (2,4-D and dicamba) applications, although targeted for V3 soybean growth stage, were applied between V2 (PD3) to V5 (PD1) for Enlist variety (Table 3.4), and VC (PD3) to V3 (PD1) for Xtend variety (Table 3.5 and Table 3.6), whereas acifluorfen + clethodim + rhizobia treatment was applied between V1 (PD3) to V5 (PD1) growth stages (Table 3.5 and Table 3.6). Herbicides were applied with a CO_2-pressurized bicycle-type sprayer calibrated to deliver 187 L ha^{-1} at 207 kPa at a ground speed of 4.5 km hr^{-1}. The nozzles were set at 46 cm above the crop. Table 3.7 and 3.8 shows the local environmental conditions at time of herbicide applications.

Table 3.4. Postemergence herbicide (+/- rhizobia) applications to Enlist E3 soybeans evaluated for weed control and nodulation potential at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing seasons.

Postemergence applications	Herbicide applied	Rate (kg a.e.or a.i./ha)	Location	Planting date (PD)	POST herbicide application date	Soybean growth stage	Harvest date
Enlist E3 herbicide treatments (2019 season)							
2,4-D + clethodim 2,4-D + glufosinate	Enlist One™ + Select Max® Enlist One™ + Liberty® 280 SL	0.54 + 0.13 0.54 + 0.30	Northeast	May 15 May 30 June 15	July 15	V5 V3 V2	October 30
			East-central	May 15 June 2 June 19	July 15	V5 V3 V2	October 29
			Southeast	May 7 June 5 June 19	July 16	V5 V3 V2	October 19
Enlist E3 herbicide treatments (2020 season)							
2,4-D + clethodim 2,4-D + glufosinate	Enlist One™ + select Max® Enlist One™ + Liberty® 280 SL	0.54 + 0.13 0.54 + 0.30	East-central	May 20 June 3 June 16	July 19	V5 V3 V2	October 9
			Southeast	May 15 May 29 June 12	July 22	V5 V3 V2	October 15

* Herbicide treatments (2,4-D + clethodim and 2,4-D + glufosinate) were applied with +/- rhizobia. Bradyrhizobia (USDA 110) inoculant was applied at the rate of 1 L ha^{-1}. Adjuvant [AMS at 3 kg ha^{-1} or Class Act® Ridion® at 1.2 L ha^{-1}] and non-ionic surfactant [Chemsurf 90 at 3.0 L ha^{-}1] were added into the spray tank to enhance coverage and improve herbicide uptake on plant surfaces.

* Soybeans took about 10 days from planting to emergence, 5days from VE – VC stage, and about 10 days in between growth stages.

Table 3.5. Postemergence herbicide (+/- rhizobia) applications to Xtend soybeans evaluated for weed control and nodulation potential at northeast, east-central, and southeastern South Dakota in 2019 growing seasons.

Xtend herbicide treatments in 2019 growing season							
Postemergence applications	Herbicide applied	Rate (kg a.e. or a.i./ha)	Location	Planting date (PD)	POST herbicide application date	Soybean growth stage	Harvest date
Dicamba + glyphosate	XtendiMax®+PowerMAX®	0.28 + 0.34	Northeast	May 15 May 30 June 15	June 27 (dicamba)	V3 V1 VC	October 30
Acifluorfen + clethodim	Acifin™ 2L + Select Max®	0.18 + 0.13			July 15 (acifin)	V5 V3 V1/V2	
			East-central	May 7 June 5 June 19	June 27 (dicamba)	V4 V1 VC	October 29
					July 15 (acifin)	V5 V2/V3 V1	
			Southeast	May 15 June 2 June 19	June 25 (dicamba)	V3 V1 VC	October 19
					July 16 (acifin)	V5 V3 V1	

* Herbicide treatments (Dicamba + glyphosate and acifluorfen + clethodim) were applied with +/- rhizobia. Bradyrhizobia (USDA 110) inoculant was applied at the rate of 1 L ha^{-1}. Adjuvant [Class Act® Ridion® at 1.6 Lha^{-1}] and surfactant [Strike Zone® LC at 3.0 Lha^{-}1] were added into the spray tank to enhance coverage and improve herbicide uptake on plant surfaces.

* Soybeans took about 10 days from planting to emergence, 5days from VE – VC stage, and about 10 days in between growth stages.

Table 3.6. Postemergence herbicide applications to Xtend soybeans evaluated for weed control and nodulation potential at east-central, and southeastern South Dakota in 2020 growing season.

Xtend herbicide treatments in 2020 growing season							
Postemergence applications	Herbicide applied	Rate (kg a.e. or a.i./ha)	Location	Planting Date (PD)	Date applied	Soybean growth stage	Harvest date
Dicamba + glyphosate	XtendiMax® + PowerMAX®	0.28 + 0.34	East-central	May 15 May 29 June 12	June 24 (dicamba)	V3 V1 VC	October 9
Acifluorfen + clethodim	Acifin™ 2L + Select Max®	0.18 + 0.13			July 16 (acifin)	V5 V3 V2	
			Southeast	May 20 June 3 June 16	June 24 (dicamba)	V2/V3 V1 VE	October 15
					July 22 (acifin)	V5 V3 V2	

* Herbicide treatments (Dicamba + glyphosate and acifluorfen + clethodim) were applied with +/- rhizobia. Bradyrhizobia (USDA 110) inoculant was applied at the rate of 1 L ha^{-1}. Adjuvant [AMS at 3 kg ha^{-1} or Class Act® Ridion® at 1.6 Lha^{-1}] and OnTarget™ at 1.0 L ha^{-}1] were added into the spray tank to enhance coverage and improve herbicide uptake on plant surfaces.

* Soybeans took about 10 days from planting to emergence, 5days from VE – VC stage, and about 10 days in between growth stages.

Table 3.7. Environmental conditions at time of herbicide applications at northeast, southeast and east-central South Dakota in 2019.

Location	Date	Variety	Treatments	Time Range	Weather Start (Temp., Wind, RH)	Weather Finish (Temp., Wind, RH)
Northeast	6/27/2019	RR2X	Dicamba	11:00 - noon	19, 10 SE, 84%	22, 16 SE, 73%
	7/15/2019	RR2X	Acifluorfen	3:40-4:30	32, 14 NW, 59%	32, 13 WNW, 48%
	7/15/2019	Enlist	2,4-D	5:00-6:45	32, 13 WNW, 48%	31, 14 WNW, 48%
	7/23/2019	Enlist	2,4-D (PD3)	1:30 - 3:20	24, 10 NW, 48%	25, 10 var, 46%
Southeast	6/19/2019	RR2X	Dicamba	noon - 1:25	22, 3 S, 52%	22, 6 NE, 57%
	6/25/2019	RR2X	Dicamba	3:00-4:00	27, 16 NW, 43%	27, 13 NW, 41%
	7/16/2019	RR2X	Acifluorfen	4:00-5:00	31, 11 SE, 53%	30, 11 SE, 57%
	7/16/2019	Enlist	2,4-D	2:30-3:30	29, 11 SE, 63%	30, 11 SE, 57%
	7/24/2019	Enlist	2,4-D (PD 3)	1:15 - 3:15	27, 24 SE, 47%	27, 24 SE, 47%
East-central	6/18/2019	RR2X	Dicamba	4-5 pm	24, 8 E, 46%	24, 11 NNE, 46%
	6/26/2019	RR2X	Dicamba	10:00-11:00 am	22, 14 SE, 68%	23, 13 S, 64%
	7/12/2019	RR2X	Acifluorfen	8:00-8:30 am	22, 13 SSW, 81%	23, 13 SSW, 76%
	7/12/2019	Enlist	2,4-D	9:00-11:15 am	23, 13 SSW, 76%	23, 5 SW, 85%
	7/22/2019	Enlist	2,4-D (PD3)	1:45 - 3:15	23, 14 N, 49%	23, 10 NE, 53%

*Weather: temperature (^{0}C), wind speed (kph), and relative humidity (%).

*RR2X: Roundup Ready 2 Xtend soybean variety.

Table 3.8. Environmental conditions at the time of herbicide applications at southeast and east-central South Dakota in 2020 season.

Location	Date	Variety	Time Range	Weather Start (Temp., Wind, RH)	Weather Finish (Temp., Wind, RH)
Southeast	6/24/20	RR2X	6:00 - 7:00 PM	28, 5 SW, 37%	27, 5 S, 38%
	7/22/20	Enlist	4:00-7:45 PM	29, 16 E, 48%	27, 14 SE, 57%
	7/28/20	RR2X (acifin)	3:00-5:00 PM	31, 13 SW, 57%	32, 8 SW, 54%
East-central	6/4/20	RR2X & Enlist	4-5 PM	28, calm, 41%	23, calm, 37%
	6/19/20	RR2X & Enlist (PD1)	4-5 PM	23, 8 var, 55%	23, 8 N, 53%
	6/24/20	RR2X	1-3 PM	26, 16 NNW, 37%	26, 16 WNW, 38%
	7/16/20	RR2X	6:30 - 8:30 PM	27, 14 SW, 74%	25, calm, 79%
	7/19/20	Enlist	9:45 - 11:00 AM	24, 23 W, 52%	26, 24 W, 48%
	7/20/20	Enlist	8:45 - 10:30 AM	22, 13 SE, 66%	24, 19 SE, 62%

Weather: temperature (^{0}C), wind (kph), and relative humidity (%).
*RR2X: Roundup Ready 2 Xtend soybean varieties.

Experimental design

Treatments at all locations were arranged by soybean variety (Enlist E3 or Xtend) in a split plot design with four replications. Planting date (early-PD1, mid-PD2, or late-PD3) was the main plot whereas herbicide treatments were the sub-plots. Soybeans were planted at 0.76 m row spacing. Individual plots were 4 rows wide by 9 meters long. An untreated buffer of 15 m was also left between the two soybean varieties planted.

Data collection

Weed density (plant m^{-2}) was collected between the middle soybean rows at 2, and 6 weeks after POST herbicide treatment applications. Weed species per plot were identified, and samples dried in the oven [GRIEVE-model WRH6106-500] at 60 ^{0}C for 5 days, and biomass quantified. At R5 stage of soybean growth (between late-July and early August), plant greenness index was measured using the chlorophyll meter SPAD-502 plus [Konica Minolta] from four soybean plants within each plot and the average value recorded. The plants that were measured for leaf greenness were cut 2 cm above the soil, dried at 60 ^{0}C and biomass quantified. Also, soil samples (500 cm^3) were collected from the two plants using a 11- cm diameter golf hole cutter centered over the stem at a depth of 0.08 m, with samples stored in a cooler (3 ^{0}C) for root nodule evaluation.

In the laboratory, soil was removed from soybean roots by washing with Liquinox, a soap that consists of a homogeneous blend of sodium linear alkylaryl sulfonate, sodium xylene sulfonate, and ethoxylated alcohol. Nodules from the washed roots were counted and thereafter sliced to determine activity. When cut, a red/pink color indicated an active nodule, whereas green, white, and black coloration indicated inactive, immature, and dead nodules, respectively. At crop physiological maturity stage (when one pod on main stem reached mature pod color), the aboveground weed density was collected from the field, dried to constant weight at 60 ^{0}C and biomass quantified. The middle two rows of the plots were harvested using a small plot combine and seeds dried at 60^{0}C for 7 days. Grain yield, seed oil and seed protein contents were reported at 13% moisture. A 100-seed weight was also quantified

Statistical analysis

Data obtained from each variety/maturity group, location and year were analyzed independently using the R – statistical software package (www.r-project.org). Square root transformation of weed densities were performed to improve homogeneity of variances. Transformed data were subjected to ANOVA using the linear mixed effect procedure in R. Herbicide and planting dates parameters were fixed effects, whereas blocks were random. The fixed effects of herbicide and planting date were tested using the type II statistics. Treatment means were separated ($p \leq 0.05$) using the Fisher's Least Significant Difference (LSD) (Steel et al. 1997) and back transformed data are reported.

3.3. Results and Discussion.

3.3.1. Climatic conditions of study locations

Growing degree days from planting to POST herbicide application, soil sampling for root nodule evaluation, and harvest are presented in Tables 3.9 and 3.10. Also, temperature and rainfall data for northeast (Southshore), southeast (Beresford), and east-central (Brookings) locations for 2019 and 2020 growing seasons are presented in Tables 3.11 and 3.12, respectively. At each location, growing degree days and temperatures were near the 30-year average (1981-2010) for both years. Monthly total rainfall ranged from 5 cm (southeast) to 17 cm (east-central). The summer of 2019 was wetter than normal. Rainfall in July at northeast, southeast, and east- was 103, 31, and 95 % above normal. However, in 2020, rainfall amounts were 50 % below normal at the two study locations (southeast and east-central) for all months, except July at east-central location where precipitation was 23 % above normal. Total growing season rainfall at east-central and southeast was 32 cm and 22 cm, which were 34 % and 112 % below the 30-year average and considered dry to drought conditions.

Table 3.9. Growing degree days (GDD) from planting to POST herbicide application (July), soil sampling for root nodule evaluation (August), and harvest (October) at northeast, east-central, and southeastern South Dakota in 2019 growing season.

Planting date	GDD POST Application	GDD Soil Sampling	GDD Harvest	Date of Harvest	30-year average temperature (^oC)
Northeast					
May 15 (PD1)	687	1001	1178	Oct. 30	17.8
May 30 (PD2)	572	1015	1136		
June 15 (PD3)	416	887	1008		
East-central					
May 15 (PD1)	701	1101	1279	Oct. 29	17.6
June 2 (PD2)	598	1023	1198		
June 19 (PD3)	473	915	1047		
Southeast					
May 7 (PD1)	1032	1310	1535	Oct. 19	19.6
June 5 (PD2)	980	1211	1378		
June 19 (PD3)	562	1101	1236		

* A base temperature of 10 ^0C was used to calculate growing degree days for the period from soybean planting to postemergence herbicide application, soil sampling for root nodule evaluation, and harvest.

Table 3.10. Growing degree days (GDD) from planting to POST herbicide application (July), soil sampling for root nodule evaluation (August), and harvest (October) at northeast, east-central, and southeastern South Dakota in 2020 growing season.

Planting date	GDD POST Application	GDD Soil Sampling	GDD Harvest	Date of Harvest	30-year average temperature (^{o}C)
East-central					
May 20 (PD1)	1100	1245	1420	Oct. 9	17.6
June 3 (PD2)	1013	1210	1314		
June 16 (PD3)	800	953	1166		
Southeast					
May 15 (PD1)	1179	1326	1553	Oct. 15	19.6
May 29 (PD2)	1011	1285	1458		
June 12 (PD3)	814	1002	1292		

* A base temperature of 10 ^{0}C was used to calculate growing degree days for the period from soybean planting to postemergence herbicide application, soil sampling for root nodule evaluation, and harvest.

Table 3.11. Monthly average temperature (^{0}C) at northeast, east-central and southeastern South Dakota (2019 and 2020), and 30-year average (1981-2010).

Location	*2019 growing season*				
	May	June	July	August	September
Northeast[a]	11 (13)	19 (19)	21 (22)	18 (20)	16 (15)
East-central[b]	11 (13)	19 (19)	22 (21)	19 (20)	18 (15)
Southeast[c]	13 (15)	21 (21)	23 (23)	21 (22)	19 (17)
	2020 growing season				
East-central[b]	13 (13)	22 (19)	23 (21)	22 (20)	15 (15)
Southeast[c]	14 (15)	24 (21)	24 (23)	22 (22)	17 (17)

https://mesonet.sdstate.edu/archive).

Table 3.12. Monthly rainfall (cm) at northeast, east-central, and southeastern South Dakota, and 30-year average (1981-2010).

2019 growing season						
Location	May	June	July	August	September	Total
Northeast[a]	8.6 (8.1)	6.2 (10.2)	16.9 (8.7)	10.5 (7.8)	10.0 (6.6)	52.2 (41.4)
East-central[b]	12.9 (8.7)	7.9 (10.5)	16.2 (8.4)	7.9 (8.4)	16.0 (7.5)	60.9 (43.5)
Southeast[c]	15.7 (10.2)	9.8 (11.7)	10.9 (8.7)	8.2 (9.0)	7.4 (7.8)	52.0 (47.4)
2020 growing season						
East-central	7.5 (8.7)	8.0 (10.5)	10.2 (8.4)	4.3 (8.4)	2.4 (7.5)	32.4 (43.5)
Southeast	5.2 (10.2)	8.8 (11.7)	4.7 (8.7)	2.9 (9.0)	0.8 (7.8)	22.4 (47.4)

[a]study was conducted at northeastern location in 2019 only

[b]study was conducted at east-central location in 2019 and 2020 growing seasons

[c]study was conducted at southeastern location in 2019 and 2020 growing seasons

*30-year long-term averages from 1981-2010 in parentheses (climate data were retrieved from https://mesonet.sdstate.edu/archive).

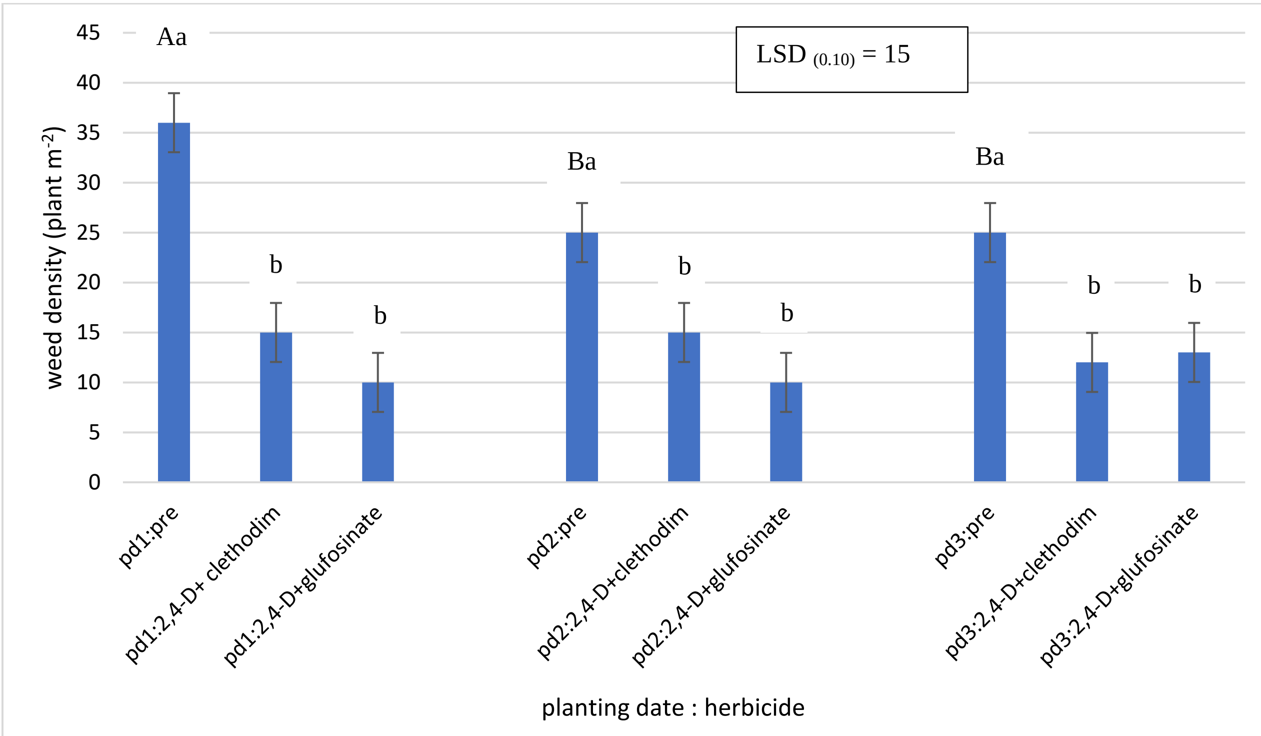
3.3.2. Enlist E3 soybean evaluated for weed control and nodulation potential at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

<u>Weed control</u>

The average weed density at 2 weeks after herbicide treatment application at all locations was about 20 plants m^{-2} for all sites in 2019, and in 2020 east-central had 3 plants m^{-2}, and southeast had 40 plants m^{-2}, and the weed species found were volunteer corn (east-central and southeast) and volunteer wheat and woolly cupgrass (northeast). No planting date by herbicide interaction was found for weed density at two weeks following the POST application of treatment in either year (Table A1). However, at northeast location only, an interaction was observed between planting date and herbicide treatment [$p \leq 0.01$] at 6 weeks after POST treatment application (Table A2). Weed density for PD1/pre-only herbicide treatment had more weeds (average 37 plants per m^{-2}) than either PD2 or PD3 combination (average < 20 plants per m^{-2}). Furthermore, pre-only treatments for all planting dates had greater weed density than the post applied treatments ($\leq$ 15 plants m^{-2}) when compared within a planting date (Figure 3.1).

An interaction between herbicide treatment and planting date was observed [$p \leq 0.01$] for end-of-season weed biomass at the northeast location (Table A3). Pre-only treatments at PD1 and PD3 had greater weed biomass (consisting of both grass and broadleaf weeds) than the pre-only at PD2 and any of the POST herbicide treatments (2,4-D + clethodim, and 2,4-D + glufosinate) (Figure 3.2). The 2,4-D + clethodim, and 2,4-D + glufosinate provided excellent weed control no weeds present in most of these treatments (Figure 3.2). Although the auxin herbicide (2,4-D) was combined with clethodim or glufosinate, the uncontrolled weeds were mostly grasses (including barnyardgrass, volunteer wheat, and large crabgrass), and were found in the PD3 treatment. The combined application of rhizobia with herbicide had no impact on weed density and biomass in the study and data were combined across rhizobia treatment. Dominant weed species present at end-of-season biomass sampling are presented in Table 3.13. Numerous weed species, both grasses and broadleaf were observed in the pre-only treatment across planting dates (Table 3.13). At east-central and southeast, and for both growing seasons, volunteer corn was found in the early planting date, whereas volunteer wheat was present at northeast. Overall, plots that received pre-only treatment had more weeds than any other treatment at all three locations (Figure 3.3.).

Figure 3.1. Interaction effect of planting date and herbicide (averaged over rhizobia treatment) on weed density at 6 weeks after POST treatment application in Enlist soybean evaluated at northeastern South Dakota in 2019.

