SOYBEAN WATER STRESS DEVELOPMENT AND MITIGATION IN WEST-CENTRAL NORTH DAKOTA

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

In recent years, soybean [*Glycine max. (*L.) *Merr*.] production has moved into west-central North Dakota, an area known for common deficits between potential plant water use and annual rainfall. Soybean seed yield reductions due to water stress are the greatest during reproductive stages of growth. In a year of limited rainfall, foliar-applications of five different water use modulating chemicals applied at early reproductive growth stages commonly reduced seed yields, while improvements were few and inconsistent. Seed-applied plant growth regulators (PGRs) were recognized as possible seed treatments to conserve soil water by reducing vegetative plant growth, thus improving water dynamics later in the growing season. In the following field study, late-terminated, fall-seeded cover crops were shown to significantly reduce spring soil water levels. However, favorable rainfall throughout the growing season buffered any cover crop or seed-applied PGR treatment effects on growing season soil water, plant water status, and soybean seed yield.

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INTRODUCTION/LITERATURE REVIEW

Soybean Acreage in North Dakota

Soybean [*Glycine max* (L.) Merr.] moved into the top spot for number of hectares (ha) planted in North Dakota in 2017, passing wheat (*Triticum aestivum* L.) which was previously the dominant crop grown in the state (USDA NASS, 2017). Soybean has been a common crop on the eastern side of North Dakota for many years, but its westward expansion is a recent trend. Between 2013 and 2016, McLean County (located in west-central North Dakota) reported an increase of 132% in soybean hectares harvested, from 18,332 to 42,613 ha. Whereas, Cass County (located in eastern North Dakota) only saw an increase of 3% between the same years, from 186,965 to 192,428 ha (USDA-NASS, 2019). The expansion of soybeans into the west-central part of the state can be explained by: higher prices in recent years despite large supplies worldwide (Good, 2015), nitrogen fixation rotational benefits (Badaruddin and Meyer, 1994; Long, 1989), the development of shorter season cultivars (Kumudini et al., 2001), and other advances in agricultural management like improved weed control (Gianessi, 2005).

Annual Precipitation and Soybean Water Use

West-central North Dakota is in a semi-arid region, which on average, receives significantly less total precipitation than the eastern side of the state during the growing season. Based on a 30-year average of normal growing season precipitation, the eastern and west-central parts of North Dakota receive approximately 450 mm and 370 mm of precipitation between April 1 and October 31, respectively (Figure 1). This reduced amount of precipitation leads to deficits between available water and potential crop water use, which contributes to lower production potential in the western region. This is exemplified by a four-year study done in

Mandan, ND on soybean row spacing by Alessi and Power (1982), where soybean seed yield during two of the study years was less than 800 kg ha^{-1}, due to limited rainfall.

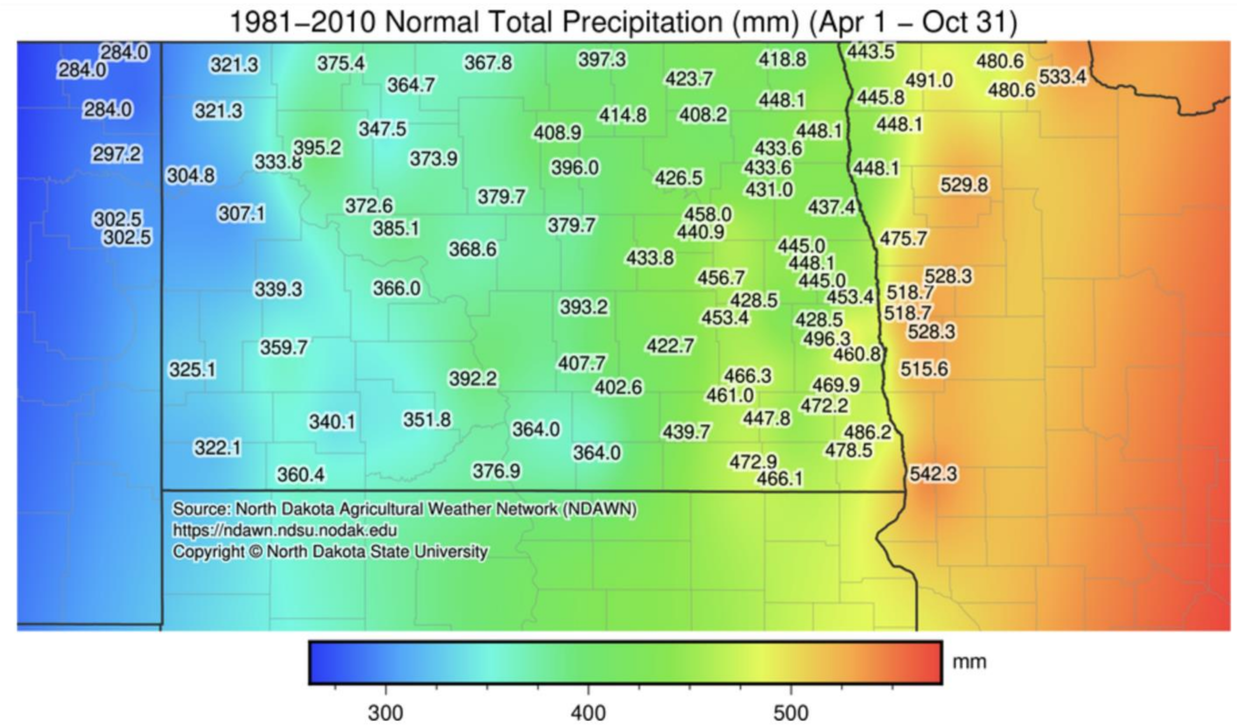

Figure 1. 30-year normal total precipitation (mm) for North Dakota and surrounding areas between 1 April and 31 October, 1981-2010. (NDAWN, 2019). Accessed 2/19/2019.

Historically, small grains, like wheat, have been the dominant crops grown in western North Dakota because of their shorter growing seasons and lower requirements of seasonal water. In 1977 and 1978, when soybean was a relatively new crop in North Dakota, Bauder and Ennen (1981) quantified crop water use in eastern North Dakota with rain gauges and the measurement of soil water depletion with neutron probes. Their results indicate that wheat used 302 mm (11.9 inches) of water, which is below the 30-year average rainfalls listed above for both the eastern and western regions. However, soybean used 429 mm (16.9 inches), which would produce a deficit in available water in the western portion of North Dakota in most years. Furthermore, they found a strong correlation ($r = 0.97$, $r \leq 0.05$) between total water use and days from crop emergence to maturity: soybean was 131 days, while wheat was only 74 days, indicating that growing season length has a larger impact on total crop water use than morphology (Bauder and Ennen, 1981).

Common terms for crop water use include evapotranspiration (ET; water evaporated from the soil surface and transpired through growing plants calculated through a soil water mass balance or energy balance approach) and potential evapotranspiration (PET; crop water use estimated by calculations on climatic or weather data and crop coefficients). Using weighing lysimeters, Brun et al. (1985) conducted a study at North Dakota State University in the 1982 growing season to quantify ET from soybean. Their results reported a total growing season (25 May to 26 September) ET value of 395 mm (15.6 inches) when soybean was planted into standing wheat stubble from the previous growing season.

Rijal et al. (2012) calculated daily ET of a soybean crop in the southeastern part of North Dakota to be approximately 4 mm day^{-1} throughout months of June, July, and August, which are the major growth months for soybean. In west-central Minnesota, Reicosky et al. (1985) calculated daily PET for the same date range to be approximately 5 mm day^{-1}. These papers would agree that daily ET is commonly lower in the early parts of the growing season and also in later parts of the year, fluctuating greatly with weather and precipitation events. If the average of these two values (4.5 mm day^{-1}) is carried over the 91 day-range between 1 June and 31 August, the growing season water use for a soybean crop would be 409 mm (16.1 inches). Without considering the water that is lost outside 1 June and 31 August, a deficit would already occur in the western region of North Dakota for both the 395 and 409 mm scenarios described above when compared with the 30-year normal growing season precipitation of 370 mm.

It should be noted that the 30-year average for normal growing season precipitation does not include any precipitation received outside of the growing season, but it is not likely the inclusion of off-season precipitation would satisfy the deficit. A portion of the precipitation that falls as snow will be lost directly to the atmosphere through sublimation and some would run-off of the field on the soil surface after melting, and thus be unavailable for crop use during the subsequent growing season. Field research was conducted on water storage efficiencies of non-growing season precipitation (from harvest to planting in the following year) in Minot, ND between 1977 and 1982. The total precipitation received outside the growing season that was stored in the soil for the following growing season averaged 56% under no-till management (Deibert et al., 1986).

It is impractical to provide irrigation for large regions of land in western North Dakota to satisfy water deficits. Consequently, efforts to combat annual water deficits in semi-arid regions have shifted to the management of soil water through conservation and other agricultural practices. The success of soybean growers to this point is partly due to increasing water infiltration and retention through the adoption of reduced tillage practices, which has been proven to decrease surface run-off (Dick et al., 1989) and overall evaporation losses by leaving crop residues on the soil surface (van Donk et al., 2012). However, water availability is still the major limiting factor in yield potential for this drier region of North Dakota, and efforts to improve average yields and yield predictability are merited.

Soil, Plant, and Water Relations

Water is essential for plant function and growth. Its roles in photosynthesis, organic compound and nutrient transport, plant structure, and temperature moderation through evaporative cooling have been well documented. All of these processes require the plant to take up water from the soil and transport it to various tissues, where it can be utilized, or lost to the atmosphere as water vapor, a process called transpiration. Generally speaking, transpiration by plants is governed by a continuous water potential gradient with restrictive mechanisms between the soil, plant, and atmosphere. This gradient must be increasingly negative to facilitate vertical water movement. If this gradient is broken at any point inside the plant (i.e. soil matric potential becomes too resistive for the plant to overcome), wilting will occur, followed by reductions in photosynthetic potential (Hillel, 1998).

There are three general strategies through which plants respond to the onset of low soil matric potential, or more commonly referred to as drought stress: escape, avoidance, and tolerance (Kooyers, 2015). The escape strategy attempts to speed up the plant's life cycle, in an effort to complete a reproductive stage and produce viable seed before the onset of drought causes plant death. Drought avoidance involves physical and morphological changes like reductions in leaf surface area, the closing of stomata, or increases in root growth in an effort to maintain favorable water status conditions within the plant. Whereas drought tolerance is associated with the adjustment of osmotic potential within cells, to maintain proper plant and cellular function despite limited soil water conditions (Lisar et al., 2012; Kooyers, 2015).

The alteration of guard cell turgor pressure to change the size of stomatal apertures mentioned above is a complex process primarily moderated by the formation and translocation of abscisic acid (ABA), but can also be affected by environmental factors like sunlight and high concentrations of carbon dioxide inside plant tissues (Zeiger, 1983). The closing of the stomata reduces the surface area through which water can escape from the plant, helping the plant maintain its current internal water status until drought stress is relieved. Osmotic adjustment as a drought tolerance strategy is the process through which plants accumulate quantities of osmolytes, either organic solutes in the cytosol of the cell (amino acids, glycerol, sugars) or inorganic ions inside the cell's vacuole (Na^+, K^+, Ca^{2+}, and Cl^-). These accumulations of osmolytes lower the osmotic potential in plant cells, and therefore water follows the flux of osmolytes into the cell (Chen and Jiang, 2010). These stomatal and osmotic adjustments allow dynamic adaptation to limited water supplies for maintaining metabolic activity during high water stress and also enable continuation of growth when conditions improve (Morgan, 1984).

Effects of Water Stress on Soybean

Soybean responds to drought stress through all three above-mentioned strategies (De Souza et al., 1997; Mutava et al., 2015; Mwenye et al., 2016), and numerous studies have reported significant negative impacts on soybean seed yield which is reviewed by Mwenye et al. (2016), especially when the stress is induced during reproductive stages when pods are beginning to develop (R3) and when a pod at one of the four uppermost nodes on the main stem contains full seed (R6) (Fehr, 1971). The results in Table 1 were adapted from a review on the effects of drought stress on soybean (Mwenye et al., 2016, p. 247) and outline the impacts of limited water on soybean seed yield at various growth stages.

As demonstrated in Table 1, reduced seed yields when water stress was applied across all growth stages commonly range from 12 to 50% from control, with an extreme of 87% under severe drought stress (Cox and Jolliff, 1986). Variations in seed yield reduction are also shown across varying growth stages, with the mid-reproductive stage (R3 to R6) exhibiting the greatest reductions ranging from 19 to 88% depending on stress severity. These reductions in yield can also be seen by looking at differences between the eastern and western portions of North Dakota. Averaged over the 2014 to 2017 growing seasons, Ward County (located in west-central North Dakota) and Cass County reported yields of 2230 kg ha^{-1} and 2700 kg ha^{-1}, respectively, which is a 17.4% lower yield in the west-central county. (USDA-NASS, 2019).

Table 1. Adapted table from Mwenye et al., (2016) outlining the effect of water stress timing on soybean seed yield.

Reference	Location	Climate/ rainfall	Irrigation method/ strategy	Stress intensity/ duration	Yield range kg ha^{-1}	Yield reduction by growth stage (%)				
						Ve	R1-R2	R3-R6	R7-R8	All growth stages
Cox and Jolliff (1986)	Oregon, USA	-	Deficit irrigation, line source	Well-watered kept above -0.05 Mpa	Dryland: 400 Deficit: 2400 Control: 3290	-	-	-	-	87 27 -
Eck et al. (1987)	Texas, USA	Semi-arid 360 mm	Deficit irrigation	40-80% less than well-watered	370 - 3130	-	12-28	27-88	8	-
Specht et al. (2001)	Nebraska, USA	-	Deficit irrigation, sprinkler	0, 20, 40, 60, 80, 100% replenishment of ET	933-2085	-	-	-	-	50
Karam et al. (2005)	Teal Amara, Lebanon	Semi-arid 592 mm	Deficit irrigation 700-800 mm	7 days (R2, R5, R7)	2300-3500	-	15	35	15	-
Dogan et al. (2007)	Sanliurfa, Turkey	Semi-arid 450 mm	Deficit irrigation 440-690 mm	8-12 days	1955-3684	-	13	19-47	-	-
Kobraee et al. (2011)	Iran	Semi-arid 278 mm	Deficit irrigation	R1, R3, R6	1688-3173	-	45	30-47	-	-
Candogan et al. (2013)	Turkey	Temperate	Deficit irrigation at 0-90 cm profile	-	2070-3760	-	-	-	-	12-45
Kobraee et al. (2014)	Kermanshah, Iran		Deficit irrigation	-	1637-2274	-	29	22-28	-	-

†Adapted from "The role of proline and root traits on selection for drought-stress tolerance in soybeans: a review," by Mwenye et al., 2016, South African Journal of Plant and Soil, 33(4) p. 247.
‡ All experiments listed were performed in a field setting

The agronomic benefits of nitrogen-fixation (N_2 fixation) by soybean in a cash crop rotation were outlined by Badaruddin and Meyer (1994), and these benefits have contributed to the west-ward expansion of soybean in North Dakota. However, significant decreases in symbiotic N_2 fixation have been related to soybean water stress, leading to decreased soybean yield. Furthermore, this process has been shown to be more sensitive to drought stress than photosynthesis or respiration (Serraj et al., 1999; Todd et al., 2006).

Ureides are nitrogenous molecular products of nitrogen fixation that are transported from soybean nodules throughout the plant, and under normal water conditions are catabolized in plant leaf tissues to be used in protein synthesis (Winkler et al., 1987). However, under water stressed conditions, these transport molecules accumulate in soybean tissue and are related to a feedback inhibition of symbiotic N_2 fixation in root nodules (Purcell et al., 2004; King and Purcell, 2005). The exact pathway by which the accumulation of these molecules in plant tissues inhibit N_2 fixation in root nodules before photosynthetic rates are affected is under debate, and may be due to a combination of processes, including manganese deficiency in leaf tissues (Todd et al., 2006; King and Purcell, 2005). Once photosynthetic rates are reduced due to water stress, less carbohydrate substrates are supplied to root nodules, which leads to greater reductions in nitrogen fixation rate (Serraj et., 1999). Therefore, ureide accumulation in soybean tissue appears to be both an early and sensitive indicator of drought stress.

