Cold Temperature and Water Effect on Germination and Emergence of Soybean [*Glycine max* (L.) Merr.]

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Shashini Anuththara Neththasinghe Appuhamilage

Graduate Program in Horticulture and Crop Science

The Ohio State University

2024

Thesis Committee

Dr. Alexander J. Lindsey, Advisor

Dr. Laura E. Lindsey

Dr. Aaron B. Wilson

Abstract

The occurrence of warm spring temperatures in the Midwestern United States has increased over the last century, and farmers are interested in planting soybean early to capitalize on the benefits of the longer growing season. However, risks of cold precipitation and/or soil temperatures after planting may hinder crop establishment. Environmental factors and management decisions along with cold temperatures may also affect the chilling response in soybean. Therefore, the objectives of this research were to (1) investigate how duration of exposure to cold conditions during the imbibition and early germination phases affect seed germination in two soybean cultivars, (2) quantify how soil moisture content at planting, planting depth, temperature, and form of precipitation affect soybean emergence and early growth, and (3) quantify the effect of moisture content at planting and temperature/form of precipitation during germination on soybean emergence and *Fusarium graminearum* infection. Three controlled environment studies were conducted from 2023-2024 in Columbus, OH using two untreated soybean varieties. The first experiment assessed temperature and duration of exposure to cold temperatures during germination. Across temperature pairings, exposure for 12 to 24h in cold temperature immediately after starting the germination assay resulted in the greatest reduction (7%) in germination compared to the untreated check. Cultivar 3.4 RM was more affected by cold temperature exposure than the 3.7 RM. Regardless of treatment, germination was always

over 83% after 10 days. The second experiment highlighted that water application (1.7 or 10℃) or application of ice within the first 11 hours of planting negatively affected emergence and early season growth (30-65% reduction in biomass). Cold temperatures alone (1.7℃) did not affect emergence percentage or biomass. Planting at 3.8 cm depth under drier soil conditions (20% AWC) increased biomass compared to wetter soil conditions (60% AWC), though differences were not observed when planted at 2.5 cm depth. In the third experiment 1.7℃ liquid water application or the same quantity of water as ice when inoculum was applied reduced total emergence by 6% compared to same water treatments without inoculum. Inoculated media produced more dead seeds under 60% moisture content compared to 80% moisture content. These results suggest cold temperatures alone may not fully explain stand reductions in field environments. Other abiotic factors like soil texture and moisture as well as biotic factors like pests and diseases could be associated with stand losses in early planted soybean and their association with cold temperatures during germination and early growth need to be further studied. It is possible that the duration of cold temperatures and timing of initial exposure may also play a role in germination and emergence should be evaluated in future trials.

Dedication

Dedicated to my parents, Ranjith Neththasinghe and Kumari Samaradhiwakara, and my husband, Prabath Senevirathna.

Acknowledgments

I would like to express my deepest gratitude to everyone who contributed to the success of this project. My heartfelt thanks go to my lab members - Victor de Vasconcelos Gomes, Wanderson Novis, Bayron Hernandez, Ben Domin, and Pedro de Menezes - for their unwavering support and assistance in the lab and greenhouse.

I want to give a special thank you to Dr. Alexander Lindsey for dedicating a lot of time, and for being a great advisor, in addition to guiding me for research procedures. Also, thank you to my committee members Dr. Laura E. Lindsey and Dr. Aaron Wilson, for taking the time to support and guide me through the process, and providing very useful feedback for my project.

I extend my sincere thanks to Zak Ralston, the lab manager, and Jenna Moore from the Department of Plant Pathology for their assistance with inoculum study. My gratitude also goes to Dr. Horacio Lopez-Nicora for his help with the inoculum study. I am thankful to Isaac Knowles, the Kottman greenhouse manager, for facilitating access to all the greenhouse facilities. I would also like to acknowledge Stacy Cochran and James Davis from the Ag Tech Innovation Hub, Nationwide, and Taylor Dill for capturing the project procedures on video. Thanks to Pioneer ® and Dekalb ® for providing the seeds used in this study.

I would also like to thank the Ohio Soybean Council for funding this project, as well as the Nationwide AgTech Innovation Hub and the OSU Soybean Research Center for their partial funding support. Finally, thanks are extended to the Department of Horticulture and Crop Science, The Ohio State University for the opportunity to initiate and conduct this research seamlessly.

Vita

June 2020……. BSc. Agricultural Technology and Management: Advanced Field Crop Production Specialization, University of Peradeniya, Sri Lanka

March 2021 – November 2022……. Research Assistant, Department of Crop Science, University of Peradeniya, Sri Lanka

Jan 2023 – Present……Graduate Research Associate, Department of Horticulture and Crop Science, The Ohio State University, USA

Publications

Neththasinghe, N. A. S. A., Dissanayaka, D. M. S. B., & Karunarathna, A. K. (2023). Rhizosphere nutrient availability and nutrient uptake of soybean in response to biochar application. Journal of Plant Nutrition, 46:17, Pp: 4085-4095. https://doi.org/10.1080/01904167.2023.2220740

Sugathas, S., Neththasinghe, N. A. S. A., Sirisena, D. N., Thilakasiri, R., Ariyarathna, M., Kadupitiya, H. K., ... & Suriyagoda, L. D. B. (2024). Effects of agro-climatic zone, soil orders, and irrigation types on the exchangeable cadmium in paddy soils. Soil & Environmental Health, 100078. https://doi.org/10.1016/j.seh.2024.100078

Fields of Study

Major Field: Horticulture and Crop Science

Concentration: Agronomy

Table of Contents

List of Tables

List of Figures

Chapter 1. Literature Review

1.1Soybean history and production overview

Soybean [*Glycine max* (L.) Merr] is an important legume crop cultivated in the United States and worldwide. Soybean is the dominant oilseed crop accounting for about 90% of oilseed production within the country (USDA -ERS, 2024). Over 70% of the soybeans cultivated in the United States are used for animal feed, especially for poultry, hogs, dairy, beef, and aquaculture. Around 15% of the U.S. soybeans are used to produce foods for human consumption, especially for vegetable oil (USDA, Fact sheet, 2015). Most of the soybean oil produced (68% of total) is reserved for human consumption (USDA, Fact Sheet, 2015), with the biodiesel industry utilizing 25% of the oil produced. The remaining 7% of the soybean oil produced is allocated for other industrial uses.

Large-scale soybean production in the U.S. began in the 20th century, with the area planted expanding rapidly due to increased planting flexibility, increased corn-soybean rotations, low production costs, and periodic adoptions of improved agronomic practices such as narrow row cultivation (Rowntree et al., 2013; USDA- ERS, 2024). Currently, the U.S. ranks as the second largest soybean producer and exporter in the world after Brazil. After corn, soybean is the second major agricultural crop sown in the U.S. Over the last two decades, soybean cultivation has increased by 15% of the total land area cultivated in the U.S. accounting for 35 million ha in 2024 (USDA- ERS, 2024). In 2023, national soybean production totaled 113.3 million metric tons, an increase of 35% compared to the last two decades (USDA- NASS, 2024). Production value of

soybean stood at about 52.8 billion U.S. dollars in 2023, a 75% increase compared to 2000 (USDA-NASS, 2024). In 2023, 49 million metric tons of soybean has exported to the outside market and 49% of the total production in the U.S. as whole grain, meal, or oil (United Soybean Board, 2024; USDA- FAS, 2023). However, soybean export has plateaued since 2016 due to high domestic demand.

More than 80% of the cultivated area is concentrated in the U.S. Midwest where soybean yield is highest (USDA- ERS, 2023). This region of the U.S. benefits from favorable climate conditions, well-established agricultural infrastructure, and good soil conditions that support large-scale soybean production. In 2024, the top soybean producing states were Illinois, Iowa, and Minnesota, accounting for more than 37% of total U.S. production. (USDA- ERS, 2024). In Ohio, soybean is the most widely cultivated field crop over two million ha planted in 2023 (USDA-NASS, 2024). In Ohio, 7.47 million metric tons of soybeans were produced in 2023, with an average yield of 3.9 metric tons per hectare, a 24% increase compared to the last two decades. It is the fifth largest soybean-producing state in the U.S. as of 2023 (USDANASS, 2024). Twenty-six thousand farmers cultivate soybeans in Ohio, and the annual economic impact is 5.3 billion U.S. dollars from soybean production, which is approximately 33% of Ohio's agricultural economy (Ohio Soybean Council, 2024). To ensure Ohio farmers remain competitive in soybean production markets, it is crucial to focus on the management decisions surrounding soybean cultivation. These decisions play a key role in maintaining and enhancing production levels, especially in the face of changing climate conditions.

1.2 Evaluation of planting dates of soybean

Climate change and weather patterns significantly influence agricultural decision-making, particularly in the Midwestern United States, which accounts for two-thirds of the nation’s land

and produces 65% of its soybean and corn. Given the region's agricultural prominence, it is crucial to consider climate fluctuations when making farming decisions. Over the past century, the average air temperature in the Midwest has risen by 1.1℃ and is projected to increase by another 5℃ by the end of the century (Kunkel et al., 2013; Pryor et al., 2013; National Climate Assessment, 2024). Furthermore, Abendroth et al. (2019) also found that spring temperatures have been increasing from 1950 to 2017 in most counties in Midwestern U.S. This warming trend has led to earlier spring temperatures, causing the final frost of the spring season to occur approximately seven days earlier than it did a century ago (Schrage, 2018). Climate models have predicted that wet springs may become more frequent in Ohio due to climate change (Baule et al., 2017; O'Neal et al., 2005). Annual precipitation has increased by 10 to 20% during the last century and is projected to continue with more intense precipitation events in the future (Schoof et al., 2010; Grady et al., 2021). Hence, days suitable for fieldwork in the spring have been declining since 1995 with most weeks only having two or four days suitable for Ohio farmers. Farmers may decide to plant more hectarage before a weather front because the precipitation that falls would prevent future fieldwork, even if it is early in the season (Lindsey et al., 2024).

Given all these changes in climate and weather patterns over past years, decision making related to farming practices also change overtime. Among those practices, planting date is an important management decision, and timely planting is important to achieve higher grain yield and quality of soybeans (Egli & Cornelius, 2009; Hu & Wiatrak, 2012; Rowntree et al., 2013). Soybean is a warm-season annual crop planted in spring and harvested in autumn. Soil temperature at the time of planting, soil moisture content, and weather forecast are important considerations when deciding a planting date for soybeans. It is recommended to plant soybeans when soil temperatures reach 10°C and adequate moisture is present at the planting depth of 2.5 to 3.75 cm to facilitate

germination (Bruin & Pedersen. 2008; Lindsey et al., 2017). Soybeans have traditionally been planted in late April and early May throughout much of the Midwest. However, this can vary depending on the growing region and the maturity group of the soybeans. In southern Ohio, it is currently recommended to plant soybeans after April 15, while in northern Ohio, planting is advised to commence in the last few days of April, provided soil conditions are suitable (Lindsey et al., 2017). However, there has been a consistent trend toward earlier planting dates in the Midwestern United States (Rowntree et al., 2013). The Risk Management Agency has adjusted the replant crop insurance dates, allowing farmers in northern and southern Ohio to begin planting soybeans as early as April 15th and April 5th, respectively, while still maintaining eligibility for insurance coverage.

The shift in soybean planting dates in the U.S. has been driven by several factors, including advancements in crop management practices, genetic improvements, and evolving environmental conditions. Planting soybeans early leads to increased yields by extending the vegetative growing season. This extension allows the soybean plants to absorb more heat units, which in turn promotes the production of more nodes on the main stem (De Bruin & Pedersen, 2008; Robinson et al., 2009; Hu & Wiatrak, 2012; Staton, 2023). Soybean crops can produce a new main stem node every 3.7-day interval until the seed development stage (Conley et al., n.d.). Similarly, production in greater biomass and close canopy also helps to control weed growth (Conely et al., n.d.). Numerous studies have highlighted the benefits of early planting (Conely et al., n.d.; Hu & Wiatrak, 2012; Staton, 2018). Specht et al. (2017) recorded that soybean planting dates in the Midwestern US have persistently shifted towards early calendar dates at a rate of 0.5 days per year. According to Mourtzinis et al. (2019), by planting 8–10 days earlier than normal, growers in Iowa and Ohio could have had a 10.8 and 29.9 kg ha^{-1} yield increase, respectively, during the past

decades. It was reported that the early planting of soybeans produced on average, an extra 269 kg ha^{-1} (Sonnenberg, 2020). A study conducted by Southworth et al. (2002) explained that early planting brings yield advantage in the Midwestern US because of future changes in climate and climate variability. A recent study conducted by Kannberg et al. (2024) summarized that, in west central Ohio, soybean produced higher yields when planted in early April without a cover crop compared to mid-May planting dates. The study conducted by Robinson et al. (2009) summarized that soybean planting in mid-May produced only 76% yield from seed planted in late March.

Apart from weather pattern changes, trends towards larger farms, planting equipment capable of planting more area per day, and availability of effective seed treatments to combat seedling diseases have also prompted a shift towards earlier planting of soybeans (USDA-ERS, 2024). MacDonald et al. (2013) reported that, from 1987 to 2007, soybean plant hectarage has doubled in size. Those factors also aided further early planting decisions taken by farmers. Discussions are always underway among farmers about the advantages of late versus early soybean planting in terms of yield. Consequently, planting date decisions can fluctuate based on these perceived yield advantages (Licht, 2021). Studies conducted in Midwestern regions have shown yield penalties due to late planting of soybean. Research conducted by Hankinson et al. (2015) found that in certain regions of Ohio, soybean yield decreased by up to 38 kg ha^{-1} day^{-1} when planting occurred after May 1. Similarly, a study by Knott et al. (2019) noted a 0.5% yield reduction for each day of planting delay after May 8 in Kentucky. A recent study conducted in Iowa reported that a 30-day delay in planting date results in a reduction in seed filling duration by 5 to 10 days (Conely et al., n.d.).

1.3 Risk of early planting

Even though these warm spring temperatures allow farmers to go with early planting dates, there is a chance of adverse weather events that can negatively affect the planting decisions. Although early planting produces yield advantage, it also is associated with certain risks driven by environmental conditions such as low temperature and frost or freeze (chilling) damage (Specht et al., 2012). Soybean is a chilling-sensitive plant that requires a temperature of soil above 10℃ and the optimal temperature is 25℃ for successful germination (Haider et al., 2023). Most past studies observed that the greatest chilling sensitivity occurs during seed germination (Hobbs and Obendrof, 1972; Bramlage et al., 1978). Hence, it is important to understand the physiological and biochemical changes during seed germination.

1.3.1 Seed germination

Seed germination is the first condition of growth for soybeans, beginning with water uptake (aka imbibition) and ending with radicle extrusion (El-Maarouf-Bouteau, H. 2022; Haidar et al., 2023). It occurs over three phases and combines both physical and physiological processes. Imbibition is the first phase of germination and starts with rapid water uptake by cell walls and colloidal compounds. Total water uptake and rate of uptake depend on the water potential between seed and the environment, soil properties, and seed composition (Woodstock, 1988; Haider et al., 2023). Within the first few minutes of the imbibition, the seed coat is wetted and absorbed gases are released (Parrish & Leopold, 1977; McDonald, 1994). During this phase, the seed starts to swell and hydrolyze seed tissues which induces the transformation of the cell membrane from a gel state to a liquid crystalline state (Nonogaki et al., 2010; Szczerba et al., 2021; El-Maarouf-Bouteau, H. 2022). During imbibition, temporary damage occurs to the cell membrane, leading to

leakage of various substances from the seed. This highlights the crucial role of the cell membrane's resilience and repair mechanisms in mitigating such damage (Duke et al., 1983; McDonald, 1994). This leakage becomes more pronounced when the seeds deteriorate. The second phase of germination is referred to as the lag or activation phase and is marked by slowed rates of water uptake and the initiation of respiration metabolism by activating ATP synthesis, hormone metabolism and signalization, and mobilization of metabolic reserves (Suo et al., 2022), and preparation of elongation and cell division (Szczerba et al., 2021; El-Maarouf-Bouteau, H. 2022). During the third phase, growth, water uptake resumes to ensure root and radicle elongation and energy reserve mobilization. Plant hormones such as gibberellic acid (GA), abscisic acid (ABA), play a major role to regulate this germination process directly or indirectly (Nonogaki et al., 2010; Haider et al., 2023).

Many studies have attempted to explain the physiological and biological responses of seeds during imbibition. McDonald et al. (1988) explained seed water potential and the process of initial wetting of seed coat and tissues. Webster and Leopold (1977) reported an increase in respiratory activity and extensive membrane reorganization of cellular organelles occur during the same time. Duke et al. (1983) observed about solute leakage from soybean seeds during seed germination. Planting into cool wet soil could impair the germination process and be associated with certain risks. Recent studies conducted by Suo et al. (2022) and Haider et al. (2023) found more about biochemical and genetic changes happening inside seeds associated with physiological responses. Both studies investigated the seed's tolerance and resistance pathways to cold temperatures and waterlogging conditions, focusing on several genes and molecular pathways. Haider et al. (2023) specifically examined how cold tolerance develops within the seed by exposing it to temperatures of only for 4℃ and 20℃ for approximately 16 hours.

1.3.2 Germination and cold stress during emergence

Imbibition is the most critical stage of seed germination (Hobbs and Obendrof, 1972). Imbibitional chilling, a risk that occurs when dry seeds absorb water below 10°C during the initial phase of germination (Duke et al., 1977; Leopold & Musgrave, 1979; Szczerba et al., 2021), often coincides with planting into cold soils or potentially could occur in a field if planted shortly prior to a cold front. This not only brings colder temperatures but could also cause cold rainfall within 24 hours of planting, potentially causing injury to the seeds or seedlings (Licht n.d.). Chilling injury is another physiological disorder that occurs when soil temperature changes after imbibition (Herner, 1986). Suo et al. (2022) recorded that the optimal temperature for soybean seed germination is 25°C, and the base temperature is 4°C. They mentioned that germination does not occur at temperatures below this base temperature. Furthermore, they highlighted that temperature is not the sole determinant of germination. The duration for which the seeds are exposed to specific temperatures also plays a crucial role in the germination process. Cold stress during imbibition impairs the reorganization of cell membrane and selective permeability resulting loss of important cellular components including proteins, lipids, and ions (Bramlage et al., 1978; Cheng et al., 2010). This membrane leakage could potentially increase pre-emergence damping off promote microbial growth and disease pressure and cause further adverse effects for the germination (Bohner, 2003). When dry seeds absorb water at temperatures below 10°C, known as chilling temperatures, but still above freezing, the phospholipids in the cell membrane are unable to change rapidly into a hydrated phase due to rigid molecular shape (Simon, 1974). Visual symptoms of chilling injury included transverse cracking (cotyledons displaying outer layers of dead tissue) and browning of cotyledons (Hobbs and Obendorf, 1972; Tully et al., 1981). Long-term exposure to cold temperatures can slow down the rate of germination by slowing growing degree day (GDD)s

accumulation and soil crusting (Herner, 1990). The study conducted by Casteel, 2021 revealed that 50% of soybean emergence occurs with the accumulation of 140 to 160 GDDs (Fahrenheit (°F)). Moreover, they have highlighted that if soybeans were planted in cold, wet soil conditions and fail to emerge after 160 GDDs, it may be necessary to consider replanting. Injuries to the seeds as a result of cold temperatures manifest as poor emergence, increased decay, production of abnormal seedlings, poor seedling vigor, and seed death (Hobbs & Obendorf, 1972; McDonald et al., 1988; Herner, 1990; Suo et al., 2022). Cool and wet soil conditions, often a consequence of early planting, can lead to the movement of soil aggregates into open pores. This process can subsequently result in soil crusting (Vann & Stokes, 2020).

Poor emergence is of major agricultural risk since germination in cold soil can markedly reduce productivity. Reduced emergence due to imbibitional chilling or cold injury comes at a high cost. If a field needs to be re-planted, there is an additional cost of seed, labor, fuel, and equipment. Conely et al. (2012) reported that early-planted soybeans yielded maximally with final stands between 240,000 to 330,000 plants per hectare. However, when stand counts drop to less than 120,000 plants per hectare, replanting becomes a feasible option, but other costs and added late planting yield penalties also need to be considered (Gaspar & Conley, 2015). Some regions of Ohio recorded lower stand counts due to early planting, which was attributed to a combination of factors such as disease, slug damage, and frost damage (Sonnenberg, 2023). In some Ohio farm fields in 2021, even when adequate stands emerged, the seedlings exhibited reduced vigor and appeared sickly due to slug damage due to cold temperature driven by snow fall.

1.3.3. Factors affecting seed germination and their response to chilling temperature.

One of the crucial factors for maximizing soybean production is the establishment of an optimum seedling stand following planting (Wuebker et al., 2001). Germination and emergence of soybeans is a function of the interaction of the seed with its surrounding environmental factors including temperature, moisture content, and growth media composition (Hatfield & Egli, 1974). The extent of cold injuries depends on temperature, the timing of low-temperature exposure, stage of germination, seed moisture content, seed coat integrity, rate of imbibition, and seed vigor (Herner, 1990).

Temperature is an abiotic factor that significantly impacts soybean growth and yield and plays a major role controlling plant morphological and physiological process. Exposure to cold temperature is one temperature stress that soybean seeds and seedlings face due to early planting practices (Alsajri, 2018). Soil temperature is an important factor in germination success rate and seedling establishment (Haider et al., 2023). Some research has shown that the rate of hypocotyl elongation increases as temperature increases during seed germination (Wuebker et al., 2001) and imbibitional water uptake is faster with warmer compared to colder temperatures (Roskruge and Smith, 1997). Hatfield & Egli (1974) found that as temperature increased from 10 to 25℃, the seedling elongation rate for soybean increased.

The rate of water uptake is also affected by seed coat characteristics and integrity. Seeds with damaged seed coats imbibe water rapidly and damages increase under low temperature. Study conducted by McDonald et al. (1988) studied that water uptake by different parts of a seed when exposed to cold temperatures. The findings revealed that the embryonic axis was the most hydrated part during imbibition. Seed vigor and cultivar differences also affect the rate of imbibitional chilling injury. Low-vigor seeds showed more injuries by imbibition at low temperatures. Injuries

to the chilling-sensitive seed during cold temperature conditions are driven by the membrane composition of the seed (Szczerba et al., 2021). Several studies have been conducted to identify the correlation between membrane composition and susceptibility to chilling injury during seed germination. Some reports have detected a correlation between higher unsaturated and saturated fatty acid ratios and susceptibility to chilling injuries during low temperatures (Dogras et al., 1977). Some reports suggest the difference in the type of phospholipid synthesized in cotyledonary and axis tissue, and the activity of membrane-bound enzymes during germination and early seed growth under low temperatures (Yu et al., 2015; Dhaliwal & Angeles-shim, 2022).

Moisture content in the growing media has a significant effect on soybean emergence. Planting into a moist seedbed with good seed-to-soil contact is necessary for germination to occur. According to Staton (2021), soybean seed must imbibe 50% of its weight in moisture for the germination process to begin and remain above 20% moisture after the seed swells and the seed coat splits. Furthermore, lack of oxygen in saturated soil and formation of a soil crust during soybean germination may cause reduced or uneven emergence. Results from a laboratory study conducted by Wuebker et al. (2001) were that soybean germination was reduced by 15% after only one hour of flood conditions.