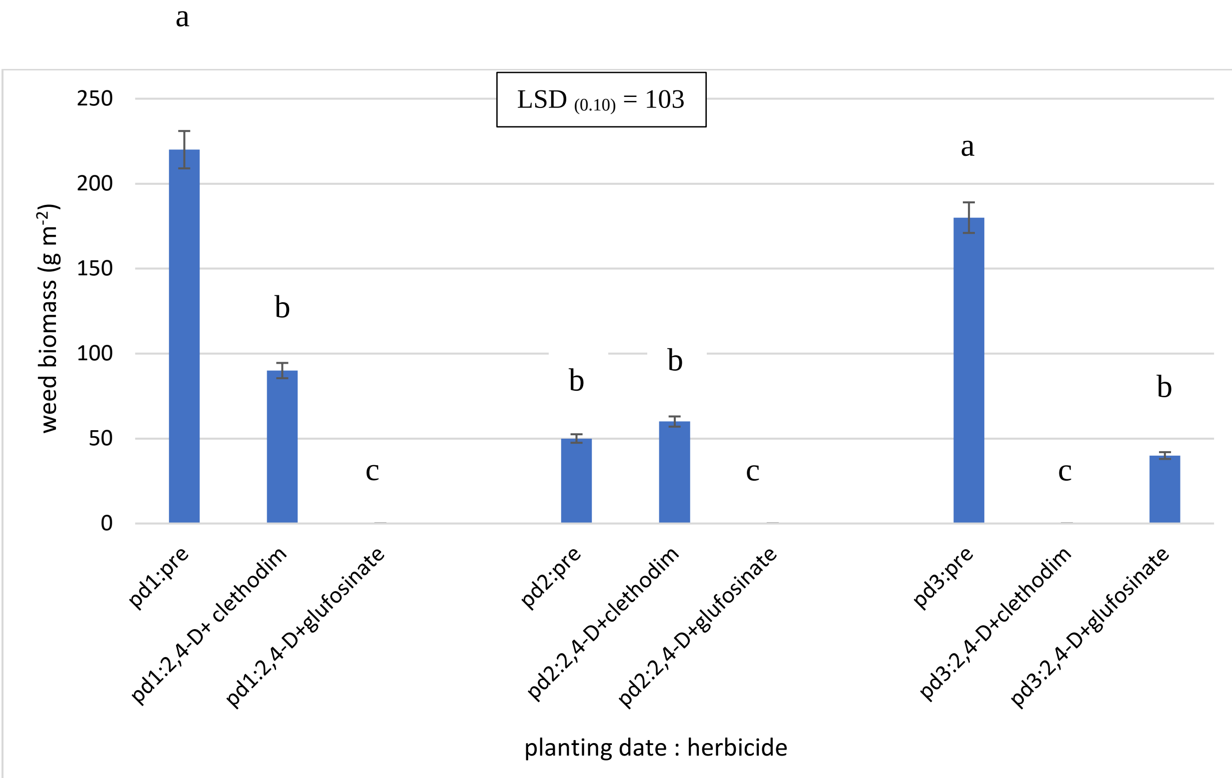


* Different letters above the bars indicate significant differences among the planting date by herbicide treatment using the Fisher's test for significance at $p \leq 0.10$. Uppercase letters show the comparison between planting date by herbicide treatment interactions, whereas lowercase letters show comparison within each planting date by herbicide treatment interactions. Error bars show the standard deviation of planting date by herbicide treatment.

* Planting dates: pd1–May 15; pd2–May 30; pd3–June 15.

* Weed density values for all treatment +/- rhizobia were averaged. Average values are presented.

Figure 3.2. Effect of planting date by herbicide interaction on weed biomass at end-of-season in Enlist soybean averaged over rhizobia treatment at northeastern South Dakota in 2019 season



* Different letters above the bars indicate significant differences among the planting date by herbicide treatment using the Fisher's test for significance at $p \leq 0.10$. Error bars show the standard deviation of planting date by herbicide treatment.

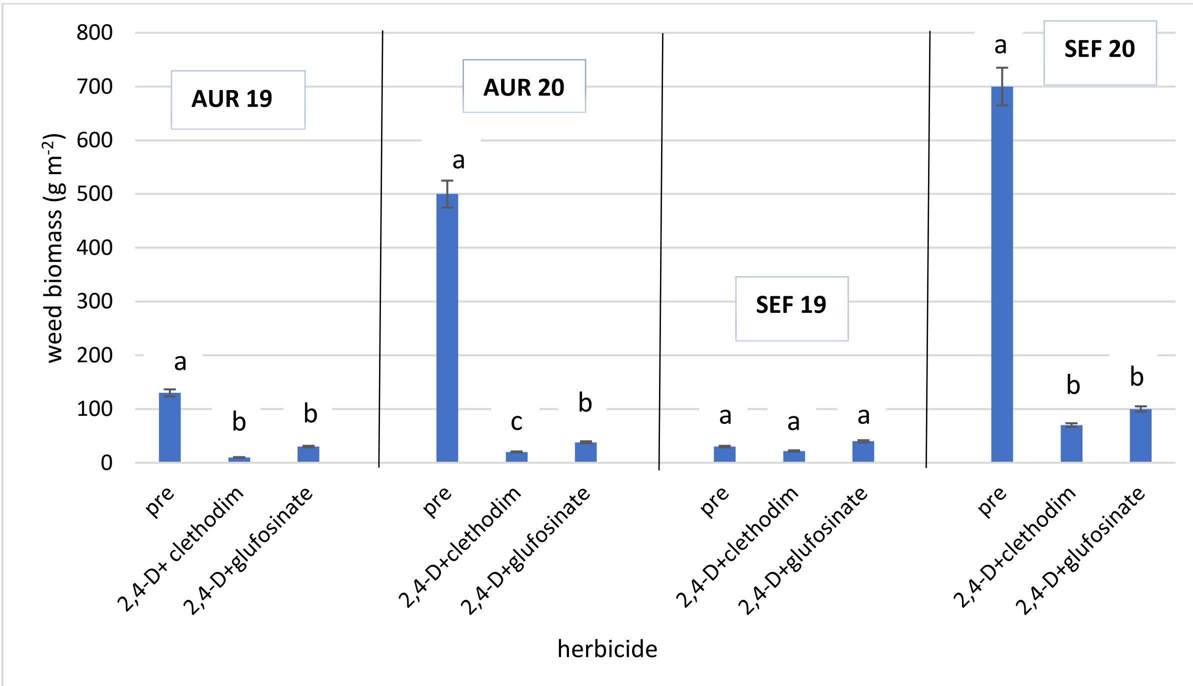
* Planting dates (pd1: May 15, pd2: May 30, pd3: June 15).

Table 3.13. Weed species present during end-of- season weed biomass sampling (September) in Enlist soybean at northeast, east-central and southeastern South Dakota.

	Pre-only Treatment		
Location	Early planting (PD1)	Mid-season planting (PD2)	Late planting (PD3)
Northeast[a] 2019 only	green foxtail, yellow foxtail, common lambsquarters, woolly cupgrass, redroot pigweed, large crabgrass, prostrate pigweed, dandelion, volunteer wheat	green foxtail, yellow foxtail, dandelion, volunteer wheat	volunteer wheat, dandelion, woolly cupgrass, common lambsquarters, barnyardgrass, large crabgrass
East-central[b] 2019 & 2020	volunteer corn, dandelion, barnyardgrass, wild buckwheat, common lambsquarters	quackgrass, barnyardgrass, lady's thumb, velvetleaf, green and yellow foxtail	wild buckwheat, lady's thumb, quackgrass, barnyardgrass, woolly cupgrass, velvetleaf, common lambsquarters, green and yellow foxtail, redroot pigweed, dandelion, wild four o' clock,
Southeast[c] 2019 & 2020	volunteer corn, redroot pigweed, barnyardgrass, waterhemp, marestail	green and yellow foxtail, foxtail barley, fall panicum, field sandbur, large crabgrass, waterhemp, marestail	barnyardgrass, green and yellow foxtail, foxtail barley, fall panicum, field sandbur, large crabgrass, dandelion, common waterhemp, redroot pigweed, marestail, wild buckwheat,
	2,4-D + clethodim treatment		
	Early planting (PD1)	Mid-season planting (PD2)	Late planting (PD3)
Northeast[a] 2019 only	barnyardgrass, volunteer wheat	common lambsquarters, volunteer wheat	
East-central[b] 2019 & 2020		volunteer corn, green foxtail	volunteer corn
Southeast[c] 2019 & 2020	green foxtail, volunteer corn	large crabgrass, green foxtail, and barnyardgrass	large crabgrass, barnyardgrass
	2,4-D + glufosinate treatment		
	Early planting (PD1)	Mid-season planting (PD2)	Late planting (PD3)
Northeast[a] 2019 only			barnyardgrass and volunteer wheat
East-central[b] 2019 & 2020	green foxtail, volunteer corn	volunteer corn	
Southeast[c] 2019 & 2020	large crabgrass and barnyardgrass.		large crabgrass

[a]Northeast experiment location was sampled on September 19, 2019.
[b]east-central experiment location was sampled on September 9, 2019, and September 25, 2020.
[c]southeast experiment location was sampled on September 15, 2019, and September 16, 2020.

Figure 3.3. Impact of herbicide on weed biomass at end-of-season (g m^{-2}) averaged over planting date and rhizobia treatment in Enlist soybean evaluated at east-central and southeastern South Dakota in 2019 and 2020.



* Different letters above the bars indicate significant differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$

Soybean greenness index (SPAD)

Chlorophyll meter readings has been shown to be positively correlated with leaf nitrogen concentration (Wood et al. 1993). In 2019, SPAD values at R5 growth stage averaged about 41 among all treatments at northeast and east-central locations. However, at southeast location, SPAD values from Enlist soybean variety was impacted by herbicide treatment (Table A4) with the pre-only averaging 27 and the 2,4-D treatments averaging 38 (Table 3.14). In 2020 and at east-central location, the late planting date (June 16) had a lower SPAD value (40.2) than the earlier planting date (May 20) which had a SPAD value of 41.5 (Table 3.14). The low SPAD values obtained from the pre-only treatment was probably due to weed stress. The application of herbicide treatments had no impact on SPAD values of Enlist soybean at east-central location.

Soybean aboveground biomass

Average biomass for Enlist soybean over all herbicide treatment was about 25 g/plant (Northeast-2019), 17 g/plant (east-central), and 16 g/plant (southeast). No herbicide by planting date interaction was observed at any location in 2019 and 2020 seasons (Table A5). In 2020, planting date, but not herbicide treatment, impacted soybean biomass at southeast ($p \leq 0.01$) and east-central ($p \leq 0.05$) locations. Delaying planting from May 15 (early) to June 12 (late) at southeast resulted in 38 % loss in soybean biomass. For each day of a delay in planting at southeast, the Enlist variety lost on average 8g of soybean biomass per plant. Also, delaying planting from May 20 to June 16 at east-central in 2020 resulted in 13% reduction in soybean biomass (Table 3.15), and for each day that planting was delayed, the Enlist soybean lost on average 1.4g of plant biomass.

Table 3.14. Herbicide and planting date effect on SPAD/chlorophyll value of Enlist soybean evaluated at southeast (2019) and east-central (2020) South Dakota.

Southeast (2019 season)	
Herbicide treatment	***SPAD value***
2,4-D + glufosinate	38.1 a
2,4-D + clethodim	37.9 ab
Pre-only	27.4 b
LSD $_{(0.10)}$ = 0.3	
East-central (2020 season)	
Planting date	***SPAD value***
Early (May 20)	41.5 a
Mid (June 3)	41.5 a
Late (June 16)	40.2 b
LSD $_{(0.10)}$ = 0.1	

* Different letters indicate differences among herbicide and planting date using the Fisher’s test for significance at $p \leq 0.10$.

Table 3.15. Effect of planting date on aboveground biomass of Enlist soybean evaluated at southeast and east-central South Dakota in 2020 growing seasons.

Southeast (2020 season)	
Planting date	***Soybean biomass (g/plant)***
May 15	33.7 a
May 29	28.4 b
June 12	20.9 c
LSD $_{(0.10)}$ = 4.4	
East-central (2020 season)	
Planting date	***Soybean biomass (g/plant)***
May 30	17.0 a
June 3	16.5 ab
June 16	14.8 b
LSD $_{(0.10)}$ = 1.7	

* Different letters indicate differences among planting date using the Fisher's test for significance at $p \leq 0.10$.

Nodule number

In 2019, average nodule numbers per 500 cm^3 of soil were 38, 68, and 59 at northeast, east-central, and southeast locations. Only herbicide treatment at northeast influenced the number of nodules (Table A6). Total nodule number from 2,4-D + clethodim and 2,4-D + glufosinate treatment at northeast averaged about 57 nodules per 500 cm^3 soil; and was more than what was found in the pre-only plots which had 27 nodules per 500 cm^3 soil (Table 3.16) Also, the application of rhizobia to 2,4-D + clethodim negatively impacted nodule numbers as nodulation was reduced by 47 % (Table 3.16). However, in 2020 growing season, foliar rhizobia application to 2,4-D + clethodim treatment increased nodule number by 3 % at east-central site (Table 3.17). Furthermore, planting Enlist soybeans early (May 20), rather than at a late date (June 16) at east-central location increased nodule numbers by about 30 % (Table 3.18).

Active nodule number

Active nodules ranged from 29 to 93% of the total nodules. The number of active nodules were above the number suggested in literature for good N fixation (Staton, 2011). No planting date by herbicide interaction was found for active nodule number at all three study locations and in both 2019 and 2020 seasons (Table A7). However, the number of active nodules were impacted by herbicide only at east-central location in 2020 season. The 2,4-D + glufosinate treatment with an active nodule number of 31 was 34 % less than the 2,4-D + clethodim treatment (Table 3.17). Overall, the active nodule number was not influenced by application of rhizobia with herbicide.

Planting date influenced active nodule numbers at southeast (2019 and 2020) and east-central (2020) locations. In 2019, and at southeast, active nodule numbers increased by 144 % when planting was delayed from May 7 to June 19, whereas in 2020, active nodule numbers reduced by 48% when planting was delayed until June 12 (Table 3.18). Also, delaying planting at east-central location from May 20 until June 16 resulted in 50 % reduction in active nodule numbers.

Soil moisture levels from rainfall in the growing season influenced the number of active nodules. Since soybean roots were sampled in late July/early August, and rainfall amounts that month were above the 30-year average for all locations [except for southeast (2020)], the number of active nodules (regardless of the total nodule number) decreased considerably with increasing soil moisture. Having too wet or flooded soils at northeast location resulted in more nodules being decayed or rotten (http://msue.anr.msu.edu/news/evaluating_soybean_nodulation).

Table 3.16. Effect of herbicide on nodule number and active nodule number of Enlist soybean evaluated at northeast, east-central, and southeastern South Dakota in 2019 season.

Location/year	Herbicide treatment	Nodule number per 500 cm^3 soil	Active nodule number per 500 cm^3 soil
Northeast 2019	2,4-D + clethodim	64 a	15
	2,4-D + glufosinate	50 ab	14
	2,4-D + glufosinate + rhizobia	49 ab	14
	2,4-D + clethodim + rhizobia	34 bc	10
	Pre-only	29 c	8
	Pre + rhizobia	24 c	7
	LSD $_{(0.10)}$	1.3	N/S
East-central 2019	2,4-D + clethodim	68	34
	2,4-D + glufosinate	69	34
	2,4-D + glufosinate + rhizobia	66	42
	2,4-D + clethodim + rhizobia	65	33
	Pre-only	66	40
	Pre + rhizobia	71	40
	LSD $_{(0.10)}$	N/S	N/S
Southeast 2019	2,4-D + clethodim	28	13
	2,4-D + glufosinate	32	13
	2,4-D + glufosinate + rhizobia	31	14
	2,4-D + clethodim + rhizobia	31	16
	Pre-only	41	23
	Pre + rhizobia	38	22
	LSD $_{(0.10)}$	N/S	N/S

* Different letters indicate differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$.

* N/S – not significant.

Table 3.17. Effect of herbicide on nodule number and active nodule number of Enlist soybean evaluated at northeast, east-central, and southeastern South Dakota in 2020 season.

Location/year	Herbicide treatment	Nodule number per 500 cm^3 soil	Active nodule number per 500 cm^3 soil
East-central 2020	2,4-D + clethodim	61 bc	40 ab
	2,4-D + glufosinate	46 c	31 b
	2,4-D + glufosinate + rhizobia	54 bc	40 ab
	2,4-D + clethodim + rhizobia	63 a	47 a
	Pre-only	52 bc	33 b
	Pre + rhizobia	59 ab	39 ab
	LSD $_{(0.10)}$	10.1	9.1
Southeast 2020	2,4-D + clethodim	43	29
	2,4-D + glufosinate	39	19
	2,4-D + glufosinate + rhizobia	41	24
	2,4-D + clethodim + rhizobia	43	28
	Pre-only	43	26
	Pre + rhizobia	38	25
	LSD $_{(0.10)}$	N/S	N/S

* Different letters indicate differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$.

*N/S – not significant.

Table 3.18. Effect of planting date on total nodule number and active nodule number of Enlist soybean averaged over herbicide and rhizobia treatment at southeast (2019 and 2020) and east-central (2020) South Dakota.

Planting date	Nodule number per 500 cm^3 soil.	Active nodule number per 500 cm^3 soil.
Northeast 2019		
May 15	43	10
May 30	38	10
June 15	45	14
LSD $_{(0.10)}$	N/S	N/S
East-central 2019		
May 15	76	41
June 2	59	36
June 19	68	34
LSD $_{(0.10)}$	N/S	N/S
Southeast 2019		
May 7	24	9 b
June 5	37	19 ab
June 19	39	22 a
LSD $_{(0.10)}$	N/S	9.3
Southeast 2020		
May 15	49 a	33 a
May 29	44 a	25 ab
June 12	30 b	17 b
LSD	13.9	9.4
East-central 2020		
May 20	66 a	50 a
June 3	59 a	40 b
June 16	43 b	25 c
LSD $_{(0.10)}$	9.4	10.1

* Different letters indicate differences among planting date using the Fisher's test for significance at $p \leq 0.10$.

* N/S – not significant.

Grain yield

Grain yield from 2019 season averaged 3443, 3080 and 3201 kg ha^{-1} at northeast, east-central, and southeastern locations, respectively. In 2020, grain yield averaged over herbicide and rhizobia treatments at east-central and southeast locations reduced by 19 % and 30 % when compared to 2019 season. There was no interaction between planting date and herbicide treatment for grain yield at any of the three study locations (northeast, southeast and east-central SD), and for both years (2019 and 2020) (Table A8). In 2019 season, and only at northeast location, grain yield of Enlist soybean was influenced by planting date. When planting was delayed from the optimum date (May 15) to the latest date (June 15), Enlist soybean yield reduced by 12 %. Yield was more in the early and mid-planting dates (3591 and 3597 kg $ha^{-1,}$ respectively) than in the late planting date (3147 kg ha^{-1}) (Table 3.19). In 2020, soybean yields at southeast and east-central locations were reduced by 38, and 49 %, respectively when planting was delayed from early to the late dates (Table 3.19).

100-seed weight

In 2019, the weight of a hundred seed of Enlist soybean across locations averaged 15 g. There was no planting date by herbicide interaction at northeast, east-central and southeast locations. However, 100-seed weight was influenced by only herbicide at northeast (Table A9). 2,4-D + clethodim herbicide treatment increased 100-seed weight by 5% over the pre-only treatment which was heavily infested with weeds (Table 3.20). The foliar application of rhizobia inoculant with herbicide did not influence seed weight. In 2020, 100-seed weight was influenced by planting date at east-central and southeastern location with higher seed weights found in the early and mid-planting dates than in the late planting date (Table 3.20). The 100-seed weight of Enlist soybean was reduced by 6 % (southeast) and 4 % (east-central) when planting was delayed until June 12 and June 16, respectively.

Table 3.19. Grain yield of Enlist soybean evaluated at northeast (2019), east-central (2019 and 2020) and southeastern (2019 and 2020) South Dakota locations.

Location/year	**Planting date**	**GDD[a]**	**Yield (kg ha^{-1})**	**Harvest date**
Northeast 2019	May 15	1178	3591.2	October 30
	May 30	1136	3597.9	
	June 15	1008	3147.4	
	LSD $_{(0.10)}$ = 255.6			
East-central 2019	May 15	1279	2999.4	October 29
	June 2	1198	3261.7	
	June 19	1047	2993.0	
	LSD $_{(0.10)}$ = N/A			
Southeast 2019	May 7	1535	3584.5	October 19
	June 5	1378	3490.3	
	June 19	1236	2528.6	
	LSD $_{(0.10)}$ = N/A			
East-central 2020	May 20	1420	3207.9	October 9
	June 3	1314	2797.6	
	June 16	1166	1634.2	
	LSD $_{(0.10)}$ = 457.3			
Southeast 2020	May 15	1553	2770.7	October 15
	May 29	1458	2232.7	
	June 12	1292	1728.4	
	LSD $_{(0.10)}$ = 282.5			

[a]GDD – growing degree days. N/A = not applicable.

Table 3.20. Effect of herbicide (northeast, 2019 season) and planting date (east-central, 2020 season) averaged over rhizobia and herbicide treatment on 100-seed weight of Enlist soybean.

Location/year	**Herbicide treatment**	**100-seed weight (g)**
Northeast 2019	2,4-D + clethodim	16.0 a
	2,4-D + glufosinate	15.6 ab
	Pre-only	15.3 b
		LSD $_{(0.10)}$ = 0.5
Location/year	**Planting date**	**100-seed weight (g)**
East-central 2020	May 20	14.9 a
	June 3	14.7 a
	June 16	14.3 b
		LSD $_{(0.10)}$ = 0.3
Southeast 2020	May 15	14.6 a
	May 29	14.6 a
	June 12	13.7 b
		LSD $_{(0.10)}$ = 0.7

* Different letters indicate differences among herbicide and planting date treatment using the Fisher's test for significance at $p \leq 0.10$.

Seed oil content

Soybean seeds have an oil content of approximately 18-22% (Willis, 2003; Patil et al. 2018). Average seed oil in the 5-location year study was 19 %. There was no planting date by herbicide treatment interaction for seed oil [at 13 % moisture] at all study locations in 2019 and 2020 (Table A10). The seed oil of Enlist soybean was 1 to 2 % greater at east-central location with 2,4-D + clethodim treatment compared to the pre-only treatment (Table 3.21). A combined application of rhizobia with herbicide did not increase seed oil of Enlist soybean. In 2020, seed oil content was influenced by planting date at east-central and southeast locations, with the early and mid-planting dates having 2 % and 9 % greater oil content than the late planting date at southeast and east-central locations, respectively (Table 3.22).