Water Use Modulation

As previously outlined, reductions in seed yield of soybean due to water stress is most significant when the stress is present during reproductive stages of soybean growth. Therefore, the modulation and/or reduction of soybean water use during vegetative growth stages, to conserve soil water and improve water availability during reproductive growth stages may be an effective strategy for coping with seasonal water deficits. This concept was studied by He et al. (2017) on eight soybean genotypes, who reported a general increase in seed yield for all genotypes due to conserved soybean water use prior to flowering. Additionally, the authors mentioned the variation between genotypes, in particular, the two cultivars with the lowest daily water use prior to flowering were the only cultivars to produce seed in the treatment with the greatest water stress. The authors contribute the drought tolerance of these cultivars to improved root morphology, early flowering tendencies, and reductions in stomatal conductance when soil water availability was high; consequently, reducing transpiration rates and cumulative water use and thus conserving water for seed filling stages (He et al., 2017).

Several mechanisms for altering soybean water use during vegetative growth stages exist. These mechanisms can be put into two general categories: antitranspirants (ATs) and plant growth regulators (PGRs). Antitranspirants are chemical compounds, applied foliarly to reduce the transpiration rates of the treated plants (Gale and Hagan, 1966). There are two general types of leaf-applied antitranspirants: reflective coatings and physical films/barriers. Reflective antitranspirants are light in color and after an application to the leaves, reflect more sunlight, keeping the plant cooler and thus decreasing the physiological requirement of transpiration for thermoregulation (Abou-Khaled et al., 1970). Film/barrier types impose a physical layer between the plant and the atmosphere, buffering the plant from evaporative demand (Gale and Hagan, 1966).

Plant growth regulators are naturally occurring plant hormones or their synthetic substitutes that can be applied as seed treatments, directly to plant leaves, or as soil amendments to alter plant growth. In agriculture, PGRs are commonly used to improve stress tolerance, encourage ripening, or induce desirable morphological characteristics traits in cultivated plants.

The pathways by which these compounds alter plant water use are more complex than the general AT types listed above. Each PGR affects plant growth and hormone levels differently, dependent upon which hormone or hormones are affected, the applied concentration, plant growth stage, as well as the environmental conditions during application. An extensive review on the subject of PGRs and their applications in agriculture was done by Rademacher (2015). In regard to reducing plant water use, PGRs with above-ground growth inhibiting properties are of interest under the premise that a smaller plant should require less transpirational water than a larger one, as well as PGRs that optimize root length and rooting density, to effectively scavenge the soil for unutilized water resources. A direct effect on transpiration rate can also be produced by some PGRs through temporary reduction of stomatal openings, directly decreasing the effective area for vapor transfer and also limiting total plant growth, cumulatively reducing transpiration (Gale and Hagan, 1966; Mishra and Pradhan, 1972). Plant growth regulators have also been shown to induce the accumulation of osmolytes in plant cells, like proline and soluble sugars, thus maintaining proper leaf water status for physiological function under limited water conditions (Zhang et al., 2007).

Some fungicides have also been reported to have PGR effects, by reducing transpiration rates through the closing of stomatal apertures (Petit et al. 2012; Nason et al., 2007), however the direct mechanism through which this is taking place is not fully understood (Nason et al., 2007). Strobilurin fungicides have been reported to reduce water uptake by the roots in wheat plants, thus conserving soil water for later growth stages (Inagaki et al., 2009), and application of triadimefon fungicides under water stressed conditions by Fletcher and Nath (1984) reduced transpiration rates of wheat and soybean seedlings by 40 and 19%, respectively.

An important consideration for the use of ATs and PGRs, is the reduction in photosynthetic potential of the treated plant. All methods of modulating plant water use affect photosynthetic processes in their respective way (Davenport et al., 1972). For reflective ATs, light energy from the sun is required by the plant for photosynthesis; thus, reflecting usable solar energy decreases the maximum potential of photosynthesis (Davenport et al., 1969). Since photosynthesis requires the plant to capture CO_2 through its leaves, the physical layer imposed by film/barrier ATs can inhibit the influx of CO_2, consequently decreasing photosynthetic potential (Gale and Hagan, 1966). Decreased stomatal apertures from PGRs could also inhibit the diffusive transport of CO_2 into the plant, thus decreasing photosynthetic potential (Davenport et al., 1969; Nason et al. 2007). Furthermore, the reduction in plant biomass from the application of PGRs may reduce effective leaf surface area for the collection of sunlight. This reduction in biomass could also favor evaporation from the soil or increase weed pressure, both of which would not be adventitious for crop water use efficiency regarding total ET.

Research on the use of ATs to manage water use in soybean to directly increase seed yield is almost non-existent, but one study on the application of kaolin, a reflective, foliar-applied AT, reported a seed yield increase of 21% over the control under limited irrigation treatment (Javan et al., 2013). Other studies have looked at the impact on incoming solar radiation by reflective type ATs, concluding that more solar energy is indeed reflected from soybean leaves, and treated crops do exhibit reductions in transpiration rates between 10-25% (Baradas et al., 1976; Abou-Khaled et al., 1970). Utilizing PGRs to specifically reduce the negative effects of water stress and improve drought tolerance in soybean crops is more prevalent than ATs, but again current literature is limited. Results however, show promise in increasing soybean seed yield as Jing et al. (2012) found seed-applied uniconazole (PGR), at the optimum rate of 4 mg kg^{-1} of seed, to increase seed yield by 22% and 8% versus the control (no uniconazole), under soil water treatments of 75% and 45% of field water capacity, respectively. Under drought stress, Zhang et al. (2007) reported a seed yield increase between 20% versus the control, by the foliar application of uniconazole (rate of 50 mg L^{-1}) at the beginning of soybean flowering. Nason et al. (2007) found that strobilurin fungicides reduced transpiration rates in wheat, barley (*Hordeum vulgare* L.), and soybean plants. However, they also reduced net photosynthesis in the same crops, especially under water stressed conditions; concluding that the use of strobilurin fungicides to improve water use efficiency is questionable (Nason et al., 2007).

In 2014, greenhouse studies were conducted at North Dakota State University (NDSU), to evaluate the effectiveness of 18 foliar-applied chemicals to decrease soybean water use (Goos, R. J., unpublished data, 2014). The nine chemicals at various concentrations shown to significantly reduce plant water use versus their respective control at the $p = 0.05$ level of significance are listed in Table 2. Effects varied greatly over the six-day period, both quantitatively and temporally. Abscisic acid greatly reduced soybean water use shortly after application and its effect diminished over time, while Cerone, paclobutrazol, and uniconazole were exhibiting greater reductions in water use as the experiment progressed. Chitosan, Headline SC, Priaxor, Quadris, and Stratego YLD all maintained a smaller, but substantial reduction in water use versus the control throughout the experiment.

Table 2. Foliar-applied compounds shown to cause significant reductions in soybean water use over a six day period versus their respective control in a greenhouse experiment conducted by Goos, R.J. in 2014 (unpublished data).

Material tested	Chemical name
Abscisic acid	abscisic acid
Chitosan	chitosan
Headline SC	pyraclostrobin
Priaxor	pyraclostrobin + fluxapyroxad
Quadris	azoxystrobin
Stratego YLD	prothioconazole + trifloxystrobin
Cerone	ethephon
Uniconazole	uniconazole
Paclobutrazol	paclobutrazol

†Treatments listed were significantly different from the control at $p = 0.05$.

Cover Crops and Water Use

Management decisions and their effects on soil quality and ecosystem services are becoming more important in agroecosystems (Power, 2010). Cover crops are being adopted in agriculture for both their short-term and long-term (greater than five years) benefits including limiting water and wind erosion (Blanco-Canqui and Wortmann, 2017), immobilizing soluble nutrients (Dabney et al., 2001), alleviating compaction zones (Chen and Weil, 2009), improving soil structure and pore characteristics (Abdollahi et al., 2014), and suppressing weeds (Jabran et al., 2015). These benefits are easily realized in characteristically wet regions, where poor soil drainage affects crop production and cover crops are implemented to utilize excess soil water. However, in semi-arid regions like west-central North Dakota, the implementation of cover crops and the water they utilize during growth may exacerbate seasonal water deficits between annual precipitation and following cash crop water requirements.

In Iowa, Qi and Helmers (2010) quantified evaporative water loss with lysimeters under bare soil and rye (*Secale cereale* L.), as a fall-seeded cover crop, and concluded that during a month of high growth the following spring, estimated ET from the rye was 2.4 mm d^{-1}, while evaporation from bare soil was 1.5 mm d^{-1}. Leaving crop residues on the surface has been shown to reduce evaporation rates when compared with bare soil (van Donk, 2012), which in the above study would be synonymous to a no-till treatment, thus further increasing the difference in soil water depletion by cover crops versus undisturbed residue from the previous cash crop. Several studies throughout the United States have shown significant soil water depletion in the spring under fall-seeded rye versus non-cover cropped treatments (Liebl et al., 1992; Clark et al., 1997; Basche et al., 2016). However, the caveat to this result was that significant differences were highly dependent upon termination timing of the rye. Generally, these termination dates were categorized as either early (two weeks prior to the seeding of the following cash crop) or late (around planting time of the cash crop). The late termination date significantly lowered available soil water compared to the non-cover cropped control, as well as the early-terminated rye treatments; while soil water in the early-terminated rye was similar to the non-cover cropped treatments (Liebl et al., 1992; Clark et al., 1997; Krueger et al., 2011).

Reduced yields of cash crops following a rye cover crop were only mentioned by Liebl et al. (1992), where lower soybean yields were related to late cover crop termination during all four years of their study in Illinois, and by De Bruin et al., (2005) who reported that rye cover cropping in Minnesota used significant soil water, reducing soybean yield only in a year with below average rainfall. However, these negative effects were not present when rainfall was above normal (De Bruin et al., 2005). Therefore, excessive water use by cover crops in low-precipitation, semi-arid regions could cause significant yield reductions in following cash crops, which outlines the delicate balance between cover crop water use during growth and improved water dynamics after their termination (Dabney et al., 2001; Unger and Vigil, 1998).

After termination, the residue left on the surface and the decaying roots underground have a cumulative positive effect on soil water dynamics throughout the growing season by improving soil structure and porosity (Abdollahi et al., 2014), improving infiltration through reduced soil crusting and reduced surface water velocity (Pikul and Zuzel, 1994), decreasing surface evaporation (Unger and Vigil, 1998), and suppressing weeds which can decrease competition for water and nutrient resources for the subsequent cash crop (Jabran et al., 2015). The lack of negative effects on final yield of following cash crops due to low spring available water is attributed to the mulching effect of decomposing rye residue later in the growing season (Clark et al., 1997; Basche et al., 2016). This mulching effect showed improved soil water availability in later dates throughout the growing season, when cash crops are more susceptible to yield reduction due to water stress (Clark et al., 1997; Basche et al., 2016), even when severe drought conditions were present (Daigh et al., 2014). This was particularly noted for early-terminated rye treatments, which tended to lay down and create a mat over the soil surface versus later terminated rye that tend to remain standing throughout the growing season (Munawar et al., 1990; Krueger et al., 2011).

A study conducted at the Area IV Soil Conservation Districts Research Farm near Mandan, ND recognized the lack of information and research on cover crops in semi-arid regions, particularly central North Dakota, and showed the effectiveness and success of fall-seeded cover crops into no-till cropping systems was greatly affected by precipitation received within 14 days of cover crop seeding. The resulting differences in soil water were subtle in years with significant biomass growth between cover crop treatments and the no cover crop control, due to the fact that rainfall was the contributor to significant biomass production (Liebig et al., 2015). In essence, this study indicated the importance of post-seeding precipitation events on the establishment and therefore overall benefit of planting cover crops in semi-arid regions.

The long-term use of cover crops has been shown to have a positive effect on seasonal water dynamics (Basche et al., 2016), by buffering variations in rainfall through improved infiltration, water storage capacity, and water availability to subsequent plants through increases in organic matter and water-stable aggregates in the soil (Hudson, 1994; Tisdall and Oades, 1982). These long-term benefits could help overcome future seasonal water deficits in semi-arid regions. However, careful considerations need to be made regarding stored soil water depletion through cover crop termination, in the short-term, especially in semi-arid regions.

Research Objectives and Thesis Organization

Soybean production in the semi-arid region of west-central North Dakota has increased dramatically in recent years. Based on this trend, it is likely that soybean production will continue to be just as dominant in the coming growing seasons. The issue faced by all soybean growers in this region is the deficit between potential crop water use and growing season precipitation. As mentioned previously, adoption of reduced-till practices in these regions has greatly benefited farmers and has proven an effective strategy to mitigate water stress in cropping systems through reductions in surface run-off by improving infiltration and reduced evaporation through the preservation of crop residue on the soil surface (Klocke et al., 2009; van Donk et al., 2012).

Even with these advances in agricultural technology and management, water shortages are an ever-lingering issue for farmers in this region. Antitranspirants or PGRs have been shown to optimize plant growth and water use efficiency of plants and may be a solution for maximizing potential production of soybean under seasonal water deficits, by reducing vegetative growth and conserving soil water for reproductive stages. The use of cover crops for their many soil benefits has proved promising in humid areas, however their utilization of stored soil water could induce further deficits in available water for subsequent cash crops in semi-arid regions. This has been reported in the early parts of the growing season, but the mulch effect from decomposing rye residue has also been shown to improve soil water dynamics during the later stages of the growing season, when soybean exhibits the greatest reduction in yield due to water stress.

If soybeans continue their expansion across western North Dakota, solutions for common deficits in water availability throughout a growing season should be explored. Therefore, the objectives of this research were: 1) to compare the effects of different foliar-applied ATs and PGRs at various soybean growth stages on in-season soybean leaf water status, above-ground biomass production, and seed yield, 2) to compare the effects of two seed-applied PGRs at increasing concentrations and their effects on soybean growth and biomass production in a greenhouse setting, 3) to monitor the effects of fall-seeded cover crops on spring soil water availability, and 4) to compare the subsequent effects of cover crops and seed-applied PGRs on in-season soil-soybean water dynamics, as well as, soybean seed yield and quality. These objectives will be addressed through two field experiments and a greenhouse study in the following sections. First, a materials and methods section will outline each experiment separately, followed by a results and discussion section describing the findings of each experiment separately, and finally, a general conclusion will focus on the effectiveness and applicability of the studied treatments on mitigating soybean water stress in the semi-arid region of west-central North Dakota.

MATERIALS AND METHODS

Experiment 1: Foliar-Applied Water Use Modulators and Their Effects on Leaf-Water Status, Biomass Production, and Seed Yield in Soybean

The research objective of this field experiment was to compare the effects of different foliar-applied ATs and PGRs at varying soybean growth stages on soybean water status throughout the reproductive stages of growth, final biomass yield, and seed yield. This experiment was conducted at three sites during the 2017 growing season: the North Central Research Extension Center (NCREC) in Minot, ND, west of Underwood, ND, and southwest of Coleharbor, ND. Soil types varied between locations and are described in Table 3.

Table 3. Soil type variations between site locations in the 2017 summer field experiment.

Location	Soil Series	Taxonomic Class
Minot, ND	Forman-Aastad loams	Forman: Fine-loamy, mixed, superactive, frigid Calcic Argiudolls Aastad: Fine-loamy, mixed, superactive, frigid Pachic Argiudolls
Underwood, ND	Wilton silt loam	Wilton: Fine-silty, mixed, superactive, frigid Pachic Haplustolls
Coleharbor, ND	Williams-Bowbells loams	Williams: Fine-loamy, mixed, superactive, frigid Typic Argiustolls Bowbells: Fine-loamy, mixed, superactive, frigid Pachic Argiustolls

† Soil Survey Staff, NRCS, USDA. Web Soil Survey. https://websoilsurvey.sc.egov.usda.gov (accessed 6 Mar. 2019).
‡ Soil Survey Staff, NRCS, USDA. Official Soil Series Descriptions. https://soilseries.sc.egov.usda.gov/osdname.aspx (accessed 6 Mar. 2019).