The ideal planting depth for soybean is 2.5 to 3.8 cm where tillage is used and low in moisture or sand (Lawson et al., 2009; Lindsey, 2018b). It is key to plant soybeans at enough depth to imbibe moisture to have a uniform emergence (Staton, 2023). Planting depth can vary under different field conditions such as soil moisture, soil type, seed, and tillage conditions. Furthermore, the size of the seed and seedling vigor ratings also need to be considered when deciding the planting depth. Planting soybeans too shallow can result in uneven emergence due to inconsistent moisture content and the seeding depth. Shallow planting however may be used in some conditions

like early planting when adequate soil moisture conditions exist. Stucky (1976) has found that temperature was the most pronounced factor for soybean emergence and average time for emergence is low when temperature increases to 16 to 32℃. Within each temperature, shallower planting depth resulted shorter emergence time. Recent recommendations from the Midwest US, though, suggest deeper planting of soybeans (4.4-5.0 cm) may be recommended if low soil moisture levels are present at the time of planting (Ernst, 2024).

1.3.4 Early planting and *Fusarium graminearum*

Early planting can also lead to increased disease pressure. Seed and seedling disease associated with early planting due to unfavorable environmental conditions also affect rapid seed germination, emergence, and plant stand. Soybean damping-off is most common seedling disease, caused by *Phytophthora sojae, Pythium spp.*, and *Fusarium graminearum* which are some of the most common seedling pathogens in Ohio. These pathogens can cause seed and root rot, pre and post-emergence damping off during seedling stage (Broders et al., 2007; Ellis et al., 2011; Marburger et al., 2017). Among these pathogens, *F. graminearum* has been recently identified as a pathogen of soybeans (Fernandez and Fernandes, 1990). *F. graminearum* is primarily considered as an economically important pathogen for wheat and other cereal crops (Ellis et al., 2011). This is often due to some management practices of crop rotation and shifting soybean planting dates into early dates. In Ohio, farmers often practice crop rotation mainly corn-soybean and corn-soybean-wheat with no till or reduced till practices. The high volume of plant debris presents in the soil due to these reduced tillage conditions serves as a primary source of inoculum, allowing it to overwinter and affect the crop in the subsequent season. (Cruz et al., 2020).

Cool and wet soil conditions likely to occur with early planting provide favorable conditions for infection by *F. graminearum*. Additionally, they could also slow germination and emergence resulting in more time where seedlings are susceptible to infection by these pathogens. Symptoms of these infections appear as light-brown lesions on the root and shoot on emerged seedlings, then become necrotic and subsequently plants wilt and die. Disease occurrence and severity depends on three factors, including the susceptibility of the host plant, the pathogen presence, and conducive environmental conditions for infection to occur (the disease triangle; Francl, 2001). The disease's biotic causal agent interacts with a host plant under favorable environmental conditions for disease development (Scholthof, 2007). Yan and Nelson (2022) observed that significant reduction in soybean emergence occurred at 10℃ in treatment with Fusarium compared to 20 and 25℃. Brennan et al. (2003) observed that optimal mycelial growth of Fusarium and inhibition of wheat seedling growth occurred within a temperature range of 10-30℃. Therefore, it is important to understand how cool and wet soil conditions associated with early planting dates affect disease severity in soybean.

1.4. Research evidence on soybean seed germination and effect of environmental factors.

There are some research evidences that studied about effect of low temperature and other factors effect on soybean seed germination and emergence (Table 1.1). McDonald et al. (1988) demonstrated that the presence of a seed coat can delay water uptake during the first eight hours of soaking. The soybean seed coat, being extremely hydrophilic, can absorb up to 3.8 times its fresh weight in water, thereby reducing the risk of imbibitional injury from rapid water uptake. Herner (1990) found that more injuries occur when seeds are exposed to low temperatures compared to moderate temperatures. Little to no injuries were observed when imbibition started

with exposure to warm temperatures (25℃) before a cold temperature (5℃). Obendorf & Hobbs (1970) studied the effect of seed moisture and temperature sensitivity. They revealed that some soybean varieties with 6% (fresh weight basis) recorded up to a 75% reduction in survival when exposed to 5℃ compared to seeds with 16% moisture content. However, low moisture seeds initially exposed to 25℃ before exposure to cold showed an increased survival rate. Bramlage et al. (1978) investigated the impact of chilling stress on soybeans during imbibition. They analyzed solute leakage patterns of soybean seeds with different moisture content, subjected to varying durations of chilling and normal temperatures. Leopold & Musgrave (1979) observed that chilling temperatures caused respiratory changes and altered solute leakage patterns. Hence it is important to understand that how other environmental and management practices such as, moisture content at planting, planting depth and temperature affect emergence and standability of soybean. Farmers often make their planting and replanting decisions with limited knowledge of how these factors affect their seeds and stand. Improving recommendations related to these factors will help farmers make better management decisions related to planting, seeding rate, replanting, and will help reduce waste and improve efficiency.

Table 1.1. Past research evidence regarding cold temperature effect on soybean germination and emergence

Citation	Cold temperature range	duration	Seed part	Measurement	Other factors tested
Bramlage et al., 1978	2℃ to 25℃ (2,6,8,10,12,14,16,25℃)	1hr	Cotyledon or Embryo	Solute leakage	Moisture content of cotyledon
Obendorf and Hobbs, 1970	5℃ and 25℃	12h at 5 and 25℃ 24h at 5℃ 12h at 25℃ plus 24h at 5c 36h at 5℃	Seeds	Seedling weight and height (2 weeks old) Seed water uptake during imbibition	Seed moisture
Haider et al., 2023	4℃ and 20℃	16h at either 4℃ or 20℃	Seeds	Cold tolerance QTL identify. Genome-wide analysis	Soybean cultivars
Szczerba et al., 2021	10, 15 and 25℃	48h	Seeds	Amylase and dehydrogenase activity Cell membrane permeability Germination vigor Yield	Soybean cultivars
Hobbs and Obendrof, 1972	5 and 25℃	36h at 5℃ 12h at 25℃	Seeds	Survival and seedling growth Solute leakage Yield	Seed moisture
Leopold and Musgrave, 1979	Room temperature (22-24℃) Ice bath (1-4℃)	2h (continuous leakage measurement up to 2h)	Peeled cotyledon	Solute leakage Respiratory rate	
McDonald et al., 1988		72h	Seed coat Embryonic axis Cotyledons whole seed	Water uptake by seed parts	

Duke et al., 1977	10 to 30℃	72h	Mitochondria isolated from embryonic axis	Mitochondrial respiration	

1.5.Summary

Soybean ranks as the second most widely cultivated field crop in the U.S., and it holds the top spot in Ohio. Farmers, aiming to capitalize on the length of the growing season driven by warm spring temperatures, have started planting soybeans two to six weeks ahead of the recommended planting dates. However, this practice carries a risk. Cold temperatures and cold precipitation after planting can cause imbibitional chilling injuries to soybean germination and early seed growth. This can lead to poor emergence, potentially requiring producers to re-plant a field. Such a scenario would negate the yield gains from early planting and result in increased costs. In response to these concerns, the topic of imbibitional chilling has been featured in the Ohio State University Extension's Agronomic Crops Team newsletter (CORN) in the past six years.

As detailed in Table 1.1, there is a lack of recent studies on the impact of cold temperatures on soybean germination and emergence. Most of the past research has focused on micro-level changes to seeds, such as solute leakage patterns, cell membrane permeability, genome-wide analysis, and respiratory changes in response to cold temperatures. While these studies have examined a range of cold temperatures (below 10℃ but above 0℃), almost all have used a temperature range of 25 to 30℃ as a reference for warm conditions. However, in early April in Ohio, soil temperatures are more likely to fall between 10 to 15℃. This discrepancy could potentially lead to different responses in soybean seeds under imbibition and early seed germination.

It is important to note that cold temperature exposure is not the sole factor affecting imbibitional chilling. Other elements, such as the duration of exposure to specific cold temperatures, environmental factors like soil moisture, management practices such as planting depth, along with

seed vigor and cultivar characteristics, also play significant roles. Therefore, understanding how these factors affect soybean emergence and early growth is vital for making early planting decisions. Much of the existing research has focused on a limited range of exposure times to cold temperatures. Only a few studies have explored the effects of varying timing of exposure durations, and these have primarily examined micro-level changes in seeds. Moreover, there is a lack of research evidence regarding the optimum soil moisture needed for soybean emergence. The impact of planting depth on early planting and the associated risks of cold temperatures also need to further investigation. Consequently, several research gaps exist, particularly concerning how the duration of exposure to specific low-temperature ranges, soil moisture content, and planting depth affect soybean germination and emergence. This understanding can assist farmers in refining their agronomic strategies, ultimately leading to enhanced soybean productivity.

References

Abendroth, L. J., Miguez, F. E., Castellano, M. J., & Hatfield, J. L. (2019). Climate warming trends in the US Midwest using four thermal models. *Agronomy Journal*, *111*(6), 3230-3243. https://doi.org/10.2134/agronj2019.02.0118

Alsajri, F. A. (2018). *Temperature Effects on Soybean Growth, Development, Physiology, Yield and Seed Quality*. Mississippi State University.

Baule, W., Allred, B., Frankenberger, J., Gamble, D., Andresen, J., Gunn, K.M., & Brown, L. (2017). Northwest Ohio crop yield benefits of water capture and subirrigation based on future climate change projections. *Agricultural Water Management, 180*, 87-97. http://dx.doi.org/10.1016/j.agwat.2017.04.019

Bohner, H. 2003. Do soil temperatures at planting impact soybean yield? Ministry of Agriculture, Food and Rural Affairs. Available at; https://cropwatch.unl.edu/2019-CW-News/2019-images/soybean/Do-soil-temperatures-at-planting-impact-soybean-yield-Horst Bohner.pdf (accessed May 2023).

Bramlage, W.J., Leopold, A.C. and Parrish, D.J. 1978. Chilling stress to soybeans during imbibition. *Plant Physiology*, 61(4), pp.525-529. https://doi.org/10.1104/pp.61.4.525

Broders, K. D., Lipps, P. E., Paul, P. A., & Dorrance, A. E. (2007). Evaluation of Fusarium graminearum associated with corn and soybean seed and seedling disease in Ohio. Plant disease, 91(9), 1155-1160. https://doi.org/10.1094/PDIS-91-9-1155.

Cheng, L., Gao, X., Li, S., Shi, M., Javeed, H., Jing, X., ... & He, G. (2010). Proteomic analysis of soybean [Glycine max (L.) Meer.] seeds during imbibition at chilling temperature. Molecular Breeding, 26, 1-17. https://doi.org/10.1007/s11032-009-9371-y

Conley S., Holshouser D., Inman M., Lee C., Lindsey L., Licht M., Kandel H., Kleinjan J., Knott C., Naeve S., Nafziger E., Ross J., Singh M., Specht J. & Vann R., (n.d.). The best soybean planting date, The best soybean management practices by Extension researchers from across the United States, Science for Success, University of Michigan State. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.canr.msu.edu/agronomy/Science%20for%20Success-%20Planting%20Date.pdf.

Cruz, D. R., Leandro, L. F. S., Mayfield, D. A., Meng, Y., & Munkvold, G. P. (2020). Effects of soil conditions on root rot of soybean caused by Fusarium graminearum. *Phytopathology*, *110*(10), 1693-1703. https://doi.org/10.1094/PHYTO-02-20-0052-R

De Bruin, J. L., & Pedersen, P. (2008). Soybean seed yield response to planting date and seeding rate in the Upper Midwest. *Agronomy Journal*, *100*(3), 696-703. https://doi.org/10.2134/agronj2007.0115

Dhaliwal, L. K., & Angeles-Shim, R. B. (2022). Cell membrane features as potential breeding targets to improve cold germination ability of seeds. *Plants*, *11*(23), 3400.

Dogras, C. C., Dilley, D. R., & Herner, R. C. (1977). Phospholipid biosynthesis and fatty acid content in relation to chilling injury during germination of seeds. *Plant Physiology*, *60*(6), 897-902.

Duke, S. H., Kakefuda, G., & Harvey, T. M. (1983). Differential leakage of intracellular substances from imbibing soybean seeds. *Plant Physiology*, *72*(4), 919-924. https://doi.org/10.1104/pp.72.4.919.

Egli, D. B. and Cornelius, P. L. 2009. A regional analysis of the response of soybean yield to planting date. *Agronomy Journal*, 101(2), pp. 330-335. https://doi.org/10.2134/agronj2008.0148

Ellis, M. L., Broders, K. D., Paul, P. A., & Dorrance, A. E. (2011). Infection of soybean seed by Fusarium graminearum and effect of seed treatments on disease under controlled conditions. Plant disease, 95(4), 401-407. https://doi.org/10.1094/PDIS-05-10-0317.

El-Maarouf-Bouteau, H. (2022). The seed and the metabolism regulation. Biology, 11(2), 168.

Ernst, M. (2024). Seeding: Back to the Basics. *Crops & Soils*, *57*(3), 12-17. https://doi.org/10.1002/crso.20357

Fernandez, M. R., and Fernandes, J. M. C. 1990. Survival of wheat pathogens in wheat and soybean residues under conservation tillage systems in southern Brazil Can. J. Plant Pathol.-Rev. Can. Phytopathol. 12:289-294.

Gaspar, A. P., & Conley, S. P. (2015). Responses of canopy reflectance, light interception, and soybean seed yield to replanting suboptimal stands. *Crop Science*, *55*(1), 377-385. https://doi.org/10.2135/cropsci2014.03.0200

Grady, K. A., Chen, L., & Ford, T. W. (2021). Projected changes to spring and summer precipitation in the Midwestern United States. *Frontiers in Water*, *3*, 780333. https://doi.org/10.3389/frwa.2021.780333

Haidar, S., Lackey, S., Charette, M., Yoosefzadeh-Najafabadi, M., Belzile, F., Rajcan, I., ... & Samanfar, B. (2023). Genome-wide analysis of cold imbibition stress in soybean, Glycine max. Frontiers in Plant Science, 14, 1221644.

Hatfield, J.L. and Egli, D.B. 1974. Effect of temperature on the rate of soybean hypocotyl elongation and field emergence 1. *Crop Science*, 14(3), pp.423-426. https://doi.org/10.2135/cropsci1974.0011183X001400030025x

Hankinson, M. W., Lindsey, L. E., & Culman, S. W. 2015. Effect of planting date and starter fertilizer on soybean grain yield. Crop, Forage & Turfgrass Management, 1(1), 1-6. https://doi.org/10.2134/cftm2015.0178

Herner, R. C. (1986). Germination under cold soil conditions. *HortScience*, *21*(5), 1118-1122.

Herner, R. C. (1990). The effects of chilling temperatures during seed germination and early seedling growth. *Chilling injury of horticultural crops*, 51-69.

Hobbs, P.R. and Obendorf, R.L. 1972. Interaction of Initial Seed Moisture and Imbibitional Temperature on Germination and Productivity of Soybean. *Crop Science*, 12(5), pp.664-667. https://doi.org/10.2135/cropsci1972.0011183X001200050033x

Hu, M., & Wiatrak, P. 2012. Effect of planting date on soybean growth, yield, and grain quality. *Agronomy Journal*, 104(3), 785-790. https://doi.org/10.2134/agronj2011.0382

Kannberg, S., Lindsey, A. J., Chiavegato, M. B., & Lindsey, L. E. (2024). Effect of ultra-early, early, and normal soybean planting dates and rye cover crop on soybean grain yield. *Agronomy Journal*. https://doi.org/10.1002/agj2.21550

Knott, C., Herbek, J., & James, J. (2019). Early Planting dates maximize soybean yield in Kentucky. *Crop, Forage & Turfgrass Management*, *5*, 180085. https://doi.org/10.2134/cftm2018.10.0085

Kunkel, K. E., L. E. Stevens, S. E. Stevens, L. Sun, E. Janssen, D. Wuebbles, S. D. Hilberg, M. S. Timlin, L. Stoecker, N. E. Westcott, and J. G. Dobson. 2013: Regional Climate Trends and Scenarios for the U.S. National Climate Assessment: Part 3. Climate of the Midwest US. NOAA Technical Report NESDIS 142-3. 103 pp., National Oceanic and Atmospheric Administration, National Environmental Satellite, Data, and Information Service, Washington, D.C

Leopold, A. C., & Musgrave, M. E. (1979). Respiratory changes with chilling injury of soybeans. *Plant physiology*, *64*(5), 702-705. https://doi.org/10.1104/pp.64.5.702

Licht M., Integrated crop management, Extension and Outreach. Iowa state university. Imbibitional chilling or cold injury | Integrated Crop Management (iastate.edu)

Licht M. (2021). Corn, and Soybean Planting Date Considerations. Integrated crop management, Extension and Outreach. Iowa state university. Available at https://crops.extension.iastate.edu/blog/mark-licht-zachary-clemens/corn-and-soybean-planting-date-considerations. (accessed 24 February 2024).

Lindsey, L. E., Tilmon, K., Michel, A., & Dorrance, A. (2017). Soybean production. In L. E. Lindsey & P. R. Thomison (Eds.), Ohio agronomy guide (15th ed., Bulletin no. 472, pp. 56–68). The Ohio State University Extension.

Lindsey, A.J., Ortez, O.A., Thomison, P.R., Carter, P.R., Coulter, J.A., Roth, G.W., Carrijo, D.R., Quinn, D.J. & Licht, M.A. (2024). Severe storm damage and short-term weather stresses on corn: A review. *Crop Science*. https://doi.org/10.1002/csc2.21212

MacDonald, J. M., Korb, P., & Hoppe, R. A. (2013). Farm size and the organization of US crop farming. Available at https://ageconsearch.umn.edu/record/262221.

McDonald, M. B. (1994). Seed germination and seedling establishment. *Physiology and determination of crop yield*, 37-60. https://doi.org/10.2134/1994.physiologyanddetermination.c3

Mourtzinis, S., Specht, J.E. and Conley, S.P. 2019. Defining optimal soybean sowing dates across the US. *Scientific Reports*, 9(1), pp.2800. https://doi.org/10.1038/s41598-019-38971-3

Nonogaki, H., Bassel, G. W., & Bewley, J. D. (2010). Germination—still a mystery. Plant Science, 179(6), 574-581. https://doi.org/10.1016/j.plantsci.2010.02.010

Obendorf, R. L., & Hobbs, P. R. (1970). Effect of Seed Moisture on Temperature Sensitivity During Imbibition of Soybean 1. *Crop science*, 10(5), 563-566. https://doi.org/10.2135/cropsci1970.0011183X001000050034x

Ohio soybean council, fact sheet, 2024. Available at chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.soyohio.org/wp-content/uploads/2013/07/Ohio-Soybean-Council-Fact-Sheet-UPDATED.pdf. (accessed 20 March 2024).

O'Neal, M.R., Nearing, M.A., Vining, R.C., Southworth, J., & Pfeifer, R. A. (2005). Climate change impacts on soil erosion in Midwest United States with changes in crop management. Catena, 61, 165-184. https://dx.doi.org/10.1016/j.catena.2005.03.003

Parrish, D. J., & Leopold, A. C. (1977). Transient changes during soybean imbibition. *Plant Physiology*, *59*(6), 1111-1115. https://doi.org/10.1104/pp.59.6.1111

Pryor, S. C., Barthelmie, R. J., & Schoof, J. T. (2013). High-resolution projections of climate-related risks for the Midwestern USA. *Climate research*, *56*(1), 61-79.

Robinson, A. P., Conley, S. P., Volenec, J. J., & Santini, J. B. (2009). Analysis of high yielding, early-planted soybean in Indiana. *Agronomy Journal*, *101*(1), 131-139. https://doi.org/10.2134/agronj2008.0014x

Roskruge, C.L. and Smith, M.T. 1997. Peroxidative changes associated with chilling injury in soybean seeds during imbibition. *Journal of Plant Physiology*, 151(5), pp.620-626. https://doi.org/10.1016/S0176-1617(97)80239-8.

Rowntree, S.C., Suhre, J.J., Weidenbenner, N.H., Wilson, E.W., Davis, V.M., Naeve, S.L., Casteel, S.N., Diers, B.W., Esker, P.D., Specht, J.E. and Conley, S.P. 2013. Genetic

gain× management interactions in soybean: I. Planting date. *Crop Science*, 53(3), pp.1128-1138. https://doi.org/10.2135/cropsci2012.03.0157.

Scholthof, K. B. G. (2007). The disease triangle: pathogens, the environment and society. *Nature Reviews Microbiology*, *5*(2), 152-156. https://doi.org/10.1038/nrmicro1596.

Schoof, J. T., Pryor, S. C., & Surprenant, J. (2010). Development of daily precipitation projections for the United States based on probabilistic downscaling. *Journal of Geophysical Research: Atmospheres*, *115*(D13). https://doi.org/10.1029/2009JD013030

Schrage S., 2018. 115 years of data reveal longer U.S. growing season, temp trends, University communication and marketing, University of Nebraska, Lincoln. Available at https://news.unl.edu/newsrooms/today/article/115-years-of-data-reveal-longer-us-growing-season-temp-trends. (accessed 3 April 2024).

Shaun Casteel, 2021. Soybean stands:Emergence?Replant?. Extension Entomology, Purdue University. https://extension.entm.purdue.edu/newsletters/pestandcrop/article/soybean-stands-emergence-replant/. (accessed 06 June 2024).

Simon, E. (1974). Phospholipids and plant membrane permeability. *New Phytologist*, *73*(3), 377-420. https://doi.org/10.1111/j.1469-8137.1974.tb02118.x

Sonnenberg D, 2020. Ohio soybean production and research in 2020. Ohio Ag Net, Ohio's Country Journal. Available at https://ocj.com/2020/11/ohio-soybean-production-and-research-in-2020/. (accessed 21 March 2024).

Sonnenberg D, 2023. Early soybean planting and stand evaluation. Ohio Ag Net, Ohio's Country Journal. Available at; https://ocj.com/2023/05/early-soybean-planting-and-stand-evaluation/ (accessed 1 April 2024).

Southworth, J., Pfeifer, R. A., Habeck, M., Randolph, J. C., Doering, O. C., Johnston, J. J., & Rao, D. G. (2002). Changes in soybean yields in the Midwestern United States as a result of future changes in climate, climate variability, and CO 2 fertilization. *Climatic Change*, *53*, 447-475. https://doi.org/10.1023/A:1015266425630

Specht, James E., Jennifer M. Rees, Gary L. Zoubek, Keith L. Glewen, Brandy S. VanDeWalle, James W. Schneider, David L. Varner, and Allan R. Vyhnalek. (2012). Soybean planting date: When and Why? Nebraska Extension EC145. http://extensionpubs.unl.edu/publication/9000016368318/soybean-planting-date-when-and-why/

Specht, James E. and Thomas Hoegemeyer. 2017. Nebraska Soybean and Corn Pocket Field Guide, 2017 Edition.

Staton M., 2023. Soybean planting depth considerations when planting into dry soil conditions. Michigan State University Extension. Michigon State University. Available at; https://www.canr.msu.edu/news/soybean-planting-depth-considerations-when-planting-into-dry-soil-conditions (accessed 09 April, 2024).