Seed protein content

Soybean seed protein has been reported to be about 38-48% on a dry weight basis (Willis, 2003). Average seed protein content of Enlist soybean in the 5-location year study was 34 %. No planting date by herbicide interaction was found at northeast, east-central and southeast locations in 2019 season (Table A11). However, in 2020, there was an interaction between planting date and herbicide treatment at southeast ($p \leq 0.05$) and east-central ($p \leq 0.00$) locations. At both locations, seed protein content for early and mid-planting dates with auxin-based herbicide (2,4-D + clethodim or 2,4-D + glufosinate) treatment combination was greater (average protein value = 35 %) than the late planting date/herbicide combination (average protein value = 34 %). The pre-only treatment for early or mid-planting date had lesser protein values than the post applied treatment [2,4-D + clethodim or 2,4-D + glufosinate] when compared within a planting date at east-central location (Figure 3.4). The pre-only treatment for mid-planting date at southeast location had lower seed protein value (33.7 %) than the post applied treatments when compared within a planting date [(2,4-D + clethodim = 34.1 %; 2,4-D + glufosinate = 34.9 %)] (Figure 3.5). Overall, as seed protein increased, seed oil levels decreased for Enlist soybean variety planted.

Table 3.21. Herbicide effect on seed oil (at 13% moisture) of Enlist soybean evaluated at east-central South Dakota in 2019 season.

East-central (2019 season)	
Herbicide treatment	Seed oil content at 13% moisture
2,4-D + clethodim	19.1 a
2,4-D + glufosinate	19.1 a
Pre-only	18.9 b
	LSD $_{(0.10)}$ = 0.7

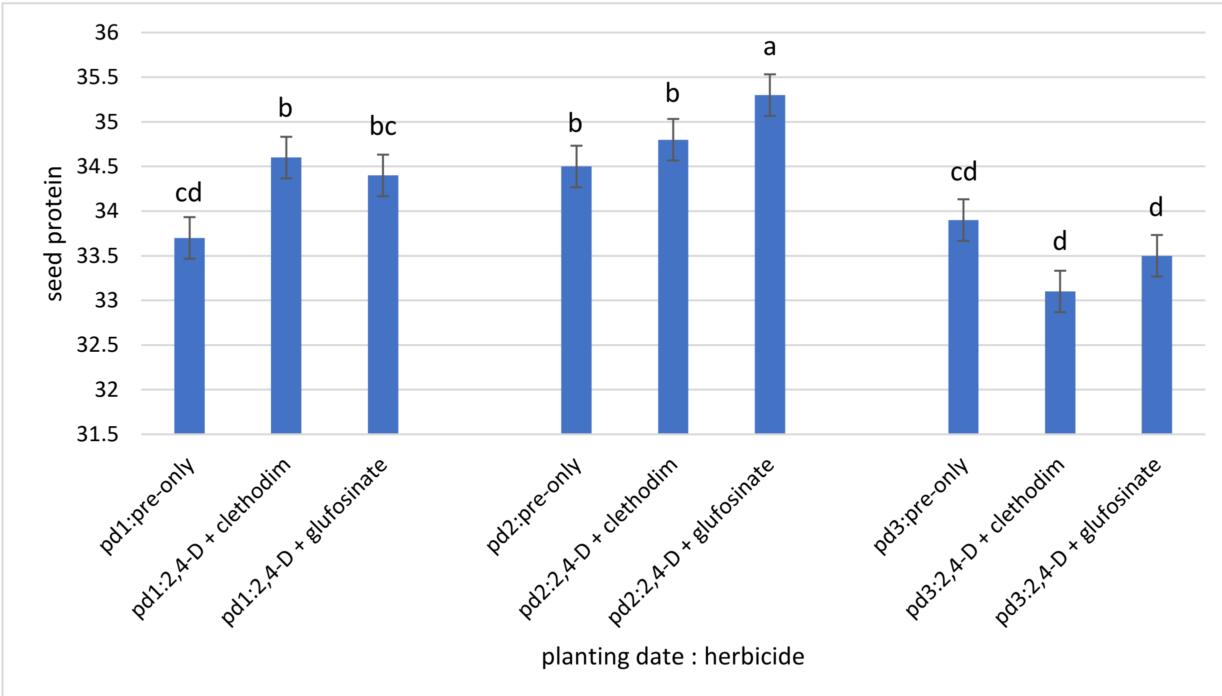
* Different letters indicate differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$.

Table 3.22. Effect of planting date on seed oil content of Enlist soybean evaluated at southeast and east-central locations in 2020 cropping season.

Planting date	Seed oil at 13% moisture
Southeast 2020	
May 15	19.3 a
May 29	19.3 a
June 12	18.9 b
LSD $_{(0.10)}$ = 0.3	
East-central 2020	
May 20	19.0 a
June 3	18.2 b
June 16	17.5 b
LSD $_{(0.10)}$ = 0.4	

Different letters indicate differences among planting date using the Fisher's test for significance at $p \leq 0.10$.

Figure 3.4. Interaction effect of planting date and herbicide (averaged over rhizobia treatment) on seed protein content [at 13% moisture] of Enlist E3 soybean evaluated at east-central South Dakota in 2020 season.



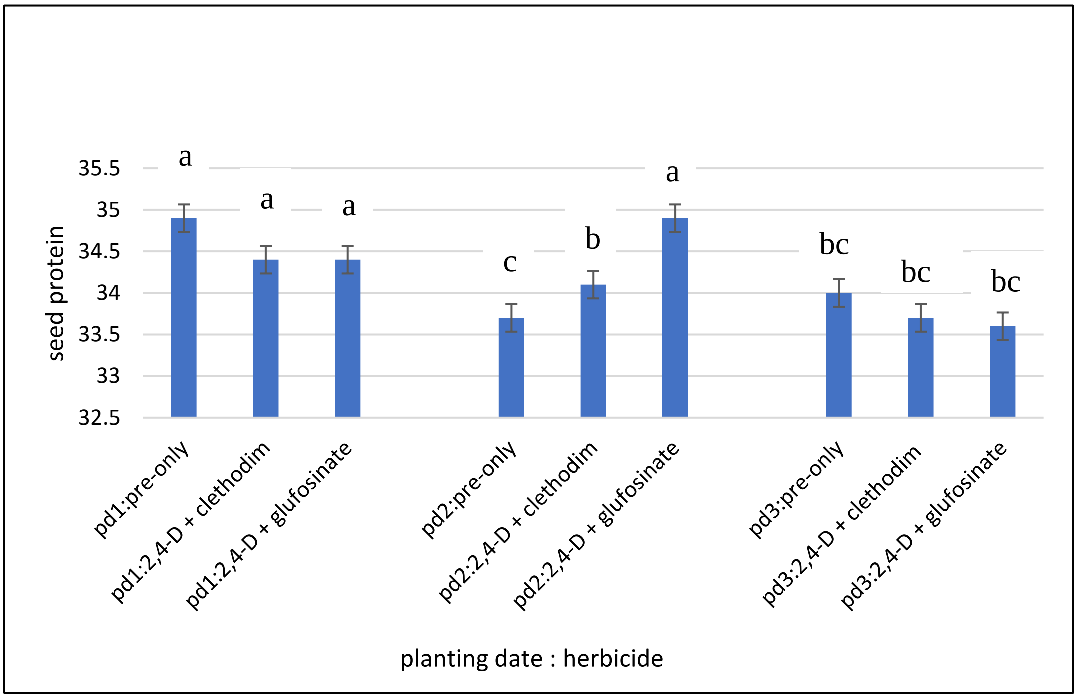
*Different letters above the bars indicate differences among the planting date by herbicide treatment using Fisher's test for significance at $p \leq 0.10$. Bars show standard deviation of treatment.

* pd1 = early planting date: May 20.

* pd2 = mid-planting date: June 3.

* pd3 = late planting date: June16.

Figure 3.5. Interaction effect of planting date and herbicide (averaged over rhizobia treatment) on seed protein content [at 13% moisture] of Enlist E3 soybean evaluated at southeastern South Dakota in 2020 season.



*Different letters above the bars indicate differences among the planting date by herbicide treatment using Fisher's test for significance at $p \leq 0.10$. Bars show standard deviation of treatment.

* pd1-early planting date: May 15, 2020.

* pd2-mid-planting date: May 29, 2020.

* pd3-late planting date: June 12, 2020.

3.3.3. Roundup ready 2 Xtend soybean evaluated for weed control and nodulation potential at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

Weed control

No planting date by herbicide interaction was found in Xtend soybean variety evaluated for weed density (plants m^{-2}) at two weeks after treatment application at northeast, east-central, and southeastern South Dakota in 2019 and 2020 seasons (Table A12). However, the number of weeds found at the three study locations were influenced by herbicide application (Table A12). Dicamba + glyphosate controlled the annual grasses [for example, woolly cupgrass at east-central location; green and yellow foxtails at northeast; and barnyardgrass at southeast locations] and the broadleaf weeds (mostly pigweed species) present. At southeast, the acifluorfen + clethodim treatment had higher densities of large crabgrass, barnyardgrass, and green foxtail. The pre-only and acifluorfen treatments had greater number of weeds than dicamba + glyphosate at 2 weeks following treatment application at all locations (Table 3.23 and Table 3.24).

An interaction between planting date and herbicide treatment was found at 6 weeks after treatment application at the northeast location (2019 season) (Table A13). At 6 weeks, all planting dates plots treated with dicamba + glyphosate had no weeds, whereas the pre-only treatment at early and mid-planting dates had greater weed densities when compared to acifluorfen + clethodim treatments (Figure 3.6). Weeds such as green foxtail, yellow foxtail, barnyardgrass, woolly cupgrass, large crabgrass, wild buckwheat, redroot pigweed, and eastern black nightshade were controlled by dicamba + glyphosate at the three study locations in 2019 and 2020. Uncontrolled weeds in acifluorfen +

clethodim treatment included waterhemp, marestail, large crabgrass, and at the southeast location, field sandbur.

Weed species present at the end-of-season weed biomass sampling at the three experiment locations are presented in Table 3.24. Green foxtail, yellow foxtail, dandelion, and redroot pigweed are weed species common to the three study locations. The analysis of variance (ANOVA) showed no planting date by herbicide interaction for weed biomass at harvest at the three study locations, and for either growing season (2019 and 2020) (Table A14). However, weed biomass was impacted by herbicide ($p<0.00$) at northeast (2019), southeast (2019 and 2020), and east-central (2020) locations (Table A14). For the two growing seasons, and across study locations, soybean plots treated with dicamba + glyphosate had fewer weeds when compared to the acifluorfen + clethodim and pre-only treatments (Table 3.25). Weed biomass at harvest was also impacted by planting date at northeast ($p=0.04$) and southeast ($p=0.01$) locations in 2019 and 2020 seasons, respectively, with the early planting date having 65 % more weed biomass than the late planting date at northeast. However, at southeast location, weed biomass was 58 % less in early planting date compared to the late planting date (Table 3.26) as soybean canopy was dense and provided shading to weeds that emerged later in the season.

Table 3.23. Herbicide treatment and planting date effect on weed density at 2 weeks after POST treatment application in Xtend soybean evaluated at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing season.

Weed density (plants m^{-2}) at 2 weeks after POST treatment application in Xtend soybean					
	2019 season			2020 season	
Herbicide (Averaged over planting date)	Northeast	East-central	Southeast	East-central	Southeast
Pre-only	79 a	16 a	66 a	32 a	72 a
Acifluorfen + clethodim	32 a	8 ab	26 b	18 ab	70 a
Dicamba 1 app. + glyphosate	4 c	3 b	4 b	4 b	13 b
Dicamba 2 apps. + glyphosate	N/A	6 b	8 b	N/A	N/A
LSD $_{(0.10)}$	7.0	3.0	13.0	6.0	7.0
Planting date (Averaged over herbicide treatment)	Northeast 2019 season				
May 15	20 a				
May 30	14 b				
June 15	11 b				
LSD $_{(0.10)}$	13.0				

*N/A – not applicable. Common lambsquarters and barnyardgrass were the weeds present at 2 weeks following POST treatment application at northeast. Barnyardgrass, redroot pigweed, wild buckwheat and volunteer corn were the weeds present at two weeks after POST treatment application at east central and southeastern locations. Planting date was significant at northeast location only.

Table 3.24. weed species present in Xtend soybean at 2 weeks and 6 weeks after herbicide treatment application, and at end-of-season (September) weed biomass sampling.

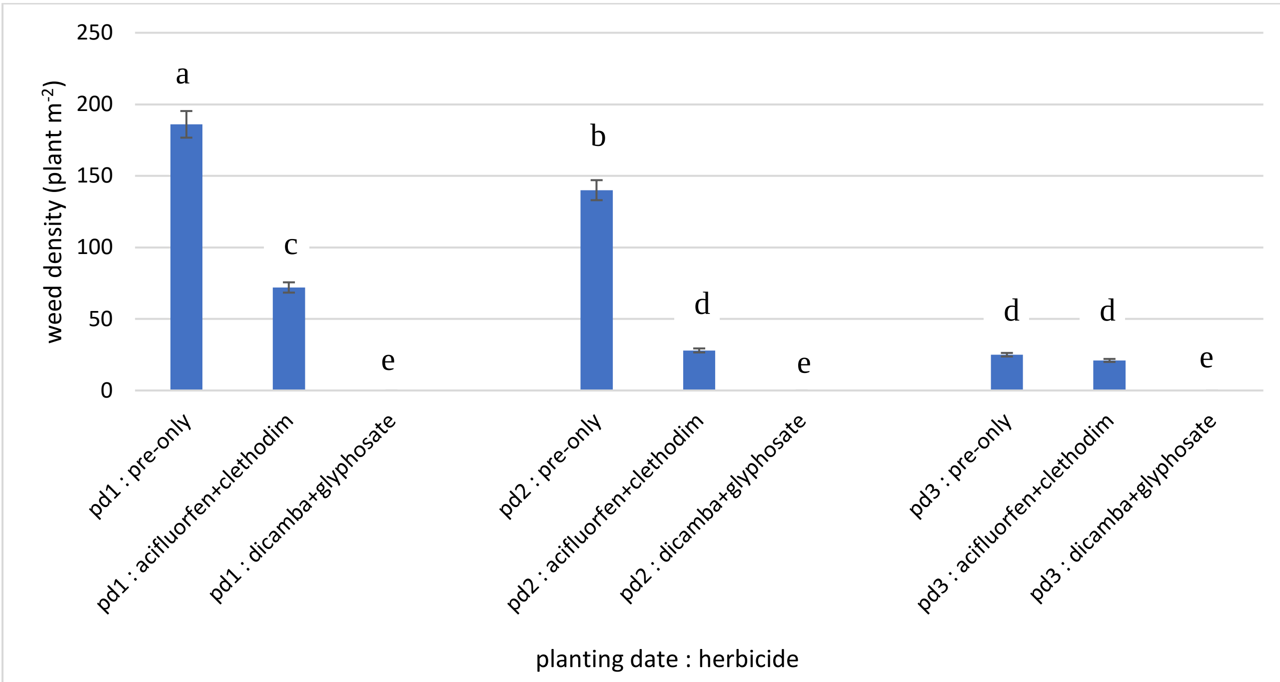
Weeds present at 2 weeks after herbicide treatment application			
	Northeast	**East-central**	**Southeast**
Pre-only	common lambsquarters, common purslane, prostrate pigweed, green foxtail, yellow foxtail	woolly cupgrass, common lambsquarters, wild buckwheat, green foxtail	barnyardgrass, large crabgrass, green foxtail,
Acifluorfen + clethodim	green foxtail, yellow foxtail	woollycupgrass	large crabgrass, barnyardgrass, and green foxtail
Dicamba + glyphosate	common lambsquarters	volunteer corn	volunteer corn
Weeds present at 6 weeks after herbicide treatment application			
Pre-only	common lambsquarters, common purslane, prostrate pigweed, dandelion, green foxtail, yellow foxtail, woolly cupgrass, wild buckwheat	woolly cupgrass, green foxtail, yellow foxtail, quackgrass, dandelion, common lambsquarters, redroot pigweed, wild buckwheat	barnyardgrass, large crabgrass, green foxtail, yellow foxtail, fall panicum, waterhemp, marestail, field sandbur
Acifluorfen + clethodim	Common lambsquarters,	Waterhemp, dandelion	Waterhemp, marestail, field sandbur
Dicamba + glyphosate	No weeds	No weeds	field sandbur

*The weed species present did not change across planting dates.

Weeds present at end-of-season weed biomass sampling			
	Pre-only Herbicide Treatment		
Location	Early planting (PD1)	Mid-season planting (PD2)	Late planting (PD3)
Northeast[a] 2019 only	similar weeds were found in all three planting dates, and they include the following: green foxtail, yellow foxtail, woolly cupgrass, barnyardgrass, large crabgrass, common lambsquarters, common purslane, prostrate pigweed, dandelion, redroot pigweed, volunteer wheat		
East-central[b] 2019 & 2020	velvetleaf, dandelion, barnyardgrass, wild buckwheat, common lambsquarters, dandelion	quackgrass, barnyardgrass, lady's thumb, velvetleaf, green and yellow foxtail, woolly cupgrass, common lambsquarters, wild buckwheat	wild buckwheat, lady's thumb, quackgrass, barnyardgrass, woolly cupgrass, velvetleaf, common lambsquarters, green and yellow foxtail, redroot pigweed, dandelion, wild four o' clock, waterhemp
Southeast[c] 2019 & 2020	volunteer corn, redroot pigweed, barnyardgrass, waterhemp, marestail	green and yellow foxtail, foxtail barley, fall panicum, field sandbur, large crabgrass, waterhemp, marestail	barnyardgrass, green and yellow foxtail, foxtail barley, fall panicum, field sandbur, large crabgrass, dandelion, common waterhemp, redroot pigweed, marestail, wild buckwheat,
	Acifluorfen + clethodim treatment		
	Early planting (PD1)	Mid-season planting (PD2)	Late planting (PD3)
Northeast[a] 2019 only	volunteer wheat	common lambsquarters, volunteer wheat	barnyardgrass, green foxtail and volunteer wheat.
East-central[b] 2019 & 2020	volunteer corn	volunteer corn, green foxtail	volunteer corn, green foxtail
Southeast[c] 2019 & 2020	volunteer corn	large crabgrass, barnyardgrass, fall panicum	Common waterhemp, marestail, field sandbur
	Dicamba + glyphosate treatment		
	Early planting	Mid-season planting	Late planting
Northeast[a] 2019 only			barnyardgrass and volunteer wheat
East-central[b] 2019 & 2020	green foxtail, volunteer corn	volunteer corn	
Southeast[c] 2019 & 2020	large crabgrass and barnyardgrass.		large crabgrass

[a]Northeast experiment location was sampled on September 19, 2019.
[b]east-central experiment location was sampled on September 9, 2019, and September 25, 2020.
[c]southeast experiment location was sampled on September 15, 2019, and September 16, 2020.

Figure 3.6. Interaction effect of planting date and herbicide on weed density [plant m⁻²] at 6 weeks after POST treatment application in Xtend soybean evaluated at northeast location in 2019.



* Different letters above the bars indicate significant differences among the planting date by herbicide treatment using the Fisher's test for significance at $p \leq 0.10$. Error bars show the standard deviation of treatment mean.

Table 3.25. Effect of herbicide on end-of-season weed biomass (g m^{-2}) averaged over planting date in Xtend soybean evaluated at northeast (2019), southeast (2019 and 2020), and east-central (2020) locations.

End-of-season weed biomass (g m^{-2})				
Herbicide treatment	Northeast 2019 ($p \leq 0.001$)	Southeast 2019 ($p \leq 0.001$)	Southeast 2020 ($p < 0.001$)	East-central 2020 ($p < 0.001$)
Dicamba 1 app. + glyphosate	23 b	37 bc	58 d	28 c
Dicamba 2apps. + glyphosate	-	33 bc	-	-
Pre-only	188a	172 a	427 a	179 a
Acifluorfen + clethodim	67 b	85 b	245 c	64 bc
$LSD_{(0.10)}$	60	104	100	62

* Different letters indicate significant differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$.

Table 3.26. Effect of planting date on end-of-season weed biomass (g m^{-2}) averaged over herbicide treatment in Xtend soybean evaluated at northeast (2019) and southeast (2020).

Location/year	Planting date	weed biomass (g m^{-2})
Northeast 2019	May 15	112 a
	May 30	110 a
	June 15	40 b
	$LSD_{(0.10)}$	56
Southeast 2020	May 15	144 b
	May 29	212 b
	June 12	341 a
	$LSD_{(0.10)}$	96

* Different letters indicate significant differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$.

Soybean greenness index (SPAD) measured at R5

The SPAD values measured at R5 growth stage averaged 41 in the 5-location year study. No planting date by herbicide interaction was found for Xtend soybean SPAD readings at the study locations [northeast, southeast, and east-central South Dakota] in 2019 and 2020 (Table A15). In 2019, and at southeast location, the Xtend soybean SPAD value was influenced by herbicide application ($p<0.05$) (Table A15). Plots treated with dicamba + glyphosate and acifluorfen + clethodim herbicide had higher SPAD values (average = 41) than the pre-only treated plots (40) (Table 3.27). SPAD values when rhizobia inoculant was applied with dicamba + glyphosate and pre-only treatments increased by 3 %, whereas rhizobia application with acifluorfen + clethodim reduced SPAD value by 1 %. However, in 2020, rhizobia application with herbicide had no impact on SPAD readings at southeast and east-central locations.

Planting date impacted the SPAD values obtained at east-central and southeast locations in 2019 and 2020 growth season, respectively (Table 3.28). Delaying soybean planting from May 15 (early) to June 19 (late) increased SPAD value by 1% at east-central location in 2019, whereas planting late reduced SPAD values by 2% at southeast location in 2020 season (Table 3.28).

Table 3.27. Effect of herbicide on the greenness index averaged over planting date in Xtend soybean evaluated at southeast (2019 and 2020) and east-central (2020) South Dakota.

Xtend soybean SPAD value taken at R5 growth stage			
Herbicide	Southeast (2019)	Southeast (2020)	East-central (2020)
Dicamba + glyphosate + rhizobia	41.8a	39.8 a	40.8 a
Acifluorfen + clethodim	41.5 a	39.3 ab	40.8 a
Dicamba 2apps. + glyphosate + rhizobia	41.1 b	N/A	N/A
Acifluorfen + clethodim + rhizobia	41.1 b	39. 1 b	41.1 a
Pre + rhizobia	41.0 b	39.3 ab	39.7 b
Dicamba + glyphosate	40.7 c	39.8 a	40.9 a
Dicamba 2apps. + glyphosate	40.6 c	N/A	N/A
Pre-only	40.1 c	39.8 a	39.2 b
LSD $_{(0.10)}$	1.3	0.5	0.6

* Different letters within the same column indicate significant differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$. *N/A means not applicable.

Table 3.28. Effect of planting date on the greenness index averaged over herbicide treatment in Xtend soybean evaluated at east-central (2019) and southeast (2020) South Dakota.