Soybeans were planted into the standing wheat residue from the previous growing season with no-till air seeders, at all three locations. The Minot location was planted with ‘ND17009GT’ soybean seed, while the Underwood and Coleharbor plots were planted with ‘ND Bison’ soybean seed. The physical plots were established in a level location with uniform stand, between V2 and V3 stages of soybean growth. At this time, 1.83 m x 9.14 m plots were laid out in a randomized block design with 12 treatments and four replicates at each location. Foliar treatments were applied with a hand-held CO_2 plot sprayer either at the first sign of flowering (R1) or when pods began developing (R3) at their respective rates and application timing listed in Table 4. Treatments of ethephon (PGR), paclobutrazol (PGR), and Priaxor (pyraclostrobin + fluxapyroxad: PGR) were selected based on results from greenhouse studies on the effectiveness of foliar-applied transpiration reducing chemicals on soybean, conducted in 2014 at North Dakota State University, Fargo, ND (Goos, R. J., unpublished data, 2014). Wilt-Pruf (pinolene: film/barrier AT) and kaolinite (kaolin: reflective AT) were added based on research in other crops (AbdAllah, 2017; Javan et al., 2013; Abou-Khaled et al., 1970).

Table 4. Plant growth regulators (PGR) and antitranspirants (AT) applied as foliar treatments in the 2017 field experiment.

Treatment	Chemical name	Mechanism	Application time‡	Rate
				---kg ha^{-1}---
Control	-	-	-	-
Ethephon	Ethephon	PGR	1st	0.28
Ethephon	Ethephon	PGR	2nd	0.28
Ethephon	Ethephon	PGR	Both	0.28
Paclobutrazol	Paclobutrazol	PGR	1st	0.041
Paclobutrazol	Paclobutrazol	PGR	2nd	0.041
Paclobutrazol	Paclobutrazol	PGR	Both	0.041
				---L ha^{-1}---
Priaxor	Pyraclostrobin	PGR	1st	0.585
Priaxor	Pyraclostrobin	PGR	2nd	0.585
Priaxor	Pyraclostrobin	PGR	Both	0.585
Wilt-Pruf	Pinolene	Film/barrier AT	Both	4.68
Kaolinite	Kaolin	Reflective AT	Both	5% suspension

† Spray volume for all applications was 206 L ha^{-1}
‡ 1st = First signs of R1, 2nd = R3, and Both = Foliar treatment was applied at both 1st and 2nd application timings.

Rainfall and Soil Water

Rainfall was quantified with rain gauges at the Underwood and Coleharbor sites, while the NDAWN weather station at the NCREC provided this information for the Minot location. Volumetric water content was measured with ECH_2O EC-5 (Decagon Devices, Pullman, WA) sensors, permanently installed at 15 and 45 cm below the soil surface in three control plots to estimate general soil water status for two depths (0-30 cm and 30-60 cm).

Relative Leaf Water Content

Relative leaf water content (RWC) is also known as the relative turgidity technique and is used to estimate water deficits in leaves (Barrs and Weatherley, 1962; Arndt et al., 2015). Approximately weekly, six trifoliate leaf samples were collected from randomly selected plants within each plot and removed where the petiole attached to the stem. These samples were enclosed in sealable plastic bags and placed in a chilled cooler to limit water loss from the leaves before the rest of the procedure could be carried out. Upon arrival to a facility with a scale and water for rehydration, the leaf samples were removed from the plastic bag and a 2 mm section was removed from the end of each petiole to remove any air gaps that had developed within the xylem. These six trifoliates were then weighed all together to get a fresh weight (FW). Next, the ends of the petioles were submerged in water to simulate a condition of unrestricted water availability, and after 4 h of rehydration, the leaf surfaces and petioles were dried off with a paper towel and weighed again to get a turgid weight (TW). Finally, the leaf samples were placed in a small paper bag and dried at 70°C for 12 h, in a dehydrator, and weighed again to get a dried weight (DW). Calculation of RWC can then be accomplished using the following:

$$\text{RWC (\%)} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100 \qquad (1)$$

Yield and Biomass Determination

Harvested area for biomass and seed yield determination included the middle-two rows in each plot x 4.57 m long. At Minot, the row spacing was 25.4 cm, while the other two plots had a row spacing of 30.5 cm. Total biomass was collected from this harvest area by hand, with a hedge trimmer at ground level and the plants were dried in large air-forced driers at 90°C for 2 d. Total biomass weights (stems, pods, and seeds) were taken, and the collected samples were fed individually into a plot combine for threshing. Seeds were then collected and weighed. Vegetative biomass was calculated by subtracting seed weight from total biomass. Finally, biomass and seed yields were calculated by their respective row spacing, as well as a seed to vegetative biomass ratio.

Statistical Analysis

Results are reported as the least significant mean of each treatment minus the control value from its respective replication. The actual mean value of each control is presented as well, to give context to the presented differences. The results are separated by location and were split into two sets within each location during analysis. Set one only included the PGR treatments (ethephon, paclobutrazol, and Priaxor) at all three application timings (1^{st}, 2^{nd}, and Both). Set two contains all five products tested (ethephon, paclobutrazol, Priaxor, Wilt-Pruf, and kaolinite), however, this only utilizes the particular treatment when the product was applied at "Both" application timings. Differing sample dates for RWC were analyzed separately, and the end of year measurements of plant biomass and seed yield were also run individually. Significant differences were determined at the 0.05 level with SAS software, Version 9.4, of the SAS System for Windows, using proc GLM, treating product and timing as fixed effects (SAS Institute, 2013).

Experiment 2: Effects of Two Seed-Applied PGRs at Increasing Concentrations on Soybean Water Use and Growth in a Greenhouse Setting

The research objective of this greenhouse experiment was to evaluate the effects of different seed applied PGRs of varying concentration on soybean growth and water use. Furthermore, the results from this experiment would be used to guide treatment selection for the 2018 field study. Uniconazole (Yan et al., 2013; Yan et al., 2010; Jing et al., 2012; Zhang et al., 2007) and paclobutrazol (Jeffers, 1986; Navarro et al., 2007) were identified as possible seed-applied PGRs to reduce water use by impeding overall plant growth and could be used to conserve water use during vegetative stages of soybean growth for reproductive seed-fill stages.

The experiment was a randomized complete block design, with 12 treatments and four replicates. It was conducted at the AES-Research Greenhouse Complex of North Dakota State University, Fargo, ND over the winter of 2017-2018. Uniconazole was diluted in ethanol (as it is water insoluble), while paclobutrazol was diluted in water so that 0.0, 0.1, 0.2, 0.4, 0.8, and 1.6 mg of active ingredient would be present in 1 mL of solution, respectively. Twelve 100 g lots of ‘ND Bison’ soybean seed were weighed into plastic bags and 1 mL of solution was added to its respective bag of seed. The seeds were agitated during the application to ensure total seed coverage. The seeds were allowed to absorb the solution and then laid out quickly air dry. This application would effectively apply 0, 1, 2, 4, 8, and 16 mg of the respective active ingredient per kg of soybean seed, as outlined in Table 5.

Table 5. Treatment list of seed-applied plant growth regulators and concentration for the greenhouse experiment.

Growth regulator	Concentration	Solvent
	mg kg^{-1} seed	
None†	-	-
Uniconazole	1	Ethanol
Uniconazole	2	Ethanol
Uniconazole	4	Ethanol
Uniconazole	8	Ethanol
Uniconazole	16	Ethanol
None‡	-	-
Paclobutrazol	1	Water
Paclobutrazol	2	Water
Paclobutrazol	4	Water
Paclobutrazol	8	Water
Paclobutrazol	16	Water

† Seed treated with ethanol only
‡ Seed treated with water only

Soil Information and Planting

Forty-eight plastic bags were filled with 1 kg of a Renshaw soil (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, frigid Calcic Hapludolls) (Soil Survey Staff, NRCS-USDA, 2019). Another 48 plastic bags were filled with 1 kg of quartz sand and mixed with a soybean rhizobium inoculant suspension (0.1 g pot^{-1}) and nutrient solutions providing: 50 mg N, 50 mg P, 25 mg S, 5 mg Zn, 5 mg Mn, 5 mg Cu, and 1 mg of B per pot. The contents of the 48 bags of soil were dumped into the bags containing the sand and mixed well before placing the bag and its contents into a pot that had been previously assigned a treatment and replicate identity (the bag remained in the pot throughout the remainder of the experiment). Two hundred mL of the soil and sand mixture was removed from each pot and placed into the bag that previously held the soil. One hundred sixty mL of water was added to the soil and sand mixture in the pot and allowed to infiltrate, while 20 mL of water was added to the 200 mL of soil and sand mixture in the bag. Eight 'ND Bison' soybean seeds, treated with their respective PGR and rate, were planted on the leveled soil surface in each pot, and then covered with the moist 200 mL of soil that was previously removed, providing a seeding depth of approximately 3 cm. A filter paper was placed over the soil surface and the entire pot was covered with a plastic bag to slow evaporation and induce germination. These evaporative barriers were removed after two days as the plants began to emerge.

Emergence and Daily Water Use

Daily emergence counts were taken from day 4 through day 12 after planting, and the seedlings were thinned to four plants per pot by cutting the stem of excess plants at the soil surface on day 13. From day 5 through day 37 (termination of the experiment), the weight of each pot was recorded, and water was added until the pot weighed 2280 g daily, until water use per pot approached 100 g per day for some treatments. At this point, weighing and watering was performed twice per day, but values were summed to maintain a daily sample period. Daily water use for each pot was determined by subtracting each original weight prior to watering from 2280 g.

Biomass Production

The experiment was terminated at 37 days after planting. Plants were cut off at the soil surface and internode lengths for each individual plant were measured and averaged for each pot. Total above-ground dry matter was determined for each pot after it was dried at 90°C for 4 d. Soil was removed and washed off of the roots for each pot and after an eight day drying period at 90°C, the dried roots were weighed.

Statistical Analysis

All the data for emergence, daily water use, internode lengths, and plant biomass were presented as mean values of each treatment. However, emergence data and daily water use values were broken down into representative day-range intervals, and each day range was analyzed separately. Data were analyzed with SAS software, Version 9.4, of the SAS System for Windows, using proc GLM, with treatment as a fixed effect at the 0.05 level of significance (SAS Institute, 2013).

Experiment 3: Fall-Seeded Cover Crops and Seed-Applied PGR Effects on Soil Water and Their Subsequent Effects on Soybean Seed Quality and Yield

The research object of this field study was to evaluate the effects of fall-seeded cover crops and seed-applied PGRs on soil water conditions throughout the growing season and their subsequent effects on soybean seed quality and yield. Three sites were laid out in the fall of 2017 for this study: west of Underwood, ND; northwest of Falkirk, ND; and at the NCREC in Minot, ND. The soil type varied between the three sites and is detailed in Table 6. These sites were located in continuous crop production fields that produced wheat in the 2017 growing season and placed within areas of uniform residue amount and distribution.

Table 6. Soil type variations between site location in the 2018 summer field experiment.

Location	Soil Series	Taxonomic Class
Underwood, ND	Wilton-Temvik silt loams	Wilton: Fine-silty, mixed, superactive, frigid Pachic Haplustolls Temvik: Fine-silty, mixed, superactive, frigid Typic Haplustolls
Falkirk, ND	Falkirk loam	Falkirk: Fine-loamy, mixed, superactive, frigid Pachic Haplustolls
Minot, ND	Forman-Aastad loams	Forman: Fine-loamy, mixed, superactive, frigid Calcic Argiudolls Aastad: Fine-loamy, mixed, superactive, frigid Pachic Argiudolls

† Soil Survey Staff, NRCS, USDA. Web Soil Survey. https://websoilsurvey.sc.egov.usda.gov (accessed 6 Mar. 2019).
‡ Soil Survey Staff, NRCS, USDA. Official Soil Series Descriptions. https://soilseries.sc.egov.usda.gov/osdname.aspx (accessed 6 Mar. 2019).

The experiment was established as a randomized complete block design with eight treatments and four replicates and is outlined in Table 7. Individual plots were 5.79 m in length and seeded with a no-till plot drill, which planted six rows at a time and had a row spacing of 19 cm, for a total width of 1.14 m. Two passes of this seeder (1.14-m wide) were intended to create a plot 2.28-m wide. However, an approximate 0.4-m gap was unintentionally placed down the middle of each plot, separating the two passes, which went unnoticed until the plants began emerging. Therefore, the total dimensions of each plot (including the gap) was 5.79 m x 2.68 m, while the harvested area used for calculating yield (not including the gap) was 5.79 m x 2.28 m.

Table 7. Treatment list of fall-seeded cover crops and seed-applied plant growth regulators in the 2018 field experiment.

Treatment	Cultivar	Seeding rate	Termination	Seed-applied PGR rate
		kg ha^{-1}		mg kg^{-1} seed
Control	-	-	-	-
Rye	ND Dylan	28	Early†	-
Rye	ND Dylan	28	Late‡	-
Rye	ND Dylan	56	Early†	-
Rye	ND Dylan	56	Late‡	-
Paclobutrazol	-	-	-	4
Uniconazole	-	-	-	2
Uniconazole	-	-	-	4

† Early means 2 weeks prior to soybean planting
‡ Late means 2 days after soybean planting

Cover Crop Planting and Rye Biomass

'ND Dylan' rye cover crop treatments at two rates, 28 kg ha^{-1} (low) and 56 kg ha^{-1} (high) were planted with the no-till plot drill mentioned above at a depth of 1.9 cm, on 23 September, 2017 at the Minot location and on 29 September, 2017 for the other two locations (Table 7).

Gravimetric soil water content for each site was collected for 0-30 cm and 30-60 cm depths at time of cover crop seeding. The rye treatments went into a dormant state over the winter (vernalization) and continued to grow in the spring of 2018.

Rye was sampled in the early-terminated plots for nutrient analysis as well as above-ground biomass yield before the respective plots were terminated by cutting four, 30.5 cm long strips in representative areas from each plot. These samples were dried for 4 d at 90 °C, and weighed for dry matter biomass, and were then sent to AgVise Laboratories in Northwood, ND for macro and micronutrient analysis (nitrogen, phosphorus, potassium, and sulfur). Glyphosate [*N*-(Phosphonomethyl)glycine] at a rate of 1538 g active ingredient ha^{-1} with ammonium sulfate treated water was applied to the early-terminated rye treatments with a CO_2 backpack sprayer at all sites on 22 May, 2018 (15 days before soybean planting).

Soybean Seeding and Spring Measurements

Based on the results from the greenhouse study performed on the effects of seed-applied PGRs at varying concentrations the previous winter, uniconazole at 2 and 4 mg kg^{-1}, as well as paclobutrazol at 4 mg kg^{-1} were applied to glyphosate tolerant soybean seed by the methods described in the greenhouse experiment. Both PGR-treated and untreated seeds were inoculated with rhizobium bacteria and individually packaged to be planted at a rate of 371,000 viable seeds ha^{-1} with the same no-till plot drill described above. Planting of 'ND17009GT' soybean seed for all treatments and at all sites took place on 6 June, 2018 at a depth of 3.5 cm, and also included additional granular rhizobium inoculum.

To estimate soil water storage differences due to cover crop growth at planting time, soil was sampled at the 0-30 cm and 30-60 cm depths with a 19-mm diameter soil probe, two times in each plot and mixed, for the control and all four rye treatments on 7 June, 2018. Composite soil samples for each location were collected after the growing season at the 0-30 and 30-60 cm depths with a 50.8 mm diameter soil probe to estimate bulk density (gravimetric to volumetric water content conversions), field capacity, and permanent wilting points for each location. Field capacity and permanent wilting points were calculated by placing saturated soil samples in their respective pressure plates for 24 h at -33 kPa (field capacity) and -1,500 kPa. These soils were then moved to metal cans for weighing prior to being heated in an oven at 105°C for 24 h. Values of gravimetric water content for the two parameters were then calculated and converted to volumetric with soil bulk density data.

Early and late-terminated rye treatments were sampled for above-ground biomass and nutrient analysis with identical methods as described above for the early-terminated rye treatments. An application of glyphosate and ammonium sulfate at the same rate noted for the early termination timing was then applied across all plots on 8 May, 2018 to terminate the late rye treatments and to control any early weed pressure in any of the treatments.

Unfortunately, the Minot location was abandoned due to several compounding factors, including herbicide drift from a neighboring experiment, ultimately leading to poor growth of soybean plants in all treatments, however the cover crop and soil water data collected for the Minot location are reliable. Any discussion of methods henceforth was only conducted at the Underwood and Falkirk locations.

Stand Counts

Soybean stand counts were taken 20 d after planting in all plots by counting the number of plants within a 50-cm long section of a row, four times per plot. These four values were then averaged for each plot. Soybean growth stage was monitored throughout the growing season.