Suo, R., Sandhu, K., Wang, M., You, F., Conner, R., Cober, E., & Hou, A. (2022). Soybean (Glycine max L.) seed germination in response to waterlogging and cold climate: a review on the genetics and molecular mechanisms of resistance to the abiotic stress. Canadian Journal of Plant Science, 103(1), 13-28. https://doi.org/10.1139/cjps-2022-0111

Szczerba, A., Płażek, A., Pastuszak, J., Kopeć, P., Hornyák, M., & Dubert, F. (2021). Effect of low temperature on germination, growth, and seed yield of four soybean (Glycine max L.) cultivars. Agronomy, 11(4), 800.

Tully, R.E., Musgrave, M.E. and Leopold, A.C. 1981. The seed coat as a control of imbibitional chilling injury. *Crop Science*, 21(2), pp.312-317. https://doi.org/10.2135/cropsci1981.0011183X002100020026x.

USDA coexistence fact sheets soybeans, (2015). United State Department of Agriculture. Available at chrome extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.usda.gov/sites/default/files/documents/coexistence-soybeans-factsheet.pdf. (accessed 22 March 2024).

USDA-ERS, (202).). Economic Research Service. U.S. Department of Agriculture. Soybean-Overview. Available at, https://www.ers.usda.gov/topics/crops/soybeans-and-oil-crops/. (accessed 19 August 2023)

USDA-ERS, (2024).). Economic Research Service. U.S. Department of Agriculture. Soybean-Overview. Available at, https://www.ers.usda.gov/topics/crops/soybeans-and-oil-crops/. (accessed 10 March 2024)

USDA-FAS, (2024). Foreign Agricultural Service, U.S. Department of Agriculture. U.S. Soybean exports in 2023. Available at https://fas.usda.gov/data/commodities/soybeans. (accessed 21 March 2024).

USDA NASS. (2024). USDA National Agricultural Statistics Service. Quick Stats. Available at https://quickstats.nass.usda.gov/. (accessed 20 February 2024).

United Soybean Board, (2024). Global market. Available at https://www.unitedsoybean.org/issue-briefs/global-markets/. (accessed 22 March 2024).

United States Environmental Protection Agency, (2024). Available at https://www.epa.gov/climate-indicators/climate-change-indicators-length-growing-season. (accessed 20 March 2024).

Vann R., and Stokes D., (2020). Soil crusting, Nebraska State University. Soybean Crusting | NC State Extension Publications (ncsu.edu)

Webster, B. D., & Leopold, A. C. (1977). The ultrastructure of dry and imbibed cotyledons of soybean. *American Journal of Botany*, *64*(10), 1286-1293. https://doi.org/10.1002/j.1537-2197.1977.tb10822.x.

Woodstock, L. W. (1988). Seed imbibition: a critical period for successful germination. Journal of Seed Technology, 1-15.

Wuebker, E.F., Mullen, R.E. and Koehler, K. 2001. Flooding and temperature effects soybean germination. *Crop Science*, 41(6), pp.1857-1861. https://doi.org/10.2135/cropsci2001.1857

Xue, A. G., Cober, E., Voldeng, H. D., Voldeng, H. D., Babcock, C., & Clear, R. M. (2007). Evaluation of the pathogenicity of Fusarium graminearum and Fusarium pseudograminearum on soybean seedlings under controlled conditions. *Canadian journal of plant pathology*, *29*(1), 35-40. https://doi.org/10.1080/07060660709507435.

Yan, H., & Nelson Jr, B. (2022). Effects of soil type, temperature, and moisture on development of Fusarium root rot of soybean by Fusarium solani (FSSC 11) and Fusarium tricinctum. *Plant Disease*, *106*(11), 2974-2983. https://doi.org/10.1094/PDIS-12-21-2738-RE.

Yu XiaoMei, Y. X., Li AiHua, L. A., & Li WeiQi, L. W. (2015). How membranes organize during seed germination: three patterns of dynamic lipid remodelling define chilling resistance and affect plastid biogenesis. doi: 10.1111/pce.12494

Chapter 2. Exposure to cold temperatures during the imbibition phase minimally affected germination in soybean.

2.1 Abstract

The frost-free period in the Midwestern United States has increased over the last century, and farmers are interested in planting soybean early to capitalize on the benefits of the longer growing season. However, risks of cold precipitation and/or soil temperatures after planting may result in imbibitional chilling and hinder crop establishment. The objective of this study was to investigate how duration of exposure to cold conditions during the early germination phases affected seed germination in two soybean cultivars. Soybean seeds were exposed to one of 21 different treatments that varied in time spent in "warm" conditions prior to "cold" temperature exposure as well as the duration of "cold" exposure before being returned to the "warm" condition. The same duration treatments were conducted at with three temperature pairings that consisted of a "colder" and "warmer" temperature combination (1.7/10.0°C, 4.4/12.8°C and 7.2/15.5°C). Across temperature pairings, exposure for 12 to 24h in cold temperature immediately after starting the assay resulted in the greatest reduction (7%) in germination compared to the treatment that was not exposed to the cold temperature. Cultivar 3.4 RM was more affected by cold temperature exposure than the 3.7 RM cultivar. The preservation of germination totals in this study (all treatments >83% germination after 10 days) suggests stand reductions in the field may not be attributable solely to low temperatures.

2.2 Introduction

Imbibition is a critical process during seed germination (McDonald et al., 1988; Yin et al., 2009). Dry seeds absorb water and commence swelling, leading to the hydration of cellular

membranes and their transition to a functional state (Bohner, 2003). The physical process of water uptake is regulated by factors such as seed coat characteristics, seed osmotic potential, and internal composition and location of energy storage compounds. The imbibition process is also directly related with the establishment of an optimum seedling stand following planting (Wuebker et al., 2001). However, the temperature of the water absorbed during imbibition can affect the subsequent phases of germination by affecting cellular re-activation activities (e.g., mitochondrial maturation, RNA translation). Soybean [*Glycine max* (L.) Merr] is considered as warm season crop and recommendations for planting in Ohio are to ensure soil temperatures of 10°C are present to facilitate germination (Bramlage et al., 1978; Lindsey et al., 2017; Szczerba et al., 2021). Soybean is chilling sensitive, and injuries can occur when dry seeds absorb water below 10°C which is called imbibitional chilling (Bramlage et al., 1978; De Bruin & Pedersen, 2008; Duke et al., 1977; Leopold & Musgrave, 1979; Nielsen, 2020).

To avoid imbibitional chilling, timely planting is crucial, as planting date optimization promotes greater seasonal growth and development as well as enhanced yield potential (Hu & Wiatrak, 2012). Poor establishment associated with imbibitional chilling is a potential risk to soybean yields. In soybean, economically optimum plant population should be at least 108,000 to 232,000 plants ha^{-1}, to obtain more than 90 percent of optimum yield (Gaspar & Conley, 2015; Lee et al., 2008). While soybean is able to compensate for the lower population density through increased branching and pod setting (Gaspar & Conley, 2015), 69,160 plants ha^{-1} is often presented as the critical stand.

Due to environmental shifts brought by climate change, the questions of when to plant and what cultivars to plant are concerns farmers are more frequently faced with. Planting after May 1^{st} can decrease soybean yield by up to 38 kg ha^{-1} day^{-1} after May 1 (Hankinson et al., 2015; Rattalino

Edreira et al., 2017). Early planting can result in greater yields by maximizing the length of the growing season which allows soybean plants to capture more heat unit to produce more nodes on the main stem (Bandara et al., 2021; De Bruin & Pedersen, 2008; Hu & Wiatrak, 2012; Staton, 2018). Early planting, though, can result in seeds being exposed to cold soils and water during imbibition. Past studies have observed range of different durations for exposing soybean seeds to a range of cold temperatures (Bramlage et al., 1978; Hobbs & Obendorf, 1972; McDonald et al., 1988; Tully et al., 1981).

Although there is a growing body of researchers that emphasizes early planting to maximize production status (De Bruin & Pedersen, 2008; Egli & Cornelius., 2009; Kucharik, 2006; Rowntree et al., 2013), there is a still a knowledge gap related to the effects of the duration of cold temperature exposure during imbibition and shortly after planting on soybean seeds that emulates early to mid-April weather in Ohio. Much of the past work investigating seed response to cold temperatures has held seeds at 25°C prior to cold exposure, but soils in early April in Ohio are more likely to be between 13-16°C. There is a gap in knowledge related to how soybean respond to imbibitional chilling and if there is a differential effect on germination as a function of the duration of exposure to cold temperatures. Therefore, the objective of this study was to investigate how the duration of cold exposure during the imbibition phase affects seed germination in soybean cultivars and its effects on the formation of normal seedlings.

2.3 Materials and Methods

2.3.1 Seed lot characteristics

Two commercially available untreated soybean cultivars [Pioneer® 'P34A65PR' (3.4 relative maturity or RM) and 'P37T51PR' (3.7 RM)] were obtained within 6 months of project initiation and kept in 10°C cold storage until initiation of the experiments. Both soybean seed lots were untreated.

Prior to conducting the experiments, each lot was analyzed for internal seed moisture content, standard warm germination (AOSA, 2018), and two vigor tests of accelerated aging (AA) and cold germination tests (rolled towel method) were performed according to the methodology by the AOSA (1983). The standard germination test (SG), as outlined in AOSA (2018), was conducted on 100 seeds per cultivar using rolled paper towels (50 seeds per towel). Temperature-controlled chambers (Darwin Chamber or Percival Scientific Chamber) set to 25°C and 24 hours of day light cycle was employed for the SG test. For the AA vigor test, 42 g of seeds were placed in the inner chamber of AA boxes ensuring that the seeds did not come into contact with the distilled water (40 mL box^{-1}) present in the box. The AA boxes were then positioned in an outer AA chamber (VWR Scientific Products) for 72 hours (hr) at 41°C; seed moisture was confirmed to be between 270 and 300 g kg^{-1} at the conclusion of the AA period. To complete the AA test, seeds were removed from the outer chamber, and the SG test procedures on 100 seeds per lot were followed to determine final germination after 7 days (AOSA, 1983). For the cold germination test, the rolled towel cold germination vigor test with non-soil version as described by Loeffler et al. (1985) was employed (AOSA, 1983). Hundradseeds of each cultivar (50 seeds per towel) were placed in a saturated paper towel (78# heavy, Anchor Paper) and covered with a wet thin paper towel (38#

regular) and placed in a chamber with cold temperature of 10°C for seven days without light. After seven days, towels were transferred to warmer chamber at 25°C for seven days. Germination was counted at 4 and 7 days after transferring to the 25°C chamber. The results of the initial seed lot evaluations are presented in Table 2.1.

Table 2.1. Initial seed moisture content, standard warm germination percentage, and germination of normal seedlings after the accelerated aging and cold germination vigor tests for each soybean cultivar lot.

Soybean cultivar	Initial Seed Moisture Content	Standard Warm Germination Test	Accelerated Aging Vigor Test	Cold Germination Vigor Test
	g kg^{-1}	%		
3.4 RM	96	94	86	86
3.7 RM	92	95	82	80

2.3.2 Experimental Procedures

For the controlled environment experiment, exposure of seeds to colder temperatures during the early phases of germination was of interest to evaluate. Three pairings of "colder" and "warmer" temperatures were tested independently, and were selected as they emulate soil temperatures that could be experienced in Ohio under early-planting conditions:

1. Colder temperature of 7.2°C and warmer temperature of 15.5°C
2. Colder temperature of 4.4°C and warmer temperature of 12.8°C
3. Colder temperature of 1.6°C and warmer temperature of 10.0°C

Two chambers (Darwin chamber and Percival scientific chamber) were maintained at the assigned 'warmer' and 'colder' temperatures, respectively. Two replications of each temperature pairing were conducted over time. Paper towels (38# regular (thin) and 78# heavy (thick), Anchor Paper)

were moistened using distilled water and held at respective "warmer" temperature prior to starting the germination tests.

Fifty seeds of each soybean cultivar were placed onto a thick paper towel using seed counter tray. A single thin moist towel was laid on top of the seeds and rolled. Moist-rolled paper towels were used as the substratum and placed inside two plastic bags to retain moisture. Each rolled towel containing seeds was exposed to one of 21 cold temperature treatments (Table 2.2). These treatments, called exposure-duration (ED) treatments, varied for when the exposure to the "colder" temperature occurred after initial placement at the "warmer" temperature (exposed to cold at 0, 2, 4, 8, or 12 hrs after initiation) as well as how long the seeds experienced the colder temperature (3, 6, 12, or 24 hrs) before being returned to the warmer temperature. By 36 hrs after initiation, all ED treatment towels had been returned to the warmer temperature and remained in the warmer temperature chamber until 48 hrs after initiation had passed. The treatment that was not moved to the colder temperature and remained in the warmer chamber for the full 48 hr period was considered the untreated check (UTC). The first 48 hrs were conducted under dark conditions.

Table 2.2 Duration and timing of exposure to warmer and colder temperatures. Dashes indicate no treatments underwent this timing combination.

Initial time at warmer temperature (hours)	Time at colder temperature (hours)				
	0 (UTC)	3	6	12	24
0	X	X	X	X	X
2	--	X	X	X	X
4	--	X	X	X	X
8	--	X	X	X	X
12	--	X	X	X	X

After 48 hrs had passed from initiation, the temperature of the warmer chamber was raised to 25°C and rolled towels were provided a 14hr/10hr day/night light cycle for a duration of another 8 days (AOSA, 2018) to conclude the germination assay.

Germination percentages were taken at 4, 7 and 10 days after trial initiation and the number of abnormal seedlings and dead seeds were assessed at 10 days. At four days after initiation, seeds with emerged radicle were counted as germinated seeds. On the seventh- and tenth-days seedlings that exhibited well-balanced, symmetrical growth across all essential parts including roots, hypocotyl, and cotyledon, were counted as normal germinated seeds (AOSA, 2018). At 10 days, seedlings with radicles measuring 1 cm or longer, showing the shoot system (hypocotyl and cotyledons), were counted as germinated. Seedlings with malformed roots, missing or damaged cotyledon and weak or decayed hypocotyl were counted as abnormal seedlings at 10 days after initiation (AOSA, 2018).

2.3.3 Statistical Analysis

The experiment was conducted using a split-plot randomized complete block design. The temperature pairings were treated as the whole plot factor, and cultivar and cold exposure treatments being the sub-plot factors. Two replications of the whole plot factor were conducted in time. Analysis of variance (ANOVA) was performed within each germination measurement date using the PROC GLIMMIX procedure in SAS 9.4 at α=.05 (SAS Institute, Cary, NC; version 9.4). The fixed factors were temperature pairings (Temp), duration of exposure to cold temperatures ED) and soybean cultivar (C) and their interactions. The random factors were the replication and replication x Temp. Mean separation was conducted as a pair-wise comparison when the global F test in ANOVA was significant (α=.05). Variation of normal and abnormal seedling and dead seed

percentages were graphically represented using surface maps using Response Surface Methodology (RSM). The figures were generated in R, utilizing the 'rsm' and 'fields' packages.

2.4 Results and Discussion

At 4 days, germination was unaffected by Temp, ED, and their interactions. At 4 days, the 3.4 RM cultivar exhibited more germinated seeds (96.4%) compared to 3.7 RM (94.7%) across all other factors ($p < 0.001$; Table 2.3).

Table 2.3. Analysis of Variance table for germinated seeds, normal seedlings, abnormal seedlings, and dead seeds percentages of soybeans for temperature treatment (Temp), duration of exposure to low temperature (ED), cultivar (C), and their interactions in 4, 7 and 10 days after initiation. P-values below 0.05 are indicated with bold.

Effect	*p*-value				
	Germinated seeds	Normal Seedling		Abnormal seedling	Dead Seeds
	4 days	7 days	10 days	10 days	10 days
Temp	0.6780	0.1539	0.7752	0.3340	0.0967
ED	0.2321	**0.0457**	**0.0013**	**0.0047**	**<.0001**
C	**<0.0001**	**<0.0001**	**0.0004**	**0.0006**	0.2221
Temp x C	0.4009	0.6307	0.1816	0.9793	**0.0126**
Temp x ED	0.0661	0.5713	0.1326	0.2872	0.3100
ED x C	0.3873	**0.0127**	**0.0476**	**0.0107**	0.1478
Temp x ED x C	0.9639	0.5699	0.8753	**0.0352**	0.5543

While both cultivars would be classified as having a strong vigor score as the values were greater than 80% at the time of project initiation (Seed laboratory, Iowa State University, n.d.; Sodak labs, inc.; Table 2.1), variation in normal seedling percentage at 7 and 10 days was evident for both cultivars in response to ED treatments (Table 2.3; Figures 2.1 and 2.2). For Cultivar 3.4 RM, the greatest 7-day germination (88% or greater) was observed when seeds were exposed to warmer temperatures for more than 8 hours prior to cold exposure for 12 or 24 hrs (Figure 2.1a; Suppl. Table 2.1). In the case of Cultivar 3.7 RM at 7 days, initial exposure to warmer temperature

of 4 hrs or longer before transfer to the colder temperatures reduced germination by 2-4 percentage points compared to immediate exposure or no exposure to colder temperatures (Figure 2.1b, Suppl. Table 2.1). Notably, the central region of the 3.7 RM plot, indicated by the lowest germination (82.5%), corresponds to 12 hours of exposure to warmer temperatures followed by 12 hours exposure to colder temperatures (Suppl. Table 2.1; Figure 2.1b). When the duration of cold temperatures increased without pre-exposure to warmer temperature, the germination percentage gradually decreased in both cultivars at 7 days (Figure 2.1). At 7 days, seven ED treatments reduced the percentage of normal seedlings in 3.4 RM compared to the UTC, while only four treatments had a similar effect on 3.7 RM (Supplementary Table 2.1).

After 10 days, the 3.4 RM seeds exposed to initial warm temperatures of 0 or 2 hours and exposed to short durations of cold (3 hours or less) generally recorded greater germination (90% or greater) than those experiencing warmer temperatures of 4 hrs or more prior to cold temperature exposure (Figure 2.2a; Suppl. Table 2.1). However, initial exposure to warmer temperatures for 12 hours followed by 12 hours or more in colder temperatures also exhibited germination exceeding 90%. Germination percentage was least (87% or less) for seeds exposed to long durations of colder temperatures after short initial periods of warmer temperatures (8 hrs or less). For Cultivar 3.7 RM, seeds exposed to 3 hours of colder temperatures regardless of the initial duration of warmer temperature exposure generally resulted in higher germination (88% or greater). However, 3 hours of cold exposure after 0 or 8 hours in warmer temperatures before exposure did result in lower germination percentages of 85 and 85.7%, respectively (Suppl. Table 2.1). In all cases where seeds were exposed to colder temperatures for more than 12 hours after warmer temperatures of less than 4 hours, lower germination occurred (Figure 2.2b). Twelve of the ED treatments produced fewer normal seedlings than the UTC in 3.4 RM, whereas only four

of the ED treatments reduced normal seedling germination values statistically compared to the UTC in 3.7 RM (Supplemental Table 2.1).

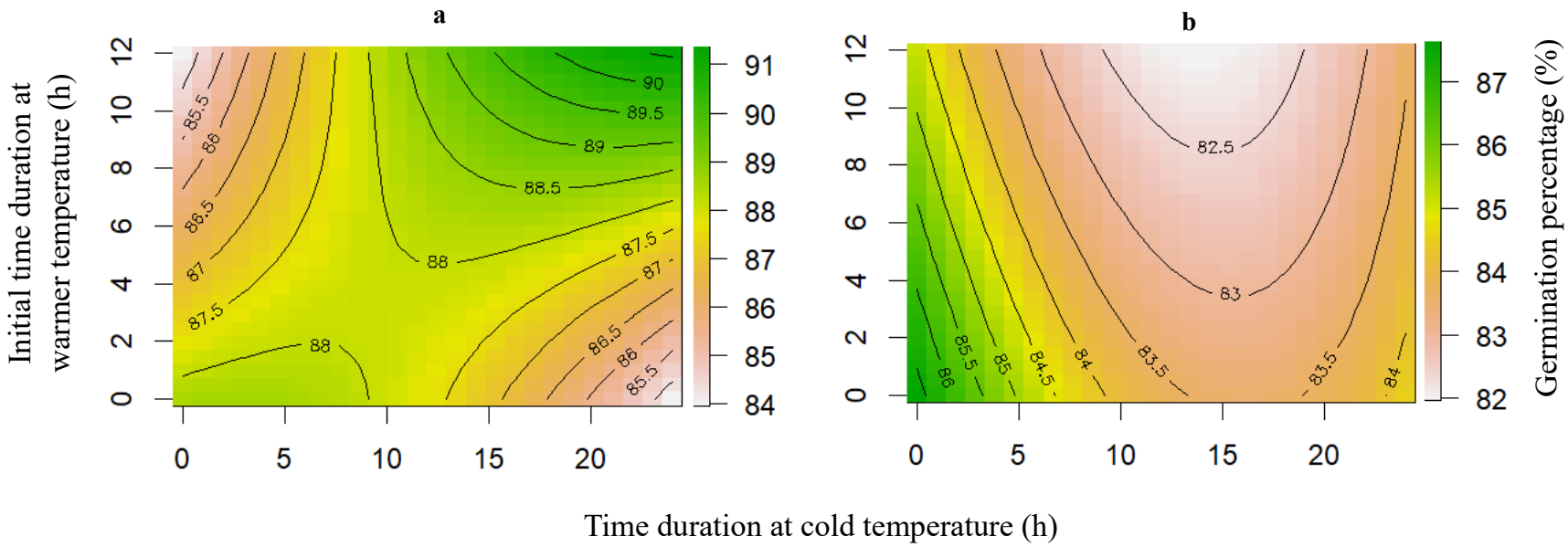


Figure 2.1. Variation in normal seedling percentage of (a) cultivar 3.4 RM and (b) cultivar 3.7 RM at 7 days after initiation for the exposure-duration treatments across temperature pairings. The Y-axis shows the initial time duration exposure to the warmer temperature, and the X-axis represents the duration of exposure to cold temperature prior to being returned to the warmer temperature. The contour lines represent the normal seedling percentage indicated on the line. The color gradient, ranging from light pink to green, visually represents the germination gradient, where light pink indicates lowest germination and green indicates higher germination.