Xtend soybean SPAD value at R5 growth stage	
East-central (2019)	
May 15	40.6 b
June 2	40.6 b
June 19	41.0 a
LSD $_{(0.10)}$	0.3
Southeast (2020)	
May 15	39.9 a
May 29	39.6 a
June 12	39.1 b
LSD $_{(0.10)}$	0.5

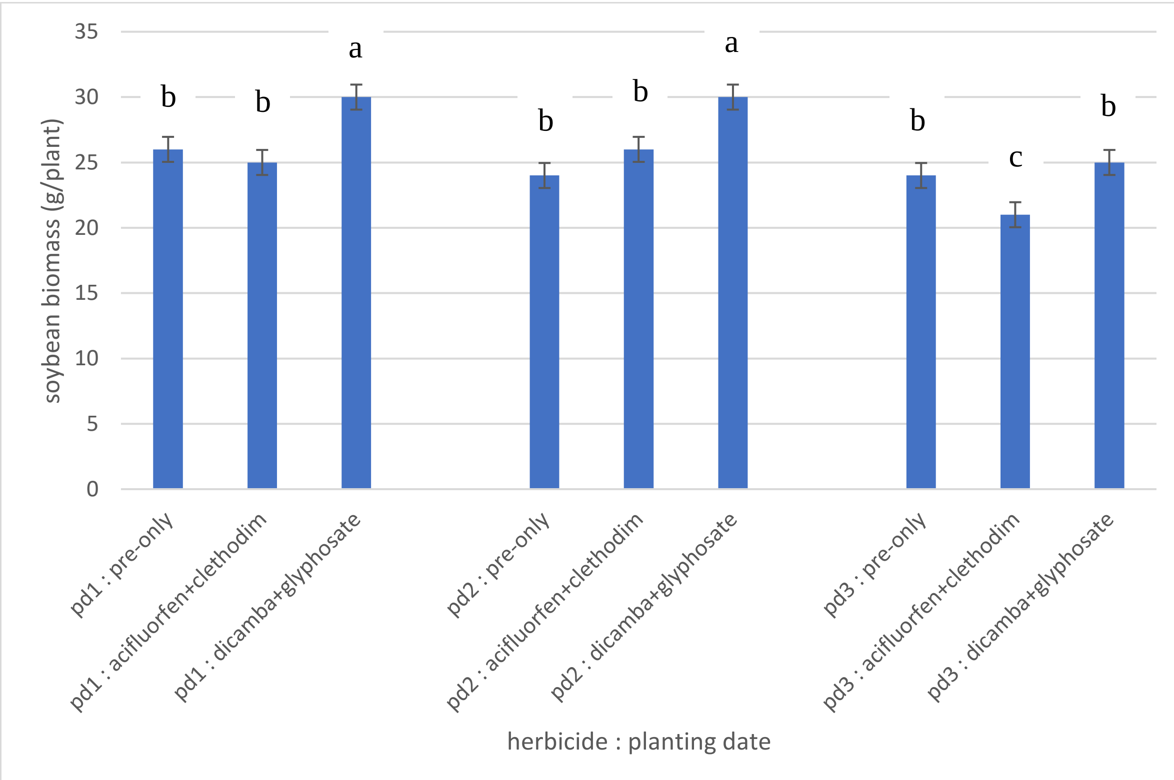
* Different letters within the same column indicate significant differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$.

Soybean aboveground biomass

Average biomass for Xtend soybean in the study was about 22 g/plant. An interaction was observed between planting date and herbicide treatment in 2019 (northeast only; $p \leq 0.05$) and 2020 (east-central only; $p \leq 0.01$) seasons (Table A16). When comparing within planting dates at northeast, soybean from plots treated with dicamba + glyphosate treatment at early (May 15) and late (June 15) planting dates had more biomass (average = 27 g/plant) than acifluorfen + clethodim (average = 24 g/plant) (Figure 3.7). Also, when comparing within the mid-planting date (May 30), dicamba + glyphosate treatment had greater biomass (18 % increase) than the pre-only treatment (Figure 3.7).

At east-central location (2020 season), dicamba + glyphosate herbicide treatment at an early (May 20) and late (June 16) planting dates had greater biomass (average = 16 g/plant) than the pre-only (average = 13 g/plant) and acifluorfen + clethodim (12 g/plant) treatments (Figure 3.8). Also, acifluorfen + clethodim treatment at late (June 16) planting date combination had the lowest soybean biomass (8 g/plant) (Figure 3.8). Delaying Xtend soybean planting from the optimum sowing date (May 15) until a later date at southeast (June 12) and east-central (June 16) sites in 2020 resulted in about 49 and 23 % loss in biomass (Table 3.29).

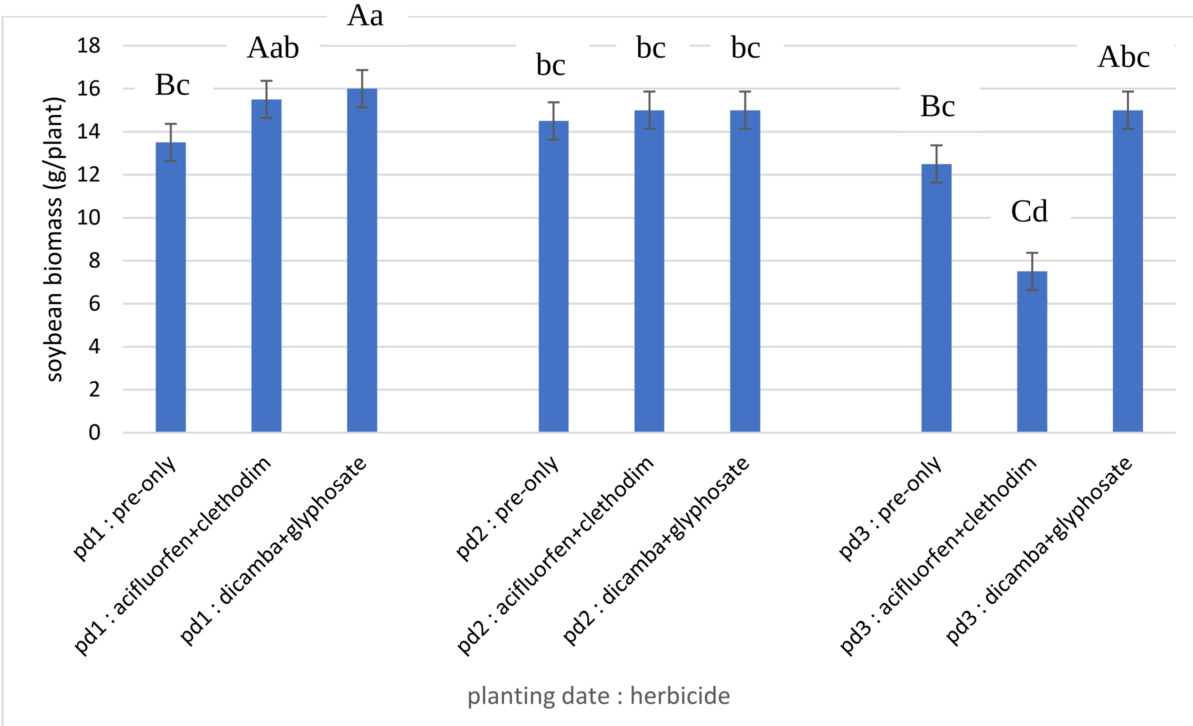
Figure 3.7. Interaction effect of planting date and herbicide treatment (averaged over rhizobia treatment) on soybean (Xtend) aboveground biomass (g/plant) evaluated at northeast location in 2019.



* Different letters above the bars indicate significant differences among the planting date by herbicide treatment using the Fisher's test for significance at $p \leq 0.10$. Error bars show the standard deviation of treatment mean.

*pd1/early planting date = May 15; pd2/mid-planting date = May 30; pd3/late planting date = June 15.

Figure 3.8. Interaction effect of planting date and herbicide on soybean (Xtend) aboveground biomass (g/plant) evaluated at east-central location in 2020 season.



* Different letters above the bars indicate significant differences among the planting date by herbicide treatment using the Fisher's test for significance at $p \leq 0.10$. Uppercase letters show the comparison between planting date by herbicide treatment interactions, whereas lowercase letters show comparison within each planting date by herbicide treatment interactions. Error bars show the standard deviation of planting date by herbicide treatment.

*pd1/early planting date = May 20; pd2/mid-planting date = June 3; pd3/late planting date = June 16.

Table 3.29. Effect of planting date (averaged over herbicide and rhizobia treatments) on aboveground soybean biomass in Xtend soybean evaluated at southeast (2019 and 2020) and east-central (2020) experiment locations

Location	Planting date	Plant biomass (g/plant)
Southeast 2019	May 7	14.4 ab
	June 5	10.0 b
	June 19	17.3 a
	LSD $_{(0.10)}$	2.0
Southeast 2020	May 15	22.3 a
	May 29	21.7 a
	June 12	11.3 b
	LSD $_{(0.10)}$	2.6
East-central 2020	May 20	15.1 a
	June 3	14.9 a
	June 16	11.7 b
	LSD $_{(0.10)}$	1.4

* Different letters within the same column indicate significant differences among planting dates using the Fisher's test for significance at $p \leq 0.10$.

Nodule number

In 2019, total nodule numbers at northeast, southeast and east-central sites averaged 38, 23 and 72 nodules per 500 cm^3 soil, respectively. However, in 2020 season, average nodule numbers at southeast and east-central sites were 42 and 48 nodules per 500 cm^3 soil. No interaction was found between planting date and herbicide treatment at all three locations, and for both years. Only herbicide treatment at east-central location influenced nodule numbers (Table A17). The number of nodules from soybean (Xtend variety) treated with dicamba + glyphosate averaged about 84 nodules/500cm^3 soil and was more than what was found in acifluorfen + clethodim treated plots which had about 50 nodules/500 cm^3 soil (Table 3.30). An application of herbicide with rhizobia had no impact on nodule numbers in 2019 growing season. However, in 2020, nodule numbers were influenced by rhizobia application (Table 3.30). Dicamba + glyphosate + rhizobia, pre-only + rhizobia, and acifluorfen + clethodim + rhizobia treatments all increased nodule number by 2, 18, and 25%, respectively at east-central site, whereas dicamba + glyphosate + rhizobia at southeast increased nodule number by 32 % (Table 3.30). Also, delaying planting from the optimum sowing date (May 15) to later in the season (June) decreased nodule number by 38 % (Table 3.31)

Active nodule number

Active nodules ranged from 22 to 94% of the total nodules. No planting date by herbicide interactions were found for number of active nodules in the Xtend soybean variety evaluated at northeast, southeast, and east-central South Dakota in 2019 and 2020 (Table A18). In 2019, only herbicide impacted active nodule numbers at east-central site. Dicamba + glyphosate treatment had greater active nodule number (average = 84 active nodules per 500 cm^3 soil) than pre-only (average = 66 active nodules per 500 cm^3 soil) and acifluorfen + clethodim (average = 48 active nodules per 500 cm^3 soil) treatments (Table 3.32). Foliar application of rhizobia with herbicide did not increase active nodule number of Xtend soybean. However, in 2020, acifluorfen + clethodim increased active nodule number by 39 % at east-central site, whereas dicamba + rhizobia treatment increased active nodule number by 55 % at southeast when compared to the pre-only herbicide treatment. (Table 3.32). Also, compared to the early planting date, active nodule numbers at southeast and east-central locations reduced by 43 % and 62 %, respectively, when Xtend soybean was planted late in the season (mid-June) (Table 3.33).

Table 3.30. Effect of herbicide on total nodule number (per 500 cm^{-3} soil) (averaged over planting date) of Xtend soybean evaluated at east-central (2019 and 2020) and southeast (2020) experiment locations.

Nodule number (500 cm^{-3} soil) at R5 stage of Xtend soybean			
Herbicide treatment	East-central 2019	East-central 2020	Southeast 2020
Dicamba 1 app. + glyphosate	90 a	51.7 a	39.5 bc
Dicamba 2apps. + glyphosate	83 a	-	-
Dicamba 2apps. + glyphosate + rhizobia	80 a	-	-
Pre-only	69 bc	42.7 b	42.4 b
Pre + rhizobia	65 bc	50.4 a	42.8 b
Acifluorfen + clethodim + rhizobia	62 cd	50.1 a	41.6 bc
Acifluorfen + clethodim	50 d	40.2 b	35.9 c
Dicamba + glyphosate + rhizobia	-	52.8 a	52.2 a
LSD $_{(0.10)}$	28.2	6.4	7.1

* Different letters within the same column indicate significant differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$.

Table 3.31. Effect of planting date on total nodule number (per 500 cm^{-3} soil) of Xtend soybean evaluated at southeast and east-central experiment locations in 2020 season.

Location	Planting date	Nodule number/500 cm^{-3} soil
Southeast 2020	May 15	49.8 a
	May 29	43.7 a
	June 12	33.7 b
	LSD $_{(0.10)}$	9.8
East-central 2020	May 20	59.1 a
	June 3	51.9 a
	June 16	32.9 b
	LSD $_{(0.10)}$	9.8

* Different letters within the same column indicate significant differences among planting dates using the Fisher's test for significance at $p \leq 0.10$.

Table 3.32. Effect of herbicide and rhizobial treatments on active nodule number (per 500 cm^{-3} soil) of Xtend soybean evaluated at east-central (2019 and 2020) and southeast (2020) experiment locations.

Active nodule number per 500 cm^3 soil			
Herbicide treatment	East-central 2019	East-central 2020	Southeast 2020
Dicamba 1 app. + glyphosate	83.7 a	32.4 ab	24.1 b
Dicamba 2apps. + glyphosate	85.9 a	-	-
Dicamba 2apps. + glyphosate + rhizobia	75.3 ab	-	-
Pre-only	66.0 bc	27.3 bc	25.5 b
Pre + rhizobia	58.1 cd	33.4 ab	25.4 b
Acifluorfen + clethodim + rhizobia	48.3 d	33.8 ab	23.8 b
Acifluorfen + clethodim	48.0 d	24.3 c	19.8 b
Dicamba + glyphosate + rhizobia	-	38.5 a	37.4 a
LSD $_{(0.10)}$	19.0	6.9	6.4

* Different letters within the same column indicate significant differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$.

Table 3.33. Effect of planting date on active nodule number (per 500 cm^{-3} soil) of Roundup Ready 2 Xtend soybean evaluated at southeast and east-central locations in 2020

Location	Planting date	Active nodule number/500 cm^{-3} soil
Southeast 2020	May 15	33.3 a
	May 29	25.7 ab
	June 12	19.0 b
	LSD $_{(0.10)}$	10.7
East-central 2020	May 20	42.3 a
	June 3	36.5 a
	June 16	16.0 b
	LSD $_{(0.10)}$	7.5

* Different letters within the same column indicate significant differences among herbicide treatment using the Fisher's test for significance at $p \leq 0.10$.

Grain yield

Average grain yields at northeast, southeast, and east-central locations were 3221, 3789, and 3006 kg ha^{-1}, respectively in 2019 growing season. However, about 32 % yield reduction was observed in 2020. There was no interaction between planting date and herbicide at the three locations for both years (2019 and 2020) (Table A19). Herbicide treatment application impacted grain yield at southeast (2019 and 2020) and east-central (2020) locations. In 2019, the pre-only treatment had the lowest grain yield (3369 kg ha^{-1}) compared to Dicamba + glyphosate (3874 kg ha^{-1}) and acifluorfen + clethodim (3880 kg ha^{-1}) treatments. (Table 3.34). In 2020, dicamba + glyphosate application increased yield by about 27 % when compared to acifluorfen + clethodim and pre-only treatments at southeast. However, at east-central site, Dicamba + glyphosate application increased grain yield by 4 and 12 % when compared with acifluorfen + clethodim and the pre-only treatments, respectively (Table 3.34). Rhizobia application with herbicide treatments did not impact grain yield (Table 3.34).

Planting date influenced the grain yield of Xtend soybean at southeast and east-central locations in both years (Table A19). Overall, the early and mid-planting dates had higher yields than late planting date (Table 3.35). At southeast, a yield loss of about 7 % (2019 season) and 32 % (2020 season) occurred when Xtend soybean was planted late (around mid-June). Also, at east-central site, delaying planting from the optimum date (mid-May) to a late date (mid-June) resulted in 13 and 71 % yield loss in 2019 and 2020 seasons, respectively.

Table 3.34. Effect of herbicide on grain yield (kg ha^{-1}) of Xtend soybean evaluated at southeast (2019 and 2020), and east-central (2020) experiment locations.

Grain yield (kg ha^{-1})			
Herbicide treatment	Southeast 2019	Southeast 2020	East-central 2020
Dicamba 1 app. + glyphosate	3954.4 a	2535.4 a	2380.7 ab
Dicamba 2apps. + glyphosate	4203.2 a	-	-
Dicamba 2apps. + glyphosate + rhizobia	3645.0 ab	-	-
Pre-only	3369.3 b	2165.5 b	2293.3 abc
Pre + rhizobia	-	1977.2 b	2340.3 ab
Acifluorfen + clethodim + rhizobia	3799.7 a	2098.2 b	2219.3 bc
Acifluorfen + clethodim	3961.1 a	2091.5 b	2098.2 c
Dicamba 1 app + glyphosate + rhizobia	3692.1 ab	2723.7 a	2454.7 a
LSD $_{(0.10)}$	300.1	252.3	347.2

* Different letters within the same column indicate significant differences among herbicide treatments using the Fisher's test for significance at $p \leq 0.10$.

Table 3.35. Effect of planting date on grain yield (kg ha^{-1}) of Xtend soybean evaluated at northeast, southeast and east-central experiment locations in 2019 and 2020 growing seasons.

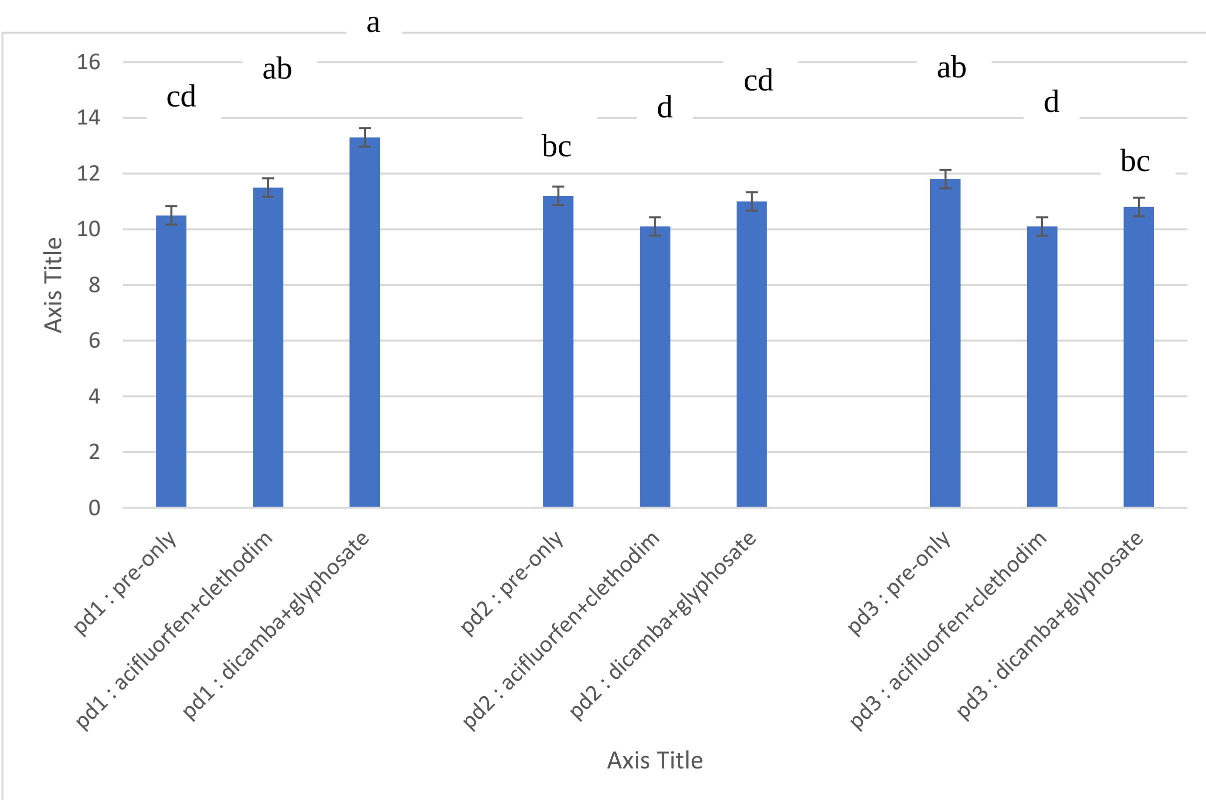
Grain yield (kg ha^{-1}) of Xtend soybean					
	Northeast 2019[a]	Southeast 2019[b]	East-central 2019[c]	Southeast 2020[d]	East-central 2020[e]
PD1	3262	3679 b	3208 a	2688 a	3093 a
PD2	3255	4250 a	3033 ab	2285 b	2905 a
PD3	3147	3437 b	2778 b	1827 c	894 b
Mean	3221	3789	3006	2267	2297

* Different letters within the same column indicate significant differences among planting dates using the Fisher's test for significance at $p \leq 0.10$. *Planting date [(Northeast/2019 season: PD1 – May 15; PD2 – May 30; PD3 – June 15); (Southeast/2019 season: PD1 – May 7, PD2 – June 5, PD3 – June 19); (East-central/2019 season: PD1 – May 15; PD2 – June 2, PD3 – June 19); (Southeast/2020 season: PD1 – May 15; PD2 – May 29; PD3 – June 12); (East-central/2020 season: PD1 – May 20; PD2 – June 3; PD3 – June 16).

100-seed weight

Average 100-seed weight in 2019 were 14.2 g (northeast), 18.3 g (southeast) and 17.8 g (east-central). In 2020, and at east-central and southeast sites, the 100-seed weight of Xtend soybean reduced by 16 and 39 %, respectively, compared to 2019 season. There was an interaction between planting date and herbicide at southeast, in 2020 (Table A20). Dicamba + glyphosate herbicide, when compared to pre-only treatment, and within the early planting date increased 100 -seed weight by 18 % (Figure 3.9). Regardless of herbicide treatment, the 100-seed weight of soybean (Xtend variety) at northeast location was 14.2 g, excluding dicamba + glyphosate + rhizobia treatment which had 4 % increase in seed weight over other treatments (Table 3.36). In 2019, and at east-central location, acifluorfen + clethodim and dicamba + glyphosate (2x application) treatments had the lowest 100-seed weight (17.5 and 17.8 g, respectively). In addition, dicamba + glyphosate (1x application) increased 100-seed weight by 2 % over a double application of dicamba + glyphosate herbicide treatment (Table 3.36). From the study, delaying soybean planting from the optimum sowing date (May 15) to June 19 increased seed weight by 5 % in 2019 season, whereas about 11 % loss occurred with a delay in planting from May 20 to June 16 in 2020 season (Table 3.37).