Soil Water Status

To monitor the general water status of each site, volumetric soil water content of four plots (two control and two high rate, late-terminated rye) was recorded approximately weekly with ECH_2O EC-5 (Decagon Devices, Pullman, WA) sensors permanently installed at 45 cm below the soil surface to provide a general indication of soil water at that depth. Volumetric soil water content from the soil surface, to a depth of 20 cm was measured in each plot approximately every seven days (weather permitting), from 28 June, 2018 through 9 September, 2018 with a FieldScout TDR 350 Soil Moisture Meter (Spectrum Technologies, Inc., Aurora, IL).

Plant Sampling and Relative Leaf Water Content

Plant samples were collected approximately weekly to evaluate plant water status. Four soybean plants were randomly selected in each plot, and the top 10-cm of each plant was cut off and sealed in a plastic bag, which was placed in a chilled cooler until returning to a location with the equipment required to complete the RWC process. Upon arrival, the most mature trifoliate from each 10-cm of stem was cut off at the petiole, just above the node. These four trifoliates were then taken through the RWC procedure previously detailed in the 2017 field experiment.

Extraction for Proline and Ureide Analyses

Any additional foliage (not including the petioles), flowers, or pods were then removed from the rest of the stems and dried at 70°C for 4 h. These stems and petioles were ground up to pass through a 0.850 mm screen, and 0.200 g of ground plant sample was added to a sealable test tube. Twenty mL of water was added to the test tube and the contents was shaken. The test tubes were heated in a water bath for 30 min at 90°C, agitated again, and then heated for another 30 min. After heating, the test tubes were cooled in a water bath and their contents were poured into funnels lined with filter paper capturing particles greater than eight micrometers in size. The filtered liquid was captured in 20 mL scintillation vials and frozen to reduce microbial degradation until aliquots of this sample could be used in proline and ureide analyses.

Proline Analysis

Standards of 0, 2, 5, and 10 mg L^{-1} of proline were prepared by dissolving 1 g of dry proline in 1 L of water, producing 1000 mg L^{-1} and diluting accordingly. A color developing reagent (acid-ninhydrin) consisting of 2.50 g of ninhydrin, 60 mL of glacial acetic acid, and 40 mL of 6 *M* phosphoric acid was produced daily.

The frozen extracts described above were thawed and 2 mL of the extract was pipetted into a sealable test tube, along with 2 mL of the acid-ninhydrin coloring reagent and an additional 2 mL of glacial acetic acid. In every batch of extracts processed, two sets of (0, 2, 5, and 10 mg L^{-1}) proline standards were also prepared in the same manner, by pipetting 2 mL of each standard instead of the plant extract. The test tubes were sealed and placed in a water bath at 90°C for 1 h under an opaque cover to exclude light, and then cooled quickly in a water bath. Percent transmittance (%T) of the extract or standard solutions was determined spectrophotometrically at 520 nm with a Spectronic 20 (Bausch and Lomb, Bridgewater, NJ). The %T value was converted to Absorbance (A) with this formula:

$$\text{Absorbance (A)} = -1 \times \log(\%T/100) \qquad (2)$$

The standards in mg L^{-1} and their respective A values were used to create a polynomial regression equation using an online equation generator (http://www.xuru.org/rt/PR.asp), to which the A from the extracts were inputted individually to calculate the mg L^{-1} of proline in the extract solution. This value was then multiplied by the 20 mL of water and divided by the recorded amount of ground plant sample (0.200 g) from the original extraction process. The result of this equation is the µg proline g^{-1} dried plant tissues (DW) at the time of sampling.

Ureide Analysis

The procedure used for determining the ureide content of soybean tissues is described by the proposed method in Goos et al. (2015), but a general outline will be provided here. Frozen plant extracts were thawed, and a 0.3 mL aliquot of plant extract or standard was pipetted into a sealable test tube, along with 0.3 mL of water, and 0.3 mL of 5 *M* NaOH. The sealed test tubes were placed in a 90°C water bath for 30 min. After quickly cooling in a water bath, 7.0 mL of a color developing reagent was added to each test tube, mixed, resealed, and again placed in the 90°C water bath for 1 h under an opaque cover. At this time, %T was recorded at 525 nm with a Spectronic 20 (Bausch and Lomb, Bridgewater, NJ) and later converted to A using EQ 2.

Similar to the proline analysis, the standards in mg L^{-1} and their respective A values were used to create a polynomial regression equation using an online equation generator (http://www.xuru.org/rt/PR.asp), to which the A from the extracts were inputted individually to calculate the mg L^{-1} of ureides in the extract solution. This value was then multiplied by the 20 mL of water and divided by the recorded amount of ground plant sample (0.200 g) from the original extraction process. The result of this equation is the µg ureides g^{-1} dried plant tissues (DW) at the time of sampling.

Percent Leaf Drop

Percent leaf drop was determined by visually estimating the proportion of leaves that had fallen to the ground versus the amount still attached to the plants for each plot on 13 September and 22 September, 2018 at both locations.

Seed Yield and Quality

Soybean plants were harvested with a plot combine, and the seed from each plot was collected and weighed individually. As mentioned previously, the gap down the middle of each plot was not included in the harvest area, so the actual harvest area used for yield calculation was 5.79 m x 2.28 m. Test weight was determined by pouring the soybean seed into a closed funnel, and opening the bottom of the funnel to empty the seed into an open-top cylindrical container of known volume. The soybean seed was leveled with a stick and the contents was weighed to calculate test weight. Soybean seed samples were analyzed for protein and oil content using near infrared reflectance spectroscopy (NIRS) at 400-2500 nm (XDS Rapid Content Analyzer, Foss, Demark).

Statistical Analysis

Results for spring soil water measurements and rye biomass are split by location due to the spatial variability between each of the three locations. This data includes the Minot location, as this data is dependable and unaffected by the issues that caused this site to be abandoned later in the experiment. The two soil depths (0-30 and 30-60 cm) were analyzed separately regarding soil water measurements. These data were analyzed with SAS software, Version 9.4, of the SAS System for Windows, using proc GLM, with treatment as the fixed effect (SAS Institute, 2013).

Results for the rest of the measurements are presented as the mean of each treatment across the remaining two locations, Underwood and Falkirk. Differing sample dates within a particular measurement were analyzed separately. All of these data were analyzed with SAS software, Version 9.4, of the SAS System for Windows, using proc GLIMMIX, with location as a random effect and treatment was the fixed effect at the 0.05 level of significance (SAS Institute, 2013).

RESULTS AND DISCUSSION

Experiment 1: Foliar-Applied Water Use Modulators and Their Effects on Leaf-Water Status, Biomass Production, and Seed Yield in Soybean

The objective of the 2017 growing season field study was to evaluate the effects of foliar-applied water use modulators on leaf water status, biomass production, and seed yield of soybean crops at three locations in west-central North Dakota. Overall, growing season precipitation was low for this area in 2017, which proved to be an excellent example of the potential water deficits in crop production. Spring soil water was favorable due to significant snow accumulations throughout the previous winter, however, rainfall events between snow melt all the way through the end of June were scattered spatially and limited in quantity. This spatial variation during the early growing season will be exhibited in the difference in yields achieved between the Underwood and Coleharbor sites, which were located 8 km apart from each other. These spatial and temporal differences in received precipitation are shown in Table 8. Cumulative rainfall throughout this period was between 100 and 120 mm for all three locations, however, the most notable difference between locations throughout this time period was the from 15 July through 31 July, where the Underwood location received 28 mm of rainfall, while the Minot and Coleharbor locations only received 8 and 13 mm respectively.

Table 8. Rainfall for the 2017 Minot, Underwood, and Coleharbor locations.

Date range	Minot	Underwood	Coleharbor
	------------------------------	mm	------------------------------
15-31 July	8	28	13
1-15 Aug.	61	66	64
16-31 Aug.	8	0	4
1-15 Sept.	30	24	22
Total	107	118	102

† Rainfall data was only collected from 15 July through 15 September at all three locations because the experiments were placed into previously established soybean production fields.

This additional in rainfall at the Underwood site led to improved growing conditions throughout the reproductive stages of growth compared to the other sites, which is displayed in the actual control mean values for the following measurements of relative leaf water content, biomass produced, and seed yield for each location. Overall, the precipitation dynamics described above provided ideal conditions to evaluate these foliar-applied chemicals in a year of overall below average precipitation, as well as at three drought severity levels. As previously mentioned, the Underwood location was the least water stressed, while the Minot location came in second, followed by the Coleharbor location with the highest severity of drought.

Ranges in relative leaf water contents for eight soybean cultivars were studied by Hossain et al. (2014). When water was not limiting, all cultivars reported values between 85-95%. However, under water stress (irrigation withheld for four weeks) values ranged from 40-70%, dependent upon cultivar was measured, with values commonly around 60-70% (Hossain et al., 2014). These values give context to the actual control means for each location throughout the experiment, as well as to the difference in values reported in the following relative water content tables.

Effects of Water Use Modulators When Applied at Both Application Timings

The following analysis and interpretation of relative leaf water content, seed yield, and biomass production includes only the experimental treatments that were applied at both application timings and contain the following water use modulators: ethephon (PGR), paclobutrazol (PGR), Priaxor (pyraclostrobin + fluxapyroxad: PGR), Wilt-Pruf (pinolene: Film/Barrier AT), and kaolinite (kaolin: Reflective AT). Results for all of these measurements are reported as the experimental treatment value minus the value of its respective control treatment. Statistical differences are presented between treatments and also against “zero” or what would be the value of the control plot for each particular measurement and sample time, as indicated in the notes for each table. The actual control mean value is also presented in the table to give context to the difference from control values reported.

Relative Leaf Water Content

Minot Location

Differences between the analyzed treatments that were applied at both application times were found to be insignificant at all sampling dates for the Minot location. However, throughout the sampling dates, particular treatments were significantly different from the control and are displayed in Table 9. The most notable differences between the treatments and the control are found in the August 10 sample date, when the soybeans were in the R4 growth stage, which was six days after the second application of each chemical was applied. All treatments reported higher RWC values than the control for this sampling date, and ethephon, paclobutrazol, and Priaxor values were significantly higher than the control value. A 29 mm rainfall event happened on 12 August and these significant differences almost disappear in the subsequent sampling date on 17 August, except for ethephon. Throughout the first three sampling dates, ethephon and Priaxor consistently reported higher leaf water content values than the control plot, indicating that these chemicals improved the water status of soybean leaves on a relative mass basis compared to the control. However, caution should be taken when interpreting a two or three percent difference to be noteworthy in context of the actual control mean value, which ranged from 80-90% throughout the sampling dates for this location.

Table 9. Effect of foliar-applied water use modulators on percent relative leaf water content when applied to soybean leaves at R1 and R3 growth stages, reported as treatment minus control for the 2017 Minot location.

			Soybean growth stage and sampling date				
			R2	R3	R4	R5	R6
Treatment	Mechanism	Timing†	27 July	1 Aug.	10 Aug.	17 Aug.	27 Aug.
			---------------------------------%----------------------------------				
Ethephon	PGR	Both	1.77	2.53	2.33Ψ	1.14Ψ	-0.31
Paclobutrazol	PGR	Both	0.93	-0.34	1.51Ψ	0.06	0.85
Priaxor	PGR	Both	2.15	2.77Ψ	1.98Ψ	-0.16	-0.09
Wilt-Pruf	Film/Barrier AT	Both	0.62	0.26	1.34	0.09	-2.74Ψ
Kaolinite	Reflective AT	Both	-0.70	1.53	0.54	0.99	-0.19
	Actual Control Mean		83.25	81.34	84.99	87.14	86.69
		Sig. of F	ns	ns	ns	ns	ns
		C.V., %	309	186	82	223	366

† Both = Treatment was applied at 13 July and 4 August.
‡ Greek letter "Ψ" indicates the experimental treatment mean was significantly different from control at the 0.05 level, using Tukey's, within each sampling date.

Underwood Location

Significant differences were not found between any treatments on any of the sampling dates at the Underwood location. Similarly to the Minot location though, ethephon consistently improved relative leaf water contents and exhibited a significant increase when compared to the control on August 31, as shown in Table 10. This sampling was performed six days after the second application of each chemical was applied. Overall, this site shows less variation than the Minot location, possibly indicating that as water availability improves differences in leaf water contents become harder to detect. Again, a difference from control with a magnitude around one or two percent is likely negligible in context with the actual control mean value around 87 percent.

Table 10. Effect of foliar-applied water use modulators on percent relative leaf water content when applied to soybean leaves at R1 and R3 growth stages, reported as treatment minus control for the 2017 Underwood location.

			Soybean growth stage and sampling date			
			R2	R2	R4	R6
Treatment	Mechanism	Timing†	8 Aug.	17 Aug.	31 Aug.	7 Sept.
			------------------------%-------------------------			
Ethephon	PGR	Both	1.79	2.45	1.31Ψ	3.22
Paclobutrazol	PGR	Both	0.83	-1.13	-0.34	-0.46
Priaxor	PGR	Both	1.70	0.65	0.03	1.16
Wilt-Pruf	Film/Barrier AT	Both	0.32	-0.39	0.65	0.33
Kaolinite	Reflective AT	Both	-0.37	-0.02	0.98	1.41
		Actual Control Mean	95.06	92.62	87.46	83.80
		Sig. of F	ns	ns	ns	ns
		C.V., %	212	723	207	276

† Both = Treatment was applied at 21 July and 24 August.
‡ Greek letter Ψ" indicates the experimental treatment mean was significantly different from control at the 0.05 level, using Tukey's, within each sampling date.

Coleharbor Location

The Coleharbor location experienced the greatest drought severity and therefore expressed larger values of variation between treatments. Especially after the second treatment application, and as water became more limited over time, which is shown by the continuous decline in the actual control mean values. This trend and the values for each treatment and sample date are displayed in Table 11. The effect ethephon has on relative leaf water content is dramatic when water is limiting, which is exhibited in the final sample date on 9 September, where ethephon was significantly greater than any other treatment as well as significantly greater than the control with a value of 9.39%. This is a substantial increase in water content, especially considering the fact that plants were entering later reproductive stages when the greatest differences were expressed. This shows that the application of ethephon was allowing the plant to maintain improved leaf water status versus the other treatments, including the control. For the first time, Wilt-Pruf expressed a discernible trend at this location and improved leaf water content values for the last three sampling dates.

Table 11. Effect of foliar-applied water use modulators on percent relative leaf water content when applied to soybean leaves at R1 and R3 growth stages, reported as treatment minus control for the 2017 Coleharbor location.

			Soybean growth stage and sampling date			
			R2	R3	R5	R6
Treatment	Mechanism	Timing†	14 Aug.	27 Aug.	3 Sept.	9 Sept.
			---------------------------%---------------------------			
Ethephon	PGR	Both	0.11	1.16	5.44Ψ	9.39aΨ
Paclobutrazol	PGR	Both	2.21	0.74	-0.05	-1.26b
Priaxor	PGR	Both	-2.08	0.88	2.12	-1.18b
Wilt-Pruf	Film/Barrier AT	Both	-0.83	1.68	2.49	1.73ab
Kaolinite	Reflective AT	Both	-1.98	0.56	1.93	-1.05b
	Actual Control Mean		91.60	85.19	74.75	70.28
		Sig. of F	ns	ns	ns	0.0061
		C.V., %	269	166	144	287

† Both = Treatment was applied at 8 August and 28 August.
‡ Greek letter "Ψ" indicates the experimental treatment mean was significantly different from control at the 0.05 level, using Tukey's, within each sampling date.
§ Different letters indicate significant differences among experimental treatments at the 0.05 level, using Tukey's, within each sampling date.

Seed Yield and Biomass Production

Minot Location

At this location, all treatments except kaolinite reduced seed yields compared to the control treatment; however, none of these changes were significantly different from each other or from the control. Biomass production results were not as definitive and again showed no significant differences, as shown in Table 12. Ethephon and kaolinite increased vegetative biomass yield over the control, while the other three treatments decreased biomass production. With an actual control seed yield of 1212 kg ha^{-1} and a control biomass yield of 1576 kg ha^{-1} the reported difference magnitudes, although insignificant, represent substantial yield differences. Within this context, a difference of 200 kg ha^{-1} represents a 17% change in seed yield, and a 13% change in biomass yield.

Table 12. Effect of foliar-applied water use modulators on seed and plant biomass production when applied to leaves at R1 and R3 growth stages, reported as treatment minus control for the 2017 Minot location.