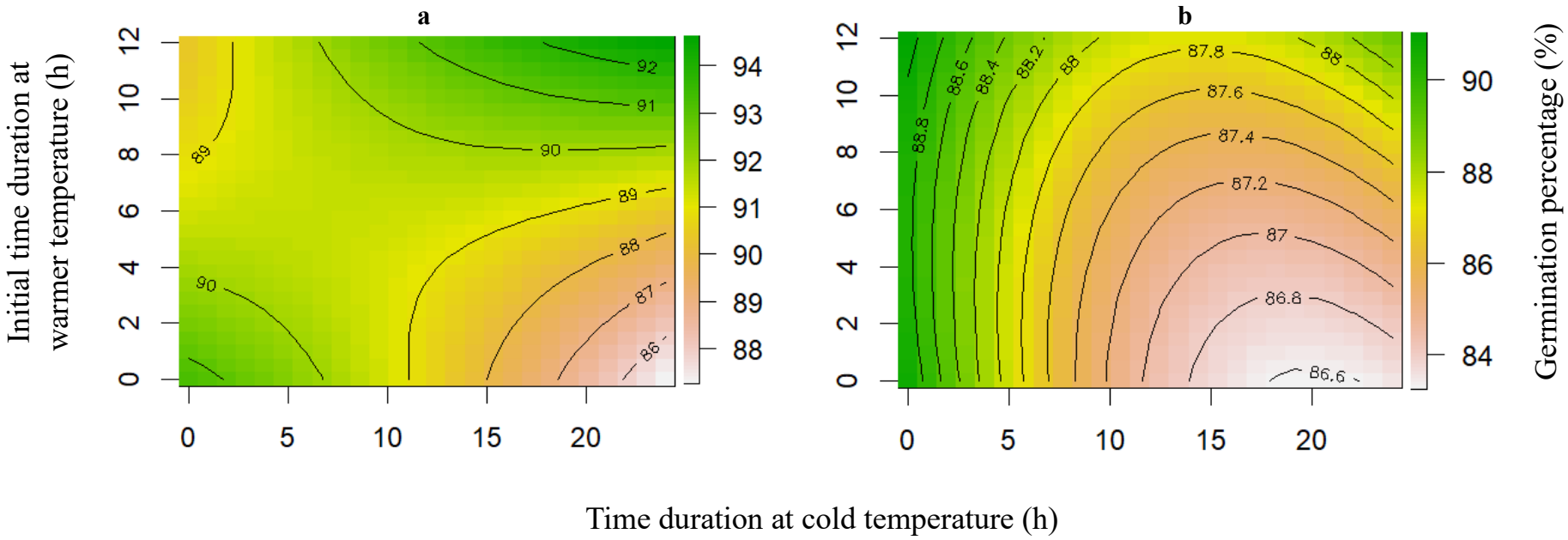


Figure 2.2. Variation in normal seedling percentage of (a) cultivar 3.4 RM and (b) cultivar 3.7 RM at 10 days after initiation for the exposure-duration treatments across temperature pairings. The Y-axis shows the initial time duration exposure to the warmer temperature, and the X-axis represents the duration of exposure to cold temperature prior to being returned to the warmer temperature. The contour lines represent the normal seedling percentage indicated on the line. The color gradient, ranging from light pink to green, visually represents the germination gradient, where light pink indicates lowest germination and green indicates higher germination.

When examining the ED x C interaction at 7 days, the 3.7 RM cultivar consistently produced lower counts of normal seedlings compared to the 3.4 RM cultivar for most time durations (Supplementary Table 2.1). At 10 days for the ED x C interaction, normal seedling counts were statistically similar in both cultivars for most ED treatments. However, in the few ED treatments where they differed, the normal seedling percentage of the 3.7 RM cultivar was statistically lower at 10 days than the 3.4 RM cultivar (Supplementary Table 2.1). This could be attributed to the higher germination percentage in initial cold germination and the high initial seed vigor score of the 3.4 RM cultivar evaluated in this trial. The impact of Temperature (Temp) and its interactions did not show significance ($p \geq 0.13$) concerning the percentage of normal seedlings at 7 and 10 days (Table 2.3).

Existing literature commonly suggests that seeds need to be exposed to cold temperatures for at least 6 to 12 hours to induce imbibitional damage (Bramlage et al., 1978; Cheng et al., 2010). Some studies highlight the initial minutes of imbibition as crucial (Bramlage et al., 1978; Cheng et al., 2010), while others, such as Pollock and Toole (1966), argue that the first 24 hours are most critical for inducing imbibitional damage. Contrary to these findings, our results consistently demonstrated that 6 hours of cold exposure was not as damaging as durations of 3, 12, and 24 hours within the first 48 hours of germination. Duke et al. (1978) found that under cold temperatures (10°C), dehydrogenase activity initially decreased during the first 3 hours due to cold water imbibition stress, then increased to combat oxidative stress caused by the cold temperature. This may help to explain why less damage was observed with the 6-hour exposure treatments, though damage was greater in the other durations studied. The 3-hour duration may have been too short to fully induce benefits from this upregulating process. Extended exposure to cold temperatures in our study resulted in increased seed injuries, leading to a decline in germination

counts after 12 hours. This aligns with the notion that temperature and the rate of water entering dry seeds are crucial factors affecting imbibitional chilling (Cheng et al., 2010). Rapid water uptake during the initial hours may contribute to lower germination, as observed by Bramlage et al. (1978).

Direct exposure to cold temperatures for 12 and 24 hours without pre-exposure to warmer temperatures resulted in lowest germination (Figures 2.1 and 2.2, Suppl. Table 2.1). Imbibition at cold temperatures (≤ 10°C) negatively impacts membrane structure and function, mitochondrial respiration, leading to solute leakage and disruption of metabolic activities in seeds (Bramlage et al., 1978; Duke et al., 1978). Plants can adapt to cold stress by regulating gene expression and producing proteins that maintain membrane stability. Changes in dehydrogenase (Glucose-6-Phosphate) activity may contribute to this defense strategy (Duke et al., 1978), though this was not quantified in our current study.

Past research has often maintained seeds at 25°C before and after cold exposure. Obendorf & Hobbs (1970) noted that imbibition of 60 g kg^{-1} moisture seeds at 5°C reduced survival, (25 to 87%), but no reduction was observed with 160 g kg^{-1} moisture seeds. It is possible the initial moisture content of seeds in this study (92-96 g kg^{-1}) may have contributed to partial reductions in germination in the current study compared to the results reported by Obendorf & Hobs (1970). Additionally, an initial 8-hour exposure to warmer temperature (25°C) reduced imbibition damage when seeds were subsequently subjected to chilling at 7°C (Bohner, 2003). In the current study, germination was marginally reduced after 10 days, even with the 8 hours pre-exposure to warmer temperatures. This could be attributed to the initial warmer temperature being lower than that in the referenced work. Rees & Specht (2021) reported increased soybean germination from 47% to 65% when seeds were switched from 15°C to 2.2°C for at least 6 hours compared to 2 or 4 hours.

They suggested that the first eight hours were the most critical for imbibition. However, our results indicate that exposure to cold temperatures after 8 to 12 hours at a warmer temperature (10-15.5℃) still resulted in marginal germination reduction after 10 days. Direct exposure to cold temperatures for 12 or 24 hours led to the greatest decline in germination. Nevertheless, normal seedling counts of 87% or greater were recorded 10 days after initiation. The differences in observed values in the current study may also have been influenced by the use of germination paper towels as opposed to the use of field soils in the cited studies.

Injury to seeds subjected to cold temperatures during germination manifests as reduced seedling vigor, diminished emergence, and an increase in dead seeds (Bramlage et al., 1978). In our study, seed injuries were quantified by evaluating the percentage of abnormal seedlings and dead seeds. The three-way interaction of Temp x ED x C was significant for the percentage of abnormal seedlings ($p=0.03$; Table 2.3). This interaction was primarily driven by differences in magnitude of ED response, with the largest differences between treatments coming from the 4/12.8℃ temperatures within cultivar 3.4 RM (ranging from 3% abnormal in UTC up to 15% abnormal in 24 hours cold exposure after 2 hours in the warmer temperature). Similar increases of lesser magnitude were evident in the other two temperature pairings as well as for the 3.7 RM cultivar. As such, the interaction of ED×C will be discussed from this point forward (Figure 2.3).

Greater numbers of abnormal seedlings were produced for the 3.4 RM cultivar when seeds were exposed to colder temperatures for 24 hours after an initial exposure to warmer temperature of more than 2 hours. However, 12 hours of warmer temperatures prior to 24 hours of cold exposure resulted in similar abnormal seedlings as the UTC (Figure 2.3a). In cultivar 3.7 RM, the 24 hours cold temperature exposure increased abnormal seedling number regardless of the warmer temperature duration prior to exposure (Figure 2.3b). In the ED×C interaction, eleven ED

treatments showed greater abnormal seedlings in 3.4 RM, compared to only three ED treatments for 3.7 RM (Supplementary Figure 2.1). This could be attributed in part to the lower percentages of abnormal seedlings recorded for 3.4 RM in the UTC (Supplementary Figure 2.1). The ED treatments that resulted in significantly greater abnormal seedling percentages for 3.7RM over the UTC differed than those for 3.4 RM and suggest differential responses to cold temperature exposure existed for the varieties evaluated in this assay.

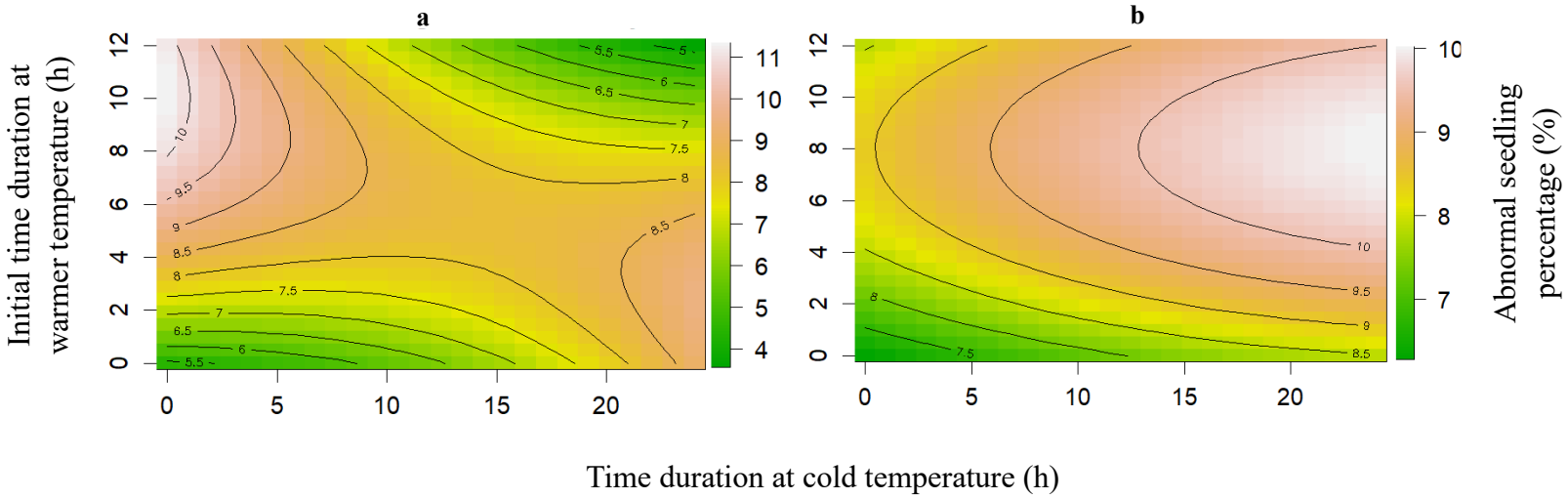


Figure 2.3. Variation in abnormal seedling percentage of (a) cultivar 3.4 RM and (b) cultivar 3.7 RM at 10 days for the exposure-duration treatments across temperature pairings. The Y-axis shows the initial time duration exposure to the warmer temperature, and the X-axis represents the duration of exposure to cold temperature prior to being returned to the warmer temperature. The contour lines represent the abnormal seedling percentage indicated on the line. The color gradient, ranging from green to light pink, visually represents the abnormal seedling gradient, where light pink indicates highest abnormal seedling and green indicates lowest abnormal seedling..

Imbibition of cold water disrupts the membrane reorganization of seed during rehydration, and the release of cellular components due to the loss of selective permeability results in dead seed (Suo et al., 2022). It was evident that direct exposure to colder temperatures led to an increase in dead seeds percentages (Figure 2.4). Additionally, exposure to cold temperature after initial exposure to warmer temperature less than 4h also resulted higher dead seed count.

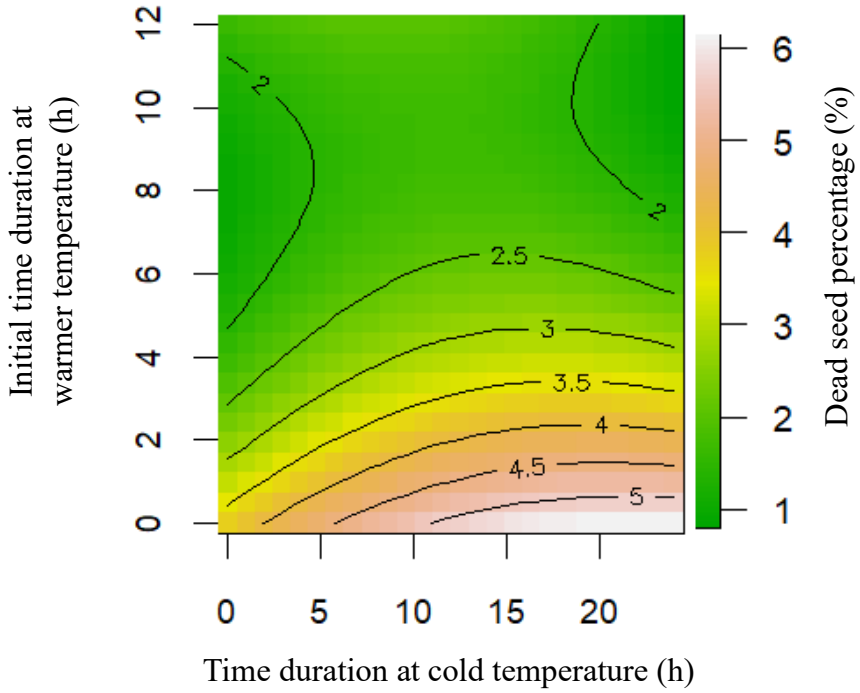


Figure 2.4. Variation in dead seed percentage at 10 days for exposure-duration treatments across temperature pairings and cultivars. The Y-axis shows the initial time duration exposure to the warmer temperature, and the X-axis represents the duration of exposure to cold temperature prior to being returned to the warmer temperature. The contour lines represent the dead seed percentage indicated on the line. The color gradient, ranging from green to light pink, visually represents the dead seed gradient, where light pink indicates highest dead seeds and green indicates lowest dead seed.

The interaction of Temp x C was significant for the percentage of dead seeds ($p = 0.0126$; Table 2.3), as depicted in Figure 2.5. Notably, the lowest temperature pairing (1.6/10°C) resulted in higher counts of dead seeds compared to the higher temperature treatment pairing (7.2/15.5°C) for both cultivars. This discrepancy could be attributed to the varying levels of cold tolerance

exhibited by each cultivar, evident in their initial vigor test results (Table 2.1), with 3.4 RM and 3.7 RM showing 4% and 8% dead seeds, respectively. Additionally, the emergence scores from company literature, reflecting the cultivars' ability to germinate under suboptimal conditions, were 8 and 6 for 3.4 RM and 3.7 RM, respectively. These scores are on a scale where 9 signifies excellent germination and 1 indicates poor germination. This indicates differences in their overall germination potential under challenging conditions. Supporting this, Rees & Specht (2021) observed that no seeds emerged when exposed to 2℃ for 72 hours. Hence, the lower temperature treatment in our study may have compromised the germination potential of both cultivars, contributing to the higher counts of dead seeds, even though the duration was less than 72 hours.

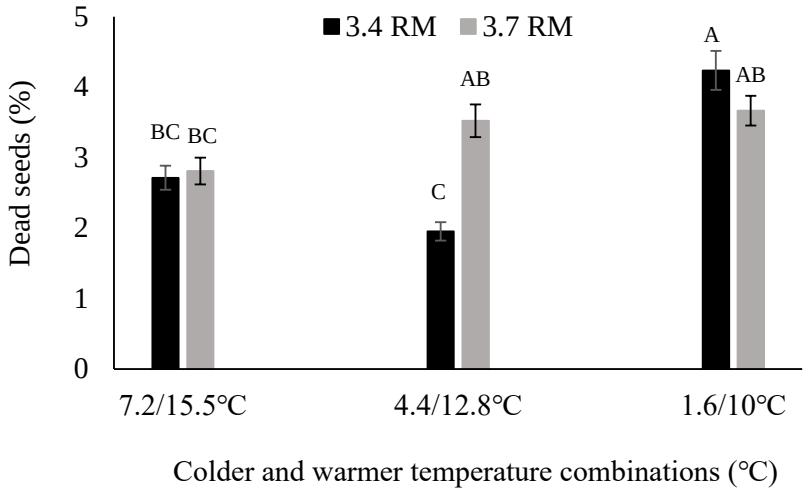


Figure 2.5. Dead seeds (%) of two soybean cultivars exposed to low temperatures during the imbibition phase within each low temperature pairing. Means followed by the same letter are not significantly different according to the paired t-test ($p < 0.05$).

2.5 Summary

Initial exposure to cold temperatures for 12 to 24 hours was most damaging to soybean seeds, resulting in 6-7% lower germination, than the untreated check after 10 days. However, all tested ED treatments produced normal germination percentages of 83% or greater. All germination

assays were conducted using rolled towel methods in controlled temperature conditions, but these results suggest cold temperatures alone may not fully explain stand reductions in field environments. Other abiotic factors like soil texture and moisture as well as biotic factors like pests and diseases could be associated with stand losses in early planted soybean and their association with cold temperatures during germination and early growth need to be further studied. It is possible that the duration of cold temperatures and timing of initial exposure may also play a role in germination, especially for soybeans, and should be evaluated in future trials.

References

Andrade, F. H., Otegui, M. E., & Vega, C. (2000). Intercepted Radiation at Flowering and Kernel Number in Maize. *Agronomy Journal*, *92*(1), 92–97. https://doi.org/10.2134/agronj2000.92192x

Anderson, M., Vittetoe, R., Licht, M. A., & Rieck-Hinz, A. (n.d.). *Stand Assessments—Soybean | Integrated Crop Management*. Retrieved February 23, 2024, from https://crops.extension.iastate.edu/encyclopedia/stand-assessments-soybean

Association of Official Seed Analysis (AOSA) (1983) Seed Vigor Testing Handbook, Contribution No. 32 to the handbook on Seed Testing.

Association of Official Seed Analysts (AOSA). (2018). Rules for testing seeds. Association of Official Seed Analysts.

Bandara, A.Y., Weerasooriya, D.K., Bell, T.H. and Esker, P.D. 2021. Prospects of alleviating early planting-associated cold susceptibility of soybean using microbes: New insights from microbiome analysis. Journal of Agronomy and Crop Science, 207(2), pp.171-185.

Bohner, H. 2003. Do soil temperatures at planting impact soybean yield? Ministry of Agriculture, Food and Rural Affairs. https://cropwatch.unl.edu/2019-CW-News/2019-images/soybean/Do-soil-temperatures-at-planting-impact-soybean-yield-Horst Bohner.pdf accessed November 2023.

Bramlage, W.J., Leopold, A.C. and Parrish, D.J. 1978. Chilling stress to soybeans during imbibition. Plant Physiology, 61(4), pp.525-529. https://doi.org/10.1104/pp.61.4.525

Cheng, L., Gao, X., Li, S., Shi, M., Javeed, H., Jing, X., ... & He, G. (2010). Proteomic analysis of soybean [Glycine max (L.) Meer.] seeds during imbibition at chilling temperature. Molecular Breeding, 26, 1-17. https://doi.org/10.1007/s11032-009-9371-y

De Bruin, J.L. and Pedersen, P. 2008. Soybean seed yield response to planting date and seeding rate in the Upper Midwest. Agronomy Journal, 100(3), pp.696-703. https://doi.org/10.2134/agronj2007.0115

Duke, S.H., Schrader, L.E. and Miller, M.G. 1977. Low temperature effects on soybean (Glycine max [L.] Merr. cv. Wells) mitochondrial respiration and several dehydrogenases during imbibition and germination. Plant Physiology, 60(5), pp.716-722. https://doi.org/10.1104/pp.60.5.716

Egli, D. B. and Cornelius, P. L. 2009. A regional analysis of the response of soybean yield to planting date. Agronomy Journal, 101(2), pp. 330-335. https://doi.org/10.2134/agronj2008.0148

Gaspar, A. P., & Conley, S. P. (2015). Responses of canopy reflectance, light interception, and soybean seed yield to replanting suboptimal stands. *Crop Science*, *55*(1), 377-385. https://doi.org/10.2135/cropsci2014.03.0200

Greaves, J. A. (1996). Improving suboptimal temperature tolerance in maize- the search for variation. Journal of Experimental Botany, 47(3), 307–323. https://doi.org/10.1093/jxb/47.3.307

Hankinson, M. W., Lindsey, L. E., & Culman, S. W. 2015. Effect of planting date and starter fertilizer on soybean grain yield. Crop, Forage & Turfgrass Management, 1(1), 1-6. https://doi.org/10.2134/cftm2015.0178

Hobbs, P.R. and Obendorf, R.L. 1972. Interaction of Initial Seed Moisture and Imbibitional Temperature on Germination and Productivity of Soybean. Crop Science, 12(5), pp.664-667. https://doi.org/10.2135/cropsci1972.0011183X001200050033x

Hu, M., & Wiatrak, P. 2012. Effect of planting date on soybean growth, yield, and grain quality. Agronomy Journal, 104(3), 785-790. https://doi.org/10.2134/agronj2011.0382

Iowa State University. (n.d.). Accelerated Aging. *ISU Seed Lab*. Retrieved February 23, 2024, from https://seedlab.iastate.edu/portfolio-items/accelerated-aging/

Kucharik, C. J. (2006). A multidecadal trend of earlier corn planting in the central USA. Agronomy Journal, 98(6), 1544-1550. https://doi.org/10.2134/agronj2006.0156

Lee, C. D., Egli, D. B., & TeKrony, D. M. (2008). Soybean response to plant population at early and late planting dates in the Mid-South. *Agronomy Journal*, *100*(4), 971-976. https://doi.org/10.2134/agronj2007.0210

Leopold, A. C. (1980). Temperature effects on soybean imbibition and leakage. Plant Physiology, 65(6), 1096-1098. https://doi.org/10.1104/pp.65.6.1096

Leopold, A. C., & Musgrave, M. E. (1979). Respiratory changes with chilling injury of soybeans. Plant physiology, 64(5), 702-705. https://doi.org/10.1104/pp.64.5.702

Licht, M. (2023). Imbibitional chilling or cold injury Integrated Crop Management. https://crops.extension.iastate.edu/encyclopedia/imbibitional-chilling-or-cold-injury

Lindsey, L. E., Tilmon, K., Michel, A., & Dorrance, A. (2017). Soybean production. In L. E. Lindsey & P. R. Thomison (Eds.), Ohio agronomy guide (15th ed., Bulletin no. 472, pp. 56–68). The Ohio State University Extension. https://stepupsoy.osu.edu/sites/hcs soy/files/472%20Ohio%20Agronomy%20Guide%2015%20Ed%20red_0.pdf

Loeffler, N. L., Meier, J. L., & Burris, J. S. (1985). Comparison of two cold test procedures for use in maize drying studies. Seed Science and Technology, 13(3), 653-658.