Figure 3.9. Interaction effect of planting date and herbicide on 100-seed weight of Xtend soybean evaluated at southeast location in 2020 season.



* Different letters above the bars indicate significant differences among the planting date by herbicide treatment using the Fisher's test for significance at $p \leq 0.10$. Error bars show the standard deviation of planting date by herbicide treatment. *Planting date (pd1- May 15, pd2 – May 29, pd3 – June 12).

Table 3.36. Effect of herbicide on 100-seed weight (g) of Xtend soybean evaluated at northeast and east-central experiment locations in 2019 growing seasons.

100-seed weight (g) of Xtend soybean		
Herbicide treatment	Northeast 2019	East-central 2019
Dicamba 1 app. + glyphosate	14.2 b	18.2 a
Dicamba 2apps. + glyphosate	-	17.8 bc
Pre-only	14.2 b	17.9 ab
Acifluorfen + clethodim	14.2 b	17.5 c
Dicamba 1 app. + glyphosate + rhizobia	14.8 a	-
LSD $_{(0.10)}$	0.4	0.3

* Different letters within the same column indicate significant differences among herbicide treatments using the Fisher's test for significance at $p \leq 0.10$.

Table 3.37. Effect of planting date on 100-seed weight (g) of Xtend soybean evaluated at east-central experiment locations in 2019 and 2020 growing seasons.

Location/Year	Planting date	100-seed weight (g)
East-central 2019	May 15	17.4 c
	June 2	17.8 b
	June 19	18.2 a
	LSD $_{(0.10)}$	0.3
East-central 2020	May 20	15.7 a
	June 3	15.3 a
	June 16	13.9 b
	LSD $_{(0.10)}$	1.0

* Different letters within the same column indicate significant differences among planting dates using the Fisher's test for significance at $p \leq 0.10$.

Seed oil content at 13 % moisture

Average seed oil of Xtend soybean evaluated across location (northeast, southeast, and east-central), and seasons (2019, 2020) was 19 %. No planting date by herbicide interaction was observed at any location, and year (Table A21). In 2019, only herbicide influenced seed oil content at the east-central location (Table A21). Acifluorfen + clethodim treatment (with seed oil content of 19.8) had greater seed oil than the other two treatments (dicamba + glyphosate and pre-only), and it increased seed oil by 1% (Table 3.38).

Planting date impacted seed oil at northeast (2019), southeast (2019) and east-central (2020) locations (Table A21). In 2019, and at northeast location, seed oil content was reduced by 2 % when planting was delayed from May 15 to June 15. However, at southeast, a 2 % increase in seed oil content occurred with a delay in planting from May 7 to June 19. In 2020 at east-central location, 11 % reduction in seed oil content occurred when soybean was planted at a late date (June 16) (Table 3.39).

Seed protein content at 13 % moisture

Seed protein content of Xtend soybean averaged 35 %. No planting date by herbicide interaction was observed at any location for either year (Table A22). Herbicide treatment application influenced seed protein levels at east-central (2019) and southeast (2020) locations (Table A22). Dicamba + glyphosate and the pre-only treatments had greater seed protein (average = 35 %) than acifluorfen + clethodim (Average = 34 %) (Table 3.40). Compared to acifluorfen + clethodim, the pre-only treatment increased seed protein by 2 and 5 %, whereas dicamba + glyphosate treatment increased seed protein by 1 and 3 %, at east-central and southeast locations, respectively (Table 3.40).

Also, seed protein levels were impacted by planting date at east-central location in 2019 and 2020 seasons (Table A22). In 2019, seed protein content increased by 1 % in the late planting date (June 19) compared to the early (May 15) and mid-planting (June 2) dates (Table 3.41). In 2020, delaying soybean planting from May 20 to June 3 resulted in about 2 % increase in seed protein. However, when planting was further delayed to June 16, seed protein content was reduced by 2 % (Table 3.41). Overall, as seed protein increased, seed oil levels decreased for Xtend soybean variety planted.

Table 3.38. Effect of herbicide on seed oil content of Xtend soybean variety evaluated at east-central experiment location in 2019 growing season.

Herbicide treatment	Seed oil at 13 % moisture
Acifluorfen + clethodim	19.8 a
Dicamba + glyphosate	19.7 b
Pre-only	19.7 b
Dicamba 2apps. + glyphosate	19.7 b
$LSD_{(0.10)}$	0.4

* Different letters within the same column indicate significant differences among herbicide treatments using the Fisher's test for significance at $p \leq 0.10$.

Table 3.39. Effect of planting date on seed oil content of Xtend soybean evaluated at northeast (2019), southeast (2019), and east-central (2020) experiment locations.

Location/Year	Planting date	Seed oil content at 13% moisture
Northeast 2019	May 15	18.2 a
	May 30	18.0 b
	June 15	17.9 b
		$LSD_{(0.10)} = 0.3$
Southeast 2019	May 7	19.4 b
	June 5	19.5 ab
	June 19	19.7 a
		$LSD_{(0.10)} = 0.4$
East-central 2020	May 20	20.2 a
	June 3	19.4 b
	June 16	17.9 c
		$LSD_{(0.10)} = 0.2$

* Different letters within the same column indicate significant differences among planting date using the Fisher's test for significance at $p \leq 0.10$.

Table 3.40. Effect of herbicide on seed protein content of Xtend soybean variety evaluated at east-central (2019) and southeast (2020) experiment locations.

Seed protein at 13 % moisture		
Herbicide treatment	East-central location 2019	Southeast location 2020
Acifluorfen + clethodim	33.9 b	34.3 b
Dicamba + glyphosate	34.4 a	35.5 a
Pre-only	34.5 a	35.9 a
LSD $_{(0.10)}$	0.2	0.1

* Different letters within the same column indicate significant differences among herbicide treatments using the Fisher's test for significance at $p \leq 0.10$.

Table 3.41. Effect of planting date on seed protein content of Xtend soybean evaluated at east-central experiment location in 2019 and 2020 growing seasons.

Location/Year	Planting date	Seed protein content at 13% moisture
East-central 2019	May 15	34.1 b
	June 2	34.2 b
	June 19	34.6 a
		LSD $_{(0.10)}$ = 0.1
East-central 2020	May 20	32.8 b
	June 3	33.3 a
	June 16	32.3 c
		LSD $_{(0.10)}$ = 0.2

* Different letters within the same column indicate significant differences among planting date using the Fisher's test for significance at $p \leq 0.10$.

3.4. Discussion

Postemergence herbicide applications to Enlist™ and Xtend® soybean varieties evaluated in the present study were tank-mixed with either clethodim (2,4-D, acifluorfen), or glyphosate (dicamba). Overall, grass weeds present at the three study locations (northeast, southeast, and east-central South Dakota) were poorly controlled by the auxin-based herbicide treatments (2,4-D + clethodim, 2,4-D + glufosinate and dicamba + glyphosate) whereas excellent control of broadleaf weeds, except for glyphosate resistant waterhemp and marestail at southeast location, was achieved by auxin herbicides. The poor grass weed control obtained could be due to the antagonism between auxin herbicide, 2,4-D and clethodim or dicamba and glyphosate in the tank-mix at application, which has been reported to reduce translocation of clethodim and glyphosate herbicides (Merritt et al. 2020). Herbicide antagonism is a phenomenon wherein two or more herbicides in a tank mix produce poorer weed control than individual herbicide components would supply alone (Colby S.R., 1967). Evidence of synthetic auxin herbicide (2,4-D and dicamba) antagonism with clethodim and glyphosate is documented in literature (Merritt et al. 2020). 2,4-D antagonism with clethodim has been reported in the control of volunteer wheat (*Triticum aestivum*) (Blackshaw et al. 2006). Dicamba applied POST with clethodim has also been found to result in lesser control of glyphosate-resistant volunteer corn in dicamba-tolerant soybean (Underwood et al. 2016). Poor control of Johnson grass (*Sorghum halepense*) and kochia (*Bassia scoparia*) have been reported when dicamba herbicide was applied in a tank-mix with glyphosate (Flint and Barrett, 1989; Ou et al. 2018).

The postemergence application of 2,4-D + glufosinate has been reported to provide an effective control (about 85%) of annual grasses and broadleaf weeds, including the control of barnyardgrass and common waterhemp, compared to when either 2,4-D or glufosinate was applied alone (Craigmyle et al. 2013). The study also reports a poor control of large crabgrass with an application of 2,4-D + glufosinate compared to when glufosinate was applied alone. Similar to our findings, Frane et al. (2018) reported about 98% control of glyphosate resistant weeds with POST applications of Enlist Duo (2,4-D choline + glyphosate) and Enlist One (2,4-D + glyphosate + glufosinate) in Enlist soybean. These reports support a component of our hypothesis that auxin-based herbicide programs will reduce weeds (broadleaf + grass) and provide weed control alternatives in auxin-tolerant soybeans.

Planting date by herbicide interactions were not found to be significant for chlorophyll values, soybean biomass, nodule number, and active nodule number of Enlist and Xtend soybean evaluated in the present study. Like our findings, Silva et al. (2021) reported no herbicide effects of 2,4-D choline, glyphosate and glufosinate on chlorophyll indices of E3 soybean. However, Albrecht et al. (2018) reported reductions in chlorophyll indices when higher rates (2,880 g ae ha^{-1}) of glyphosate were applied at V4 growth stage in Roundup Ready soybeans. Previous studies also suggest that an adequate supply of energy by photosynthesis is required for an efficient nodule initiation and development in soybean crop (Fransisco and Harper, 1995; Schultze and Kondorosi, 1998). Therefore, any reduction in soybean biomass result in a corresponding decrease in the supply of photosynthate to the nodules (Walsh, 1995), which will in turn impact nodulation and nodule function.

Auxin herbicide applications, in some cases, increased nodule number and active nodule number of Enlist and Xtend soybeans in the current study. This result however contrast with that of Turner et al. (2013) who reported that higher auxin concentrations inhibited root nodulation in soybean. The indirect effect of weed competition coupled with the negative allelopathic effects of some weeds to crop roots may inhibit nodulation and nodule function. Recent studies have shown that weed stress can inhibit root nodulation in soybean (Irawati et al 2012; Gal et al. 2015; Tortosa et al. 2021). Number of nodules were reported to be reduced when weeds were allowed to compete with soybean crop in field (Gal et al. 2015). Reduction in nodule number observed by Gal et al. (2015) was partly ascribed to the down regulation of the GmN93 gene caused by weed stress to crop. These findings provide an explanation for the higher nodule numbers obtained from the weed-free plots in the present study.

By using weed extracts in the laboratory, Irawati et al. (2012) was able to show that allelopathic weeds like nutgrass (*Cyperus rotundus*), Powell's amaranth (*Amaranthus powellii*) and paspalum (*Paspalum dilatatum*) inhibited root nodulation and nodule function in soybean by reducing nitrogenase enzyme activity. Similar nodule inhibition by plant residues and their extracts, leaf leachates and root exudates are found in literature. The extracts of common lambsquarters (at 1% level) was found to be inhibitory to soybean root nodulation as it reduced nodule numbers by 60% (Mallik and Tesfai, 1985). Our study recorded higher nodule number with auxin-based herbicide treatments as they provided a better control of weeds (some of which may have allelopathic trait) present at the three study locations.

Planting date in the present study influenced the yields of Enlist and Xtend soybeans evaluated at all study locations. Early and mid-planting dates had higher yields than the late planting dates. Early sowing allows more nodes to accumulate throughout the growing season. Across locations and for both study years, we observed that the late-planted soybean was shorter and had fewer nodes compared to the soybean planted early in the growth season (data not collected for statistical analysis). Past study reports a strong correlation between soybean nodes and yield (Ball et al. 2001). Soybean nodes have also been found to develop at a consistent rate of 0.27 nodes per day regardless of weather conditions (Bastidas et al. 2008). Therefore, delaying planting from the optimum planting date (which is May 15 in South Dakota) to later dates reduces the duration of vegetative and reproductive phases of growth available to crop. The morphological changes observed in the present study when soybean planting was delayed to mid-June may be a possible reason for some, but not all, of the yield differences observed. Data from multiple universities and grower's experiences suggests a potential yield gain when soybean is planted early in the season, and their findings corroborates our results (Bastisdas et al. 2008; Staton, 2011; Roozeboom, 2012; Licht et al. 2013; and Nleya et al. 2020).

The 2019 season was a very wet year with rainfall amounts exceeding the 30-year average by 50% (https://mesonet.sdstate.edu/archive). Early rains in 2019 season prevented the planting of most crops as fields were too wet thereby leaving most fields uncultivated. Also, the mid-summer rains in 2019 drowned out many areas and the few acres that were planted got flooded in July thus resulting in low crop yields. In 2020, low rainfalls and higher temperatures that occurred within the months of July and September at southeast, and August to September at east-central study location may have caused some drought stress on soybean planted in the summer of 2020, and this could be the reason for lower yields obtained in the second growing season. Soybean plants are most sensitive to drought during flowering and early pod fill growth stages. During water stress in soybean plant, floral abortion, reduced pod number, fewer seeds, and reduced seed size occur. A moderate drought stress has also been reported to reduce or stop nitrogen fixation, and this disrupts the seed development process (Lenssen, 2012). Drought conditions during R4 through R6 (full pod through full seed) stages of soybean growth can have devastating effect on yield potential as flowering stops and plants cannot compensate for lost pods (Hall and Twidwell, 2002). Early drought stress occurring during seed fill can reduce the number of seeds per pod, whereas later drought stress result in a reduced seed weight (Desclaux et al. 2000). However, if weather conditions improve, soybean flowering will re-initiate into the early seed filling stage and pod setting can occur into mid seed filling stage. Hence, rains in August could benefit soybean yields.

The literature reports on the response of soybean seed protein content to planting date vary. Some studies found no improvement in seed protein concentration of soybean when planting was delayed (Bajaj et al. 2008; Nleya et al. 2020), whereas others reported a decrease (Muhammad et al. 2009) or an increase (Mourtzinis and Conley, 2017); Tremblay et al. 2006) in soybean seed protein contents. A previous study conducted in Arkansas reports an increase in soybean seed protein concentration when planting was done in early May (Jaureguy et al. 2013). The present study found variable seed protein levels across study location and planting dates. This is similar to the findings of Nleya et al. (2020) who reported variable seed protein level among planting dates and between soybean maturity groups. Overall, as protein concentration in seed increased, seed oil levels decreased for both soybean varieties evaluated in the study.

3.5. Conclusion

Synthetic auxin herbicides [Weed Science Society of America (WSSA) and Herbicide Resistance Action Committee (HRAC) Group 4] provided excellent broadleaf weed control in auxin-tolerant soybean. A renewed concern of 2,4-D and dicamba trait technologies (Enlist and Xtend) in soybean may be that of antagonistic response with tank-mixes of common grass herbicides (for example, clethodim, ACCase inhibitor – WSSA/HRAC Group 1) with 2,4-D and dicamba herbicides. Although tank-mixing herbicides is an effortless way to apply multiple herbicides at one time, controlling both grasses and broadleaf weeds may be difficult if antagonism occurs in the plant. Applying herbicides separately with a specified interval between applications may prevent antagonism and increase herbicide activity for optimum control of weeds.

Results from our study found decreased grass weed control when grass or broad-spectrum herbicides were applied in a tank-mix with auxin herbicides. Since all postemergence treatment applications were tank-mixed with clethodim, antagonism may have reduced grass weed control. Also, hard water used for mixing herbicides can antagonize glyphosate in the plant. Cations like calcium (Ca2+) or magnesium (Mg2+) binds to negatively charged 2,4-D molecule and form large spray molecules that are less efficient in penetrating the waxy leaf cuticle of target plants, thereby resulting in poor control of weeds as seen in our study for Enlist soybean.

Dicamba can cause both metabolic and physical reactions to plants within hours of application and can inhibit plant's growth. Like the dicamba herbicide, glyphosate is transported through the phloem. However, dicamba herbicide can cause phloem plugging thus restricting glyphosate movement within the plant. If the ability of glyphosate to be translocated within the plant is restricted, then the optimum control of the plant may be reduced. Glyphosate can equally reduce the translocation of dicamba through the plant by inhibiting the EPSPS enzymes. This affects amino acid production and may indirectly influence phloem function (Amrhein et al. 1980; De Maria et al. 2006). Like glyphosate, dicamba needs the phloem to move throughout the plant and if the phloem tissue is damage, dicamba translocation to target locations in plant is cutoff. Therefore, the antagonism theory of dicamba and glyphosate can be the reason for the poor control of grasses (green foxtail, large crabgrass, volunteer wheat and volunteer corn) and broadleaf weeds (marestail and common waterhemp) observed in Xtend soybean variety evaluated in the study.

Foliar application of rhizobia combined with synthetic auxins, in most cases, did not increase nodule number, active nodule number, and grain yield as hypothesized in the study. A combination of factors including weather and soil conditions (temperature, rainfall, and pH) in field may account for reduced or no impact of rhizobia on soybean performance found in the study. A water-logged field condition, temperatures above or below the optimum requirement (27 to 35^{0}C) with highly acidic or alkaline soil pH will reduce rhizobia performance in field.

3.6. Recommendations for future research.

Herbicides and their surfactant and adjuvants used to control unwanted vegetation on croplands may have an indirect effect on the activities of beneficial soil microorganisms. Since weeds pose a serious threat to the income of farmers and may only be effectively managed (both in time and in space) using these chemicals, the sale and application of herbicides will continue to rise on a global scale. Therefore, there is need to engage in cutting edge research that investigates how to circumvent herbicide resistance (a global dilemma facing today's growers, the agroindustry, and other agricultural stakeholders) and promote agricultural sustainability. This current research studied the effect of herbicides, adjuvant, and surfactant on the growth of soybean rhizobacteria (Bradyrhizobium japonicum - USDA 110). We saw a growth increase when bacteria were treated with herbicide mixture that contained glyphosate, dicamba, AMS, Duce and Strike Zone, and cultured in both deionized water and yeast extract media. However, the rate of growth of the bacteria was influenced by the concentration of the herbicide, adjuvant, or surfactant. The effect of surfactant alone on the growth of soybean bacteria was not carried out in the present study and is recommended for further research.

The field study investigated the effect of auxin-based herbicides and rhizobia application to auxin-tolerant soybean in eastern South Dakota locations. Climate and soil conditions, in addition to COVID-19 outbreak influenced our result. High rainfall and soil moisture promoted decay of nodules and thus reduced nodule activity in 2019. The rhizobia application effect was too variable across location to account for yield increase, and travel restrictions made timely POST applications impossible. Therefore, a follow up study on rhizobia and herbicide application effect to soybean is recommended to corroborate the results in the present study.

References

Abendroth L.J., Elmore R.W., and Ferguson R.B. (2006). G06-1622 Soybean inoculation – applying the facts to your fields (part 2 of 2). Historical materials from university of Nebraska-Lincoln extension. https://digitalcommons.unl.edu/extensionhist/2077/

Adigun J.A., Daramola O.S., Adeyemi O.R., Olorunmaiye P.M., Osipitan O.A., and Idiodemise O. (2017). Effect of row spacing and period of weed interference on growth and yield of cowpea (*Vigna unguiculata* L. Walp). Nigeria Journal of Ecology 16: 88–110.

Afifi M., and Swanton C.J. (2012). Early physiological mechanisms of weed competition. Weed Science, 60:542-551.

Albrecht A.J.P., Albrecht L.P., Barroso A.A.M., Cesco V.J.S., Krenchinski F.H., Silva A.F.M., and Victoria F.R. (2018). Glyphosate tolerant soybean response to different management systems. Journal of Agricultural Science, 10:204-216.

Ali N. (2010). Soybean processing and utilization. In: G. Singh (Ed.) The soybean. botany, production and uses. Wallingford, UK: CAB International, pp. 345– 374.

Allen E.K. and Allen O.N. (1950). Biochemical and symbiotic properties of the rhizobia. Bacteriology Review, 14:273.2.

Alms J., Moechnig M., Vos D., and Clay. S. (2016). Yield loss and management of volunteer corn in soybean. Weed Technology, 30:254-262.

American Society of Agronomy. (2018). Fixing soybean's need for nitrogen: spraying beneficial bacteria increases yield, sustainability. Science Daily. Retrieved November 18, 2021. www.sciencedaily.com/releases/2018/03/180321090851.htm

Amrhein N., Deus B., Gehrke P., and Steinrucken H. C. (1980). The site of inhibition of the shikimate pathway by glyphosate II. Interference of glyphosate with chorismate formation in vivo and in vitro. Plant Physiology, 66:830–834.

Andersen S.M., Clay S.A., Wrage L.J., and Matthees D. (2004). Soybean foliage residues of dicamba and 2,4-D and correlation to application rates and yield. Agronomy Journal, 96:750–760.

Anonymous (2014a). 2,4-D Amine herbicide label. http://www.cdms.net/LDat/ld4JF000.pdf. Accessed: December 9, 2020.

Anonymous (2014b). Roundup PowerMax®herbicide label. http://www.monsanto.com/products/documents/msdslabels/roundup_powermax_label.pdf. Accessed: February 22, 2020.

Anonymous (2017a). Enlist One with Colex-D Technology herbicide product label. Dow AgroSciences Publication No. D02-427-002. Indianapolis, IN: Dow AgroScience. 7p

Anonymous (2017b). Enlist Duo with Colex-D Technology herbicide product label. Dow AgroSciences Publication No. D02-407-003. Indianapolis, IN: Dow AgroScience. 7p

Arias R.N., and Fabra de Peretti A. (1993). Effects of 2,4-dichlorophenoxyacetic acid on *Rhizobium sp*. growth and characterization of its transport. Toxicology Letters, 68: 267–273.

Auch D.E., and Arnold W.E. (1978). Dicamba use and injury on soybeans (*Glycine max*) in South Dakota. Weed Science, 26:471–475.

Bælum J., Jacobsen C.S., and Holben W.E. (2010). Comparison of 16S rRNA gene phylogeny and functional tfdA gene distribution in thirty-one different 2,4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid degraders. Systematic and Applied Microbiology, 33:67–70.

Bajaj S., Chen P., Longer D., Hou A., Shi A., Ishibashi T., Zhang B., and Brye K. (2008). Planting date and irrigation effects on seed quality of early-maturing soybean in the Mid-South USA. Journal of New Seeds, 9: 212-233.

Balagué C., Stürtz N., Duffard R., and Evangelista de Duffard A.M. (2001). Effect of 2,4-dichlorophenoxy acetic acid herbicide on *Escherichia coli* growth, chemical composition, and cellular envelope. Environmental Toxicology, 16:43-53.

Ball R.A., McNew R.W., Vories E.D., Keisling T.C., and Purcell L.C. (2001). Path analysis of population density effects on short-season soybean yield. Agronomy Journal, 93:187–19.