Treatment	Mechanism	Timing†	Seed yield	Biomass yield‡
			----------------kg ha^{-1}	------------------
Ethephon	PGR	Both	-208	332
Paclobutrazol	PGR	Both	-139	-92
Priaxor	PGR	Both	-189	-270
Wilt-Pruf	Film/Barrier AT	Both	-165	-118
Kaolinite	Reflective AT	Both	217	225
	Actual Control Mean		1212	1576
		Sig. of F	ns	ns
		C.V., %	402	5179

† Both = Treatment was applied at 13 July and 4 August.
‡ Biomass yield does not include seed yield.

Underwood Location

Seed yield at the Underwood location was reduced by every treatment compared to the control, and no significant differences were seen between treatments or when compared to the control. Biomass production was also decreased by all treatments compared to the control, except for Priaxor, which actually increased above-ground biomass by a magnitude similar to the reductions expressed by the other treatments. However, none of these differences were statistically significant between each other or against the control as shown in Table 13. Overall yields were greatly improved at this location compared to the others, likely due to higher amount of precipitation earlier in the growing season. Difference from control magnitudes reported for this location are similar to the Minot location, however due to the higher overall production, these differences are less important. A change in yield of 200 kg ha^{-1}, represents a 9% change in seed yield and a 7% change in biomass production.

Table 13. Effect of foliar-applied water use modulators on seed and plant biomass production when applied to leaves at R1 and R3 growth stages, reported as treatment minus control for the 2017 Underwood location.

Treatment	Mechanism	Timing†	Seed yield	Biomass yield‡
			---------------kg ha^{-1}	----------------
Ethephon	PGR	Both	-177	-242
Paclobutrazol	PGR	Both	-273	-136
Priaxor	PGR	Both	-143	119
Wilt-Pruf	Film/Barrier AT	Both	-191	-98
Kaolinite	Reflective AT	Both	-239	-230
	Actual Control Mean		2122	2747
		Sig. of F	ns	ns
		C.V., %	167	286

† Both = Treatment was applied at 21 July and 24 August.
‡ Biomass yield does not include seed yield.

Coleharbor Location

Results at the Coleharbor location do not resemble the results at the other locations, which is likely due to the higher water stress severity. Wilt-Pruf significantly increased seed yield when compared to the control, however, no significant differences were seen between experimental treatments. Biomass yield resulted in significant differences between treatments with the greatest increase over the control shown by Wilt-Pruf, which was also significantly different from the control. These results are shown in Table 14. This 125 kg ha^{-1} increase in seed yield represents a 17% improvement, while the 193 kg ha^{-1} increase in biomass production for Wilt-Pruf is a 16% increase when compared to their control values.

Table 14. Effect of foliar-applied water use modulators on seed and plant biomass production when applied to leaves at R1 and R3 growth stages, reported as treatment minus control for the 2017 Coleharbor location.

Treatment	Mechanism	Timing†	Seed yield	Biomass yield‡
			----------------kg ha^{-1}-------------------	
Ethephon	PGR	Both	-5	-108ab
Paclobutrazol	PGR	Both	-6	71ab
Priaxor	PGR	Both	9	-118b
Wilt-Pruf	Film/Barrier AT	Both	125Ψ	193aΨ
Kaolinite	Reflective AT	Both	63	82ab
	Actual Control Mean		739	1198
		Sig. of F	ns	0.0325
		C.V., %	227	620

† Both = Treatment was applied at 8 August and 28 August.
‡ Biomass yield does not include seed yield.
§ Greek letter "Ψ" indicates the experimental treatment mean was significantly different from control at the 0.05 level, using Tukey's, within each sampling date.
¶ Different letters indicate significant differences among experimental treatments at the 0.05 level, using Tukey's, within each sampling date.

RWC and Seed Yield Relationship

Minor alterations in relative leaf water content values were exhibited by the application of these plant water use modulators to soybean leaves at R1 and R3 growth stages for all three locations of varying water stress. The only exception was ethephon, which consistently improved relative leaf water status over the control as well as the other treatments, especially at the Coleharbor location which endured the highest water stress severity. However, it doesn't appear that any relation exists between improved leaf water status and higher seed yield, which was the overarching goal of this study. The only instance where improved water status throughout several RWC sampling dates ended up improving seed and biomass yields was for Wilt-Pruf at the Coleharbor location. This treatment improved leaf water status versus the control from R3 to

R6 soybean growth stages at this location and represented significant improvements in seed and biomass yield compared to the control.

Effects of Application Timing for Foliar-Applied PGRs

The following analysis and interpretation of relative leaf water content, seed yield, and biomass production will include only the chemicals that were applied at all three application timing protocols (1^{st}, 2^{nd}, and both) and contain the following PGRs: ethephon, paclobutrazol, and Priaxor (pyraclostrobin + fluxapyroxad). Similarly to the results above for the both application timing treatments only, results will be expressed as difference from their respective control, with analysis performed on those differences between individual treatments, as well as a comparison versus the control value or "zero." The actual control mean value will also be presented to give context to each reported differences at each location.

Relative Leaf Water Content

Minot Location

The interaction between the three PGR chemicals applied at three timing variations was insignificant at all sampling timings except the final sample date of 27 August, however significant differences from control were most noted at the 10 August sample date, as shown in Table 15. The only noticeable trend regarding timing of application within each chemical throughout the range of sample dates is exhibited by Priaxor when applied only at the first timing. This treatment, although never significantly different, was consistently higher than the control value. Commonly, ethephon, when applied at any timing improved leaf water status versus the other treatments as well as the control. On the 10 August sample date, which was six days after the second application date, all of the treatments reported leaf water contents higher than the control value, with ethephon at any timing and paclobutrazol applied at both application timings being significantly different than the control. However, these differences are lost due to a rainfall event on 12 August of 29 mm.

Table 15. Effects of treatment timing of foliar-applied plant growth regulators (PGR) on percent relative water content of soybean leaves, reported as treatment minus control for the 2017 Minot location.

			Soybean growth stage and sampling date				
			R2	R3	R4	R5	R6
Treatment	Mechanism	Timing†	27 July	1 Aug.	10 Aug.	17 Aug.	27 Aug.
			--------------------------------		%		---------------------------------
Ethephon	PGR	1st	0.86	1.07	2.07Ψ	1.20	-0.23ab
		2nd	-0.68	1.42	2.71Ψ	1.91Ψ	3.39aΨ
		Both	1.77	2.53Ψ	2.33Ψ	1.14	-0.31ab
Paclobutrazol	PGR	1st	-0.15	-1.03	1.22	0.76	0.81ab
		2nd	-2.12	0.53	0.99	1.53	1.15ab
		Both	2.15	2.77Ψ	1.98Ψ	-0.16	-0.09ab
Priaxor	PGR	1st	2.02	1.21	1.6	1.47	0.73ab
		2nd	-0.82	0.5	1.23	0.44	-2.36bΨ
		Both	0.62	0.26	1.35	0.09	-2.74bΨ
	Actual Control Mean		83.25	81.34	84.99	87.14	86.69
		Sig. of F	ns	ns	ns	ns	0.0463
		C.V., %	970	234	104	163	5267

† 1st = 13 July, 2nd = 4 August, and Both = Foliar treatment was applied at both 1st and 2nd application timings.
‡ Greek letter "Ψ" indicates the experimental treatment mean was significantly different from the control at the 0.05 level, using Tukey's, for each sampling date.
§ Different letters indicate significant differences among experimental treatments at the 0.05 level, using Tukey's, within each sampling date.

Underwood Location

The chemical and timing interaction was found to be insignificant for all sampling dates. Generally, values are significantly different than the control were either found in ethephon or Priaxor for the entire range of sampling dates, which are shown in Table 16. These significant differences from the control were exhibited as time progressed, and were the greatest at the final sampling date (9 September), where all treatments produced improved leaf water status compared to the control. Ethephon, regardless of application timing protocol, significantly improved leaf water contents over the control at this date, while Priaxor expressed its greatest effect in the 31 August sampling date, where the 1st and 2nd only application timings significantly improved leaf water content over the control. This magnitude however, was not seen in the "both" application treatment of Priaxor for this sampling date.

Table 16. Effect of treatment timing of foliar-applied plant growth regulators (PGR) on percent relative water content of soybean leaves, reported as treatment minus control for the 2017 Underwood location.

			Soybean growth stage and sampling date			
			R2	R2	R4	R6
Treatment	Mechanism	Timing†	8 Aug.	17 Aug.	31 Aug.	9 Sept.
			---------------------------%---------------------------			
Ethephon	PGR	1st	0.16	1.07	0.56	2.87Ψ
		2nd	-0.17	-0.32	0.42	3.18Ψ
		Both	1.79	2.45Ψ	1.31Ψ	3.22Ψ
Paclobutrazol	PGR	1st	-0.18	-0.68	-0.50	1.45
		2nd	1.17	-1.48	0.11	1.96
		Both	1.70	0.65	0.03	1.16
Priaxor	PGR	1st	-0.34	-0.86	1.18Ψ	4.22Ψ
		2nd	0.35	-0.38	1.19Ψ	1.55
		Both	0.32	-0.39	0.73	0.45
	Actual Control Mean		95.06	92.62	87.46	83.8
		Sig. of F	ns	ns	ns	ns
		C.V., %	326	26286	165	90

† 1st = 21 July, 2nd = 24 August, and Both = Foliar treatment was applied at both 1st and 2nd application timings.
‡ Greek letter "Ψ" indicates the experimental treatment mean was significantly different from the control at the 0.05 level, using Tukey's, within each sampling date.

Coleharbor Location

The chemical and timing interaction was found to be insignificant for all sampling dates, however values significantly different from the control were found throughout the experiment for various treatments at this location, and are shown in Table 17. Interestingly, the value of paclobutrazol 2nd timing on sampling date 14 August even reported a significantly lower value than the control when no application of chemical had yet been applied to those particular plots. The second application timing of ethephon appears to have a larger effect on leaf water contents than the first application for this location. This effect is validated and further extended by the values reported for the both application timing. The 2nd application and "both" applications of ethephon significantly improved leaf water contents versus the control. The most notable reduction in leaf water contents is exhibited by Priaxor applied at the second timing at sampling date 9 September, which reports a value significantly lower than the control value. The last two sampling dates at this location exhibit difference magnitudes from the control exceeding three or four percent for ethephon and Priaxor, which are substantial differences when considered in context of the actual control values, which declined over time.

Table 17. Effect of treatment timing of foliar-applied plant growth regulators (PGR) on percent relative water content of soybean leaves, reported as treatment minus control for the 2017 Coleharbor location.

			Soybean growth stage and sampling date			
			R2	R3	R5	R6
Treatment	Mechanism	Timing†	14 Aug.	27 Aug.	3 Sept.	9 Sept.
			----------------------------%----------------------------			
Ethephon	PGR	1st	-1.83Ψ	0.99	1.25	0.74
		2nd	0.1	0.46	4.68Ψ	4.38Ψ
		Both	0.11	1.16	5.44Ψ	8.77Ψ
Paclobutrazol	PGR	1st	-1.4	0.98	0.29	-0.53
		2nd	-1.75Ψ	-0.8	-1.32	-1.93
		Both	-2.08Ψ	0.88	2.12	-1.18
Priaxor	PGR	1st	0.88	0.96	3.12	-0.51
		2nd	0.26	-0.048	-3.17	-4.02Ψ
		Both	-0.83	1.68Ψ	2.49	1.73
	Actual Control Mean		91.60	85.19	74.75	70.28
		Sig. of F	ns	ns	ns	ns
		C.V., %	226	182	192	648

† 1st = 8 August, 2nd = 28 August, and Both = Foliar treatment was applied at both 1st and 2nd application timings.
‡ Greek letter "Ψ" indicates the experimental treatment mean was significantly different from control at the 0.05 level, using Tukey's, within each sampling date.

Seed Yield and Biomass Production

Minot Location

Statistically significant differences were not found for the chemical and timing interaction for both seed yields and biomass yields. As shown in Table 18, no significant differences were found when compared to the control either. However, every experimental treatment combination reported reductions in seed yield compared to the control, which ranged from -17 to -352 kg ha^{-1}. No discernable trend is present for biomass yield measurements. Astonishingly, the application of ethephon, a plant growth inhibitor, at both application timings increased biomass production compared to the control, while most other treatments either decreased overall above-ground biomass yield or reported values similar to the control.

Table 18. Effect of treatment timing of foliar-applied plant growth regulators (PGR) on seed and plant biomass production, reported as treatment minus control for the 2017 Minot location.

Treatment	Mechanism	Timing†	Seed yield	Biomass yield‡
			--------------------kg ha^{-1}----------------------	
Ethephon	PGR	1st	-126	41
		2nd	-352	-250
		Both	-171	352
Paclobutrazol	PGR	1st	-77	92
		2nd	-263	-274
		Both	-162	-91
Priaxor	PGR	1st	-135	-138
		2nd	-17	-51
		Both	-189	-270
	Actual Control Mean		1212	1576
		Sig. of F	ns	ns
		C.V., %	239	588

† 1st = 13 July, 2nd = 4 August, and Both = Foliar treatment was applied at both 1st and 2nd application timings.
‡ Biomass yield does not include seed yield.

Underwood Location

The interaction of chemical and timing for both seed yield and biomass yield was found to be insignificant for the Underwood location. Details of these findings are presented in Table 19. All treatment combinations expressed decreased seed yields compared to the control, with ethephon applied at only the 2nd application timing and Priaxor applied only at the 1st application timing significantly reducing seed yield compared to the control. No trends are apparent in the biomass yield, however most treatment combinations reported values similar or less than the control. In context, the reductions in seed yield reported for ethephon applied only at the 2nd

timing and Priaxor applied at only the 1st timing represent a 17 and 20% from the actual control mean, respectively.

Table 19. Effect of treatment timing of foliar-applied plant growth regulators (PGR) on seed and plant biomass production, reported as treatment minus control for the 2017 Underwood location.

Treatment	Mechanism	Timing†	Seed yield	Biomass yield‡
			------------------kg ha^{-1}	----------------------
Ethephon	PGR	1st	-195	-45
		2nd	-365Ψ	-251
		Both	-177	-243
Paclobutrazol	PGR	1st	-180	-130
		2nd	-285	25
		Both	-173	-136
Priaxor	PGR	1st	-415Ψ	-267
		2nd	-172	-8
		Both	-196	93
	Actual Control Mean		2122	2747
		Sig. of F	ns	ns
		C.V., %	112	210

† 1st = 21 July, 2nd = 24 August, and Both = Foliar treatment was applied at both 1st and 2nd application timings.
‡ Biomass yield does not include seed yield.
§ Greek letter "Ψ" indicates the experimental treatment mean was significantly different from control at the 0.05 level, using Tukey's, within each measurement.

Coleharbor Location

Similar to the results for the "both applications" experiment, the Coleharbor location responded differently to the application of foliar water use modulators than the other two locations. No significant differences were found between the interaction of chemical and application timing for seed yield or biomass yield, as shown in Table 20. Ethephon applied only at the 1st timing however, reported a significant increase of 144 kg ha^{-1} over the control, which represents a 19% increase over the actual control mean. Priaxor applied at only the 2nd timing significantly increased biomass yield by 189 kg ha^{-1} over the control, which is a 16% increase over the actual control mean. Even though no significant differences were found, regardless of treatment, the both application timing induced greater reductions in seed yield than either of the other two application timing protocols within their respective chemical.

Table 20. Effect of treatment timing of foliar-applied plant growth regulators (PGR) on seed and plant biomass production, reported as treatment minus control for the 2017 Coleharbor location.

Treatment	Mechanism	Timing†	Seed yield	Biomass yield‡
			---------------------kg ha^{-1}	--------------------------
Ethephon	PGR	1st	144Ψ	72
		2nd	26	-38
		Both	-5	-108
Paclobutrazol	PGR	1st	20	5
		2nd	-9	-13
		Both	-6	71
Priaxor	PGR	1st	77	52
		2nd	66	189Ψ
		Both	9	-118
	Actual Control Mean		739	1198
		Sig. of F	ns	ns
		C.V., %	275	1409

† 1st = 8 August, 2nd = 28 August, and Both = Foliar treatment was applied at both 1st and 2nd application timings.
‡ Biomass yield does not include seed yield.
§ Greek letter "Ψ" indicates the experimental treatment mean was significantly different from control at the 0.05 level, using Tukey's, within each measurement.