McDonald Jr, M.B., Vertucci, C.W. and Roos, E.E.1988. Soybean seed imbibition: water absorption by seed parts. Crop Science, 28(6), pp.993-997. https://doi.org/10.2135/cropsci1988.0011183X002800060026x

Nielsen, R. L. (2020, April 6). When Should Maize Planting Begin? (Purdue University). https://www.agry.purdue.edu/ext/Maize/news/articles_20/PltDateStart_0406.html

Obendorf, R. L., & Hobbs, P. R. (1970). Effect of Seed Moisture on Temperature Sensitivity During Imbibition of Soybean 1. Crop science, 10(5), 563-566. https://doi.org/10.2135/cropsci1970.0011183X001000050034x

Pollock, B., & Toole, V. K. (1966). Imbibition period as the critical temperature sensitive stage in germination of lima bean seeds. Plant physiology, 41(2), 221-229. https://doi.org/10.1104/pp.41.2.221

Rattalino Edreira, J.I., Mourzinis, S., Conley, S.P., Roth, A.C., Ciampitti, I.A., Licht, M.A., Kandel, H., Kyveryga, P.M., Lindsey, L.E., Mueller, D.S., Naeve, S.L., Nafziger, E., Specht, J.E., Stanley, J., Staton, M.J., and Grassini, P. (2017). Assessing causes of yield gaps in agricultural areas with diversity in climate and soils. Agricultural and Forest Meteorology. 247:170-180. https://doi.org/10.1016/j.agrformet.2017.07.010

Rees, J., and Specht, J. 2021. Demonstrating critical imbibitional time for soybean. University of Nebraska-Lincoln Cropwatch Newsletter. https://cropwatch.unl.edu/2021/demonstrating-critical-imbibitional-time-soybean (accessed November 2023).

Rowntree, S.C., Suhre, J.J., Weidenbenner, N.H., Wilson, E.W., Davis, V.M., Naeve, S.L., Casteel, S.N., Diers, B.W., Esker, P.D., Specht, J.E. and Conley, S.P. 2013. Genetic gain× management interactions in soybean: I. Planting date. Crop Science, 53(3), pp.1128-1138. https://doi.org/10.2135/cropsci2012.03.0157

Sodak labs inc. Accelerated Aging (AA) test for soybean seed V.72021. Retrieved March 6, 2024, from https://www.sodaklabs.com/media/1576/22-accelerated-aging-aa-test-for-soybean-seed-ver-72021.pdf

Staton M, 2018. Michigan State University Extension, 2018. https://www.canr.msu.edu/news/things_to_consider_when_planting_soybeans_early (accessed April 2023)

Suo, R., Sandhu, K., Wang, M., You, F., Conner, R., Cober, E., & Hou, A. (2022). Soybean (Glycine max L.) seed germination in response to waterlogging and cold climate: a review on the genetics and molecular mechanisms of resistance to the abiotic stress. Canadian Journal of Plant Science, 103(1), 13-28. https://doi.org/10.1139/cjps-2022-0111

Szczerba, A., Płażek, A., Pastuszak, J., Kopeć, P., Hornyák, M., & Dubert, F. (2021). Effect of low temperature on germination, growth, and seed yield of four soybean (Glycine max L.) cultivars. *Agronomy*, *11*(4), 800. https://doi.org/10.3390/agronomy11040800

Tully, R.E., Musgrave, M.E. and Leopold, A.C. 1981. The seed coat as a control of imbibitional chilling injury. Crop Science, 21(2), pp.312-317.

Wuebker, E.F., Mullen, R.E. and Koehler, K. 2001. Flooding and temperature effects soybean germination. Crop Science, 41(6), pp.1857-1861. https://doi.org/10.2135/cropsci2001.1857

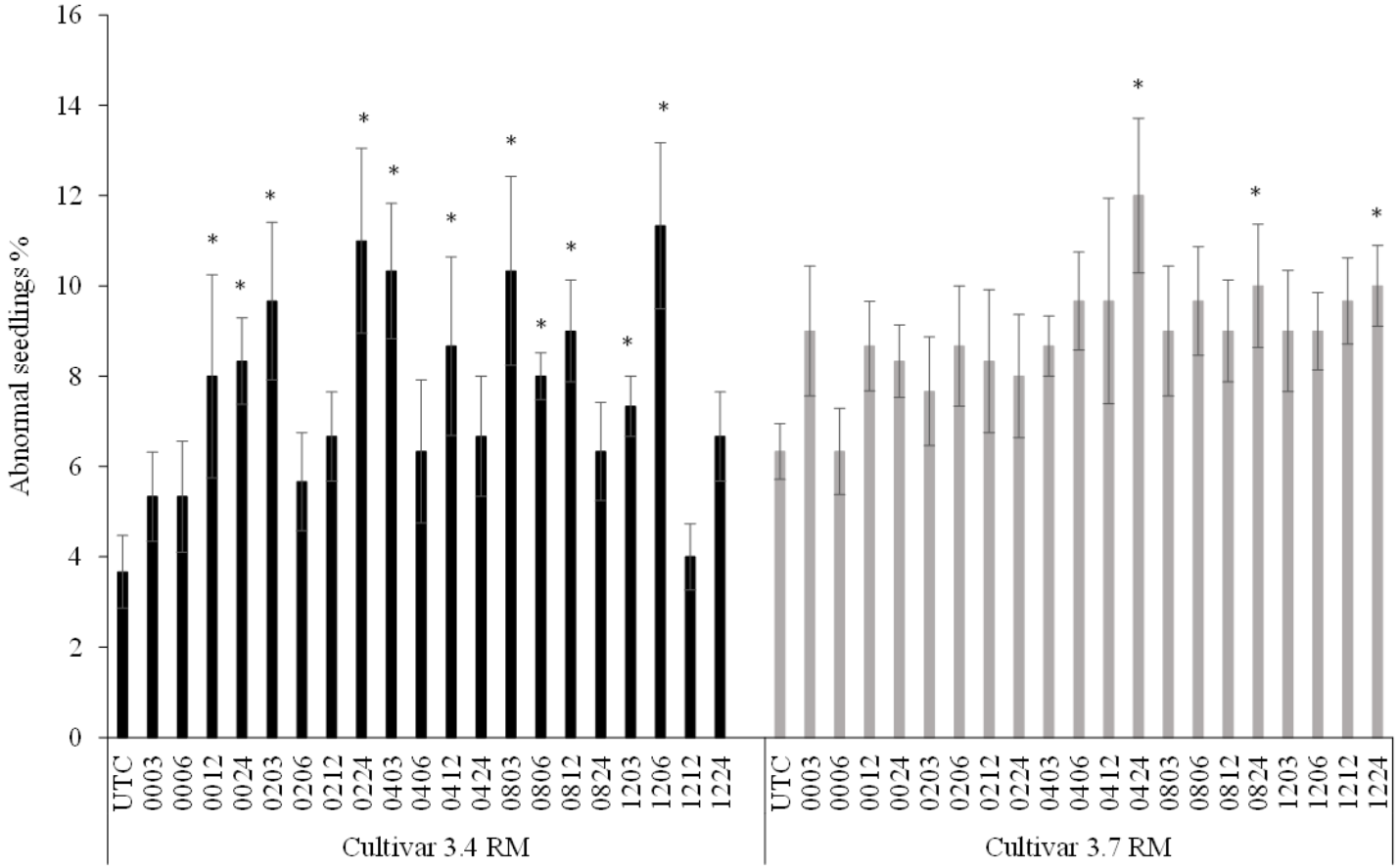
Yin, G., Sun, H., Xin, X., Qin, G., Liang, Z. and Jing, X. 2009. Mitochondrial damage in the soybean seed axis during imbibition at chilling temperatures. Plant and Cell Physiology, 50(7), pp.1305-131. https://doi.org/10.1093/pcp/pcp074

Supplemental Tables and Figures

Supplemental Table 2.1 Germination (%) of normal seedlings at 7 and 10 days for two soybean cultivars as a function of the interaction between cultivar and cold exposure treatments.

Initial hours at warmer temperature	Hours at colder temperature	Normal seedling % at 7 days		Normal seedling % at 10 days	
		3.4 RM	3.7 RM	3.4 RM	3.7 RM
0	0	91.3^{A}	$87.3^{ABCDEFGH}$	94.0^{AB}	91.0^{ABCDE}
0	3	89.0^{ABC}	83.0^{HIJ}	91.3^{ABCD}	85.0^{GH}
0	6	$85.7^{BCDEFGHI}$	$87.7^{ABCDEFG}$	88.3^{CDEFG}	90.0^{BCDEF}
0	12	88.0^{ABCDEF}	78.7^{J}	88.3^{CDEFG}	83.3^{H}
0	24	84.0^{EFGHI}	$84.7^{CDEFGHI}$	85.0^{GH}	87.0^{EFGH}
2	3	84.0^{EFGHI}	$87.7^{ABCDEFGH}$	87.3^{DEFGH}	89.0^{CDEFG}
2	6	90.3^{A}	$85.3^{BCDEFGHI}$	91.7^{ABC}	88.0^{CDEFG}
2	12	88.3^{ABCDE}	84.0^{EFGHI}	88.7^{CDEFG}	87.3^{DEFGH}
2	24	84.3^{DEFGHI}	$85.7^{BCDEFGHI}$	85.3^{GH}	88.7^{CDEFG}
4	3	$85.3^{BCDEFGHI}$	84.3^{DEFGHI}	87.3^{DEFGH}	88.7^{CDEFG}
4	6	89.0^{ABC}	$85.0^{BCDEFGHI}$	91.3^{ABCD}	89.0^{CDEFG}
4	12	88.0^{ABCDEF}	83.0^{HIJ}	88.7^{CDEFG}	87.7^{CDEFG}
4	24	89.3^{AB}	83.0^{HIJ}	90.3^{BCDE}	86.0^{FGH}
8	3	$84.7^{CDEFGHI}$	81.3^{IJ}	89.0^{CDEFG}	85.7^{GH}
8	6	89.3^{AB}	83.3^{GHI}	90.7^{ABCDE}	87.7^{CDEFG}
8	12	$87.7^{ABCDEFG}$	82.3^{IJ}	89.0^{CDEFG}	87.3^{DEFGH}
8	24	88.7^{ABCD}	84.0^{EFGHI}	89.0^{CDEFG}	87.7^{CDEFG}
12	3	88.3^{ABCDE}	83.7^{FGHI}	90.0^{BCDEF}	88.0^{CDEFG}
12	6	84.3^{DEFGHI}	83.0^{HIJ}	87.0^{EFGH}	89.0^{CDEFG}
12	12	90.3^{A}	$85.0^{BCDEFGHI}$	94.7^{A}	90.0^{BCDEF}
12	24	89.3^{AB}	82.0^{IJ}	91.3^{ABCD}	87.3^{DEFGH}

**Means followed by the same letter within each 7 and 10 days were not significantly different according to the paired t-test ($p < 0.05$).



Supplementary Figure 2.1. Abnormal seedlings (%) at 10 days for two soybean cultivars as a function of the interaction between cultivar and duration of exposure to low temperatures. Asterisk (*) above bars indicates the time durations that were significantly different from UTC within each cultivar. UTC indicates towel that remained at the warmer temperature for the first 48 hours after initiation. Four-digit codes in the X axis represent the 21 cold exposure treatments; first two digits indicates the time duration at warmer temperatures in hours before cold exposure, and the last two digits indicates the duration of the colder temperature exposure in hours.

Chapter 3. Cold temperature and water effect on emergence of soybean in soil

3.1 Abstract

Low soil temperatures and the chance of cold precipitation falling shortly after planting pose significant risks for imbibitional chilling and cold injury to soybean seedlings. Severity of injury or death from cold temperature depends on duration and timing of exposure to cold temperature, soil moisture conditions, and other management decisions such as planting depth. However, few studies have attempted to study the interaction of these factors to more clearly understand how climatic conditions and management decisions interact to affect soybean germination and emergence. Therefore, the objectives of this research were to quantify how soil moisture content at planting, planting depth, temperature, and form of precipitation affect soybean emergence and early growth. A controlled environment experiment was conducted using split-split-split plot randomized complete block design with four factors and was repeated three times. Main plot factor was the soil available water content (AWC of 20% and 60%) at the time of planting. The sub plot factor was six treatments consisting of varied water application and temperatures imposed (10℃ or 1.7℃; water, ice application, or no water added 1 or 11 hours after planting equivalent to 1.25 cm precipitation). The sub-sub plot factor was planting depth [2.5cm (shallow) and 3.8cm (recommended)], and cultivar [3.4RM and 3.7RM] was randomized within each planting depth. Daily emergence was measured through 11 days after initiation, and biomass was quantified at day 11. Application of 10℃ or 1.7℃ liquid water or ice within 11 hours after planting resulted in 30% to 64% lower normal seedling biomass than the treatments that did not

receive an application. Treatments placed at 1.7℃ 1 hour after planting for 10 hours before returning to 10℃ showed similar biomass and emergence of normal seedlings to those kept at 10℃ for the same periods. Both planting depths resulted in similar emergence and biomass within an AWC treatment. Avoidance of precipitation within the first 25 hours regardless of temperature resulted in the greatest biomass and emergence. Liquid water at 1.7℃ applied 1 hour after planting with 10 hours of 1.7℃ temperature exposure was the most damaging to soybean emergence and early growth.

3.2 Introduction

The decision of when to plant soybeans is a significant factor that influences both the yield and growth of the crop (Egli & Cornelius, 2009; Hu & Wiatrak, 2012). Farmers often grapple with this decision due to the potential yield implications and the unpredictability of weather patterns. Planting soybean early increased yield by extending the vegetative growing season (De Bruin & Pedersen, 2008; Robinson et al., 2009; Hu & Wiatrak, 2012).Late planting can result in yield penalties, prompting farmers to consider early planting (Licht, 2021). This decision is further aided by the warming trend of spring temperatures and declining of suitable days for field work with most weeks only having two or four days suitable for Ohio farmers due to heavy precipitation. Hence, farmers may decide to plant more hectarage before a weather front, even if it is early in the season (Field work capacity Tool, 2024; Lindsey et al., 2024).

Warm spring temperatures in late March and early April may result in suitable conditions for ultra-early soybean planting, adverse weather events that occur post-planting can negatively affect establishment. Specially, chance of having cold precipitation and temperature, as well as wet and cool soil conditions can negatively affect soybean plant (Specht et al., 2012). Furthermore,

current climatic models suggest an increase in the frequency of cold temperature occurrences, even in scenarios where the planting date is considered to be more typical (Baum et al., 2020; Lindsey et al., 2024). Exposure to such conditions can hinder soybean germination and emergence given that soybeans are sensitive to chilling. One field study conducted at the Nebraska research extension center in 2019 documented a reduction in soybean yield when planted in early April due to cold temperatures ranging from 0 to 7℃. Therefore, while early planting can have its advantages, it's crucial to consider the potential risks associated with subsequent weather patterns shortly after planting (Specht et al, 2019).

For soybean germination, the soil temperature threshold for facilitating germination is considered to be 10℃ (Haider et al., 2023). Temperatures below this threshold, but above freezing, are classified as cold temperatures (Duke et al., 1977; Leopold & Musgrave, 1979). When dry seeds absorb water below 10℃, soybean seeds can experience imbibitional chilling and chilling injuries. The imbibition phase can be impaired by the uptake of cold water, resulting in cellular membrane damage, impaired respiration, and solute leakage, all of which contribute to imbibitional chilling injuries (Duke et al., 1977; McDonald et al., 1988). The extent of the damage to the seeds is associated with the rate of water uptake, the duration of exposure to cold temperatures, seed moisture content at the time of exposure, seed coat characteristics, and soil moisture content (Hatfield & Egli, 1974). McDonald et al. (1988) found that intact seeds show better resistance to chilling damage than the seeds without or damaged seed coat. Hobbs and Obendrof, (1972) observed that 13 to 14% moisture content (dry weight basis) was the lowest seed moisture content that minimized solute leakage during imbibition with cold water in tested soybean varieties. Soil moisture at the time of planting may also affect chilling response, as seeds in soil with greater initial water content may imbibe more quickly. When the imbibition phase is over,

seeds exposed to non-freezing cold temperatures can result in chilling injuries. Visual symptoms of chilling injury included transverse cracking (cotyledons displaying outer layers of dead tissue), browning of cotyledons and dead seeds (Hobbs and Obendorf, 1972; Tully et al., 1981). These injuries can lead to poor emergence may require producers to re-plant a field (Szczerba et al., 2023; Wang et al., 2023). This would negate the yield gains from early planting and result in greater costs. Apart from the planting date, other management decisions of soybean planting that could influence crop sensitivity to cold temperatures include planting depth and variety selection. Stucky (1976) has found that temperature was the most pronounced factor for soybean emergence and average time for emergence is low when temperature increases to 16 to 32℃. Within each temperature, shallower planting depth resulted lower emergence time.

There is lack of recent studies conducted regarding cold temperature and effect early seed germination and emergence. Much of the existing research focuses on micro-level changes in seed characteristics, such as solute leakage patterns, seed coat characteristics, and seed moisture content, following the imbibition of cold water. Much of the past work examining exposure to cold temperatures has held seeds at 25℃ prior to cold exposure but soils in early April in Ohio are more likely to be between 11-15℃ which may differentially affect chilling injury tolerance of planted seeds (Hobbs & Obendrof, 1972; Webster & Leopold, 1977; Duke et al., 1983; McDonald et al., 1988). Most existing studies have focused on the impact of low temperatures, but not specifically on the effects of frost or cold precipitation on germination and emergence. Our current gap in knowledge relates to how seed planted into soil under conditions that emulate early to mid-April weather would be affected by a cold front or exposure to cold precipitation shortly after planting. The time spent by seeds imbibing under warm temperatures prior to exposure to cold temperatures can affect chilling tolerance of soybean seeds (Bohner, 2003; Obendorf & Hobbs, 1970; Rees &

Specht, 2019). Conducting field studies involving cold temperatures (with or without water addition), and exposure time to cold temperatures presents its own set of challenges. Therefore, it is urgent to improve our management recommendations for anticipated damage associated with cold water uptake or post-frontal weather patterns that will help farmers make better management decisions related to planting, seeding rate, replanting, reduce waste and improve efficiency. The objectives of this research were to quantify how soil moisture content at planting, planting depth, and cultivar all influence soybean emergence and early growth response to temperature, and form of precipitation.

3.3 Materials and Methods

3.3.1 General experimental details and soil collection

The study was conducted at The Ohio State University in Columbus, Ohio, from November 2023 to February 2024. Soil (Crosby silt loam) was collected from the Waterman Agricultural and Natural Resources Center (Columbus, OH) in December 2023. Soil was sieved using 1.5-1.6 cm^2 mesh screens to remove plant debris and large soil particles. A subsample of soil analyzed for soil bulk density, available moisture content boundaries (field capacity and permanent wilting point), soil texture and initial soil nutrient levels (Table 3.1 and Table 3.2). Soil was stored in closed containers at room temperature (21-23°C) until being used.

Table 3.1. Soil physical properties of bulk density (g cm^{-3}), available water content boundaries on gravimetric basis of field capacity (FC) and permanent wilting point (PWP), and soil textural classification.

Soil physical property	Method of quantification	values	
Soil bulk density (BD)	Core method		1.07 g cm^{-3}
Available Water Content Boundaries	Pressure Chamber	FC (0.03 MPa)	0.26 g/g
		PWP (1.5 MPa)	0.16 g/g
Soil texture	Hydrometer Analysis	Soil texture	silt loam
		clay	14%
		silt	68%
		sand	18%

Table 3.2. Soil organic matter, mineral nutrient content, soil pH, buffer index and cation exchange capacity (CEC) of soil used for the experiment.

Soil chemical property	method	values
Organic matter	Gravimetric determination with high temperature oxidation	38 g kg^{-1}
Phosphorus	Weak Bray Extraction (1:7) / Colorimetric	21 mg kg^{-1}
Potassium	Ammonium Acetate extraction	115 mg kg^{-1}
Magnesium	Ammonium Acetate extraction	283 mg kg^{-1}
Calcium	Ammonium Acetate extraction	1661 mg kg^{-1}
Nitrate-N	Potassium Chloride extraction	61 mg kg^{-1}
Soil pH	pH 1:1 (Soil: Water)	6
Buffer index	pH 1:1:1 (Soil: Water: Sikora Buffer)	6.7
Cation Exchange Capacity		12.9 meq/100g

3.3.2. Soybean varieties and initial seed lot quality parameters

Two commercially available untreated soybean cultivars [Pioneer® 'P34A65PR' (3.4 relative maturity or RM) and 'P37T51PR' (3.7 RM)] were obtained within 8 months of project initiation and kept in 10°C cold storage until initiation of the experiments. Emergence scores, under suboptimal conditions from company literature, were 8 and 6 for 3.4 RM and 3.7 RM, respectively (9 signifies excellent germination and 1 indicates poor germination). Prior to conducting the experiments, each lot was analyzed for internal seed moisture content, standard

warm germination (AOSA, 2018), and two vigor tests of accelerated aging (AA) and cold germination tests (rolled towel method) according to the methodology by the AOSA (1983). Seed moisture was tested using a grain moisture tester (miniGAC 200, Dickey-John).

The standard germination test (SG), as outlined in AOSA (2018), was conducted on 100 seeds per cultivar using rolled paper towels (50 seeds per towel). Temperature-controlled chambers (Darwin Chamber or Percival Scientific Chamber) set to 25°C and 24 hours of day light cycle were employed for the SG test. Preliminary seed vigor assessment was conducted using the accelerated aging (AOSA, 1983) and (AOSA, 1983). For the accelerated aging test, 42 g of seeds were placed in the inner chamber of AA boxes, ensuring that the seeds did not come into contact with the distilled water (40 mL box^{-1}) present in the box. The AA boxes were then positioned in an outer AA chamber (VWR Scientific Products) for 72 hours (hr) at 41°C; seed moisture was confirmed to be between 270 and 300 g kg^{-1} at the conclusion of the AA period. To complete the AA test, seeds were removed from the outer chamber, and the SG test procedures on 100 seeds per lot were followed to determine final germination after 7 days (AOSA, 1983). For the cold germination test, the rolled towel cold germination vigor test with non-soil version as described by Loeffler et al. (1985) was employed (AOSA, 1983). 100 seeds of each cultivar (50 seeds per towel) were placed in a saturated paper towel (78# heavy, Anchor Paper) and covered with a wet thin paper towel (38# regular) and placed in a chamber with cold temperature of 10°C for seven days without light. After seven days, towels were transferred to warmer chamber at 25°C for seven days. Germination was counted at 4 and 7 days after transferring to the 25°C chambers. The results of the initial seed lot evaluations are presented in Table 3.3.

Table 3.3. Initial seed moisture content (g kg^{-1}), standard warm germination (%), accelerated aging vigor (%) and cold germination (%) results for soybean cultivar 3.4 RM and 3.7 RM.

Soybean cultivar	Initial Seed Moisture Content	Standard Warm Germination Test	Accelerated Aging Vigor Test	Cold Germination Vigor Test
	g kg^{-1}		%	
3.4 RM	70	90	80	86
3.7 RM	69	87	75	82

3.3.3 Experimental design

The experiment was conducted using controlled-environmental chambers in Kottman Hall to impose initial treatments, with experimental units being relocated to the Kottman Hall greenhouse to complete data collection. The experimental design was a split-split-split plot randomized complete block design with three replications in time of the whole plot factor. The main plot factor was the initial soil available water content (AWC) at the time of planting with two levels (section 3.3.4.1). The subplot factor consisted of six treatments consisting of varied water application and temperatures imposed (WT) (section 3.3.4.3). The sub-subplot factor was planting depth with two levels (section 3.3.4.2), and the two soybean cultivars (3.4RM and 3.7RM; described in section 3.3.2) were randomized within each planting depth.