Barnes E.R., Jhala A.J., Knezevic S.Z., Sikkema P.H., and Lindquist J.L. (2018). Common ragweed (*Ambrosia artemisiifolia* L.) interference with soybean in Nebraska. Agronomy Journal, 110:646-653.

Barnett K.A., Mueller T.C., and Steckel L.E. (2013). Glyphosate-resistant giant ragweed (*Ambrosia trifida*) control with glufosinate or fomesafen combined with growth regulator herbicides. Weed Technology, 27:454–458.

Bastidas A.M., Setiyono T.D., Dobermann A., Cassman K.G., Elmore R.W., Graef G.L., and Specht J.E. (2008). Soybean sowing date: The vegetative, reproductive, and agronomic impacts. Crop Science, 48:727– 740.

Benbrook C.M. (2012). Impacts of genetically engineered crops on pesticide use in the U.S.—the first sixteen years. Environmental Sciences Europe, 24:24. https://doi.org/10.1186/2190-4175-24-24.

Bensch C.N., Horak M.J., and Peterson D. (2003). Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Science, 51:37–43.

Bentley R. (1990). The shikimate pathway--a metabolic tree with many branches. Critical reviews in Biochemistry and Molecular Biology, 25:307-384.

Bergey D.H., Harrison F.C., Breed R.S., Hammer B.W., and Huntoon F.M. (1923). Bergey's manual of determinative bacteriology, 1st edn. Baltimore, USA: Williams & Wilkins.

Berner D.K., Berggren G.T., and Snow J.P. (1991). Effects of glyphosate on *Calonectia crotalariae* and red crown rot of soybean. Plant Disease, 75: 809–813.

Blackshaw R.E., Harker K.N., Clayton G.W., and O'Donovan J.T. (2006). Broadleaf herbicide effects on clethodim and quizalofop efficacy on volunteer wheat (*Triticum aestivum*). Weed Technology, 20:221–226.

Bohm G.M.B., Alves B.J.R., Urquiaga S., Boddey R.M., Xavier G.R., Hax F., and Rombaldi C.V. (2009). Glyphosate and imazethapyr induced effects on yield, nodule mass and biological nitrogen fixation in field-grown glyphosate-resistant soybean. Soil Biology and Biochemistry, 41: 420-422.

Bradley K.W. (2017a). Off-target movement of dicamba in Missouri. Where do we go from here? https://ipm.missouri.edu/IPCM/2017/10/fnal_report_dicamba_injured_soybean. Accessed December 21, 2020.

Bradley K.W. (2017b). A final report on dicamba-injured soybean acres. https://ipm.missouri.edu/IPCM/2017/10/final_report_dicamba_injured_soybean. Accessed December 21, 2020.

Brender J.D. (2020). Human health effects of exposure to nitrate, nitrite, and nitrogen dioxide. In: Sutton M.A. et al. (eds) Just Enough Nitrogen. Springer, Cham.

Cafaro La Menza N., Monzon J.P., Specht J.E., and Grassini P. (2017). Is soybean yield limited by nitrogen supply? Fields Crops Research, 213:204-212.

Cardina J., Hartwig N.L., and Lukezic F.L. (1986). Herbicidal effects on crown vetch rhizobia and nodule activity. Weed Science, 34:338–343.

Carpenter P.L. (1977). Microbiology, 4th edition. London: W.B. Saunders.

Chahal G.S., and Johnson W.G. (2012). Influence of glyphosate or glufosinate with growth regulator herbicides and other agrochemicals in controlling glyphosate-resistant weeds. Weed Technology, 26:638–643.

Chahal P.S., Kruger G., Blanco-Canqui H., and Jhala A.J. (2014). Efficacy of preemergence and postemergence soybean herbicides for control of glufosinate, glyphosate, and imidazolinone-resistant volunteer corn. Journal of Agricltural Science, 6:131–140.

Chahal P.S., and Jhala A.J. (2015). Herbicide programs for control of glyphosate-resistant volunteer corn in glufosinate-resistant soybean. Weed Technology, 19:431–443.

Christoffoleti P.J., Galli A.J.B., Carvalho S.J.P., Moreira M.S., Nicolai M., Foloni L.L., Martins B.A.B, and Ribeiro D.N. (2008). Glyphosate sustainability in South American cropping systems. Pest Management Science, 64:422–427.

Clark S.A., and Mahanty H.K. (1991). Influence of herbicides on growth and nodulation of white clover, *Trifolium repens*. Soil Biology and Biochemistry, 23:725–730.

Clay D.E., Carlson C.G., Clay S.A., Wagner L., Deneke D.L., and Hay C.H. (2013). iGrow Soybeans: best management practices for soybean production. Agronomy, Horticulture, and Plant Science Books. 2.

Clay S.A., Kleinjan J., Clay D.E., Forcella F., and Batchelor W. (2005). Growth and fecundity of several weed species in corn and soybean. Agronomy Journal, 97:294.

Clay S.A., Kreutner B., Clay D.E., Reese C., Kleinjan J., and Forcella F. (2006). Spatial distribution, temporal stability, and yield loss estimates for annual grasses and common ragweed (*Ambrosia artimisiifolia*) in a corn/soybean production field over nine years. Weed Science, 54:380–390.

Clay S.A., Clay D.E., Horvath D.P., Pullis J., Carlson C.G., Hansen S., and Reicks G. (2009). Corn response to competition: growth alteration vs yield limiting factors. Agronomy Journal, 101:1522-1529.

Clay S.A. (2013). Common broadleaf weeds of South Dakota. In Clay, D.E., C.G. Carlson, S.A. Clay, L. Wagner, D. Deneke, and C. Hay (eds). iGrow Soybeans: Best management practices for soybean production. South Dakota State University, SDSU Extension, Brookings, SD.

Colby S.R. (1967). Calculating synergistic and antagonistic responses of herbicide combinations. Weeds, 15: 20–22.

Coetzer E., Al-Khatib A., and Peterson D.E. (2002). Glufosinate efficacy on amaranthus species in glufosinate-resistant soybean. Weed Technology, 16:326–331.

Costa E.M., Jakelaitis A., Zuchi J., Pereira L.S., Ventura M.V.A., Oliveira G.S., Sousa D., and Silva J.N. (2020). Simulated drift of dicamba and 2,4-D on soybeans: effects of application dose and time. Bioscience Journal, 36:857-864.

Cox W.J., Orlowski J., Ditommaso A., and Knoblauch W. (2012). Planting soybean with a grain drill inconsistently increases yield and profit. Agronomy Journal, 104:1065-1073.

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

Daramola O.S., Adigun J.A., Olorunmaiye P.M., and Adeyemi O.R. (2020). Efficacy and economic comparison of weed management in tomato. International Journal of Vegetable Science, 63:64–90.

Dart P. (1977). Infection and development of leguminous nodules. In a treatise on dinitrogen fixation, Section III: Biology ed. Hardy, R.W.F. and Silver, W.S. pp. 367–472. New York: John Wiley.

De Bruin J.L., and Pedersen P. (2008a). Effect of row spacing and seeding rate on soybean yield. Agronomy Journal, 100:704-710.

De Bruin J.L., and Pedersen P. (2008b). Soybean seed yield response to planting date and seeding rate in the upper Midwest. Agronomy Journal, 100: 696– 703.

De Maria N., Becerril J.M., Garcia-Plazaola H.A., Felipe M., and Fernandez-Pascual M. (2006). New insights of glyphosate mode of action in nodular metabolism: Role of shikimate accumulation. Journal of Agricultural and Food Chemistry, 54:2621–2628.

Desclaux D., Huynh T.T., and Roumet P. (2000). Identification of soybean plant characteristics that indicate the timing of drought stress. Crop Science, 40: 716-722.

Dill G.M., Sammons R.D., Feng P.C.C., Kohn F., Kretzmer K., Mehrsheikh A., Bleeke M., Honegger J.L., Farmer D., Wright D., and Haupfear E.A. (2010). Glyphosate: discovery, development, applications, and properties. Chapter 1. In: Nandula V.K., editor.

Glyphosate resistance in crops and weeds: history, development, and management. New York: Wiley; pp. 1–33.

Ding Y., Kalo P., Yendrek C., Sun J., Liang Y., Marsh J.F., Harris J.M., and Oldroyd G.E. (2008). Abscisic acid coordinates nod factor and cytokinin signaling during the regulation of nodulation in *Medicago truncatula*. Plant Cell, 20:2681–2695.

Donaldson D., Kiely T., and Grube A. (2002). Pesticides industry sales and usage, 1998 and 1999 market estimates. Washington, DC: Environmental protection agency office of pesticide programs.

Drouin P., Sellami M., Pre´vost D., Fortin J., and Antoun H. (2010). Tolerance to agricultural pesticides of strains belonging to four genera of *Rhizobiaceae*. Journal of Environmental Science and Health part B, 45:780–788.

Duke S.O., and Powles S.B. (2008). Glyphosate: A once in a century herbicide. Pest Management Science, 64:319-325.

Duke S.O. (2014). Perspectives on transgenic, herbicide-resistant crops in the USA almost 20 years after introduction. Pest Management Science, 71:652–657.

Eaton B.J., Russ O.G., and Feltner K.C. (1976). Competition of velvetleaf, prickly sida and Venice mallow in soybeans. Weed Science, 24:224–228.

Eberbach P., and Douglas L. (1989). Herbicide effects on the growth and nodulation potential of *Rhizobium trifolii* with *Trifolium subterraneum* L. Plant and Soil, 119: 15-23.

Esmailpour A., Hassanzadehdelouei M., and Madani A. (2012). Impact of livestock manure, nitrogen and biofertilizer (azotobacter) on yield and yield components of wheat (*Triticum aestivum* l.). Agronomical Research, 46:1-11.

Fabra A., Duffard R., and Evangelista de Duffard A. (1997). Toxicity of 2,4-dichlorophenoxyacetic acid to Rhizobium sp. in pure culture. Bulletin of Environmental Contamination and Toxicology, 59:645- 652.

Fabra de Peretti A.I., Mori de Moro G.B., Ghittoni N., Evangelista de Duffard A.M., and Duffard R.O. (1987). Effects of 2,4-D on *Rhizobium sp*. in pure culture. Toxicity Assessment, 2:217-228.

Fageria N.K., and Baligar V.C. (2005). Enhancing nitrogen use efficiency in crop plants. Advances in Agronomy, Academic Press, 88:97-185.

Fanous A., Weiland F., Lück C., Görg A., Friess A., and Parlar H. (2007). A proteome analysis of *Corynebacterium glutamicum* after exposure to the herbicide 2,4-dichlorophenoxy acetic acid (2,4-D). Chemosphere, 69:25-31.

Fehr W.R., and Caviness C.E. (1977). Stages of soybean development. Spec. Rep. 80. Cooperative Extension Service., Iowa State Univ., Ames, IA. 11pp.

Ferguson B.J., Ross J.J., and Reid J.B. (2005). Nodulation phenotypes of gibberellin and brassinosteroid mutants of pea. Plant Physiology, 138:2396–2405.

Fletcher W.W. (1956). Effect of hormone herbicides on the growth of *Rhizobium trifolii*. Nature, 177:1244.

Flint J.L. and Barrett M.B. (1989). Antagonism of glyphosate toxicity to johnsongrass (*Sorghum halepense*) by 2,4-D and dicamba. Weed Science, 37: 700–705.

Flores M., and Barbachano M. (1992). Effects of herbicides gramoxone, diuron and totacol on growth and nodulation of three strains of *Rhizobium meliloti*. Science of the Total Environment, 124:249–260.

Fogarty A.M., and Tuovinen O.H. (1995). Microbiological degradation of the herbicide dicamba. Journal of Industrial Microbiology, 14: 365–370.

Frane R., Simpson D., Buchanan M., Vega E., Ravotti M., and Valverde P. (2018). Enlist E3™ soybean sensitivity and Enlist™ herbicide-based program control of sumatran fleabane (*Conyza sumatrensis*). Weed Technology, 32: 416-423.

Francisco P.B. and Harper J.E. (1995). Translocatable leaf signal autoregulates soybean nodulation. Plant Science, 107: 167–176.

Franz J.E., Mao M.K., and Sikorski J.A. (1997). ACS Monograph. 189:653.

Gal J., Afifi M., Lee E., Lukens L., and Swanton C.J. (2015). Detection of neighboring weeds alters soybean seedling roots and nodulation. Weed Science, 63: 888-900.

Ganie Z.A., and Jhala A.J. (2017). Interaction of 2, 4-D or dicamba with glufosinate for control of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in glufosinate-resistant maize (*Zea mays* L.). Frontiers in Plant Science, 8:1207.

Gibson A.H. (1977). The influence of the environment and managerial practices on the legume-Rhizobium symbiosis. In ed. Hardy, R.W.F. and Gibson, A.H. A treatise on dinitrogen fixation, Section IV: Agronomy and Ecology pp. 393–450.

Gibson A.H., and Jordan D.C. (1983). Ecophysiology of nitrogen-fixing systems. In ed. Lange, O.L., Nobel, P.S., Osmond, C.B. and Zeigler, H. Physiological Plant Ecology III. Responses to the chemical and biological environment pp. 301–390. Springer-Verlag, Berlin.

Goldsmith P. (2008). Economics of soybean production, marketing, and utilization. In L.A. Johnson, P. J. White, & R. Galloway (Eds.), Soybeans: Chemistry, production, processing, and utilization (pp.117-150). Urbana: AOCS.

Gonzalez A., Gonzalez-Murua C., and M. Royuela. (1996). Influence of imazethapyr on Rhizobium growth and its symbiosis with pea (*Pisum sativum*). Weed Science, 44:31–37.

Gresshoff P.M. (1990). Molecular biology of symbiotic nitrogen fixation (1st ed.) pp. 278. CRC Press, Boca Raton, Florida.

Grossman J.M. (2010). Auxin herbicides: current status of mechanisms and modes of action. Pest Management Science, 66:113–120.

Hager A.G., Wax L.M., Stoller E.W., and Bollero G. (2002). Common waterhemp (*Amaranthus rudis*) interference in soybean. Weed Science, 50: 607-610.

Halford C., Hamill A.S., Zhang J. and Doucet C. (2001). Critical period of weed control in no-till soybean (*Glycine max*) and corn (*Zea mays*). Weed Technology, 15: 737-744.

Hall R.C. and Twidwell E.K. (2002). Effects of drought stress on soybean production. South Dakota State University. Extension 8034.

Hall R.G., Kirby K.K., and Hawks S. (2012). Annual report: soybean variety performance trials results. SDSU Extension.

Harris T.C. and Ritter R.L. (1987). Giant green foxtail (*Setaria viridis* var. major) and fall panicum (*Panicum dichotomiflorum*) competition in soybeans (*Glycine max*). Weed Science, 35:663–668.

Hartman G.L., West D.E. and Herman T.K. (2011). Crops that feed the world 2. Soybean – worldwide production, use, and constraints caused by pathogens and pests. Food Security, 3: 5– 17.

Heap I.M. (2014). Global perspective of herbicide-resistant weeds. Pest Management Science, 70:1306–1315.

Heap I, and Duke S.O. (2018). Overview of glyphosate-resistant weeds worldwide. Pest Management Science, 74:1040-1049.

Heap I (2021). The International Herbicide-Resistant Weed Database. Online. Tuesday, March 2, 2021.

Hernandez, A., J.I. Garcia-Plazzola, and J.M. Becerril. 1999. Glyphosate effects on phenolic Metabolism of nodulated soybean (*Glycine max* L. Merr.). J. Agric. Food Chem. 47:2920–2925.

Horvath D.P., Hansen S.A., Moriles-Miller J., Pierik R., Yan C., Clay D., Scheffler B., and Clay S. (2015). RNAseq reveals weed-induced PIF3-like as a candidate target to manipulate weed stress response in soybean. New Phytologist 207: 196–210.

Horvath D.P., Bruggeman S., Miller J., Anderson J.V., Dogramaci M., Scheffler B.E., Hernandez A.G., Foley M.E., and Clay S. (2018). Weed presence altered biotic stress & light signaling in maize even when weeds were removed early in the critical weed-free period, Plant Direct, 10:1002.

Illinois Department of Agriculture. Pesticides: Uses & Misuses. https://www2.illinois.gov/sites/agr/Pesticides/Pages/Pesticides-Uses-Misuses.aspx

Irawati C., Acram T., Paul K., and Robin J. (2012). Some weed species affecting soybean nodulation and nodule function. AGRIVITA Journal of Agricultural Science, 34: 166-174.

Jaureguy L., Rodriguez F., Zhang L., Chen P., Brye K., Oosterhuis D., Mauromoustakos A., and Clark J. (2013). Planting date and delayed harvest effects on soybean seed composition. Crop Science, 53: 2162– 2175.

Jaworski E.G. (1972). Mode of action of N-phosphonomethyl-glycine inhibition of aromatic amino acid biosynthesis. Journal of Agricultural and Food Chemistry, 20:1195–1198.

Jofré E., Mori G., Castro S., Fabra A., Rivarola V., and Balegno H. (1996). 2,4-Dichlorophenoxy acetic acid affects the attachment of *Azospirillum brasilense* Cd to maize roots. Toxicology, 107:9-15.

Johal G.S., and Rahe J.E. (1984). Effect of soil borne plant-pathogenic fungi on the herbicidal action of glyphosate on bean seedlings. Phytopathology, 74: 950–955.

Johnson V.A., Fisher L.R., Jordan D.L., Edmisten K.E., Stewart A.M., and York A.C. (2012). Cotton, peanut, and soybean response to sublethal rates of dicamba, glufosinate, and 2,4-D. Weed Technology, 26:195–206.

Katan J., and Eshel Y. (1973). Interactions between herbicides and plant pathogens. Residue Reviews, 45, 145–177.

Kegode G.O., Forcella F. and Clay S.A. (1999). Influence of crop rotation, tillage, and management inputs on weed seed production. Weed Science, 47:175–183.

Kissmann K.G. (1997). Adjuvantes para caldas de produtos fitossa-nita´rios. In: SBCPD (Ed.), Congresso Brasileiro da Cieˆncia dasPlantas Daninhas (21:1997: Caxambu, MG). Palestras e mesasredondas, pp. 188–189.

Knezevic S.Z., Evans S.P., Blankenship E.E., Van Acker R.C., and Lindquist J.L. (2002). Critical period of weed control; the concept and data analysis. Weed Science, 50:773-786.

Krueger J. P. (1989). Development of microbes for degradation of the herbicide dicamba (3, 6-dichloro-2-methoxybenzoic acid) (Doctoral dissertation, Illinois Institute of Technology).

Krueger J.P., Butz R.G., Atallah Y.H., and D.J. Cork. (1989). Isolation and identification of microorganisms for the degradation of dicamba. Journal of Agriculture and Food Chemistry, 37: 534-538.

Krueger J.P., Butz R.G., and Cork D.J. (1991). Use of dicamba- degrading microorganisms to protect dicamba susceptible plant species. Journal of Agriculture and Food Chemistry, 39: 1000-1003.

Lambert D.M., and Lowenberg-DeBoer J. (2003). Economic analysis of row spacing for corn and soybean. Agronomy Journal, 95:564-573.

Lee G.A., Crawford G.W., Liu L., Sasaki Y., and Chen X. (2011). Archaeological soybean (*Glycine max*) in east Asia: does size matter? PLoS ONE 6, e26720.

Leggett M., Diaz-Zorita M., Koivunen M., Bowman R., Pesek R., Stevenson C., and Leister T. (2017). soybean response to inoculation with *Bradyrhizobium japonicum* in the United States and Argentina. Agronomy Journal, 109:1031–1038.

Lenssen A. (2012). Soybean response to drought. Iowa State University. https://crops.extension.istate.edu/.

Lepping M.D., Herman R.A., and Potts B.L. (2013). Compositional equivalence of DAS-444Ø6-6 (AAD-12 + 2mEPSPS + PAT) herbicide-tolerant soybean and non-transgenic soybean. Journal of Agriculture and Food Chemistry, 61:11180-11190.

Li Y.H., Wang R., Sui X.H., Wang E.T., Zhang X.X., Tian C.F., and Chen W.X. (2019). *Bradyrhizobium nanningense sp. nov., Bradyrhizobium guangzhouense sp. nov. and Bradyrhizobium zhanjiangense sp. nov.,* isolated from effective nodules of peanut in Southeast China. Systematic and Applied Microbiology, 42: 126002.

Licht M.A., Wright D., and Lenssen A.W. (2013). Planting soybean for high yield in Iowa. Agriculture and Environment Extension Publications. 193.

Liu K. (2008). Food use of whole soybeans. In L. Johnson, P. J. White, & R. Galloway (Eds.), Soybeans: chemistry, production, processing, and utilization (pp. 441–482). Urbana, IL: AOCS Press

Maekawa T., Maekawa-Yoshikawa M., Takeda N., Imaizumi-Anraku H., Murooka Y., and Hayashi M. (2009). Gibberellin controls the nodulation signaling pathway in *Lotus japonicus*. Plant Journal, 58:183–194.

Malkones H.P. (2000). Comparison of the effects of differently formulated herbicides on soil microbial activities—a review. Journal of Plant Disease and Protection, 8:781–789.

Mallik M.A.B., and Tesfai K. (1985). Pesticidal effect of soybean-rhizobia symbiosis. Plant and Soil, 85:33-41.

Manderscheid R., and Wild A. (1986). Studies on the mechanism of inhibition by phosphinothricin of glutamine synthetase isolated from *Triticum aestivum* L. Journal of Plant Physiology, 123:135–142.

Manuchehri M., Dotray P., and Keeling J. (2017). Enlist^{TM} weed control systems for Palmer Amaranth (*Amaranthus palmeri*) management in Texas high plains cotton. Weed Technology, 31: 793-798.

Marron E., Ruiz N., and Rubio C. (2006). 2,4-D degrading bacterial consortium. Isolation, kinetic characterization in batch and continuous culture and application for bio-augmentating an activated sludge microbial community. Process Biochemistry, 41:1521–1528.

Mårtensson A.M., and Nilsson A.K. (1989). Effects of chlorsulfuron on Rhizobium grown in pure culture and in symbiosis with alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*). Weed Science, 37:445–450.

Mårtensson A.M. (1992). Effects of agrochemicals and heavy metals on fast-growing rhizobia and their symbiosis with small-seeded legumes. Soil Biology and Biochemistry, 24:435–445.

Martin F.W. (1988). Soybean. *ECHO Technical Note* no. 17.

Mckenzie-Gopsill A.G., Lee E., Lukens L., and Swanton C.J. (2016). Rapid and early changes in morphology and gene expression in soya bean seedlings emerging in the presence of neighbouring weeds. Weed Research, 56:267–273.

Merritt L.H., Ferguson J.C., Brown-Johnson A.E., Reynolds D.B., Tseng T. and Lowe J.W. (2020). Reduced herbicide antagonism of grass weed control through spray application technique. Agronomy Journal, 10:1131-1135.