RWC and Seed Yield Relationship

Relative leaf water content value changes induced by varying application timing dates for the three PGRs: ethephon, paclobutrazol, and Priaxor (pyraclostrobin + fluxapyroxad) were exhibited at all three locations of varying water stress. The differences from the control were less than five percent at all sample dates for each location, with the exception of the Coleharbor location with the highest water stress severity. The greatest differences at this location were seen in the later sampling dates, but were still within 10 percent of the control, and only expressed by ethephon applied at either the 2nd application timing or at both application timings. Interestingly, these improvements in relative leaf water content appear to be negatively correlated with the seed yield for the Coleharbor location. Furthermore, this same relation is seen with the two significant decreases in seed yield versus the control at the Underwood location. The later sampling dates for ethephon applied only at the 2nd timing and Priaxor applied only at the 1st timing reported significantly higher relative leaf water contents versus the control, but these treatments exhibited the greatest reductions in seed yield over the entire experiment.

Conclusion

In conclusion, the foliar application of water use modulators in soybean during reproductive growth stages does not improve leaf water status or seed yield, based on the inconsistent performance of these foliar-applied chemicals under varying levels of drought stress. In fact, when water stress was mild, most applications of these water use modulators actually reduced seed yields compared to the control treatment, with percent reductions ranging from 9 to 17% of the actual control value. The only treatments that substantially improved seed yields were kaolinite (Reflective AT) at the Minot location, Wilt-Pruf (Film/Barrier AT) at the Coleharbor location, and the application of ethephon (PGR) when applied at the 1st application timing only at the Coleharbor location, which increased seed yields by 18, 9, and 20% over the control at their respective locations. However, as mentioned before, these positive results were inconsistent, as exhibited by substantial reductions (around 17%) in seed yield at the Underwood location for most treatments. Furthermore, the variation in both seed and biomass yields within each treatment is also noted and adds to the unreliability of these water use modulating chemicals in soybean production in the semi-arid region of west-central North Dakota.

Experiment 2: Effects of Two Seed-Applied PGRs at Increasing Concentrations on Soybean Water Use and Growth in a Greenhouse Setting

The objective of this greenhouse experiment was to evaluate the effects of varying concentrations of seed-applied uniconazole and paclobutrazol on soybean emergence, growth, and water use, and to utilize this information to guide the selection of seed-applied PGR treatments for the 2018 field study. As hypothesized, increases in concentration of both uniconazole and paclobutrazol caused significant reductions in overall plant growth and effectively reduced daily water use, and consequently, cumulative water use.

Emergence

Daily emergence counts were taken from 4 to 13 days after planting (dap). Results are reported in plants emerged per pot, averaged for each treatment, and then again averaged over three respective day ranges: 4-6, 7-9, and 10-13 dap. The treatment effect was highly significant ($p < 0.0001$) in day ranges 4-6 and 7-9; however, no significant differences were seen between the treatments in day range 10-13, which is shown in Table 21. The control treatments for both uniconazole and paclobutrazol had 6.4 plants emerged in the 4-6 day range and by the 7-9 day range exhibited emergence levels similar to the 10-13 day range.

As hypothesized, increasing concentrations of both uniconazole and paclobutrazol delayed emergence. However, the effects of these two plant growth inhibitors differed over time. As concentration increased, uniconazole maintained a negative trend in plants emerged for day ranges 4-6 and 7-9. Whereas, paclobutrazol exhibited similar emergence counts within each day range, regardless of concentration. These trends display differences in how these two PGRs inhibit soybean seedling growth rates when applied at similar concentrations. Soybean seedlings appear to be more sensitive to paclobutrazol at the concentrations studied, while uniconazole requires a concentration of at least 4 mg kg^{-1} of seed to delay growth rates and emergence to levels similar of paclobutrazol at any concentration. Despite these delays in emergence, effects between any treatments are negligible in the 10-13 day range, and each pot produced around seven plants per pot before the plants were thinned down to four to normalize the results for the remainder of the experiment. Thus, indicating that neither chemical at any concentration studied reduced overall soybean stand or seed viability.

Table 21. Effects of seed-applied plant growth regulators at varying concentration on soybean emergence.

		Day range (days after planting)		
Growth regulator	Concentration	4-6	7-9	10-13
	mg kg^{-1} seed	------------------------plants pot^{-1}--------------------------		
None†	-	6.4a	7.3ab	7.3
Uniconazole	1	3.2b	7.4a	8.0
	2	1.0c	6.8abc	7.4
	4	0.4cd	5.6abcd	7.5
	8	0.3cd	3.9d	6.8
	16	0.0d	3.8d	6.9
None‡	-	6.4a	7.3ab	7.6
Paclobutrazol	1	0.2cd	5.8abcd	7.4
	2	0.3cd	4.7cd	7.0
	4	0.3cd	5.2bcd	7.6
	8	0.0d	4.8cd	7.1
	16	0.1cd	5.9abcd	7.9
	Sig. of F	<.0001	<.0001	ns
	C.V., %	25.81	14.95	9.12

† Seed treated with ethanol only

‡ Seed treated with water only

§ Letters indicate significant differences at the 0.05 level, using Tukey's, within each treatment day range.

Daily Water Use

Daily water use was calculated from five to 37 days after planting (dap) and is reported as a mean in g of water used day^{-1} pot^{-1} for each treatment averaged within four day ranges: 5-12, 13-20, 21-28, and 29-37 dap. No significant difference was seen between any treatment in the 5-12 dap range, however treatments differences were highly significant ($p < 0.0001$) in the 13-20, 21-28, and 29-37 dap ranges, as shown in Table 22. In the 13-20 day range, both control treatments were significantly higher in water use versus a PGR treatment of any concentration by around 20 g of water per day, but the water use values for all PGR treatments were similar to each other. Differences in concentration among PGR treatments became noticeable in the 21-28 day range, as water use values decreased with increasing concentration of both PGRs, leading to an approximate two-fold difference between the highest concentrations of each PGR and their respective control. These treatment differences widened in the 29-37 day range as the control for uniconazole used three times the amount of water than the 16 mg kg^{-1} uniconazole treatment, while the control for paclobutrazol used twice the amount of water compared to the 16 mg kg^{-1} paclobutrazol treatment.

The decreases in daily water use by soybean plants with seeds treated with paclobutrazol are similar to the findings of Navarro et al. (2007) and the reduction in daily water use reported for strawberry tree (*Arbutus unedo* L.) seedlings after a foliar application of paclobutrazol, and the same effect was seen in the uniconazole treatments. Over time, a trend in daily water use is similar to the emergence results for the two PGRs. Long-term effects of both PGRs are related to concentration, however greater reductions in water use are seen by the higher concentrations of uniconazole versus paclobutrazol. In fact, the highest concentrations of uniconazole hardly changed throughout the entire experiment, whereas daily water use for the highest paclobutrazol treatment began to climb in the 29-37 day range. This indicates that the effects of paclobutrazol on soybean water use appear to diminish earlier than similar concentrations of uniconazole.

Table 22. Effects of seed-applied plant growth regulators at varying concentration on daily water use.

Growth regulator	Concentration	Day range (days after planting) 5-12	13-20	21-28	29-37
	mg kg^{-1} seed	-------------------g day^{-1} pot^{-1}-------------------			
None†	-	49	74a	99a	154a
Uniconazole	1	47	53b	81bc	133abc
	2	46	49b	58de	121bc
	4	49	49b	50ef	94de
	8	44	48b	48f	61fg
	16	47	48b	46f	49g
None‡	-	47	70a	90ab	140ab
Paclobutrazol	1	48	53b	79c	144ab
	2	47	52b	68d	137abc
	4	47	48b	54ef	115cd
	8	47	47b	49ef	84ef
	16	48	48b	48f	76ef
	Sig. of F	ns	<.0001	<.0001	<.0001
	C.V., %	6.62	4.65	6.11	8.80

† Seed treated with ethanol only
‡ Seed treated with water only
§ Letters indicate significant differences at the 0.05 level, using Tukey’s, within each treatment day range.

Internode Lengths

The internode length results reported in Table 23 are the treatment means and represent these following internode intervals: the soil surface to the cotyledon node, the cotyledon node to the unifoliate node, the unifoliate node to the first trifoliate, the first to the second trifoliate, the second to the third trifoliate, and the third to the fourth trifoliate. It should be noted that in some instances, the fourth trifoliate did not develop for some of the plants exposed to the higher PGR concentrations, and therefore these values were inputted as zero.

Table 23. Effects of seed-applied plant growth regulators at varying concentration on soybean internode lengths.

Growth regulator	Concentration	Soil surface-cotyledon	Cotyledon-unifoliate	Unifoliate-1st trifoliate	1st trifoliate-2nd trifoliate	2nd trifoliate-3rd trifoliate	3rd trifoliate-4th trifoliate	Total plant height
	mg kg^{-1} seed	---cm---						
None†	-	4.7a	4.0a	2.1cd	2.6bc	2.6ab	1.4a	17.3a
Uniconazole	1	1.8b	3.0b	3.3ab	3.0ab	2.7a	1.3ab	15.1ab
	2	1.1bcd	1.1d	3.3ab	3.6a	2.1abcd	0.9abcd	12.1c
	4	0.9cd	0.6d	2.0cd	3.1ab	1.7cde	0.6cdef	8.8d
	8	0.8cd	0.5d	1.3de	1.9c	0.9fg	0.2fg	5.5e
	16	0.6d	0.4d	0.6e	0.6d	0.3g	0.1g	2.6f
None‡	-	4.7a	4.4a	2.1cd	2.6bc	2.2abc	1.1ab	17.0a
Paclobutrazol	1	1.4bc	4.0a	3.5ab	2.9abc	2.1abc	1.0abc	14.9ab
	2	1.1bcd	1.9c	3.9ab	3.1ab	2.3abc	1.0abc	13.3bc
	4	0.9cd	1.1cd	4.2a	3.7a	1.9bcde	0.9bcde	12.7bc
	8	0.8cd	0.7d	2.8bc	3.4ab	1.3cde	0.5defg	9.5d
	16	0.8cd	0.4d	2.0cd	2.6bc	1.2ef	0.4efg	7.4de
	Sig. of F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	C.V., %	18.5	17.38	16.81	14.56	17.76	26.10	8.83

† Seed treated with ethanol only
‡ Seed treated with water only
§ Letters indicate significant differences between experimental treatments at the 0.05 level, using Tukey's, within each internode.

Significant differences ($p < 0.0001$) were seen between all of the growing intervals, with the greatest differences noted in the soil surface to cotyledon node and the cotyledon node to unifoliate node, as concentrations of applied PGR increased. Sixteen mg kg^{-1} applications of both uniconazole and paclobutrazol significantly decreased the length of every internode interval compared to their respective control treatments. The unifoliate to first trifoliate and first to second trifoliate intervals show interesting trends, which are expressed in both uniconazole and paclobutrazol treatments. At uniconazole concentrations of 1, 2, and 4 mg kg^{-1}, internode lengths from the unifoliate to first trifoliate and the first to second trifoliate were actually equal to or greater than the control treatment. This is even more pronounced in the same concentrations for paclobutrazol, as well as the 8 mg kg^{-1} paclobutrazol treatment. Notably, the internode lengths of subsequent intervals in all of the abovementioned treatments appear to return closer to the control. Overall, the 1, 2, and 4 mg kg^{-1} rates of uniconazole restricted soybean plant growth up until the first trifoliate, while the 8 and 16 mg kg^{-1} rates limited growth through the fourth trifoliate. For paclobutrazol, 1 mg kg^{-1} only limited growth through the cotyledon node, while the 2, 4, and 8 mg kg^{-1} concentrations inhibited growth until the first unifoliate, and 16 mg kg^{-1} decreased growth through the fourth trifoliate.

Total plant height decreased with increasing concentration within each PGR, however, 16 mg kg^{-1} paclobutrazol resulted in plants with total heights between the 4 and 8 mg kg^{-1} uniconazole treatments. This trend in total plant height is highly correlated to the plant water use per day in day range 29-37 ($r^2 = .95$) as shown in Figure 2. This correlation demonstrates the hypothesis for this experiment, which is that a small plant will utilize less water than a larger one, and that reducing growth in early soybean growth stages should therefore conserve soil water for later growth stages.

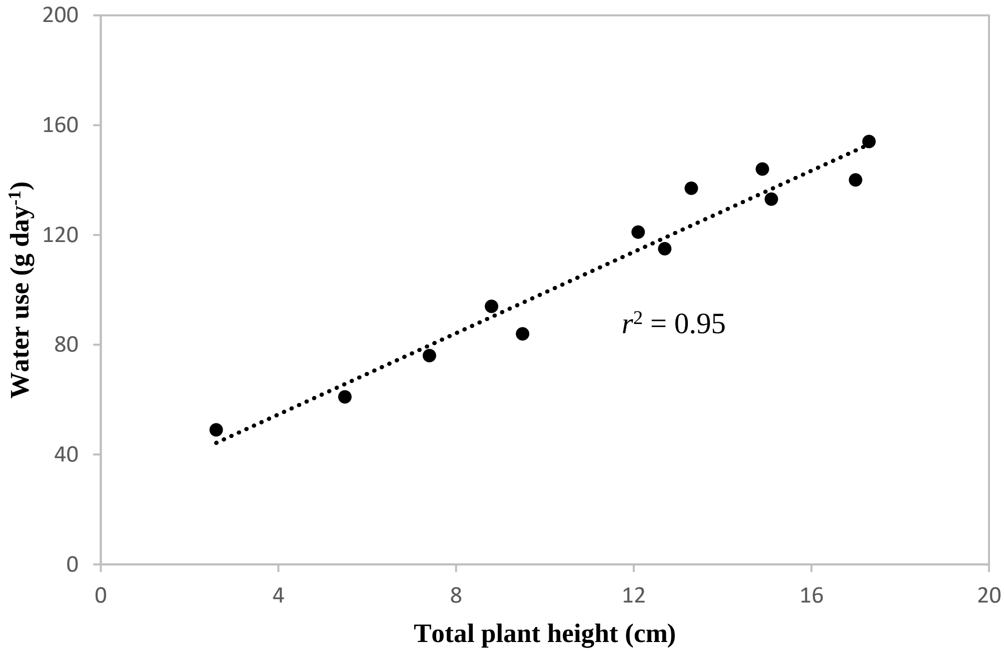

Figure 2. Relationship between total soybean plant heights plotted with daily water use in the 29-37 day range.

Plant Biomass

Both above and below ground dry biomass production was measured in grams, and are presented in Table 24, along with total produced biomass and a root to shoot ratio. The treatment effect was highly significant for all measurements ($p < 0.0001$). Dry root mass, dry shoot mass, and subsequently total biomass decreased as concentration increased for both PGRs, however when the two chemicals are compared at similar concentrations, uniconazole reduced growth more effectively than paclobutrazol. This is expected and agrees with the trends exhibited in internode lengths, but does not relate the results of Yan et al. (2010), who found seed-applied uniconazole at the 2 and 4 mg kg^{-1} concentrations to increase shoot dry weights in a field setting.

Table 24. Effects of seed-applied plant growth regulators at varying concentration on soybean biomass production.

Growth regulator	Concentration	Dry root mass	Dry shoot mass	Total biomass	Root:shoot ratio
	mg kg^{-1} seed	---------------------g pot^{-1}		-------------------	g root g^{-1} shoot
None†	-	3.86a	6.99a	10.85a	0.56c
Uniconazole	1	3.32ab	5.57bc	8.88ab	0.60c
	2	2.60bc	4.31de	6.91bcd	0.62c
	4	2.36bcd	3.26ef	5.62cdef	0.72bc
	8	1.87cd	1.96gh	3.83fg	0.98ab
	16	1.38d	1.29h	2.67g	1.08a
None‡	-	4.21a	6.50ab	10.71a	0.66c
Paclobutrazol	1	3.24ab	5.85abc	9.09ab	0.56c
	2	3.31ab	5.06cd	7.62bc	0.65c
	4	2.34bcd	4.10de	6.44cde	0.57c
	8	2.26bcd	2.83fg	5.08def	0.80abc
	16	1.68cd	2.67fg	4.36efg	0.63c
	Sig. of F	<.0001	<.0001	<.0001	<.0001
	C.V., %	18.07	11.12	13.10	18.32

† Seed treated with ethanol only
‡ Seed treated with water only
§ Letters indicate significant differences between experimental treatments, at the 0.05 level, using Tukey's, within each measurement.