3.3.4. Experiment 1 procedures.

3.3.4.1. Adjustment for soil available water content

Soil was amended with distilled water until either 20% or 60% AWC was achieved. Each water content was maintained individually and repeated three times for each specific water content levels. The soil data from Table 4.1 was used to determine the key soil moisture content levels to

achieve the 20% and 60% AWC levels through Eq. 1-3. In all cases, 100% AWC was assumed at field capacity, and 0% AWC was assumed at the permanent wilting point of the soil.

Soil moisture content at 60% AWC $$MC_{60\%}\left(\frac{W}{W}\right) = PWP + 0.60 \times (FC - PWP)$$ Eq:1

Soil moisture content at 20% AWC $$MC_{20\%\left(\frac{W}{W}\right)} = PWP + 0.20 \times (FC - PWP)$$ Eq:2

Volumetric water content $$VWC\ \left(\frac{V}{V}\right) = \mathrm{MC}\left(\frac{W}{W}\right) \times BD_{\mathrm{soil}} / \mathrm{BD}_{\mathrm{water}}$$ Eq:3

In all equations FC is soil moisture content at field capacity, PWP was soil moisture content at the permanent wilting point, BD was bulk density, respectively. The $\left(\frac{\mathrm{W}}{\mathrm{W}}\right)$ represented weight-basis and $\left(\frac{\mathrm{V}}{\mathrm{V}}\right)$ represented volume-basis. The bulk density of water was also assumed to be 1.0 g cm^{-3}.

3.3.4.2. Tray preparation

Trays measuring 50×37.5×12.5 cm were filled with soil to a depth of 8.75 cm. Each tray was designed with drainage openings at the bottom to facilitate the drainage of excess water. Volumetric water content of the soil (VWC) was initially quantified using Bluetooth soil moisture and temperature data loggers (HOBO MX 2306, HOBO MX2307). Based on current VWC, the amount of water to add to the soil in milliliters to achieve the target AWC levels was calculated using Eq.4 and Eq.5 (values reported in Supplemental Table 3.1).

Amount of water added at 20% AWC Eq:4

$$Water_{add} = \frac{(VWC_{20\%} - VWC) \times V_{soil}}{100}$$

Amount of water added at 60% AWC Eq:5

$$Water_{add} = \frac{(VWC_{60\%} - VWC) \times V_{soil}}{100}$$

Where V_{soil} is volume of soil in cubic centimeters.

Water was added to each tray individually to achieve the respective AWC. Two rows were prepared for each 2.5cm (shallow) and 3.8cm (recommended) planting depths. Eight soybean seeds from 3.4RM and 3.7RM cultivars per row was planted within each planting depth, and furrows were closed after seed placement was completed (Figure 3.1a). Bluetooth soil moisture and temperature sensors were installed in each tray at a depth of 3.75 cm to monitor soil temperature and moisture for the first 25 hours (Figure 3.1b). Then, trays were placed into a 10℃-temperature controlled chamber (Darwin Chamber) for 1 hour prior to implementing the WT treatments (Figure 3.1c).

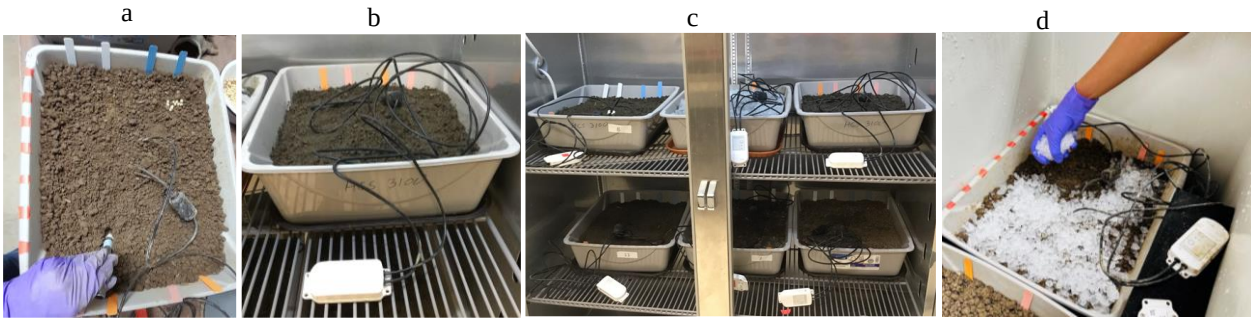


Figure 3.1. (a) Planting soybean seeds in the soil, (b) installing bluetooth soil moisture and temperature sensor, (c) trays were in the 10℃ chambers (Darwin chamber) prior to water treatments., (d) Applying ice treatment

3.3.4.3 Water-temperature (WT) treatment-Sub-plot factor

There were six WT as described in Table 3.4. Treatments consisted of the combination of two variables – timing/temperature of water addition, and cold temperature exposure and were each assigned a unique treatment ID number for ease of reference. Two temperature-controlled chambers (Darwin chamber and Percival scientific chamber) were maintained at 10℃ or 1.7℃ without light to provide temperature treatments. For applied water treatments, water was applied

as liquid water or as ice (Figure 3.1d), with total volume applied equating to 1.25 cm depth of precipitation using equations 6 and 7:

Volume of water $$V_w = width_{tray} \times length_{tray} \times 1.25$$ Eq:6

Mass of ice $$M_{ice} = width_{tray} \times length_{tray} \times 1.25 \times BD_{ice}$$ Eq:7

Each tray with water treatments received 1700 cm^3 of water. The applied liquid water temperature was maintained at either 10℃ or 1.7℃ prior to application. When providing liquid water treatments, the total volume of water was split-applied to provide enough time to percolate into the soil and avoid a long duration of standing surface water on each tray. Trays with ice treatment received 1550g of ice (treatment 3) Water and ice were applied across the entire surface area of the tray.

For the temperature component, trays were transferred in between 10℃ and 1.7℃ chambers within first 25 hours, either 1 hour after planting (phase 1, table 3.4) or 11 hours after planting (called phase 2) as prescribed (Table 3.4). Some trays (treatments 1, 2) were kept within the same chamber throughout first 25 hours based on the treatment design.

Table 3.4. Water-temperature treatments imposed during the experiment.

Treatment ID	Air Temperature (℃) and Water Treatment (cm, temperature, and form)	
	Phase 1: 1 hour post planting	Phase 2: 11 hours post planting
1	10℃, No water	10℃, No water
2	10℃, 1.27 cm as 10℃ water	10℃, No water
3	1.7℃, 1.27 cm as ice	10℃, No water
4	1.7℃, 1.27 cm as 1.7℃ water	10℃, No water
5	1.7℃, No water	10℃, 1.27 cm as 10℃ water
6	1.7℃, No water	10℃, No water

Twenty-five hours after planting (once all WT treatments completed), temperature-moisture sensors were removed from the trays. Then trays were moved to a temperature-controlled greenhouse (25℃ setpoint) (Figure 9a). Day/night conditions in greenhouse were set to 14d/10 night to emulate daylength in late April in Ohio. Beginning 72 hr after planting, trays were watered regularly in the greenhouse.

3.3.5. Experiment 2 procedures.

A separate experiment was conducted to evaluate three additional WT treatments only at 20% AWC (Table 3.5). Treatment 7 and 8 were identical to treatment 1 and 2 from Experiment 1 (Table 3.4), but treatment 9 was added to look at adding water during phase 2 after previously exposing seeds to warm temperature. All other experimental procedures and experiment design and implementation were the same as described previously.

Table 3.5. Water-temperature treatments imposed during the experiment.

Treatment ID	Air Temperature (℃) and Water Treatment (cm, temperature, and form)	
	Phase 1: 1 hour post planting	Phase 2: 11 hours post planting
7	10℃, No water	10℃, No water
8	10℃, 1.27 cm as 10℃ water	10℃, No water
9	10℃, No water	10℃, 1.27 cm as 10℃ water

3.3.6. Data collection and measurement

Daily emergence total was collected in each row in each tray separately for 11 days after initiation (10 days in the greenhouse; Figure 3.2a-b). Plants with two cotyledons totally emerged above the soil surface were counted as emerged. After emergence on day 11 was quantified, soybean plants were uprooted carefully from soil. If a plant was not present where a seed was

planted, the seed was recovered and assessed for condition as abnormal or dead. Uprooted plants were washed gently to remove all adhere soil particles in root system and then plants were assessed for the conditions and phenological stage. Biomass and number of normal seedlings and abnormal seedlings were taken each rows separately. Seedlings that exhibited a well-balanced, symmetrical growth across all essential parts including roots, hypocotyl, and cotyledon, were counted as normal seedlings (AOSA, 2018) and were pooled prior to measurement (Figure 3.2c). Seedlings with malformed roots, missing or damaged cotyledon and weak or decayed hypocotyl were counted as abnormal seedlings were also combined and weighed (Figure 3.2d). The count data of normal seedlings, abnormal seedlings and dead seeds was converted to percentages values prior to analysis.

Figure 3.2. (a) and (b) Trays after transferring to green house, (c) Normal seedlings, (d) Abnormal seedlings.

3.3.7. Data analysis

Biomass data were analyzed using analysis of variance (ANOVA) using the PROC GLIMMIX procedure in SAS 9.4 at α=.05 (SAS Institute, Cary, NC; version 9.4). The fixed factors were soil AWC at the time of planting, WT treatments, planting depth (depth), cultivar, and their interactions. The random factors were replication, replication x AWC (whole-plot error term), replication x AWC x WT (sub-plot error term), and replication x AWC x WT x depth (sub-sub

plot error term). For Experiment 2, the AWC factor was removed from the model to facilitate analysis. Mean separation was conducted using pair-wise comparisons when the global F test in was significant (α=.05).

The analysis for daily emergence patterns was conducted using sigmoid model (Equation 9) with the NLIN procedure in SAS 9.4. The difference between actual maximum germination and G_{max} (G_{diff}) also were calculated.

$$y = \frac{a}{1 + e^{(b-cx)}} + d$$

Eq:9

Total emergence percentage from days 5 to 11 were used to create descriptive model for each sampling unit (each row within a tray) with days of counts (x) as an independent variable and germination percentage as the dependent variable (y). Using Eq.10, maximum emergence (E_{max}) and 10%, 50% and 90% of the maximum emergence values were calculated (E_{10}, E_{50}, E_{90}). The time in days spent to achieve the respective E_{10}, E_{50}, and E_{90} values (T_{10}, T_{50}, and T_{90}), as well as time in days difference in between achieving 10 and 90% total emergence ($T_{10\text{-}90}$) were derived from Eq.11using the model parameters and emergence values (y) for each experimental unit.

$$x = \frac{\left(\ln\left(\frac{a}{y-d}\right) - 1\right) - b}{-c}$$

Eq:10

The values generated from Eq.10 (T_{10}, T_{50}, T_{90} and $T_{10\text{-}90}$) were then analyzed using the same analysis of variance (ANOVA) model and parameters as described for biomass data analysis.

3.4. Results and Discussion

3.4.1. Experiment 1 – Soil moisture and temperature sensor data

Figure 3.3 illustrates the variations in soil moisture and temperature within the first 25 hours for the six WT treatments at AWC of 20% (Figure 3.3a, b) and 60% (Figure 3.3c, d). Regardless of the AWC, both soil moisture and temperature exhibited similar patterns. Treatments 2, 4, and 5, where liquid water was applied 1 or 11 hours post-planting, there was a drastic increase in soil moisture immediately after water application (Figure 3.3a, c). Conversely, treatment 3, which involved ice application, showed a gradual increase in soil moisture. As for soil temperature, treatments 1 and 2 displayed a similar pattern of gradual decrease to 10°C (Figure 3.3.b, d). Treatments 3, 4, 5, and 6 (all placed in 1.7°C 1h post-planting) also showed comparable soil temperature patterns to one another. However, the ice application in treatment 3 resulted in a greater drop in temperature compared to the other three treatments followed by a more gradual return to 10°C during phase 2 (11 to 25h post-planting). These patterns suggest imposed treatments did influence soil moisture and temperature at the location of the seeds in this experiment.

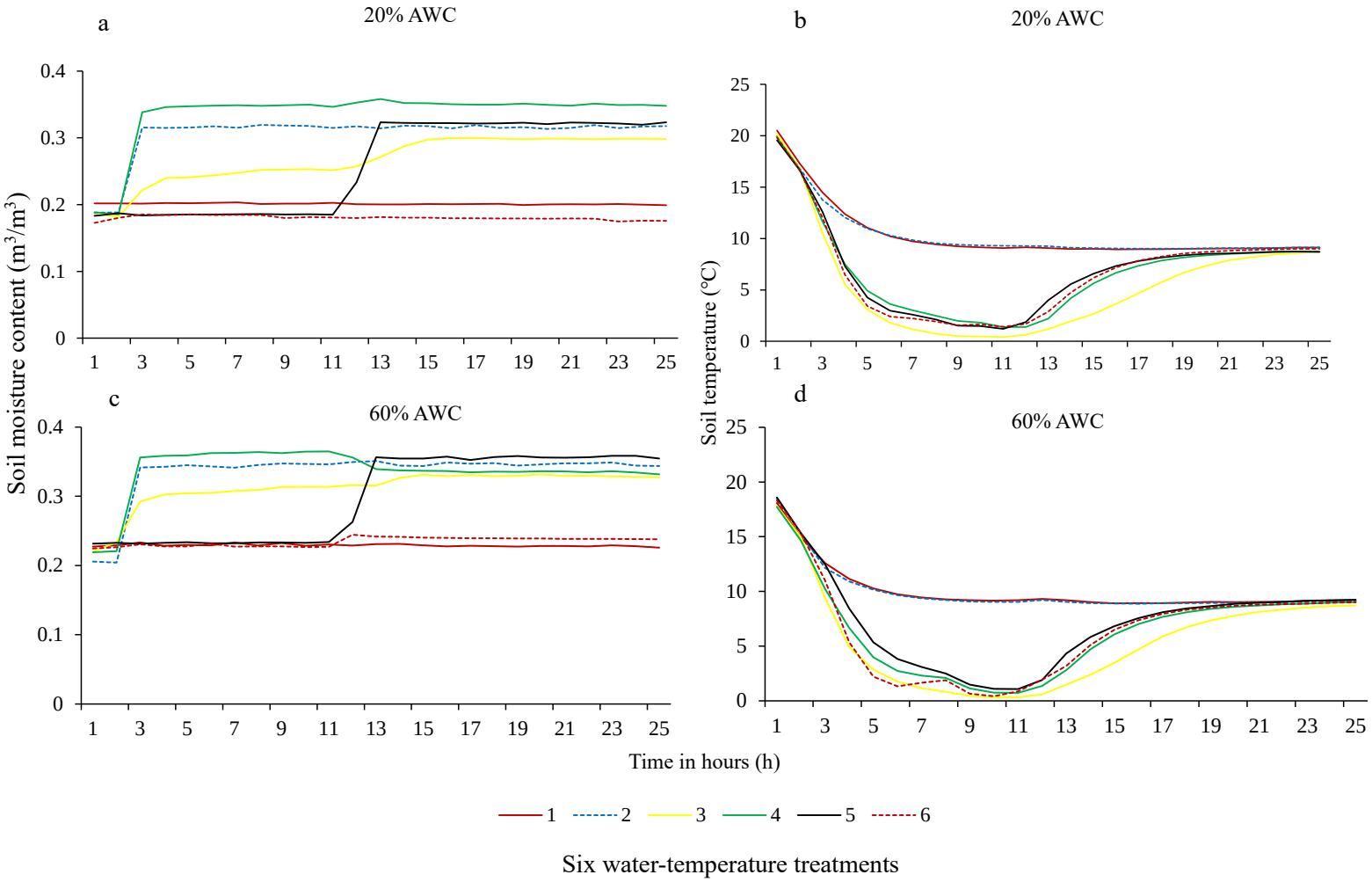


Figure 3.3. Soil moisture content (m^3 m^{-3}) (a, c) and soil temperature (°C), (b,d) for the six water-temperature treatments in the 20% available water content soil (top row panels a-b) and the 60% available water content soil (bottom row panels c-d). Water-treatment numbers in the legend correspond to the treatment ID values presented in Table 3.4.

3.4.2. Seedling biomass and total emergence

Normal seedlings biomass, emergence percentage and percentage of dead seeds were significantly affected by imposed WT treatments, cultivars, and interaction between depth and AWC (Table 3.6). The four-way and three-way interactions of seedling biomass and percentages were not significant ($p>0.05$) (Table 3.6). Abnormal seedling biomass and percentage were not affected by imposed factors ($p>0.05$).

Table 3.6. Analysis of variance results for biomass and emergence (percentage) of normal seedlings, abnormal seedlings, dead seeds, total biomass, and total emergence of soybean for available water content (AWC), water-temperature treatment (WT), planting depth (Depth), cultivar, and their interactions in 11 days after initiation. P-values below 0.05 are indicated with bold.

Effect	*p*-value						
	Normal seedling		Abnormal seedling		dead seeds	Total biomass	Total emergence %
	biomass	%	biomass	%	%		
AWC	0.2049	0.2143	0.5769	0.7045	0.0557	0.1331	**0.0340**
WT	**0.0025**	**0.0004**	0.5054	0.8822	**0.0002**	**0.0008**	**0.0003**
Depth	0.8384	0.4876	0.4977	0.8926	0.4869	0.5645	0.6750
Cultivar	**<.0001**	**0.0002**	0.7858	0.6694	**0.0010**	**<0.0001**	**0.0015**
AWC × WT	0.7805	0.9367	0.3440	0.2544	0.9494	0.9085	0.9442
AWC × Depth	**0.0315**	**0.0230**	0.2880	0.2315	**0.0282**	**0.0074**	**0.0066**
WT × Depth	0.3369	0.5761	0.9930	0.9918	0.9647	0.3466	0.8613
AWC × Cultivar	**0.0035**	**0.0089**	0.7740	0.6694	**0.0038**	**0.0017**	**0.0059**
WT × Cultivar	0.5751	0.5546	0.6351	0.4370	0.3935	0.7102	0.4515
Depth × Cultivar	0.4971	0.7409	0.9808	0.6694	0.3344	0.4723	0.5033
AWC × WT × Depth	0.7232	0.6932	0.7891	0.8725	0.5540	0.6384	0.6098
AWC × WT × Cultivar	0.8668	0.8335	0.0758	0.1043	0.0734	0.2744	0.0797
AWC × Depth × Cultivar	0.3756	0..6436	0.9720	0.8867	0.8641	0.3475	0.5836
WT × Depth ×Cultivar	0.4540	0.6612	0.7559	0.6703	0.8478	0.6575	0.7411
AWC ×WT × Depth × Cultivar	0.6740	0.5401	0.1954	0.3283	0.2183	0.4962	0.3461

Application of liquid water at 10℃ or 1.7℃ or ice, regardless of timing (treatment 2,3,4 and 5), resulted in 30% to 64% lower normal seedling biomass than the treatment with no water and ice application (treatments 1 and 6) (Figure 3.4). This pattern was likely driven by similar changes in normal seedling emergence, where only 20 to 34% of normal seedlings had emerged at 11 days after post-planting with treatments 2-5 (Figure 3.5). The reduction in normal seedling emergence was associated with greater percentages of dead seeds, rather than abnormal seedling formation (Figure 3.5). Trays placed at the lower temperature (1.7℃) for the initial 10h that did not have water applied (treatment 6) produced similar biomass and emergence of normal seedlings as trays were at 10℃ for the initial 10h without water applied (treatment 1) (Figures 3.4-3.5). When examining the temperature fluctuations in Figure 3.3, it was observed that treatments 2, 3, 4, and 5 dropped to temperatures below 10℃ after 5 hours, and then returned to 10℃ after 22 hours post-planting. This consistent temperature fluctuation could be a contributing factor to the observed results. The significant effect of WT on total biomass and total emergence was mainly driven by differences in normal seedlings. Higher biomass of normal seedlings for treatments 1 and 6 were mainly driven by greater numbers of normal seedlings.

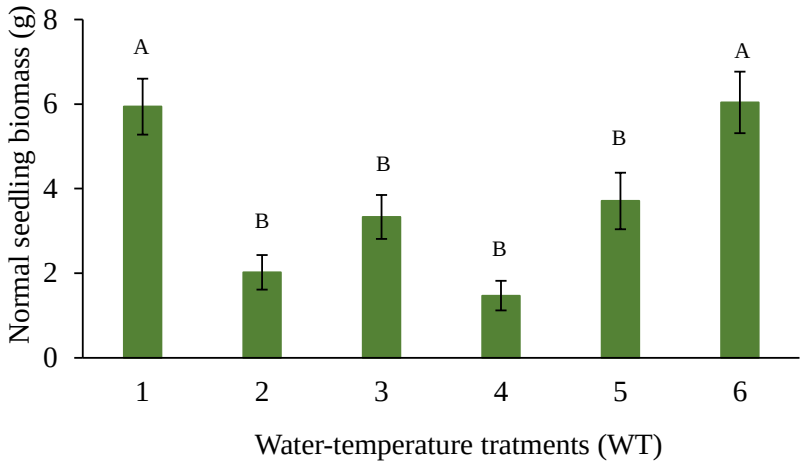


Figure 3.4. Normal seedling biomass of soybean as a function of six water-temperature (WT) treatments. The Treatment ID numbers assigned in Table 3.4 are used to differentiate the WT treatments. Means followed by the same letter are not significantly different according to the paired t-test ($p < 0.05$). Error bars are depicting the standard error of the WT treatment mean.

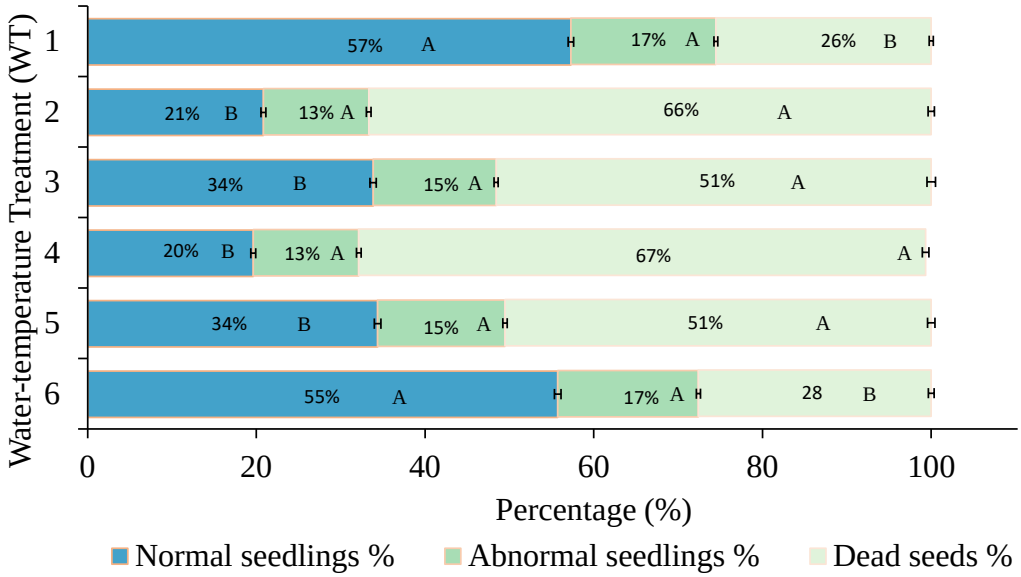


Figure 3.5. Percentage of normal seedlings, abnormal seedlings, and dead seeds as function of water- temperature (WT) treatments. The Treatment ID numbers assigned in Table 3.4 are used to differentiate the WT treatments. Means followed by the same letter in same color palette are not significantly different according to the paired t-test ($p < 0.05$). Error bars within each series are depicting the standard error of the means.