Meyer C.J., Norsworthy J.K., Young B.G., Steckel L.E., Bradley K.W., and Johnson W.G. (2015). Herbicide program approaches for managing glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus* and *Amaranthus rudis*) in future soybean-trait technologies. Weed Technology, 29:716–729.

Miller P., and Westra P. (1996). Herbicide surfactants and adjuvants. Bulletin No. 0.599, Crop Series. Colorado State University Cooperative Extension, Fort Collins, Co.

Miller M.R., and Norsworthy J.K. (2016) Evaluation of herbicide programs for use in a 2,4-D-resistant soybean technology for control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*). Weed Technology, 30:366–376.

Moechnig M., Clay S., and Deneke D. (2013). Herbicide-resistant weeds in soybeans. In Clay D.E., C.G. Carlson, S.A. Clay, L. Wagner, D. Deneke, and C. Hay (eds). iGrow Soybeans: best management practices for soybean production. South Dakota State University, SDSU Extension, Brookings, SD.

Moolani M.K., Knake E.L., and Slife F.W. (1964). Competition of smooth pigweed with corn and soybeans. Weeds, 12:126–128.

Moorman T.B. (1986). Effects of herbicides on the survival of *Rhizobium japonicum* strains. Weed Science, 34: 628–633.

Moorman T.B., Becerril J.M., Lydon L., and Duke S.O. (1992). Production of hydroxybenzoic acids by *Bradyrhizobium japonicum* strains after treatment with glyphosate. Journal of Agriculture and Food Chemistry, 40:289–293.

Moriles J., Hansen S., Horvath D.P., Reicks G., Clay D.E., and Clay S.A. (2012). Microarray and growth analyses identify differences and similarities of early corn response to weeds, shade, and nitrogen stress. Weed Science, 60:158–166.

Mortensen D.A., Egan J.F., Maxwell B.D., and Ryan M.R. (2012). Navigating a critical juncture for sustainable weed management. Bioscience, 62:75–84.

Mourtzinis S. and Conley S. (2017). Delineating soybean maturity groups across the United States. Agronomy Journal, 109. 1-7.

Mourtzinis S., Specht J.E., Lindsey L.E., Wiebold W.J., Ross J., Nafziger E.D., Kandel H.J., Mueller N., Devillez P.L., Arriaga F.J., and Conley S.P. (2015). Climate-induced reduction in US-wide soybean yields underpinned by region-and in-season-specific responses. Nature Plants, 1, article 14026.

Mueller N., Carlson C.G., and Hall R. (2013). Selecting soybean varieties. In Clay D.E., Carlson C.G., Springer, Cham Clay S.A., Wagner L., Deneke D., and Hay C. (eds). iGrow soybeans: best management practices for soybean production. South Dakota State University, SDSU Extension, Brookings, SD.

Muhammad A., S.K. Khalil K.B., Marwat A.Z., Khan I.H., Khalil Amanullah and Arifullah S. (2009). Nutritional quality and production of soybean land races and improved varieties as affected by planting dates. Pakistan Journal of Botany, 41:683–689.

Mulugeta D. and Boerboom C. M. (2000). Critical time of weed removal in glyphosate-resistant *Glycine max*. Weed Science.

Munns D.N. (1977). Mineral nutrition and the legume symbiosis. in ed. Hardy R.W.F. and Gibson A.H. A treatise on dinitrogen fixation, section IV: Agronomy and Ecology, pp. 353–391.

Nakagawa T., and Kawaguchi M. (2006). Shoot-applied meja suppresses root nodulation in *Lotus japonicus.* Plant Cell Physiology, 47:176–180.

Nave W.R., and Wax L.M. (1971). Effect of weeds on soybean yield and harvesting efficiency. Weed Science, 19:533–535.

Nestor A.R., and de Peretti, A. F. (1993). Effects of 2,4-dichlorophenoxyacetic acid on Rhizobium sp. growth and characterization of its transport. Toxicology Letters, 68:267–273.

Nleya T., Schutte M., Clay D., Reicks G., and Mueller N. (2020). Planting date, cultivar, seed treatment, and seeding rate effects on soybean growth and yield. Agrosystems, Geosciences and Environment, 3:e20045.

Norsworthy J.K., Rair D., Jha P., and Scott R.C. (2011). Confirmation, control, and physiology of glyphosate-resistant giant ragweed (*Ambrosia trifida*) in Arkansas. Weed Technology, 25:430– Springer, Cham 435.

Norsworthy J.K., Ward S.M., Shaw D.R., Llewellyn R.S., Nichols R.L., Webster T.M., Bradley K.W., Frisvold G., Powles S.B., Burgos N.R., Witt W.W., and Barrett M. (2012). Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Science, 60:31-62.

Oerke E.C. (2006). Crop losses to pests: centenary review. Journal of Agricultural Science, 144:31-43.

Office of Indiana State Chemist and Seed Commissioner (2019). OISC Announces 2020 Dicamba Restriction. https://www.oisc.purdue.edu /pesticide/pdf/dicamba 24c press release 111319.

OpenStax. (2021). Lipids. https://chem.libretexts.org/@go/page/5308. Retrieved March 15, 2021.

Osipitan O.A., Adigun J.A., Lagoke S.T.O., and Afolami S.O. (2013). Effect of inter-row spacing and weed control methods on growth and yield of cowpea (*Vigna unguiculata* L. Walp) in South- Western Nigeria. Nigeria Journal of Plant Protection, 27: 97–111.

Osipitan O.A. (2016). Weed interference and control in cowpea production: A review. Journal of Agricultural Science, 9: 11–20.

Osipitan O.A., and Dille J.A. (2017). Fitness outcomes related to glyphosate resistance in kochia (*Brassia scoparia*): What life history stage to examine? Front. Plant Science, 8:1090.

Osipitan O.A., Scott J.E., and Knezevic S.Z. (2019). Glyphosate-resistant soybean response to micro-rates of three dicamba-based herbicides. Agrosystems, Geosciences and Environment, 2:180052.

Ou J., Thompson C.R., Stahlman P.W., Bloedow N. and Jugulam M. (2018). Reduced translocation of glyphosate and dicamba in combination contribute to poor control of *Kochia scoparia*: Evidence of herbicide antagonism. Scientific Reports, 8:5330.

Owen M.D., Beckie H.J., Leeson J.Y., Norsworthy J.K., and Steckel L.E. (2014). Integrated pest management and weed management in the United States and Canada. Pest Management Science, 71:357–376.

Padgette S.R., Kolacz K.H., Delannay X., Re D.B., Lavallee B.J., Tinius C.N., Rhodes W.K., Otero Y.I., Barry G.F., Eichholtz D.A., Peschke V.M., Nida D.L., Taylor N.B., and

Kishore G.M. (1995). Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Science, 35:1451-1461.

Patil G., Vuong T.D., Kale S., Valliyodan B., Deshmukh R., Zhu C., Wu X., Bai Y., Yungbluth D., Lu F., Kumpatla S., Shannon J.G., Varshney R.K., Nguyen H.T. (2018). Dissecting genomic hotspots underlying seed protein, oil, and sucrose content in an interspecific mapping population of soybean using high-density linkage mapping. Plant Biotechnology Journal, 16:1939–1953.

Pedersen P., and Lauer J.G. (2003). Soybean agronomic response to management systems in the Upper Midwest. Agronomy Journal, 95:1146– 1151.

Penmetsa R.V., Uribe P., Anderson J., Lichtenzveig J., Gish J.C., Nam Y.W., Engstrom E., Xu K., Sckisel G., Pereira M., (2008). The Medicago truncatula ortholog of Arabidopsis EIN2, sickle, is a negative regulator of symbiotic and pathogenic microbial associations. Plant Journal, 55:580–595.

Rafael V., André R., Pablo A.K., Leandro V., Ana C.C.F., and Franciele M. (2013). Weed management in soybean. Issues and practices, soybean - pest resistance, Hany A. El-Shemy, IntechOpen.

Reddy K.N. (2001). Glyphosate-resistant soybean as a weed management tool: Opportunities and challenges. Weed Biology and Management 4: 193-202.

Richards T.A., Dacks J.B., Campbell S.A., Blanchard J.L., Foster P.G., McLeod R., and Roberts C.W. (2006). Evolutionary origins of the eukaryotic shikimate pathway: gene fusions, horizontal gene transfer, and endosymbiotic replacements. Eukaryotic Cell, 5:1517-1531.

Robinson A., Simpson D., and Johnson W. (2013a). Response of glyphosate-tolerant soybean yield components to dicamba exposure. Weed Science, 61: 526-536.

Robinson A.P., Davis V.M., Simpson D.M., and. Johnson W.G. (2013b). Response of soybean yield components to dicamba. Weed Science, 61:68–76.

Roozeboom K. (2012). Soybean planting dates: Is earlier a good Idea? AG Professional. https://www.agprofessional.com/article/soybean-planting-dates-earlier-good-idea.

Ross M.A., and Childs D.J. (1996). Herbicide mode-of-action summary. Purdue University, Department of Botany: Plant Pathology, West Lafayette IN. Report No. WS-23-W.

Salvagiotti F., Cassman K.G., Specht J.E., Walters D.T., Weiss A., and Doberman A. (2008). Nitrogen uptake, fixation, and response to fertilizer N in soybeans: A review. Field Crops Research, 108:1-13.

Salvagiotti F., Specht J.E., Cassman K.G., Walters D.T., Weiss A., and Doberman A. (2009). Growth and nitrogen fixation in high-yielding soybean: Impact of nitrogen fertilization. Agronomy Journal, 101: 958-970.

Sandoval-Carrasco C.A., Ahuatzi-Chaco´n D., Galı´ndez-Mayer J., (2013). Biodegradation of a mixture of the herbicides ametryn, and 2,4-dichlorophenoxyacetic acid (2,4-D) in a compartmentalized biofilm reactor. Bioresource Technology, 145:33–6.

Santos J.B. dos, Ferreira E.A., Kasuya M.C.M., Silva A.A. da, and Sergio de. O.P. (2005). Tolerance of Bradyrhizobium strains to glyphosate formulations. Crop Protection, 24: 543-547.

Sauer H., Wild A. and Rühle W. (1987). The effect of phosphinothricin (Glufosinate) on photosynthesis II. The causes of inhibition of photosynthesis. *Zeitschrift für Naturforschung C*, *42*, 270-278.

Sawada Y., Nagay Y., and Ueama M. (1988). Probable toxicity of surface-active agent in commercial herbicide containing glyphosate. The Lancet 1, 299–299.

Schmidt J.P., Schmitt M.A., Randall G.W., Lamb J.A., Orf J.H., and Gollany H. (2000). Swine manure application to nodulating and non-nodulating soybeans. Agronomy Journal, 92: 987-992.

Schönbrunn E., Eschenburg S., Shuttleworth W. A., Schloss J. V., Amrhein N., Evans J. N., and Kabsch W. (2001). Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. PNAS, 98:1376-1380.

Schortgen G.P. (2017). Enhancing weed control by reducing hard water antagonism of 2,4-D in spray tank mixtures. Master's Thesis, Purdue University, West Lafayette, IN, USA.

Schuls A., Kruper A., and Amrhein N. (1985). Differential sensitivity of bacterial S–enolpyruvyl shikimate–3–phosphate synthases to the herbicide glyphosate. FEMS Microbiology Letters, 28:297–301.

Schultze M., and Kondorosi A. (1998). Regulation of symbiotic root nodule development. Annual Review of Genetics, 32: 33–57.

Schutte M. and Nleya T. (2018). Row spacing and seeding rate effects on soybean seed yield. In book: Soybean - Biomass, Yield and Productivity. Available from: https://www.intechopen.com

Scott W.O. and Aldrich S.R. (1983). Modern Soybean Production. 2nd ed. S. and A. Publications, P.O. Box 2660, Station A, Champaign, Illinois 61820, 1983.

Shaner D.L. (2014). Herbicide Handbook. 10th ed. Lawrence, KS: Weed Science Society of America.16-19.

Silva A. F.M., Lucio F. R., de Marco L. R., Giraldeli A. L., Albrecht A. J. P., Albrecht L. P., Victoria Filho R., and Nunes F.A. (2021). Herbicides in agronomic performance and chlorophyll indices of Enlist E3 and Roundup Ready soybean. Australian Journal of Crop Science, 15: 305-311.

Simpson D.M., Rosenbaum K., Ellis J.M., Richburg J.S., Haile F., Granke L.L., Thompson G.D., Haygood R.A., and Walton L.C. (2014). Palmer amaranth weed control with Enlist weed control systems. Page 68 *in* Proceedings of the 2014 North Central Weed Science Society. Las Cruces, NM: North Central Weed Science Society.

Singh B., and Singh K. (2014). Microbial degradation of herbicides. Critical Reviews in Microbiology, 1–17.

Singh G., and Wright D. (2002). In vitro studies on the effects of herbicides on the growth of rhizobia. Letters in Applied Microbiology, 35:12–16.

Smith A.E. (1973). Transformation of dicamba in Regina heavy clay. Journal of Agricultural and Food Chemistry, 21: 708-710.

Smith A.E., and Cullimore P.G. (1975). Microbial degradation of the herbicide dicamba in moist soils at different temperatures. Weed Research, 15: 59-62.

Solomon C.B., and Bradley K.W. (2014). Influence of application timings and sublethal rates of synthetic auxin herbicides on soybean. Weed Technology, 28:454–464.

Soltani N., Dille J.A., Burke I.C., Everman W.J., Van Gessel M.J., Davis V.M., and Sikkema P.H. (2017). Perspectives on potential soybean yield losses from weeds in North America. Weed Technology, 31:148–115.

Somasegaran P., and Hoben H.J. (1994). Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. New York: Springer-Verlag.

SoyStats (2021). A reference guide to important soybean facts and figures. http://soystats.com/international-adoption-of-biotech-enhanced-seedstock/

Speed M.L. (1990). Growth of Pseudomonas fluorescens on the herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid). MS Thesis, Illinois Institute of Technology, Chicago.

Sprout S.L., Nelson L.M., and Germida J.J. (1992). Influence of metribuzin on the Rhizobium leguminosarum-lentil (*Lens culinaris*) symbiosis. Canadian Journal of Microbiology, 38: 343–349.

Spyridon M., and Conley S.P. (2017). Delineating soybean maturity ugroups across the United States. Agronomy Journal, 109:1397–1403.

Staton M. (2011). Planting soybeans early offers many benefits. Michigan State University Extension.

https://www.canr.msu.edu/news/planting_soybeans_early_offers_many_benefits

Staton M. (2011). Evaluating soybean nodulation. Michigan State University Extension. https://www.canr.msu.edu/news/evaluating_soybean_nodulation.

Statista. (2021.) Leading soybean producing countries worldwide from 2012/2013 to 2020/21. https://www.statista.com/statistics/263926/soybean-production-in-selected-countries-since-1980/ Accessed Dec. 19, 2020.

Steel R.G.D., Torrie J.H., and Dickey D.A. (1997). Principles and procedures of statistics: biometrical approach, 3rd ed. McGraw-Hill, USA.

Steinrücken H.C. and Amrhein N. (1980). The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. Biochemical and Biophysical Research Communications, 94:1207-12.

Stoller E.W., Harrison S.K., Wax L.M., Regnier E.E., and Nafziger E.D. (1987). Weed interference in soybeans (*Glycine max*). Reviews of Weed Science, 3:155–181.

Strachan S.D., Casini M.S., Heldreth K.M., Scocas J.A., Nissen S.J., Bukun B., Lindenmayer R.B., Shaner D.L., Westra P., and Brunk G. (2010). Vapor movement of synthetic auxin herbicides: aminocyclopyrachlor, aminocyclopyrachlor-methyl ester, dicamba, and aminopyralid. Weed Science, 58:103–108.

Taylor R. (2012). Nitrogen fertilization and irrigated soybean production. Weekly Crop Update. Volume 20, Issue 23. University of Delaware Cooperative Extension. Online. Accessed Nov. 24, 2019.

Thies J.E., Singleton P.W., and Bohool B.B. (1995). Phenology, growth, and yield of field-grown soybean and bush bean as a function of varying modes of N nutrition. Soil Biology and Biochemistry, 27: 575-583.

Tortosa G.P.S., Cabrera J.J., Bedmar E.J., and Mesa. S (2021). Oxidative stress produced by paraquat reduces nitrogen fixation in soybean-bradyrhizobium diazoefficiens symbiosis by decreasing nodule functionality. Nitrogen, 2: 30-40.

Tremblay G.J., Beausoleil J.M., Filion P, and Saulnier M. (2006). Response of three soybean cultivars to seeding date. Canadian Journal of Plant Science, 86:1071–1078.

Tu M., Hurd C., and Randall J.W. (2001). Weed Control Methods Handbook: Tools and techniques for use in natural areas. All U.S. Government Documents (Utah Regional Depository). The Nature Conservancy.

Turner M., Nizampatnam N.R., Baron M., Coppin S., Damodaran S., Adhikari S., Arunachalam S.P., Yu O., and Subramanian S. (2013). Ectopic expression of miR160 results in auxin hypersensitivity, cytokinin hyposensitivity, and inhibition of symbiotic nodule development in soybean. Plant Physiology, 162:2042-55.

Ulzen J., Abaidoo R.C., Mensah N.E., Masso C., and AbdelGadir A.H. (2016). Bradyrhizobium inoculants enhance grain yields of soybean and cowpea in Northern Ghana. Frontiers in Plant Science, 7.

Underwood M., Soltani N., Hooker D., Robinson D., Vink J., Swanton C., & Sikkema P. (2016). The addition of dicamba to POST applications of quizalofop-p-ethyl or clethodim antagonizes volunteer glyphosate-resistant corn control in dicamba-resistant soybean. Weed Technology, 30: 639-647.

Underwood M., Soltani N., Robinson D.E., Hooker D.C., Swanton C., Vink J., and Sikkema P.H. (2017). Weed control, environmental impact, and net revenue of two-pass weed management strategies in dicamba-resistant soybean. Canadian Journal of Plant Science, 98:370–379

Uscanga-Mortera E., Clay S.A., Forcella F., and Gunsolus J. (2007). Common waterhemp growth and fecundity as influenced by emergence date and competing crop. Agronomy Journal, 99:1265-1270.

USDA. (2014). National Agriculture Statistics Service. Accessed: Dec.18, 2020 http://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Ag_Resource_Management/ARMS. Soybean_Factsheet/index.asp.

USDA-ERS. (2014). Adoption of genetically engineered crops in the U.S. Accessed: Dec.18, 2020.

USDA-ERS. (2019a). Soybean farmers in States with more glyphosate-tolerant weed species appeared more likely to use dicamba-tolerant seeds. Accessed on Dec.18, 2020.

USDA-ERS. (2019b). Recent trends in GE adoption. Accessed: Dec.18, 2020.

USDA-NASS. (2014) U.S. soybean industry: glyphosate effectiveness declines, NASS highlights No. 2014-1. http://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Ag_Resource_Manag

USDA-NASS. (2015). Crop Production Survey. https://www.nass.usda.gov/Statisticsby_State/South_Dakota/index.php.

USDA-NASS. (2019). https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/related-data-statistics/. Accessed: December 19, 2020.

USDA-NASS. (2020a). Quick stats. Accessed: January 15, 2021

USDA- NASS. (2020b). State Agriculture Overview for South Dakota. Accessed: January 15, 2021.

Van Acker R., Swanton C., and Weise S. (1993). The critical period of weed control in soybean [*Glycine max* (L.) Merr.]. Weed Science, 41: 194-200.

Van De Stroet B. and Clay S.A. (2019). Management considerations for Palmer Amaranth in a Northern Great Plains soybean production system. Agrosystems, Geosciences & Environment, 2:1. https://doi.org/10.2134/age2019.04.0030 pp 1-9.

Vincent J.M. (1970). A manual for the practical study of root-nodule bacteria. IBP Handbook no.15. Oxford: Blackwell Scientific Publications.

Vivian R., André R., Pablo A. K., Leandro V., Ana C.C.F. and Franciele M. (2013). Weed Management in soybean — Issues and practices, soybean - pest resistance, Hany A. El-Shemy, IntechOpen, doi: 10.5772/54595. Available from: https://www.intechopen.com/chapters/42592.

Walsh K.B. 1995. Physiology of the legume nodule and its response to stress. Soil Biology and Biochemistry 27: 637-655.

Wax L.M., Knuth L.A., and Slife F.W. (1969). Response of soybeans to 2,4-D, dicamba, and picloram. Weed Science, 17:388–393

Wesley T.L., Lamond R.E., Martin V.L., and Duncan S.R. (1998). Effects of late-season nitrogen fertilizer on irrigated soybean yield and composition. Journal of Production Agriculture, 11: 331-336.

Wild, A. and Manderscheid R. (1984). The effect of phosphinothricin on the assimilation of ammonia in plants. Z. Naturforsch, 39c:500–504.

Willey J.M., Sherwood L.M., and Woolverton C.J. (2011). Microorganisms in terrestrial ecosystems. Prescott's Microbiology. McGraw-Hill. pp. 703–706

William K.V., Robert L.N., Theodore M.W., John K.S., Carol M., Nilda R.B., William G.J., and Marilyn R.M. (2012). Herbicide resistance: Toward an understanding of resistance development and the impact of herbicide-resistant crops. Weed Science, 60:2-30.

Willis S. (2003) The use of soybean meal and full fat soybean meal by the animal feed industry. In: 12th Australian soybean conference. Soy Australia, Bundaberg

Wolf R., Clay, S.A., and Wrage l. J. (2000). Herbicide strategies for managing kochia (*Kochia scoparia*) resistant to ALS-inhibiting herbicides in wheat (*Triticum aestivum*) and soybean (*Glycine max*)1. Weed Technology, 14, 268–273.

Wood C.W., Reeves D.W., and Himelrick D.G. (1993). Relationships between chlorophyll meter readings and leaf chlorophyll concentration, N status and crop yield: A review. Proceedings Agronomy Society of New Zealand, 23:1-9.

Wortmann C.S., Krienke B.T., Ferguson R.B., and Maharjan B. (2018). Fertilizer recommendations for soybean. G859. NebGuide. http://extension.unl.edu/publications.

Wu F., Sedivy E.J., and Hanzawa Y. (2017). Soybean domestication: the origin, genetic architecture and molecular bases. New Phytologist, 214: 539–553.

Young B.G., Matthews J.L., and Whitford F. (2016). Compendium of herbicide adjuvants. 13th edition. https://ppp.purdue.edu/wp-content/uploads/2016/11/PPP-115.pdf

Yueh L.Y., and Hensley D.L. (1993). Pesticide effect on acetylene reduction and nodulation by soybean and lima bean. Journal of the American Society for Horticultural Science, 118:73–76.