Root to shoot ratio differences were also highly significant between some treatments, with uniconazole exhibiting a consistent increase in root to shoot ratio as concentration also increased, ranging from 0.56 g root g^{-1} shoot in the low concentration to 1.08 g root g^{-1} shoot in the highest concentration, which was similar to the findings of Yan et al. (2010) and Jing et al. (2012). Root to shoot ratios of paclobutrazol lack a discernable trend, with the highest ratio in the 8 mg kg^{-1} treatment of 0.80 g root g^{-1} shoot.

Yan et al. (2010) found applications of uniconazole at the 2 and 4 mg kg^{-1} to significantly increase seed yield when inter-seeded under the shading of corn, however their 8 mg kg^{-1} treatment did not increase seed yield due to excessive reductions in growth. Even though this experiment was terminated before the soybeans entered reproductive growth, a similar outcome would be expected based on the significant decreases in overall plant growth.

Water Use Efficiency

Differences between total biomass, cumulative water use, and also total biomass water use efficiency were highly significant ($p < 0.0001$). As depicted above by the correlation between above ground plant size and daily water use, cumulative water use is expected to be related to plant size, which is shown also in Table 25. However, trends in total biomass water use efficiency (WUE) decrease as concentrations increased for both PGRs, as shown in Table 25. With the greatest biomass produced L^{-1} of water exhibited in both control treatments.

Table 25. Effects of seed-applied plant growth regulators at varying concentration on total biomass production and water use efficiency (WUE).

Growth regulator	Concentration	Total biomass	Cumulative water use	Total biomass WUE
	mg kg^{-1} seed	g pot^{-1}	L pot^{-1}	g L^{-1}
None†	-	10.85a	3.16a	3.45ab
Uniconazole	1	8.88ab	2.65c	3.35ab
	2	6.91bcd	2.31de	2.98abc
	4	5.62cdef	2.02fg	2.78bc
	8	3.83fg	1.67hi	2.30cd
	16	2.67g	1.57i	1.70d
None‡	-	10.71a	2.92ab	3.68a
Paclobutrazol	1	9.09ab	2.73bc	3.35ab
	2	7.62bc	2.56cd	2.95abc
	4	6.44cde	2.22ef	2.88abc
	8	5.08def	1.9gh	2.63bc
	16	4.36efg	1.83ghi	2.38cd
	Sig. of F	<.0001	<.0001	<.0001
	C.V., %	13.10	4.71	11.98

† Seed treated with ethanol only
‡ Seed treated with water only
§ Letters indicate significant differences between experimental treatments, at the 0.05 level, using Tukey's, within each measurement.

Conclusion

When considering all of the data presented above, this experiment shows that increasing concentrations of seed-applied PGRs cause significant reductions in soybean growth rates and consequently, overall plant growth which causes predictable decreases in both daily and cumulative water use. Therefore, application of these findings towards altering vegetative water use in soybean plants to conserve soil water for reproductive stages of growth needs to find a balance between excessive growth reduction and water conservation. It is encouraging to report that seed-applied uniconazole and paclobutrazol only delayed emergence, without reducing overall long-term soybean stands. It is obvious that significant reductions in water use can be achieved in vegetative growth stages of soybean plants; however, since this experiment was not extended into plant maturity, it is difficult to anticipate the long-term effects of each treatment on seed yield.

A morphologic and agronomic approach may best suit the selection of appropriate concentrations of seed-applied PGRs to limit water stress in soybean. For example, the significant reductions in overall plant growth, especially in the lower internodes, will reduce harvest efficiencies as pods will be lower to the ground. Furthermore, reductions in overall plant growth may lead to decreases in effective leaf area for the capturing of sunlight (limiting total photosynthetic potential), increases in soil evaporation (as more of the soil surface will be exposed to greater extremes in temperature and wind speeds), or increases in weed pressure (which would cause soybean plants to compete for essential resources beyond water).

Therefore, an optimum uniconazole concentration between 2 and 4 mg kg^{-1} should reduce growth and conserve a considerable amount of water, while not causing long-term detrimental effects on overall plant growth. Furthermore, since paclobutrazol exhibited similar results in growth reduction and decreases in water use at these same concentrations, thus it is hypothesized that similar results in reduced water use could be achieved. Since these treatments exhibited significant differences in plant water use, but also allowed plant growth rates to return to normal later on in this experiment, uniconazole at 2 and 4 mg kg^{-1} and paclobutrazol at 4 mg kg^{-1} were used as seed-applied PGRs in the 2018 field study.

Experiment 3: Fall-Seeded Cover Crops and Seed-Applied PGR Effects on Soil Water and Their Subsequent Effects on Soybean Seed Quality and Yield

The objective of the 2018 growing season field experiment was two-fold. First, to evaluate the effects of various seeding rates and spring termination timings of fall-seeded cover crops on spring soil water conditions in semi-arid, west-central North Dakota, and also to quantify cover crop growth and nutrient uptake by these cover crops. The second phase was to observe the effects of these fall-seeded cover crop treatments and also seed-applied PGRs on subsequent soybean cash crop seed yield and seed quality.

Cover Crop Biomass

Limited rainfall in the 2017 growing season for the entire western part of North Dakota resulted in volumetric water contents around 21-24% in the 0-30 cm range for all three sites at the time of planting. These rye plants reached a Haun growth stage between 1.5 and 2.0 before freezing temperatures induced dormancy, indicating that the plants had 1.5 to 2.0 fully emerged main stem leaves.

Precipitation was received between the end of the 2017 and the beginning of the 2018 growing seasons and therefore, growth of the rye cover crops was significant in the spring for all treatments. Differences existed between the three sites regarding biomass accumulation and nutrient uptake which are shown in Table 26. It should be noted that the differences between treatments at each location was highly dependent upon the date of which the sample was collected. Early sampling occurred on 22 May (Haun stage of approximately 4.0), while late sampling occurred on 7 June (Haun stage of 11.0), so the late treatments had approximately 18 additional days of growth before herbicide termination. Consequently, the differences seen between the early and late termination treatments were highly anticipated. Regardless, early treatments produced similar biomass at the Underwood and Falkirk locations, while early-terminated treatment biomass production was approximately two-fold greater at the Minot location than the Underwood and Falkirk treatments. These locational trends were also anticipated since the Underwood and Falkirk locations were 8 km apart, while the Minot location was placed approximately 90 km from the other two sites. It is likely that differences in air temperature, snow melting rates, and soil temperature (none of these metrics were measured) between these two general areas induced these differences between locations, allowing the Minot location to begin growing earlier than the other two sites. However, the trends between locations disappears or is even reversed for the biomass and nutrient uptake values for the late termination treatments. This is due to soil water dynamics at each location. Between the early and late sampling dates, the Minot location only received 34 mm of rainfall, while the other two locations received approximately 42 mm of rainfall, as shown in table 26. Higher biomass accumulation inherently led to larger nutrient uptake, with easily distinguishable numerical trends between locations and termination dates. Higher seeding rates consistently produced greater biomass and consequently higher nutrient uptake values in the early termination treatments, however this trend is not repeated in the late termination treatments.

Table 26. Rainfall for the 2018 Underwood and Falkirk sites.

Date	Underwood	Falkirk
	---------------------------mm-------------------------------	
15-31 May	41	44
1-15 June	37	38
16-30 June	80	79
1-15 July	25	28
16-31 July	17	12
1-15 Aug.	5	9
16-31 Aug.	15	13
1-15 Sept.	61	28
16-30 Sept.	35	29
Total	316	281

Table 27. Rye biomass accumulation and nutrient uptake for all three locations.

Location	Seeding rate	Termination†	Biomass	N	P	K	S
	kg ha^{-1}		-------------------------kg ha^{-1}-----------------------				
Underwood	28	Early	571	25	1.3	24	1.6
	28	Late	3746	119	9.4	121	7.4
	56	Early	682	30	1.5	29	1.8
	56	Late	3638	109	9.3	117	7.3
Falkirk	28	Early	450	18	1.0	17	1.2
	28	Late	3337	95	8.7	90	6.1
	56	Early	685	15	1.6	15	1.7
	56	Late	3331	89	8.0	86	6.1
Minot	28	Early	1141	43	2.5	43	2.8
	28	Late	3202	90	7.7	87	6.2
	56	Early	1316	46	2.5	46	3.1
	56	Late	3461	91	6.8	88	6.2

† Sampling dates differed based on the timing of termination. “Early” sample date was 22 May and “Late” sample date was 7 June.

Spring Soil Water

Soil water conditions at soybean planting for each location exhibit a trend similar to the differences in rye biomass accumulation, and it is likely that soil water governed rye biomass production differences between locations which are shown in Table 28. The increased rainfall at the Underwood and Falkirk sites lead to volumetric soil water contents relatively near field capacity for both the 0-30 and 30-60 cm depths in the control and early-terminated treatments. At both depths, the late-terminated treatments were lower than the early treatments for these two locations, however the Underwood location exhibited greater decreases in water content than the Falkirk site. Overall, the water contents for all treatments at these two sites were surprising, considering the amount of rye biomass production prior to these measurements of soil water.

Overall, the Minot location was not as favorable, and a similar trend was seen in both soil depths. The control plot had the highest water contents, which decreased by the addition of an early-terminated rye cover crop, and finally the late termination of the rye reduced water contents to near permanent wilting points. These depleted water contents are what likely caused the late rye treatments at Minot to produce less biomass than the other two locations. These reductions in available soil water in the late-terminated treatments seen at the Underwood and Minot locations are similar to the findings of Liebl et al., 1992; Clark et al., 1997; and Krueger et al., 2011. Since the greatest differences in spring soil water were expressed at the Minot location, it is unfortunate that data collection at this location was stopped. Quantifying and evaluating the water dynamics induced by these treatments long-term would have been valuable.

Table 28. Spring soil water conditions following fall-seeded rye cover crops for all three locations in 2018.

Location	Seeding rate	Termination	Depth 0-30 cm	Depth 30-60 cm
	kg/ha		----- Vol. Water (%) -----	
Underwood	Control	-	26.6a	26.4a
	28	Early	26.4a	27.4a
	28	Late	19.5b	22.4b
	56	Early	26.3a	27.4a
	56	Late	18.5b	22.4b
	Field Capacity		28.8	32.1
	Permanent Wilting Point		13.5	14.8
Falkirk	Control	-	29.3	31.9
	28	Early	28.4	29.9
	28	Late	27.5	29.7
	56	Early	29.5	31.4
	56	Late	27.5	29.2
	Field Capacity		29.0	32.1
	Permanent Wilting Point		14.2	15.2
Minot	Control	-	21.0a	20.7a
	28	Early	18.2b	18.1ab
	28	Late	14.1c	15.4b
	56	Early	17.6b	17.2ab
	56	Late	13.9c	15.1b
	Field Capacity		27.4	33.4
	Permanent Wilting Point		13.5	15.8

† Sample date was 7 June, 2018 for all locations.
‡ Different letters indicate significant differences among experimental treatments at the 0.05 level, using Tukey's, within each depth, for each location.
§ No significant differences were found between experimental treatments at the Falkirk site, for both soil depths.

Stand Counts

Results from the greenhouse experiment on various concentrations of two seed-applied PGRs indicated that concentrations all the way up to 16 mg kg^{-1} of each chemical did not impact the long-term viability of soybean seeds, as demonstrated by insignificant differences between all experimental treatments in the 10-13 day after planting range for emergence counts. This result however, did not translate to the 2018 field experiment. When analyzed across both the Underwood and Falkirk locations, uniconazole, when applied at 2 mg kg^{-1} reported the highest stand count of 3.97 plants per half-meter, while paclobutrazol at 4 mg kg^{-1} significantly reduced stand counts to 2.97 plants ha^{-1} compared with the highest treatment value, as shown in Table 29. No consistent trends were apparent between the other rye treatments and the differences reported do not appear to be considerable in magnitude.

Table 29. Treatment effects on soybean stand counts across the 2018 Underwood and Falkirk locations.

Treatment	Seeding rate	Termination/ PGR rate§	Plants emerged
	kg ha^{-1}	mg kg^{-1} seed	plants 0.5 m^{-1}
Control	-	-	3.78ab
Rye	28	Early	3.28ab
Rye	28	Late	3.28ab
Rye	56	Early	3.00ab
Rye	56	Late	3.84ab
Paclobutrazol		4	2.97b
Uniconazole		2	3.97a
Uniconazole		4	3.50ab
		Sig. of F	0.0103

† Different letters indicate significant differences among experimental treatments at the 0.05 level, using Tukey's.
‡ These data were collected on 26 June, 2018, approximately 20 days after planting.
§ "Early" or "Late" refers to the termination timing of the rye treatment, while the numerical values are the rates of PGR applied to the seeds in mg kg^{-1} of seed.

Volumetric Water Content

Rainfall for the Underwood and Falkirk sites during the 2018 growing season was favorable, and differences between the two sites were minimal until later in the growing season where the Underwood location received greater amounts of rainfall, which is summarized in Table 26. These relatively consistent rainfalls throughout the growing season maintained positive volumetric soil water contents in the top 20-cm of the soil profile, leading to no significant differences between experimental treatments, within any sampling date, as shown in Table 30. The largest variation within a sampling date of these means was 3.3% at the 17 July sampling date, indicating that overall rainfall was buffering treatment differences of any significant magnitude.

Relative Leaf Water Content

Since relative leaf water contents should be related to available soil water, insignificant or miniscule differences between treatments are also exhibited in Table 31. It appears that the paclobutrazol treatment consistently produced the lowest relative leaf water contents in the final four sampling dates. Throughout all of the sampling dates, the rye treatments consistently reported some of the lowest leaf water contents, while the uniconazole 4 mg kg^{-1} treatment was near the top at every sampling time.

Table 30. Treatment effects on volumetric water content in the upper 20 cm of the soil throughout the reproductive soybean growth stages, analyzed across the 2018 Underwood and Falkirk locations.

			Soybean growth stage and sampling date						
			R1	R2	R3	R5	R6	R6	R7
Treatment	Seeding rate	Termination/ PGR rate‡	17 July	23 July‡	1 Aug.‡	13 Aug.‡	25 Aug.	31 Aug.	9 Sept.
	kg ha^{-1}	mg kg^{-1} seed	-- Vol. Water (%) --						
Control	-	-	29.3	26.6	22.1	20.2	20.4	19.3	23.8
Rye	28	Early	30.4	27.4	23.0	19.7	19.4	18.9	23.7
Rye	28	Late	31.0	27.4	23.4	19.4	19.1	19.7	24.1
Rye	56	Early	31.3	26.7	23.1	19.4	19.1	19.0	23.3
Rye	56	Late	30.9	27.3	23.3	19.0	19.2	18.5	23.4
Paclobutrazol		4	31.1	26.9	22.8	19.7	19.1	19.0	22.7
Uniconazole		2	30.6	27.3	22.4	20.4	19.9	19.9	24.3
Uniconazole		4	32.7	28.5	24.6	20.1	18.7	20.1	23.8
		Sig. of F	ns	ns	ns	ns	ns	ns	ns

† Sampling dates were the same for the two plots at all dates except the three timings when the soybean growth stages were R2, R3, and R5. The dates listed in the table for these timings were for the Underwood location. The following dates were the sampling dates for the Falkirk site: 24 July, 2 Aug., and 15 Aug. During analysis, these sampling dates were considered the same as no rainfall was received between the varying sampling dates.

‡ "Early" or "Late" refers to the termination timing of the rye treatment, while the numerical values are the rates of PGR applied to the seeds in mg kg^{-1} of seed.

Table 31. Treatment effects on relative leaf water content of upper soybean leaves throughout the reproductive soybean growth stages analyzed across the 2018 Underwood and Falkirk sites.

			Soybean growth stage and sampling date					
			R2	R3	R5	R6	R6	R7
Treatment	Seeding Rate	Termination/ PGR rate§	23 July‡	1 Aug.‡	15 Aug.	25 Aug.	30 Aug.	6 Sept.
	kg/ha	mg kg^{-1} seed	-- % --					
Control	-	-	92.5	93.0	85.4	83.4	85.1	88.6
Rye	28	Early	99.8	92.4	86.0	83.6	84.4	88.5
Rye	28	Late	95.0	92.0	85.6	82.0	84.5	88.3
Rye	56	Early	96.9	92.9	85.3	81.8	83.3	88.0
Rye	56	Late	96.8	92.4	83.8	82.1	84.1	87.8
Paclobutrazol		4	95.8	93.8	84.0	81.1	83.4	87.4
Uniconazole		2	97.4	92.8	85.9	82.4	85.0	88.1
Uniconazole		4	98.5	94.6	87.8	83.4	84.8	88.4
		Sig. of F	ns	ns	ns	ns	ns	ns

† Different letters indicate significant differences among experimental treatments at the 0.05 level, using Tukey's, within each sampling date.