Despite more gradual fluctuations in soil temperature and moisture in treatment 3 (ice application and 1.7°C 1 hour after planting) compared to the other WT treatments (Figure 3.3), similar emergence and early growth responses were observed in WT treatments 2 and 4 (liquid water application). Several theories have been proposed in the literature to explain the injuries that seeds of warm-season crops sustain during germination and emergence at cold temperatures. These include disruptions to membrane composition, pest and disease attacks, changes in seed coat characteristics, and respiratory changes (Herner, 1986; Simon & Harun, 1972). Soybean germination and emergence depends on the soil temperature, moisture, and oxygen level within

the seed zone. Exposure to cold soil can delay germination, leaving the seeds in a dormant state and increasing their vulnerability to disease. This may have been evident in the current study due to the observation of dead seeds infected with a fungal layer. The use of untreated seeds in unsterilized field soil may have both contributed to the colonization success of the seedling diseases in the current study. A study conducted by Wang et al. (2023) observed a reduction in plant dry weight ranging from 20% to 60% in different soybean cultivars when the seeds were exposed to temperatures of 4℃. Additionally, the average germination time was prolonged to between 7 and 13 days.

The interaction between AWC and planting depth revealed that planting deeper at lower AWC resulted in higher normal seedling biomass and emergence. Notably, planting at a depth of 3.8 cm under 60% AWC yielded the lowest biomass compared to other conditions (as shown in Figure 3.6). In a study conducted by Narayanan & Fallen (2019) under low moisture soil (20%), soybean seeds showed better emergence and better primary root length compared to 60, 80, and 100% moisture content. In this current study, similar biomass and emergence were recorded both 20 and 60% AWC when looking across the planting depth. Though, only 25 to 46% normal seedlings had emerged at 11 days under both AWC.

When considering the interaction between AWC and cultivar, cultivar 3.4 RM demonstrated the highest biomass and emergence under 20% AWC, outperforming cultivar 3.7 RM and 60% AWC conditions (refer to Supplementary Table 3.2). This could be attributed to the higher vigor score recorded for cultivar 3.4 RM compared to cultivar 3.7 RM. Across all other factors, the cultivar 3.4 RM exhibited greater biomass and emergence compared to cultivar 3.7 RM. A study conducted by Obendorf and Hobbs (1970) found that seeds with a higher initial moisture content showed no reduction in survival and biomass under cold temperature imbibition.

However, both cultivars used in this study exhibited similar moisture content (as detailed in Table 3.7), indicating that moisture content was not a contributing factor in this case. Though deeper planting resulting later calendar date of emergence (Lindsey et al., 2024), greater biomass in deeper planting depth under low AWC may be due to less exposure to cold air temperature compared to shallower planting depth as was documented with corn previously (Nemergut et al., 2021). A study conducted in Nebraska in 2013 concluded that planting at 4.3 cm planting depth produced higher yield compared to shallow planting depth of less than 3.1 cm with same seeding rates under cold soil conditions. Although the effect of the seeding rate was not specifically investigated in our study, we observed a similar trend of higher biomass associated with deeper planting depths under cold soil conditions.

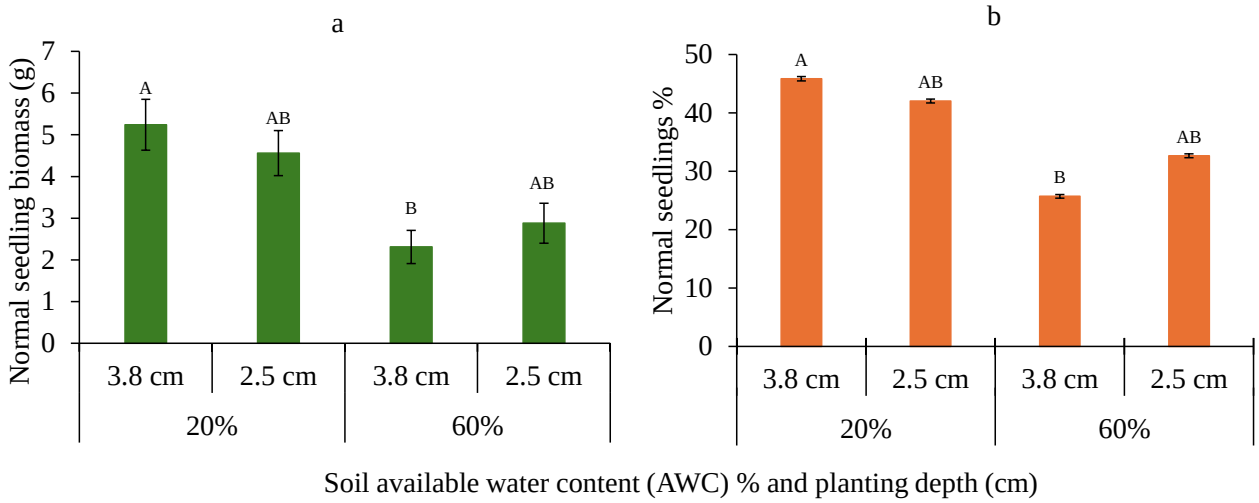


Figure 3.6. (a) Normal seedling biomass and (b) emergence of soybean seedlings as a function of soil available water content (AWC) and planting depth. Means followed by the same letter are not significantly different according to the paired t-test ($p < 0.05$). Error bars within each series are depicting the standard error of the means.

3.4.3. Daily emergence patterns

The T_{10}, T_{50}, and T_{90} values were significantly affected by WT treatments, and in most cases, the interaction between AWC and WT (Table 3.7). T_{10-90} was only affected by WT. Three-way interaction of AWC and WT and planting depth was significant for T_{10}.

Table 3.7. Analysis of variance results for time in days spent to achieve 10, 50, 90 % emergence and difference between 10 to 90 % emergence (T_{10}, T_{50}, T_{90}, T_{10-90}) of soybean for available water content (AWC), water-temperature treatment (WT), planting depth (depth), cultivar, and their interactions. P-values below 0.05 are indicated with bold.

	p-value			
Effect	T_{10}	T_{50}	T_{90}	T_{10-90}
Available Water Content (AWC)	0.1941	0.1917	0.1982	0.3050
Water-Temperature Treatment (WT)	**0.0001**	**<.0001**	**<0.0001**	**0.0434**
Depth	0.9486	0.8409	0.7953	0.5962
Cultivar	0.8072	0.5971	0.5386	0.2154
AWC × WT	**0.0051**	**0.0020**	**0.0020**	0.6028
AWC × Depth	0.5441	0.5018	0.4939	0.7432
WT × Depth	0.3416	0.2274	0.2149	0.7833
AWC × Cultivar	0.8797	0.6687	0.6102	0.2403
WT × Cultivar	0.8705	0.7414	0.6896	0.2812
Depth × Cultivar	0.7511	0.8542	0.9090	0.5144
AWC × WT × Depth	**0.0483**	0.0772	0.1053	0.8139
AWC × WT × Cultivar	0.2255	0.3206	0.3721	0.9364
AWC × Depth × Cultivar	0.2285	0.2076	0.2014	0.5455
WT × Depth × Cultivar	0.5680	0.5661	0.5718	0.7930
AWC × WT × Depth × Cultivar	0.8249	0.7792	0.7670	0.8144

Treatment 4 (which involved the application of 1.7℃ water 1 hour after planting, with an initial 10 hours in a 1.7℃ chamber) was found to be the most detrimental in terms of emergence totals, exhibiting the lowest values for E_{10}, E_{50}, and E_{90} (refer to Supplementary Figure 3.1). Discrepancies between the model-predicted maximum germination values and the normal seeding emergence values recorded were low and ranged from 0.02% to 2%. Despite treatment 4 showing the lowest emergence, it also recorded the shortest duration in days to reach T_{10}, T_{90}, and T_{10-90}

(Figure 3.7). Given the interaction between AWC and WT, treatment 4 spent the least amount of time to reach T_{10} and T_{90}, but only under 60% AWC. This was primarily because, on most occasions, the total emergence percentage of treatment 4 was 0% under 60% AWC. For T_{10}, the AWC×WT interaction varied based on the imposed planting depth. In this case, treatment 4 demonstrated the shortest time to reach E_{10}, but only at a planting depth of 2.5 cm and a moisture content of 60%.

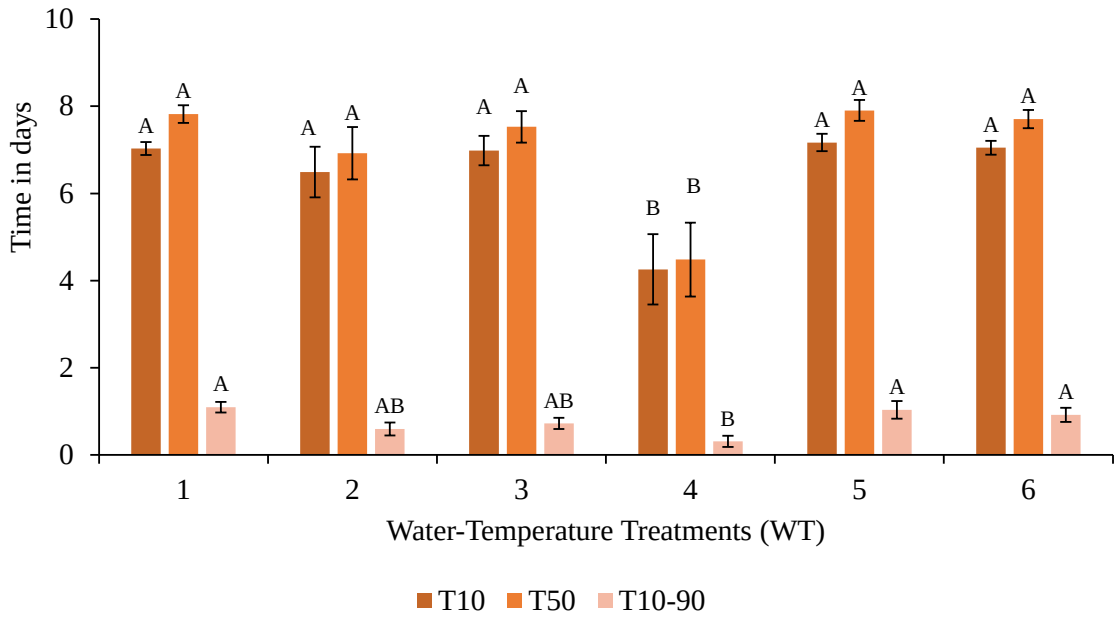


Figure 3.7. Time in days spent to achieve 10, 50% emergence and time in days difference between 10 and 90% emergence of soybean as a function of six water-temperature treatments. Means followed by the same letter are not significantly different according to the paired t-test ($p < 0.05$). Error bars within each series are depicting the standard error of the means.

Planting seeds in cold, moist soil coupled with the application of cold water can lead to rapid imbibition, resulting in water accumulation between the cotyledons and causing seed injury and poor emergence (Herner, 1986). The rate at which water enters the seed during imbibition is a crucial factor in determining seed germination. Water inside the seed coat and cotyledons can restrict the movement of oxygen between the axis tissue, creating anaerobic conditions. Furthermore, rapid water uptake can lead to swift solute leakage from the cellular compound in

the seed. If this leakage is prolonged, it can cause greater damage to the seed tissue, resulting in a high number of dead seeds.

One limitation of this study was the practice of uprooting seedlings within 11 days. Some literature suggests that germination under cold soil conditions can extend up to 14 to 16 days. Therefore, future studies might consider extending the observation period. This may have also helped increase emergence totals beyond the values reported here. It is possible for a crop like soybeans protracted periods of emergence may be beneficial, especially in early-planted scenarios where first emerged seedlings are more likely to be exposed to freezing temperatures after emergence. Having later-emerging seeds to replace those lost to freeze damage may help farmers avoid having to replant fields.

3.4.4. Experiment 2 – Biomass and normal seedling emergence.

Normal seedling and abnormal seedling emergence and biomass were affected by the imposed WT treatments (Table 3.8). Application of 10℃ water at 11 hours after planting recorded statistically similar normal seedling biomass and emergence (Supplemental Figure 3.2); application of 10℃ water 1 hour after planting reduced emergence and biomass, similar to the previous result reported in Experiment 1 (Figures 3.4-3.5). This difference was mainly driven by recording higher abnormal seedling percentage with water application. This is suggested that even in the 10℃, application of water within first 24h after planting reduced seedling biomass and emergence, though was less damaging when it occurred later in the germination phase.

Table 3.8. Analysis of variance results for biomass and emergence (percentage) of normal seedlings, abnormal seedlings, dead seeds, total biomass, and total emergence of soybean for water-temperature treatment (WT), planting depth, cultivar, and their interactions in 11 days after initiation. P-values below 0.05 are indicated with bold.

Effect	*p*-value						
	Normal seedling		Abnormal seedling		Dead seed	Total biomass	Total emergence
	biomass	%	biomass	%	%		
WT	**0.0282**	**0.0161**	**0.0436**	0.0552	0.1332	**0.0307**	0.1193
Depth	0.0795	0.1135	**0.0182**	0.0663	0.0667	**0.0138**	0.0603
Cultivar	0.4915	0.3835	0.3871	0.8164	0.6438	0.9500	0.5663
WT× Depth	0.3024	0.2963	0.5336	0.4757	0.1537	0.1536	**0.0332**
WT × Cultivar	0.7789	0.9344	0.3696	0.6067	0.4052	0.4079	0.528
Depth × Cultivar	0.3520	0.251	0.5443	0.4900	0.877	0.5685	0.7731
WT × Depth × Cultivar	0.8196	0.8547	0.8840	0.9588	0.7287	0.5498	0.8605

In WT × depth interaction, treatment with no water application recorded highest total emergence under both planting depths compared to other two treatments. Application of 10℃ water 11h after planting at 2.5cm planting depth recorded lowest total emergence. However, application of 10℃ water 11h after planting (treatment 8) showed similar total emergence with no water application treatment (treatment 7) (Figure 3.8).

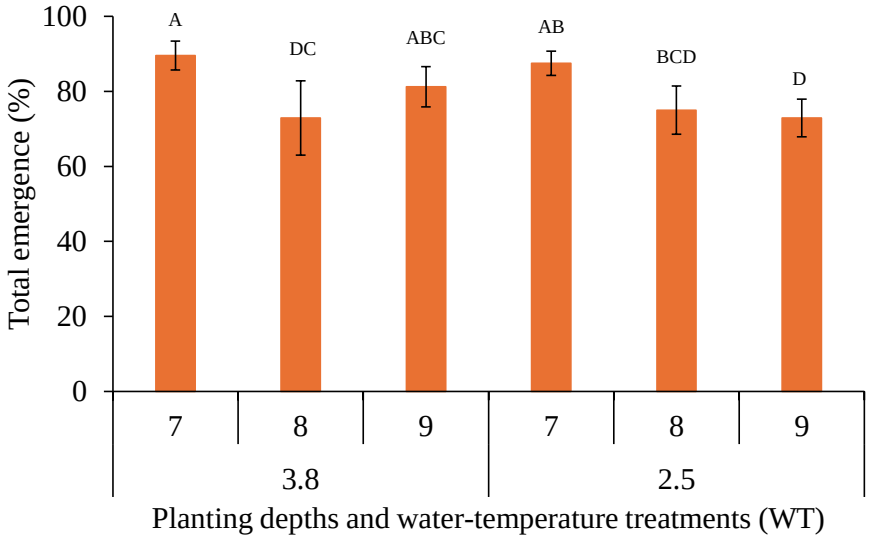


Figure 3.8. Total emergence of soybean seedlings as a function of water-temperature treatments (WT) and planting depth. Means followed by the same letter are not significantly different

according to the paired t-test ($p < 0.05$). Error bars within each series are depicting the standard error of the means.

3.5. Conclusions and recommendations

Cold precipitation within first 24 hours after planting can reduce soybean emergence and seedling biomass. However, exposure to cold temperatures (above freezing) within the first 11 hours, without any water application within the first 24 hours, does not appear to reduce soybean germination and emergence. Planting at a deeper depth in drier soil under cold temperatures can result in better biomass and emergence. In all cases, the cultivar 3.4 RM demonstrated the highest biomass and emergence. Based on the findings of this study, we recommend that farmers base their early planting decisions on the weather conditions forecasted for the next 24 hours. If there is a chance of cold rain within 24 hours after planting, it would be advisable to delay planting.

Use of treated seed may also reduce the amount of seed death and disease infection and should be investigated in future research. Furthermore, it is crucial to understand the biochemical and cellular changes that occur in seeds when exposed to cold water. This is particularly important in relation to seed moisture, seed coat characteristics, cellular component changes, and seed metabolic pathways.

References

Baum, M. E., Licht, M. A., Huber, I., & Archontoulis, S. V. (2020). Impacts of climate change on the optimum planting date of differentmaize cultivars in the central US corn belt. European Journal ofAgronomy, 119, 126101. https://doi.org/10.1016/j.eja.2020.126101.

Bohner, H. 2003. Do soil temperatures at planting impact soybean yield? Ministry of Agriculture, Food and Rural Affairs. Available at; https://cropwatch.unl.edu/2019-CW-News/2019-images/soybean/Do-soil-temperatures-at-planting-impact-soybean-yield-Horst Bohner.pdf (accessed May 2023).

De Bruin, J. L., & Pedersen, P. (2008). Soybean seed yield response to planting date and seeding rate in the Upper Midwest. *Agronomy Journal*, *100*(3), 696-703. https://doi.org/10.2134/agronj2007.0115

Duke, S. H., Kakefuda, G., & Harvey, T. M. (1983). Differential leakage of intracellular substances from imbibing soybean seeds. *Plant Physiology*, *72*(4), 919-924. https://doi.org/10.1104/pp.72.4.919

Egli, D. B. and Cornelius, P. L. 2009. A regional analysis of the response of soybean yield to planting date. *Agronomy Journal*, 101(2), pp. 330-335. https://doi.org/10.2134/agronj2008.0148

Haidar, S., Lackey, S., Charette, M., Yoosefzadeh-Najafabadi, M., Belzile, F., Rajcan, I., ... & Samanfar, B. (2023). Genome-wide analysis of cold imbibition stress in soybean, Glycine max. Frontiers in Plant Science, 14, 1221644.

Hatfield, J.L. and Egli, D.B. 1974. Effect of temperature on the rate of soybean hypocotyl elongation and field emergence 1. *Crop Science*, 14(3), pp.423-426. https://doi.org/10.2135/cropsci1974.0011183X001400030025x

Hobbs, P.R. and Obendorf, R.L. 1972. Interaction of Initial Seed Moisture and Imbibitional Temperature on Germination and Productivity of Soybean. *Crop Science*, 12(5), pp.664-667. https://doi.org/10.2135/cropsci1972.0011183X001200050033x

Hu, M., & Wiatrak, P. 2012. Effect of planting date on soybean growth, yield, and grain quality. *Agronomy Journal*, 104(3), 785-790. https://doi.org/10.2134/agronj2011.0382

Leopold, A. C., & Musgrave, M. E. (1979). Respiratory changes with chilling injury of soybeans. *Plant physiology*, *64*(5), 702-705. https://doi.org/10.1104/pp.64.5.702

Licht M. (2021). Corn, and Soybean Planting Date Considerations. Integrated crop management, Extension and Outreach. Iowa state university. Available at https://crops.extension.iastate.edu/blog/mark-licht-zachary-clemens/corn-and-soybean-planting-date-considerations. (accessed 24 February 2024).

Lindsey, A.J., Ortez, O.A., Thomison, P.R., Carter, P.R., Coulter, J.A., Roth, G.W., Carrijo, D.R., Quinn, D.J. & Licht, M.A. (2024). Severe storm damage and short-term weather stresses on corn: A review. *Crop Science*. https://doi.org/10.1002/csc2.21212

McDonald Jr, M. B., Vertucci, C. W., & Roos, E. E. (1988). Seed coat regulation of soybean seed imbibition. *Crop Science*, *28*(6), 987-992. https://doi.org/10.2135/cropsci1988.0011183X002800060025x

Robinson, A. P., Conley, S. P., Volenec, J. J., & Santini, J. B. (2009). Analysis of high yielding, early-planted soybean in Indiana. *Agronomy Journal*, *101*(1), 131-139. https://doi.org/10.2134/agronj2008.0014x

Specht, James E., Jennifer M. Rees, Gary L. Zoubek, Keith L. Glewen, Brandy S. VanDeWalle, James W. Schneider, David L. Varner, and Allan R. Vyhnalek. (2012). Soybean planting date: When and Why? Nebraska Extension EC145. http://extensionpubs.unl.edu/publication/9000016368318/soybean-planting-date-when-and-why/. (accessed 04 May 2024).

Specht J., Rees J., Glewen K., Grassini P. 2014. Soybean planting depth. Institute of Agriculture and Natural Resources, cropwatch, University of Nebraska. https://cropwatch.unl.edu/soybean-planting-depth-consider-planting-deeper. (accessed 2 June 2024.

Specht, Jim, Jenny Rees, Roger Elmore, Nathan Mueller, Keith Glewen. Considerations when Planting Soybean Early, University of Nebraska Extension Cropwatch, April 25, 2019. Considerations when Planting Soybean Early | CropWatch | University of Nebraska–Lincoln (unl.edu). (accessed 04 May 2024).

Szczerba, A., Płażek, A., Pastuszak, J., Kopeć, P., Hornyák, M., & Dubert, F. (2021). Effect of low temperature on germination, growth, and seed yield of four soybean (Glycine max L.) cultivars. *Agronomy*, *11*(4), 800.

Wang, X., Li, X., Zhou, Q., Song, S., & Dong, S. (2023). Comparison and Evaluation of Low-Temperature Tolerance of Different Soybean Cultivars during the Early-Growth Stage. *Agronomy*, *13*(7), 1716.

Webster, B. D., & Leopold, A. C. (1977). The ultrastructure of dry and imbibed cotyledons of soybean. *American Journal of Botany*, *64*(10), 1286-1293. https://doi.org/10.1002/j.1537-2197.1977.tb10822.x.

Supplemental Tables and Figures

Supplementary Table 3.1. Applied moisture content (%), Available Water Content (AWC) (%), volume of water (cm^3) and mass of ice (kg) for each tray.