Zablotowicz R.M., and Reddy K.N. (2004). Impact of glyphosate on the *Bradyrhizobium japonicum* symbiosis with glyphosate-resistant transgenic soybean: A minireview. Journal Environmental Quality, 33:825-831.

Zimdahl R.L. (1980). Weed-Crop Competition—A Review. International Plant Protection Center., Oregon State University, Corvallis, OR. Pages 83-93.

Zimdahl R.L. (1987). The concept and application of the critical weed free period. Pages 145-155 in M. A. Altieri and M. Liebman, eds. weed management in agroecosystems: ecological approaches. CRC Press, Inc., Boca Raton, FL.

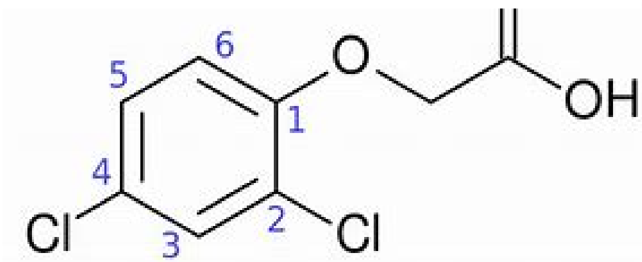
Zimdahl R.L. (2004). Weed-crop competition. A review. 2nd edition. Blackwell Publishing, Ames, IA.

https://www.weatherbase.com/weather/weather.php3?s=9037&cityname=Brookings-South-Dakota-United-States-of-America

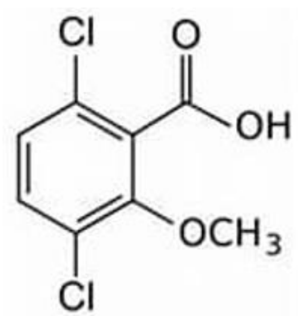
Appendix

Figure A1. Structure of herbicides.

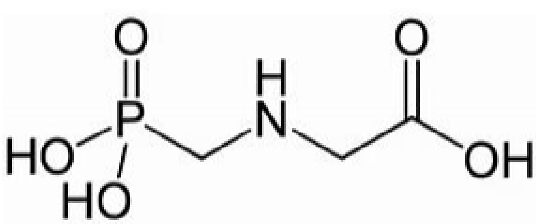
(1) 2,4-Dichlorophenoxyacetic acid



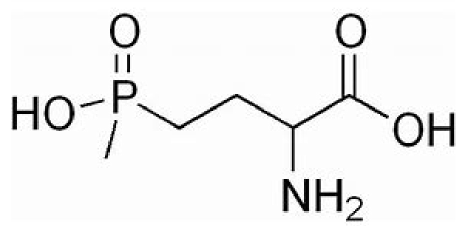
(2) Dicamba



(3) Glyphosate



(4) Glufosinate



(5) Acifluorfen

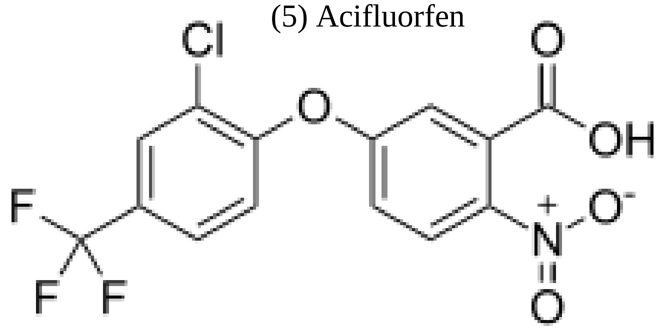


Table A1. Analysis of variance (ANOVA) of Enlist soybean variety evaluated for weed density 2 weeks after POST treatment application at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing seasons.

Weed density (plant/m^2) at 2 weeks after treatment application						
Effect	Degree of freedom	Northeast	East-central	Southeast	East-central	Southeast
		…………*2019 season* (p-value) ……….			……*2020 season* (p-value)……..	
Block	3	0.20	0.12	0.19	0.98	0.19
PD[a]	2	0.40	0.95	0.34	0.02	0.76
Ea	6					
Herbicide	5	0.00	0.01	0.22	0.39	0.80
PD:Herbicide	10	0.81	0.33	0.36	0.06	0.59
Eb	45					
CV (a)		52.4	54.2	64.1	36.9	31.7
CV (b)		61.3	48.5	58.0	19.4	35.7
R square		0.5	0.6	0.5	0.7	0.3

[a]Planting date: - [(Northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15), (East-central 2019: PD1-May 15, PD2-June 2, PD3-June 19), (East-central 2020: PD1-May 20, PD2-June 3, PD3-June 16), (Southeast 2019: PD1-May7, PD2-June 5, PD3-June 19), (Southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12).

*Weed density data were transformed using the square root transformation $\sqrt{(x)} + 1$; back transformed data are reported.

Table A2. Analysis of variance (ANOVA) of Enlist soybean variety evaluated for weed density 6 weeks after POST treatment application at northeast, east-central, and southeast South Dakota in 2019 and 2020 growing seasons.

Weed density (plant/m^2) at 6 weeks after treatment (WAT) application						
Effect	Degree of freedom	Northeast	East-central	Southeast	East-central	Southeast
		……*2019 season* (p-value)……..			……*2020 season* (p-value)……..	
Block	3	0.24	0.91	0.56	0.75	0.26
PD[a]	2	0.42	0.83	0.23	0.22	0.14
Ea	6					
Herbicide	5	0.00	0.10	0.88	0.00	0.00
PD:Herbicide	10	0.01	0.34	0.55	0.23	0.10
Eb	45					
CV (a)		62.6	60.2	118.7	55.9	24.4
CV (b)		52.7	48.8	48.6	46.9	28.0
R square		0.7	0.4	0.7	0.6	0.7

[a]Planting date: - [(Northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15), (East-central 2019: PD1-May 15, PD2-June 2, PD3-June 19), (East-central 2020: PD1-May 20, PD2-June 3, PD3-June 16), (Southeast 2019: PD1-May7, PD2-June 5, PD3-June 19), (Southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12).

*Weed density data were transformed using the square root transformation $\sqrt{(x)} + 1$; back transformed data are reported.

Table A3. Analysis of variance (ANOVA) of Enlist soybean variety evaluated for weed biomass (g/ m^2) at harvest at northeast, east-central, and southeast South Dakota in 2019 and 2020 growing seasons.

Weed biomass (g/m^2) sampled at harvest in Enlist soybean variety						
Effect	Degree of freedom	Northeast	East-central	Southeast	East-central	Southeast
		………….*2019 season* (p-value) ……….			………*2020 season* (p-value) …...	
Block	3	0.18	0.92	0.67	0.71	0.31
PD[a]	2	0.25	0.54	0.32	0.25	0.52
Ea	6					
Herbicide	5	0.00	0.01	0.63	0.00	0.00
PD:Herbicide	10	0.01	0.15	0.69	0.15	0.09
Eb	45					
CV (a)		89.1	177.3	318.4	88.8	68.9
CV (b)		99.7	161.2	210.5	65.6	74.8
R square		0.7	0.5	0.5	0.8	0.8

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A4. Analysis of variance (ANOVA) for soybean greenness (SPAD) in Enlist variety evaluated at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing seasons.

Soybean greenness at R5 stage of Enlist soybean						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	….*2019 season* (p-value) ….			….*2020 season* (p-value)…..	
Block	3	0.49	0.16	0.49	<0.05	<0.05
PD[a]	2	0.30	0.52	0.42	<0.05	0.08
Ea	6					
Herbicide	5	0.35	0.18	<0.00	0.17	0.99
PD:Herbicide	10	0.31	0.49	0.16	0.54	0.29
Eb	45					
CV (a)		5.2	3.1	6.9	3.2	1.6
CV (b)		5.8	2.5	2.9	1.9	1.6
R square		0.4	0.5	1.0	0.7	0.5

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A5. Analysis of variance (ANOVA) for soybean biomass (g/plant) in Enlist variety evaluated at northeast, east-central, and southeast South Dakota in 2019 and 2020 growing seasons.

Soybean biomass (g/plant) sampled at R5 stage of Enlist soybean						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	*2019 season* (p-value)			*2020 season* (p-value)	
Block	3	0.67	0.85	0.71	0.00	0.06
PD[a]	2	0.35	0.75	0.66	0.05	<0.01
Ea	6					
Herbicide	5	0.06	0.74	0.99	0.17	0.64
PD:Herbicide	10	0.38	0.22	1.00	0.50	0.25
Eb	45					
CV (a)		50.6	76.0	41.9	15.0	22.3
CV (b)		18.2	17.5	33.7	19.2	21.7
R square		0.7	0.8	0.3	0.5	0.7

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A6. Analysis of variance (ANOVA) of Enlist soybean variety evaluated for nodule number (500 cm^3 soil) at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing seasons.

Nodule number (500 cm^{-3} soil) at R5 stage of Enlist soybean						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	*2019 season* (p-value)			*2020 season* (p-value)	
Block	3	0.10	0.68	0.06	0.12	0.41
PD[a]	2	0.31	0.24	0.10	<0.01	<0.05
Ea	6					
Herbicide	5	<0.00	0.98	0.29	<0.05	0.68
PD:Herbicide	10	0.81	0.94	0.49	0.85	0.35
Eb	45					
CV (a)		26.9	43.8	57.8	24.1	47.9
CV (b)		26.2	29.2	24.8	21.9	22.9
R square		0.6	0.4	0.8	0.6	0.7

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A7. Analysis of variance (ANOVA) of Enlist soybean variety evaluated for active nodule number (500 cm^3 soil) at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing seasons.

Active nodule number (500 cm^3 soil) at R5 stage of Enlist soybean						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	*2019 season* (p-value)			*2020 season* (p-value)	
Block	3	0.44	0.73	<0.05	0.16	0.13
PD[a]	2	0.41	0.64	<0.05	<0.01	<0.05
Ea	6					
Herbicide	5	0.10	0.64	0.25	<0.05	0.12
PD:Herbicide	10	0.33	0.91	0.44	0.63	0.56
Eb	45					
CV (a)		93.2	73.4	78.3	37.2	52.8
CV (b)		74.9	45.1	76.4	28.9	35.1
R square		0.5	0.4	0.6	0.7	0.7

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A8. Analysis of variance (ANOVA) of Enlist soybean variety evaluated for grain yield (kg ha^{-1}) at northeast, east-central and southeastern South Dakota in 2019 and 2020 growing seasons.

Grain yield (kg ha^{-1}) of Enlist soybean evaluated at three eastern South Dakota locations.						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.26	0.73	0.89	0.11	0.72
PD[a]	2	<0.01	0.24	0.14	<0.00	<0.00
Ea	6					
Herbicide	5	0.07	0.78	0.53	0.16	0.96
PD:Herbicide	10	0.14	0.60	0.87	0.85	0.44
Eb	45					
CV (a)		10.6	16.8	48.9	26.2	17.9
CV (b)		13.1	9.1	31.4	18.3	23.1
R square		0.5	0.6	0.5	0.8	0.6

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A9. Analysis of variance (ANOVA) of Enlist soybean variety evaluated for 100-seed weight (g) at northeast, east-central and southeastern South Dakota in 2019 and 2020 growing seasons.

100-seed weight (g) of Enlist soybean evaluated at three eastern South Dakota locations.						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.09	0.68	0.32	0.00	0.85
PD[a]	2	0.30	0.07	0.09	<0.01	<0.05
Ea	6					
Herbicide	5	<0.05	0.60	0.22	0.13	0.20
PD:Herbicide	10	0.74	0.16	0.73	0.33	0.19
Eb	45					
CV (a)		3.9	3.0	12.6	3.2	6.8
CV (b)		4.0	2.4	15.8	6.4	5.7
R square		0.5	0.5	0.4	0.5	0.5

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A10. Analysis of variance (ANOVA) of Enlist soybean variety evaluated for seed oil content at northeast, east-central and southeastern South Dakota in 2019 and 2020 growing seasons.

Seed oil content (13 % moisture) of Enlist soybean evaluated at three eastern South Dakota locations.						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.42	0.08	0.46	0.42	0.31
PD[a]	2	0.13	0.26	0.38	<0.00	<0.01
Ea	6					
Herbicide	5	0.80	<0.01	0.38	0.09	0.53
PD:Herbicide	10	0.84	0.07	0.37	0.13	0.97
Eb	45					
CV (a)		3.4	1.0	27.2	2.4	1.8
CV (b)		1.3	0.8	15.9	1.3	2.1
R square		0.7	0.7	0.6	0.9	0.5

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A11. Analysis of variance (ANOVA) of Enlist soybean variety evaluated for seed protein content at northeast, east-central and southeastern South Dakota in 2019 and 2020 growing seasons.

Seed protein content (13 % moisture) of Enlist soybean evaluated at three eastern South Dakota locations.						
Effect	Degree of freedom	Northeast	East-central	Southeast	East-central	Southeast
		2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.12	0.00	0.50	0.56	0.82
PD[a]	2	<0.05	<0.00	0.46	<0.05	<0.05
Ea	6					
Herbicide	5	<0.05	0.34	0.38	0.19	<0.01
PD:Herbicide	10	0.83	0.76	0.50	<0.05	<0.00
Eb	45					
CV (a)		1.5	0.7	27.5	2.5	2.0
CV (b)		0.8	1.2	16.4	1.4	1.4
R square		0.7	0.5	0.5	0.7	0.7

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A12. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for weed density 2 weeks after POST treatment application at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing seasons.

Weed density (plants m^{-2}) at 2 weeks after POST treatment application in Xtend soybean						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.00	<0.01	0.02	0.59	0.01
PD[a]	2	<0.05	0.30	0.13	0.58	0.28
Ea	6					
Herbicide	5	<0.00	<0.05	<0.01	<0.01	<0.00
PD:Herbicide	10	0.11	0.70	0.64	0.70	0.42
Eb	45					
CV (a)		103.6	129.1	71.0	214.1	74.0
CV (b)		129.1	174.2	147.2	140.4	82.8
R square		0.7	0.6	0.5	0.5	0.6

[a]Planting date: - [(Northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15), (East-central 2019: PD1-May 15, PD2-June 2, PD3-June 19), (East-central 2020: PD1-May 20, PD2-June 3, PD3-June 16), (Southeast 2019: PD1-May7, PD2-June 5, PD3-June 19), (Southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12).

*Weed density data were transformed using the square root transformation $\sqrt{(x)} + 1$; back transformed data are reported.

Table A13. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for weed density 6 weeks after POST treatment application at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing seasons.

Weed density (plant/m^2) at 6 weeks after POST treatment application in Xtend soybean.						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.02	0.18	0.09	0.06	0.00
PD[a]	2	<0.01	0.58	0.39	0.43	0.08
Ea	6					
Herbicide	5	<0.00	0.15	0.11	<0.01	<0.00
PD:Herbicide	10	<0.01	0.87	0.73	0.49	0.89
Eb	45					
CV (a)		49.0	56.1	228.4	57.2	19.4
CV (b)		52.4	44.4	268.3	52.3	38.8
R square		0.8	0.4	0.4	0.6	0.5

[a]Planting date: - [(Northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15), (East-central 2019: PD1-May 15, PD2-June 2, PD3-June 19), (East-central 2020: PD1-May 20, PD2-June 3, PD3-June 16), (Southeast 2019: PD1-May7, PD2-June 5, PD3-June 19), (Southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12).

*Weed density data were transformed using the square root transformation $\sqrt{(x)} + 1$; back transformed data are reported.

Table A14. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for weed biomass (g/m^2) sampled at harvest at northeast, east-central, and southeastern South Dakota in 2019 and 2020 growing seasons.

Weed biomass (g/m^2) sampled at harvest in Xtend soybean						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.06	0.35	0.63	0.50	0.08
PD[a]	2	0.03	0.82	0.12	0.26	0.01
Ea	6					
Herbicide	5	<0.00	0.06	<0.00	<0.00	<0.00
PD:Herbicide	10	0.13	0.97	0.77	0.36	0.09
Eb	45					
CV (a)		91.6	244.0	131.1	118.7	58.7
CV (b)		83.8	159.7	97.5	102.2	51.2
R square		0.7	0.5	0.6	0.6	0.8

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A15. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for plant greenness (SPAD) at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

Plant greenness (SPAD) at R5 stage of Xtend soybean						
		Northeast	East-central	Southeast	East-central	Southeast
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.84	0.09	0.96	0.61	0.20
PD[a]	2	0.82	0.03	0.12	0.09	0.02
Ea	6					
Herbicide	5	0.39	0.25	0.02	0.05	0.05
PD:Herbicide	10	0.97	0.24	0.46	0.28	0.28
Eb	45					
CV (a)		3.2	1.3	1.8	3.3	1.9
CV (b)		2.4	1.5	2.2	1.7	1.6
R square		0.3	0.5	0.4	0.7	0.6

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A16. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for plant biomass (g/plant) at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

Plant biomass (g/plant) sampled at R5 stage of Xtend soybean						
		Northeast	Southeast	East-central	Southeast	East-central
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.42	0.17	0.18	0.50	0.00
PD[a]	2	0.21	0.03	0.53	0.00	0.00
Ea	6					
Herbicide	5	0.11	0.02	0.02	0.01	0.01
PD:Herbicide	10	0.05	0.10	0.25	0.08	0.01
Eb	45					
CV (a)		29.4	47.5	68.3	20.1	14.5
CV (b)		15.8	31.4	20.0	21.2	18.7
R square		0.6	0.7	0.8	0.8	0.7

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A17. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for nodule number (500 cm^3 soil) at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

Nodule number (500 cm^{-3} soil) at R5 stage of Xtend soybean						
		Northeast	Southeast	East-central	Southeast	East-central
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.09	0.04	0.00	0.93	0.20
PD[a]	2	0.82	0.89	0.18	0.02	0.00
Ea	6					
Herbicide	5	0.48	0.65	0.00	0.00	0.00
PD:Herbicide	10	0.89	0.92	0.45	0.40	0.30
Eb	45					
CV (a)		14.1	32.0	35.3	32.8	29.1
CV (b)		15.1	25.6	34.1	18.4	17.9
R square		0.3	0.5	0.7	0.7	0.8
Mean		38.0	23.1	71.1	42.4	48.0

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A18. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for active nodule number (500 cm^3 soil) at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

Active nodule number (500 cm^3 soil) at R5 stage of Xtend soybean						
		Northeast	Southeast	East-central	Southeast	East-central
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.75	0.01	0.02	0.75	0.07
PD[a]	2	0.64	0.37	0.13	0.05	<0.001
Ea	6					
Herbicide	5	0.53	0.71	<0.001	<0.001	<0.01
PD:Herbicide	10	0.92	0.75	0.06	0.42	0.48
Eb	45					
CV (a)		72.2	38.4	50.9	58.1	33.8
CV (b)		57.6	40.8	35.0	29.8	26.4
R square		0.3	0.5	0.7	0.72	0.8
Mean		23.3	5.4	66.5	26.0	31.6

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A19. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for grain yield (kg ha^{-1}) at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

Grain yield (kg ha^{-1}) of Xtend soybean evaluated at three eastern South Dakota locations.						
		Northeast	Southeast	East-central	Southeast	East-central
Effect	Degree of freedom	[2019 growing season (p-value)]			[2020 growing season (p-value)]	
Block	3	0.11	0.51	0.16	0.07	0.69
PD[a]	2	0.78	0.03	0.02	0.01	<0.001
Ea	6					
Herbicide	5	0.61	0.03	0.13	<0.001	0.01
PD:Herbicide	10	0.58	0.71	0.08	0.91	0.41
Eb	45					
CV (a)		18.8	19.8	12.1	24.9	12.2
CV (b)		14.4	13.8	11.2	19.4	10.4
R square		0.5	0.6	0.6	0.7	1.0
Mean		3221	3789	3006	2267	2300
LSD[b]		N/A	746.5	255.6	363.2	195.0

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A20. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for 100-seed weight (g) at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

100-seed weight (g) of Xtend soybean evaluated at three eastern South Dakota locations.						
		Northeast	Southeast	East-central	Southeast	East-central
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.02	0.00	0.04	0.15	0.48
PD[a]	2	0.07	0.23	<0.01	0.81	0.01
Ea	6					
Herbicide	5	<0.01	0.35	0.01	0.18	0.42
PD:Herbicide	10	0.68	0.93	0.45	0.01	0.93
Eb	45					
CV (a)		1.7	3.8	2.6	14.3	9.0
CV (b)		3.6	10.1	2.3	8.4	7.5
R square		0.5	0.3	0.7	0.6	0.5
Mean		14.2	18.3	17.8	11.2	15.0

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A21. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for seed oil content at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

Seed oil content (13 % moisture) of Xtend soybean evaluated at three eastern South Dakota locations.						
		Northeast	Southeast	East-central	Southeast	East-central
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.39	0.02	0.29	0.44	0.03
PD[a]	2	0.02	0.02	0.82	0.18	<0.001
Ea	6					
Herbicide	5	0.66	0.16	0.03	0.12	0.42
PD:Herbicide	10	0.53	0.83	0.28	0.17	0.90
Eb	45					
CV (a)		1.4	1.1	1.0	2.9	2.6
CV (b)		1.5	0.9	0.9	2.7	3.8
R square		0.5	0.7	0.5	0.5	0.8
Mean		18.0	19.5	19.7	18.5	19.2
LSD[b]		0.2	0.2	0.2	N/A	0.4

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].

Table A22. Analysis of variance (ANOVA) of Xtend soybean variety evaluated for seed protein content at northeast, southeast, and east-central South Dakota in 2019 and 2020 growing seasons.

Seed protein content (13 % moisture) of Xtend soybean evaluated at three eastern South Dakota locations.						
		Northeast	Southeast	East-central	Southeast	East-central
Effect	Degree of freedom	2019 growing season (p-value)			2020 growing season (p-value)	
Block	3	0.06	0.02	0.06	0.53	0.15
PD[a]	2	0.24	0.91	<0.05	0.73	<0.01
Ea	6					
Herbicide	5	0.43	0.24	<0.01	<0.001	0.74
PD:Herbicide	10	0.29	0.88	0.07	0.11	0.27
Eb	45					
CV (a)		1.7	1.8	1.1	3.3	1.7
CV (b)		2.1	1.1	1.2	2.4	2.7
R square		0.4	0.7	0.6	0.6	0.5
Mean		36.1	34.8	34.3	35.4	32.8
LSD[b]		0.4	N/A	0.3	0.7	0.4

[a]Planting date [(northeast 2019: PD1-May 15, PD2-May 30, PD3-June 15); (east-central 2019: PD1-May 15, PD2-June 2, PD3-June 19); (southeast 2019: PD1-May7, PD2-June 5, PD3-June 19); (east-central 2020: PD1-May 20, PD2-June 3, PD3-June 16); (southeast 2020: PD1-May 15, PD2-May 29, PD3-June 12)].