‡ Sampling dates were the same for the two plots at all dates except for the first two dates when the soybean growth stages were R2 and R3. The dates listed in the table for these timings were for the Underwood location. The following dates were the sampling dates for the Falkirk site: 24 July and 2 Aug. During analysis, these sampling dates were considered the same as no rainfall was received between the varying sampling dates.

§ "Early" or "Late" refers to the termination timing of the rye treatment, while the numerical values are the rates of PGR applied to the seeds in mg kg^{-1} of seed.

Proline Accumulation

Under water stressed conditions, plants can undergo osmotic adjustment, which is a drought tolerance strategy through which plants accumulate quantities of osmolytes, like proline, in the cytosol of the cell. This reduces the osmotic potential of cells, thus allowing greater up take and retention of water during limited water conditions (Chen and Jiang, 2010). The two sampling dates that show significant differences between experimental treatments were the 25 August and 30 August treatments, as shown in Table 32. However, during post-hoc analysis, the significant differences in 25 August were no longer present under the more conservative Tukey's mean separation testing method. These sampling times are nearing the end of the period in August that experienced the lowest rainfall throughout the experiment and may explain why differences between treatments were seen only in these dates. Overall, it appears that the PGRs reported higher proline concentrations than the control throughout the sampling dates, while all of the rye treatments were similar or slightly lower than the control, especially the late-terminated rye treatments. Zhang et al., (2007) found uniconazole as a foliar application to increase proline concentrations in soybean leaves by 7% over the control under different water stress levels, which is consistent with the results found in this study for the above mentioned sampling dates. Parvin et al., (2015) saw applications of paclobutrazol to also increase proline content in strawberry plants, so it is possible that both of these PGRs are increasing proline concentrations in leaf tissue. Since soil water contents proved to be incredibly homogenous in the top 20-cm of the soil profile, the reason for reduced proline concentrations for the late-terminated rye treatments cannot be determined through the metrics collected in this experiment. A possible explanation might be improved soil water availability in deeper depths for the late-terminated rye treatments, which would should have a greater amount of rooting networks deeper than any other treatment. This increase in rooting quantity and depth possibly improved infiltration and water retention capacities lower in the soil profile, where the following soybean crop would be acquiring water from in later stages of growth.

Table 32. Treatment effects on proline accumulation in upper soybean petioles and stems throughout the late reproductive soybean growth stages analyzed across the 2018 Underwood and Falkirk sites.

			Soybean growth stage and sampling date			
			R5	R6	R6	R7
Treatment	Seeding rate	Termination/ PGR rate§	15 Aug.‡	25 Aug.	30 Aug.	6 Sept.
	kg ha^{-1}	mg kg^{-1} seed	---------------------- µg g^{-1} DW--------------------			
Control	-	-	529	442	476ab	273
Rye	28	Early	522	401	465ab	278
Rye	28	Late	472	359	367b	293
Rye	56	Early	474	357	450ab	291
Rye	56	Late	450	354	393ab	258
Paclobutrazol		4	575	515	503ab	330
Uniconazole		2	540	458	532a	313
Uniconazole		4	515	502	529a	367
		Sig. of F	ns	0.0119¶	0.0074	ns

† Different letters indicate significant differences among experimental treatments at the 0.05 level.
‡ Sampling dates were the same for the two plots at all dates except for the first sampling date when the soybean growth stage was R5. The date listed in the table for this sample timing was for the Underwood location. The Falkirk site was sampled on 16 August. During analysis, these sampling dates were considered the same as no rainfall was received between the varying sampling dates.
§ "Early" or "Late" refers to the termination timing of the rye treatment, while the numerical values are the rates of PGR applied to the seeds in mg kg^{-1} of seed.
¶ Treatment effect was found to be significant at the 0.05 level, until post-hoc analysis with Tukey's (which is more conservative than LSD) reported no significant differences between the experimental treatments, for this sampling date.

Ureide Accumulation

The accumulation of ureides in young soybean tissue due to decreased catabolism of these transport molecules under water stressed conditions has been highly documented and appears to be both an early and sensitive indicator of limited water availability in soybean plants

(Purcell et al., 2004; King and Purcell, 2005). Since minimal differences were seen between treatments in volumetric soil water in the top 20 cm throughout the growing season, differences in ureide concentration of sampled petioles between treatments should also be absent. The results are shown in Table 33, and only show a significant difference in the first sampling date of 23 July at the Underwood site and 24 July at the Falkirk site, which were analyzed together under the assumption that conditions were not different between the two sampling dates. Trends in this sampling at the R2 stage of soybean growth indicate that the control plot had the highest accumulations of ureides, followed by the PGRs, and finally the rye treatments. However, during the August time period in which rainfall was the most limiting, the late-terminated rye treatments exhibited the highest accumulations of ureides throughout the entire experiment, as well as for each sampling date. Although insignificant, this trend in increased ureides by the late-terminated rye treatment is completely opposite of the findings for the proline contents of the identical tissue samples, and therefore contradicts the idea that soil water conditions were improved deeper in the profile due to increased rooting structures from the late-terminated rye plants. Another interesting point to make is the sudden decrease in ureide concentrations as the soybean plants entered into the R7 growth stage, when pods have been filled with seed and are beginning to mature. Ureide concentrations become relatively nonexistent in the final sampling time on 15 September, this is due to the decrease in symbiotic nitrogen fixation and subsequent transport of these molecules to the young tissues. Instead, the plant is transferring more resources to the pods, to produce viable seed.

Table 33. Treatment effects on ureide accumulation in upper soybean petioles and stems throughout the reproductive soybean growth stages analyzed across the 2018 Underwood and Falkirk sites.

Treatment	Seeding rate	Termination/ PGR rate§	Soybean growth stages and sampling date						
			R2	R3	R5	R6	R6	R7	R7
			23 July‡	1 Aug.‡	15 Aug.‡	25 Aug.	30 Aug.	6 Sept.	15 Sept.
	kg ha^{-1}	mg kg^{-1} seed	---μg g^{-1} DW---						
Control	-	-	1359a	2021	3939	2713	2716	965	115
Rye	28	Early	986b	1774	3718	2471	2333	1326	117
Rye	28	Late	1039ab	1803	4537	2891	2781	1154	202
Rye	56	Early	1058ab	1886	3754	2186	1942	612	57
Rye	56	Late	981b	1723	4738	2933	2832	747	127
Paclobutrazol		4	1186ab	1676	3859	2377	2432	733	40
Uniconazole		2	1209ab	1985	3694	2151	2455	992	43
Uniconazole		4	1188ab	1945	4339	2755	2736	1177	187
		Sig. of F	0.0104	ns	ns	ns	ns	ns	ns

† Different letters indicate significant differences among experimental treatments at the 0.05 level, using Tukey's, within each sampling date.

‡ Sampling dates were the same for the two plots at all dates except for the first three dates when the soybean growth stages were R2, R3, and R5. The dates listed in the table for these timings were for the Underwood location. The following dates were the sampling dates for the Falkirk site: 24 July, 2 Aug., and 16 Aug. During analysis, these sampling dates were considered the same as no rainfall was received between the varying sampling dates.

§ "Early" or "Late" refers to the termination timing of the rye treatment, while the numerical values are the rates of PGR applied to the seeds in mg kg^{-1} of seed.

Percent Leaf Drop

Estimated percentages of leaves dropped from the soybean plants were taken on two dates, 13 and 22 September, when soybean plants were in the R7 and R8 stages of growth. Significant differences were seen between the treatments, and the trends are quite similar between the two dates as demonstrated in Table 34. The PGRs all reported lower amounts of dropped leaves, especially the 4 mg kg^{-1} uniconazole treatment, while the rye treatment were relatively similar to the control. Since no differences were seen in soil water levels, these differences are likely due to delayed maturity by the PGRs. These differences were not significant enough to be detected in growth stage measurements, but there is clearly a physiological delay in plant maturity.

Table 34. Treatment effects on the percentage of leaves dropped at two dates analyzed across the 2018 Underwood and Falkirk sites.

Treatment	Seeding rate	Termination/ PGR rate‡	Date 13 Sept.	Date 22 Sept.
	kg ha^{-1}	mg kg^{-1} seed	------------------%	-----------------
Control	-	-	43a	81a
Rye	28	Early	40a	81ab
Rye	28	Late	41a	84a
Rye	56	Early	39ab	80ab
Rye	56	Late	43a	84a
Paclobutrazol		4	36ab	77ab
Uniconazole		2	38ab	78ab
Uniconazole		4	31b	72b
		Sig. of F	0.0009	0.0031

† Different letters indicate significant differences among experimental treatments at the 0.05 level, using Tukey's, within each sampling date.
‡ "Early" or "Late" refers to the termination timing of the rye treatment, while the numerical values are the rates of PGR applied to the seeds in mg kg^{-1} of seed.

Seed Yield and Quality

Overall, seed yields were promising and differences in yield, test weight, and protein content were statistically insignificant as well minimal in size, while a significant difference was determined for oil content as shown in Table 35. Oil contents appear to be reduced by the application of PGRs, while the rye treatments reported values similar to the control treatment.

Table 35. Treatment effects on soybean seed yield, test weight, protein content, and oil content for the 2018 Underwood and Falkirk sites.

Treatment	Seeding rate	Termination/ PGR rate‡	Seed yield	Test weight	Protein	Oil content
	kg ha^{-1}	mg kg^{-1} seed	kg ha^{-1}	kg hL^{-1}	-----------g kg^{-1}----------	
Control	-	-	2186	9.33	387	204a
Rye	28	Early	2224	9.30	388	203ab
Rye	28	Late	2185	9.33	386	204a
Rye	56	Early	2234	9.33	386	203ab
Rye	56	Late	2203	9.32	385	204a
Paclobutrazol		4	2175	9.30	386	202ab
Uniconazole		2	2282	9.33	390	200b
Uniconazole		4	2222	9.29	389	200b
		Sig. of F	ns	ns	ns	0.0004

† Different letters indicate significant differences among experimental treatments at the 0.05 level, using Tukey's, within each measurement.
‡ "Early" or "Late" refers to the termination timing of the rye treatment, while the numerical values are the rates of PGR applied to the seeds in mg kg^{-1} of seed.

Conclusion

Cover cropping in semi-arid regions has the potential to significantly reduce spring water levels as shown at the Minot location in 2018. Seeding rates do not appear to affect biomass production levels or induce differences in soil water. The greatest reductions in soil water were seen in the late-terminated rye treatments, while the rye cover crops terminated two weeks before planting of the following soybean cash crop were similar to the control treatments.

Adequate spring rainfall replenished soil water deficits and frequent rainfalls throughout the growing season at the Underwood and Falkirk locations buffered any substantial differences between treatments for all measurements taken throughout the experiment. It is possible that PGR application increases proline accumulation in soybean tissues and delayed maturity in soybean plants, but these increases did not result in boosted yields. In fact, seed yields, test weight, and protein contents of seeds were similar between all experimental treatments. Small variations were seen in oil content, with the PGRs reducing values slightly compared to the other treatments, and the opposite trend was exhibited for glucose levels. Analysis of other seed quality metrics resulted in no significant differences.

Therefore, termination timing of cover crops plays a major role in soil water depletion and without adequate rainfall, could exacerbate seasonal water deficits for subsequent cash crops. However, in 2018 (a year of adequate growing season rainfall), treatment differences between cover cropped and non-cover cropped treatments were unidentifiable. Benefits from seed-applied PGRs were not demonstrated, although in water-limiting growing seasons, reducing overall plant growth should reduce water use and possibly improve soil water dynamics later in the growing season, when potential reductions in seed yield due to water stress are the highest.

GENERAL CONCLUSION

Deficits between normal annual precipitation and potential crop water use are the greatest limiting factor for crop production in the western region of North Dakota. Therefore, the expansion of soybean production into this region commonly faces potential reductions in yield due to water stress. Since soybean crops exhibit the greatest reductions in seed yield when water stress occurs during R3-R6 reproductive stages of growth, efforts to improve water availability during this time period should benefit overall production.

The foliar-applications of plant growth regulators (PGRs) and antitranspirants (ATs) to soybean leaves at R1 and R3 growth stages did not consistently improve relative leaf water contents of soybean plants, even under three different levels of drought stress. Any improvements in leaf water status were not related to improved seed yields. In fact, under mild to moderate drought conditions, the application of water use modulators reduced soybean seed yields by 9-17% when compared to the control. Under severe drought, foliar-applications of ethephon (PGR) at the R1 stage of growth, and the two antitranspirants applied at R1 and R3: Wilt-Pruf (Film/Barrier AT), and kaolinite (Reflective AT) did significantly improve seed yields at their respective locations (9-20%), but these results were not replicated at the other sites, especially under mild drought stress. Even if foliar-applications of water use modulators could be proven to consistently improve seed yields under severe drought conditions, further analysis would need to be done to show how economical this practice would be for farmers.

Smaller plants generally use less water than larger ones, as shown in the greenhouse experiment on various concentrations of two seed-applied PGRs, uniconazole and paclobutrazol. Significant reductions in the heights of soybean plants were highly correlated to significant reductions in daily water use due to the application of these growth inhibitors. This shows that water can be conserved in vegetative stages of growth, and as the effects of the PGRs diminish, growth can continue into the reproductive stage when more soil water should be available. However, the optimal concentrations at which these chemicals should be applied is governed by the balance between water conservation and overall plant growth inhibition. Extreme reduction in growth could have negative long-term impacts such as reduced effective leaf area for photosynthesis, increased weed pressure, higher amounts of evaporation for the soil surface, or decreased harvest efficiency. Therefore, uniconazole and paclobutrazol at concentrations around 2 to 4 mg kg^{-1} seed were determined to significantly reduce growth and water use, but also allow adequate overall soybean growth. Furthermore, uniconazole treatments of the same concentration appeared to reduce growth for longer periods of time than paclobutrazol.

Cover cropping has become a key component in the soil health movement and its implementation to reduce erosion, improve soil structure, and even suppress weeds has proven effective in regions of adequate rainfall. However, the water used by the fall-seeded cover crop has been shown to significantly reduce available soil water levels in the spring. Especially when a cover crop like rye (which continues growth after a winter dormancy) is allowed to grow for an extended period of time before the planting of a cash crop. This reduction in soil water levels in the spring however has been shown to not have a long term negative effect on cash crop yields due to a mulching effect which reduces evaporation throughout the growing season and actually can provide improved soil water dynamics during reproductive stages of growth, which are commonly in parts of the year when atmospheric evaporation demands are high. This phenomenon was unable to be realized in the 2018 field experiment, where soil water levels were only substantially different at one location, which eventually had to be abandoned. The other two sites received adequate rainfall throughout the growing season, and differences between any cover cropped treatments compared to the control were negligible. Regardless, this lack of negative results on final seed yield due to reduced spring soil water by cover crops is promising and shows the potential of this practice in semi-arid regions when rainfall is normal.

The seed-applied PGRs also studied in this 2018 field experiment did not result in any significant impacts on soybean seed yield. It is possible that results could have been seen under water limiting conditions, as it is clear that soil water can be conserved with the reduction in growth of soybean plants based on the results of the greenhouse experiment. Seed quality was only influenced by the application of PGRs in reduced oil contents, while glucose levels were actually improved over the control treatment.

Future research should be performed over multiple years, on a larger scale, and across varying landscape positions to evaluate the effects of fall-seeded cover crops on soil characteristics and subsequent cash crop yields in this semi-arid region of North Dakota. Additionally, efforts to quantify cost to benefit ratios for the implementation of these practices over both short-term and long-term time frames should be pursued. It is clear from the results of this thesis, that the fear in cover crop implementation simply because the growth of the cover crop utilizes excessive amounts of soil water is unreasonable in years of adequate growing season precipitation.

The management of all water resources received in semi-arid regions requires a holistic approach to water capture, retention, utilization and efficiency. None of which matter without living plants in the soil, thus laying the foundation upon which to begin managing both scarce and valuable water resources.

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