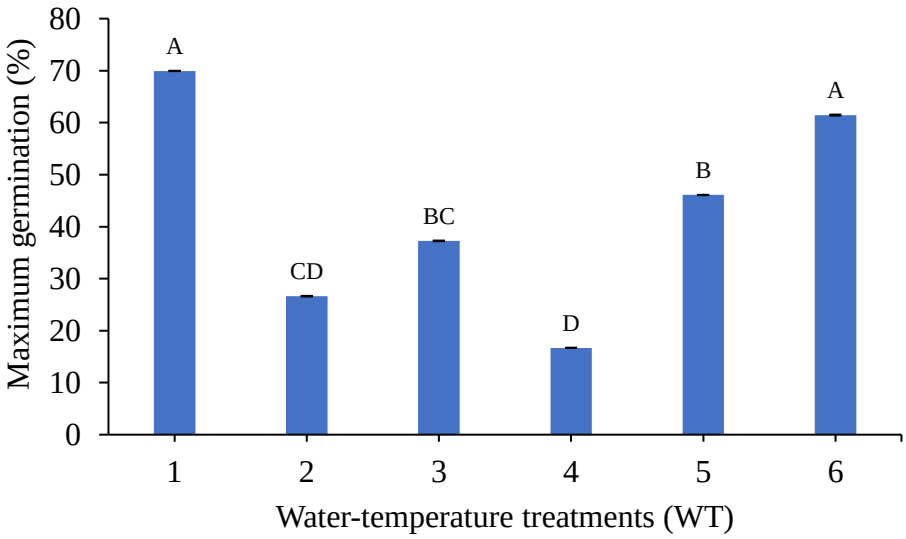
Parameter	Value	Equation/method
Soil Available Moisture content (AWC) (w/w)	10.50%	Table 4
Soil Available Moisture content (AWC) (v/v)	10.75%	Eq:4
Soil Moisture content at 60% AWC (w/w)	21.92%	Eq:2
Soil Moisture content at 60% AWC (v/v)	23.47%	Eq:4
Soil moisture content at 20% AWC (w/w)	17.90%	Eq:3
Soil Moisture content at 20% AWC (v/v)	19.15%	Eq:4
Soil moisture content (volumetric)	19.00%	Bluetooth soil moisture sensor
Amount of water added at 20% AWC	17 cm^3	Eq:5
Amount of water added at 60% AWC	500 cm^3	Eq:6
Volume of water (1.27 cm depth)	1700 cm^3	Eq:7
Mass of ice	1.55 kg	Eq:8

Supplementary Table 3.2. Normal seedling biomass and emergence as a function of interaction between available water content (AWC) and cultivar (C).

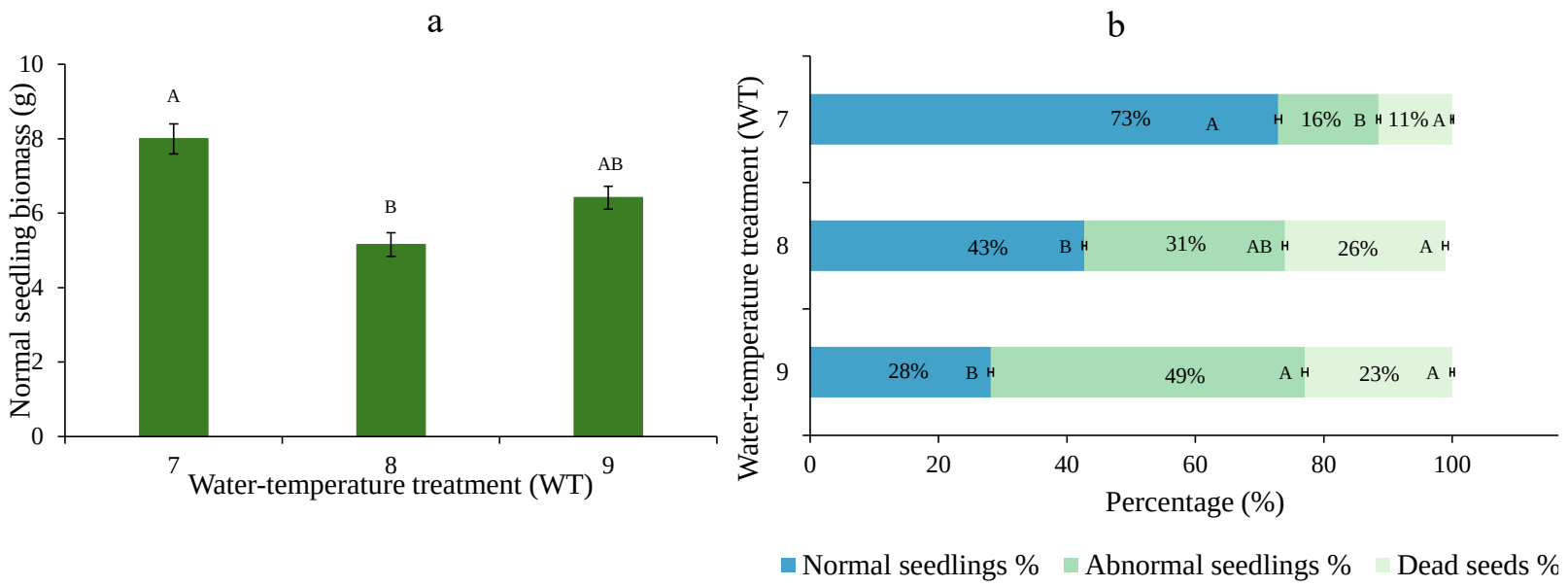
		Normal seedling	
AWC %	Cultivar	Emergence %	biomass (g)
20	3.4RM	52.78^{A}	5.96^{A}
	3.7RM	35.07^{B}	3.85^{B}
60	3.4RM	30.90^{B}	2.81^{B}
	3.7RM	27.43^{B}	2.38^{B}

Means followed by same letter in each column were not significantly different

Supplementary figures



Supplementary Figure 3.1. Model predicted maximum emergence percentage (%) varied with applied six water-temperature (WT) treatments. Treatment ID values correspond to the imposed WT treatments described in Table 3.4. These values were generated through sigmoid model (Equation 9) with the NLIN procedure in SAS 9.4. Mean followed by same letters were not significantly different ($P<0.05$). Error bars within each series are depicting the standard error of the means.



Supplementary Figure 3.2. (a) Normal seedling biomass and (b) Percentage of normal seedlings, abnormal seedlings, and dead seeds of soybean as a function of the three water-temperature (WT) treatments. Treatments have been identified by their Treatment ID values described in Table 3.5. Means followed by the same letter are not significantly different according to the paired t-test ($p<0.05$). Error bars within each series are depicting the standard error of the means.

Chapter 4. Cold temperature and water effect on *Fusarium graminearum* infection and emergence of soybean

4.1. Abstract

Fusarium graminearum is a fungus that causes damping-off in soybeans, though currently it is unclear how moisture/precipitation and temperature representative of ultra-early planting conditions (prior to April 15) affect disease incidence. This study aimed to quantify the effect of moisture content at planting and temperature/form of precipitation during germination on soybean emergence and *F. graminearum* infection. A greenhouse experiment was conducted using a split-split-split plot randomized complete block design and was repeated in times. Main plot was the water content available in the media (60% and 80%) at planting. The subplot factor consisted of four temperature/form of precipitation treatments (10℃ or 1.7℃, water applied as liquid or ice). The sub-subplot factors was presence or absence of *F. graminearum* inoculum, and soybean cultivar was randomized within inoculum level. Daily emergence was measured through 11 days after planting, and biomass and number of seedling with brown lesions (necrosis) on crown also were quantified at day 11. The result showed that 1.7℃ liquid water application or the same quantity of water as ice when inoculum was applied reduced total emergence by 6% compared to same water treatments without inoculum. Inoculated media produced more dead seeds under 60% moisture content compared to 80% moisture content. However, the presence of inoculum did not affect the total biomass accumulation in soybean seedlings. The study suggests that the activity of *F. graminearum* had some effect on seedling emergence under cold precipitation, though future research should be conducted to expand upon these results.

4.2. Introduction

Fusarium graminearum is a common pathogen affecting soybean seedlings in Ohio, causing seed decay, pre and post-emergence damping off, and root rots during the early stages of crop development (Marburger et al., 2017). Various Fusarium species have been identified as causing root rot, with the severity of different species varying from region to region (Yan and Nelson, 2022). Among these, *F. graminearum* and *F. oxysporum* are the most common causal organisms for these diseases in soybeans in Ohio (Yan and Nelson, 2022). Prior to identifying *F. graminearum* as pathogenic to soybeans, it was found in various parts of the soybean plant across the northern states in the U.S. Symptoms associated with *F. graminearum* infection during the early seedling stage begin with light-brown lesions on the roots and shoots, which become necrotic and turn black. Irregularly shaped lesions also appear on the cotyledons and leaves, subsequently causing the plant to wilt and die. Little research has been conducted to determine the potential yield loss caused by *F. graminearum* due to Fusarium root rot. A study conducted by Koenning & Wrather (2010) observed an annual yield loss ranging from 158,000 to 289,000 metric tons from 2006 to 2009 from each soybean producing states in United State.

Seedling disease caused by *F. graminearum* has most likely increased in soybean due to changes of some production practices (Broders et al., 2007). *F. graminearum* is an economically important pathogen of cereal crops, especially corn and wheat, causing substantial yield and quality loss. Most farmers in Ohio practice crop rotation with corn-soybean or corn-soybean-wheat with no tillage or reduced tillage practices (Broders et al., 2007; Ellis et al., 2011). These practices leave substantial amount of the corn and wheat crop residues on the soil surface and can act as a primary inoculum source for soybean infection. The presence of crop residues in early spring with

reduced tillage practices also provide favorable conditions for the fungus to overwinter on crop debris (Ellis et al., 2011).

F. graminearum would be most pathogenic to soybean seedlings at 25°C (Leslie and Summerell, 2008). However, cool, wet and compacted soil conditions are also favorable for this pathogen (Wrather et al., 2001; Yan and Nelson, 2022). Early planting practices could result in farmers planting soybean seeds into cool, wet environmental conditions that can delay seed germination and could facilitate favorable conditions for infection by soil borne pathogens. Furthermore, the frequency precipitation events during April and May also increased in past several years (Lindsey et al., 2024). Most of the past work only studied the effect of the *F. graminearum* ranging from 10°C to 30°C (Yan and Nelson, 2022). The impact of *F. graminearum* as a seedling pathogen of soybean in Ohio, under unexpected cold temperature and moist soil conditions are still under researched. The objective of research was to quantify the effect of media moisture content at planting, temperature/form of precipitation and presence or absence of *F. graminearum* inoculant on soybean emergence and early growth.

4.3. Materials and Methods

4.3.1. General experimental details and media preparation

The study was conducted at The Ohio State University in Columbus, Ohio, from February 2024 to April 2024. The experiment was conducted using greenhouse potting media and *Fusarium graminearum* inoculum. Potting media used was PRO-MIX BX general purpose soilless media consisting of high quality fibrous peat moss (75-85%), perlite, limestone, and wetting agent. A subsample of media analyzed for initial media nutrient levels (Table 4.1). Total porosity, water

holding capacity, air space, and bulk density of the substrate was measured using a porometer (Table 4.2). Permanent wilting point (PWP) of the substrate was measured by 1.5 MPa ceramic plate extractor method. The container capacity (CC) of the substrate was measured using the gravimetric method, and was assumed to be the maximum water holding capacity of the media.

Table 4.1. Media nutrients, pH and conductivity measurement (EC) of media used for the experiment.

Chemical property	values	Rating
Nitrate (NO_3-N)	76 mg kg^{-1}	Acceptable
Phosphorus (P)	17.1 mg kg^{-1}	High
Potassium (K)	66 mg kg^{-1}	Acceptable
Magnesium (Mg)	22 mg kg^{-1}	Low
Calcium (Ca)	115 mg kg^{-1}	Acceptable
Sodium (Na)	19 mg kg^{-1}	Optimum
pH	6.1	Optimum
Conductivity (EC)	1.01 mmho/cm	Acceptable

Table 4.2. Air space (%), water holding capacity, total porosity, bulk density (g cm^{-3}), permanent wilting point (PWP)(%), and container capacity (CC)(%) of the substrate

Air space	Porometer	0.12 g/g
Water holding capacity	Porometer	0.72 g/g
Total porosity	Porometer	0.84 g/g
CC	Gravimetric method	0.86 g/g
PWP	1.5 MPa ceramic plate extractor method	0.19 g/g
Bulk density	Porometer	0.10 g cm^{-3}

4.3.2. Soybean varieties and initial seed lot quality parameters

Two commercially available untreated soybean cultivars [Pioneer® 'P34A65PR' (3.4 relative maturity or RM) and 'P37T51PR' (3.7 RM)] were obtained within 8 months of project initiation and kept in 10°C cold storage until initiation of the experiments. Emergence scores, under suboptimal conditions from company literature, were 8 and 6 for 3.4 RM and 3.7 RM, respectively (9 = excellent germination; 1 = poor germination). Prior to conducting the experiments, each lot was analyzed for internal seed moisture content, standard warm germination (AOSA, 2018), and two vigor tests of accelerated aging (AA) and cold germination tests (rolled towel method) according to the methodology by the AOSA (1983). Seed moisture was tested using a grain moisture tester (miniGAC 200, Dickey-John). The results from these tests were previously reported in Chapter 3 Table 3.3.

4.3.3. Experimental design

The experiment was conducted using controlled-environmental chambers in Kottman Hall to impose initial treatments, with experimental units being relocated to the Kottman Hall greenhouse to complete data collection. The experimental design was a split-split-split plot randomized complete block design with three replications in time of the whole plot factor. The main plot factor was the initial available water content (AWC) at the time of planting with two levels (section 4.3.4.1). The sub plot factor consisted of four treatments consisting of varied water application and temperatures (WT) imposed (section 4.3.4.4). The sub-sub plot factor was presence or absence of *F. graminearum* inoculum (section 4.3.4.3), and the two soybean cultivars (3.4RM and 3.7RM; section 4.3.2) were randomized within two inoculum conditions.

4.3.4. Experiment arrangement.

4.3.4.1. Adjustment of soil available water content

Media was brought to 60% or 80% available water content (AWC). Each water content was maintained independently. The data from Table 2 was used to determine the key media moisture content levels to achieve the 60% and 80% AWC levels through Eq. 1-3. In all cases, 100% AWC was assumed at CC, and 0% AWC was assumed at the PWP of the media.

Media moisture content at 80% AWC $MC_{80\%}\left(\frac{W}{W}\right) = PWP + 0.80 \times (CC - PWP)$ Eq:1

Media moisture content at 60% AWC $MC_{60\%\left(\frac{W}{W}\right)} = PWP + 0.60 \times (CC - PWP)$ Eq:2

Volumetric water content $VWC\ \left(\frac{V}{V}\right) = \mathrm{MC}\left(\frac{W}{W}\right) \times BD_{\mathrm{media}}$ Eq:3

In each equation CC is container capacity of the media, PWP was media moisture content at the permanent wilting point, BD was bulk density of the media, respectively, as reported in table 4.2. The term $\left(\frac{\mathrm{W}}{\mathrm{W}}\right)$ represents weight-basis and $\left(\frac{\mathrm{V}}{\mathrm{V}}\right)$ represents volume-basis.

Moisture content of the media at the time of initial water level adjustment (VWC) was measured using Bluetooth moisture and temperature data logger (HOBO MX2306, HOBO MX2307). Based on current moisture content, the amount of water to add to the media in milliliters to achieve the target AWC levels was calculated using Eq.4 and Eq.5

Amount of water added at 80% AWC Eq:4

$$Water_{add} = \frac{(VWC_{80\%} - VWC) \times V_{media}}{100}$$

Amount of water added at 60% AWC Eq:5

$$Water_{add} = \frac{(VWC_{60\%} - VWC) \times V_{media}}{100}$$

Where V_{media} is volume of the media in cubic centimeters.

4.3.4.2. Tray preparation

Trays measuring 57.5×40×15 cm were filled with media to a depth of 11.25 cm. Each tray was designed with drainage openings at the bottom to facilitate the removal of excess water. Water was added to each tray individually to achieve the respective AWC values. Four rows with 3.75 cm were prepared in each tray, where two rows were applied *F. graminearum* inoculum and two rows were maintained without inoculum. Eight soybean seeds from 3.4RM and 3.7 RM cultivars per row was planted. Seeds were placed after adding the inoculum where rows with inoculum and furrows were closed after seed placement was completed. An additional row (5^{th} row) of soybean seeds was planted parallel to other rows in the center of the tray as a border to separate inoculated and non-inoculated rows. Bluetooth moisture and temperature sensors were installed in each tray at a depth of 3.75 cm to monitor media temperature and moisture for the first 25 hours.

4.3.4.3. *Fusarium graminearum* inoculum preparation

F. graminearum inoculated sorghum (*Sorghum bicolor)* seeds were used. Old culture plugs of *F. graminearum*, grown on potato-dextrose agar acidified with 5% lactic acid was used to inoculate the sorghum. Inoculum was stored in an air-tight bag in room temperature until been used. Approximately 5 grams (rate of 7.2 g m^{-1}) of inoculated sorghum seeds were added per row for the inoculated treatment. The inoculated sorghum seeds were placed at the same row with soybean seeds at the time of planting (Marburger et al., 2017; Figure 4.1). All trays were placed into the 10°C temperature-controlled chamber (Darwin Chamber) for 1 hour prior to application of the WT treatments.

a b c

Figure 4.1. (a) Preparation of growing media, (b) planting soybean seeds, (c) application of *F.graminearum* inoculum.

4.3.4.4. Water-temperature (WT) treatment sub-plot factor

There were four WT treatments as described in Table 4.3. Treatments consisted of the combination of two variables –timing of cold temperature exposure and form and temperature of water addition. Two temperature-controlled chambers (Darwin chamber and Percival scientific chamber) were maintained at 10℃ or 1.7℃ without light to provide temperature treatment. WT treatments 2-4 were moved to the 1.7°C chamber 1 hr after planting, while treatment 1 was retained in the 10°C conditions. For applied water treatments, water was applied as liquid water or as ice, with total volume applied equating to 1.25 cm depth of precipitation using equations 6 and 7:

Volume of water $$V_w = width_{tray} \times length_{tray} \times 1.25$$ Eq:6

Mass of ice $$M_{ice} = width_{tray} \times length_{tray} \times 1.25 \times BD_{ice}$$ Eq:7

Each tray with water treatments were received 3000 cm^3 of water. Trays with ice treatment received 2760g of ice (treatment 3). Water treatments were split applied (1500 cm^3 applied, allowed to infiltrate for 3-5 min, remaining volume applied) to provide enough time to infiltrate and avoid flooding. Water and ice were applied across the entire surface area of the tray. The

applied liquid water temperature was maintained at either 10℃ or 1.7℃ prior to application using the same chamber as the trays. After 10 hours of exposure to cold temperatures, treatments 2-4 were returned to the 10℃ chamber. Media temperature and moisture content were measured with first 25 hours using Bluetooth soil moisture and temperature sensors.

Table 4.3. Water-temperature treatments imposed during the experiment.

Treatment ID	Phase 1: 1 hour after planting	Phase 2: 11 hours after planting
1	10℃, 1.27 cm as 10℃ water	10℃, No water
2	1.7℃, 1.27 cm as ice	10℃, No water
3	1.7℃, 1.27 cm as 1.7℃ water	10℃, No water
4	1.7℃, No water	10℃, No water

Twenty-five hours after planting (once water-temperature treatments completed), temperature-moisture sensors were removed, and trays were moved to a temperature-controlled greenhouse (25℃ setpoint). Day/night conditions in greenhouse were set to 14d/10 night to emulate daylength in late April in Ohio. Beginning 72 hr after planting, trays were watered regularly in the greenhouse.

4.3.5. Data collection and measurements

Daily emergence total was collected in each row in each tray separately for 11 days after initiation (10 days in the greenhouse). When two cotyledons were totally appeared above the media surface, a plant was counted as emerged. After emergence on day 11 was quantified, soybean plants were uprooted carefully from media. If a plant was not present where a seed was planted, the seed or seedling was recovered and assessed for condition as abnormal or dead. Uprooted plants were washed gently to remove all adhere particles in root system and then plants were assessed for the conditions and phenological stage. Biomass and number of normal seedlings and abnormal seedlings were taken each rows separately. Seedlings that exhibited a well-balanced,

symmetrical growth across all essential parts including roots, hypocotyl, and cotyledon, were counted as normal seedlings (AOSA, 2018) and were pooled prior to measurement. Seedlings with malformed roots, missing or damaged cotyledon and weak or decayed hypocotyl were counted as abnormal seedlings were also combined and weighed. Number of seedlings that were showed crown necrosis (brown color lesions) from seeds that were inoculated with *F. graminearum* also measured. The count data of normal seedlings, abnormal seedlings, seedling with crown necrosis and dead seeds was converted to percentages values prior to analysis.

4.3.6. Data analysis

Biomass data were analyzed using analysis of variance (ANOVA) using the PROC GLIMMIX procedure in SAS 9.4 at α=.05 (SAS Institute, Cary, NC; version 9.4). The fixed factors were media AWC at the time of planting, WT treatments, presence or absence of inoculum, cultivar and their interactions. The random factors were replication, replication x AWC (whole-plot error term), replication x AWC x WT (sub-plot error term), and replication x AWC x WT x inoculum condition (sub-sub plot error term). Mean separation was conducted as pair-wise comparisons when the global F test in ANOVA was significant (α=.05).

The analysis for daily emergence patterns was conducted using sigmoid model (Equation 9) with the NLIN procedure in SAS 9.4. The differences between actual maximum emergence and E_{max} (E_{diff}) also were calculated.

$$y = \frac{a}{1 + e^{(b-cx)}} + d$$

Eq:9

Total emergence percentage from days 5 to 11 were used to create descriptive model for each sampling unit (each row within a tray) with days of counts (x) as an independent variable and germination percentage as the dependent variable (y). Using Eq.9, maximum emergence (E_{max}) and 10%, 50% and 90% of the maximum emergence value was calculated (E_{10}, E_{50}, E_{90}). The time in days spent to achieve the respective E_{10}, E_{50}, and E_{90} values (T_{10}, T_{50}, and T_{90}), as well as time in days difference in between achieving 10 and 90% total emergence (T_{10-90}) were calculated from model equation parameters using Eq.10 for each sample unit.

$$x = \frac{\left(\ln\left(\frac{a}{y-d}\right) - 1\right) - b}{-c}$$

Eq:10

The values generated from Eq.10 (T_{10}, T_{50}, T_{90} and T_{10-90}) were then analyzed using the same analysis of variance (ANOVA) model and parameters as described for biomass data analysis.

4.4 Results and discussion

4.4.1 Temperature and moisture sensor data

Figure 4.2 illustrates the variations in soil moisture and temperature within the first 25 hours for four WT treatments at AWC of 60% (Figure 4.3a, b) and 80% (Figure 4.2c, d). Regardless of the AWC, both soil moisture and temperature exhibited similar patterns. Treatment 1 and 3, where liquid water was applied 1 hour after planting, there was a drastic increase in soil moisture immediately after water application (Figure 4.2a, c). Treatment 2, which involved ice application,

showed a gradual increase in soil moisture. As for soil temperature, media at 60% AWC where 1.7℃ liquid water was applied 1 hour after planting (treatment 3) showed greater drop in temperature compared to other treatments. In 80% AWC, treatment 2 with ice application showed slower warming after return to 10°C compared to other treatments (Figure 4.2d)

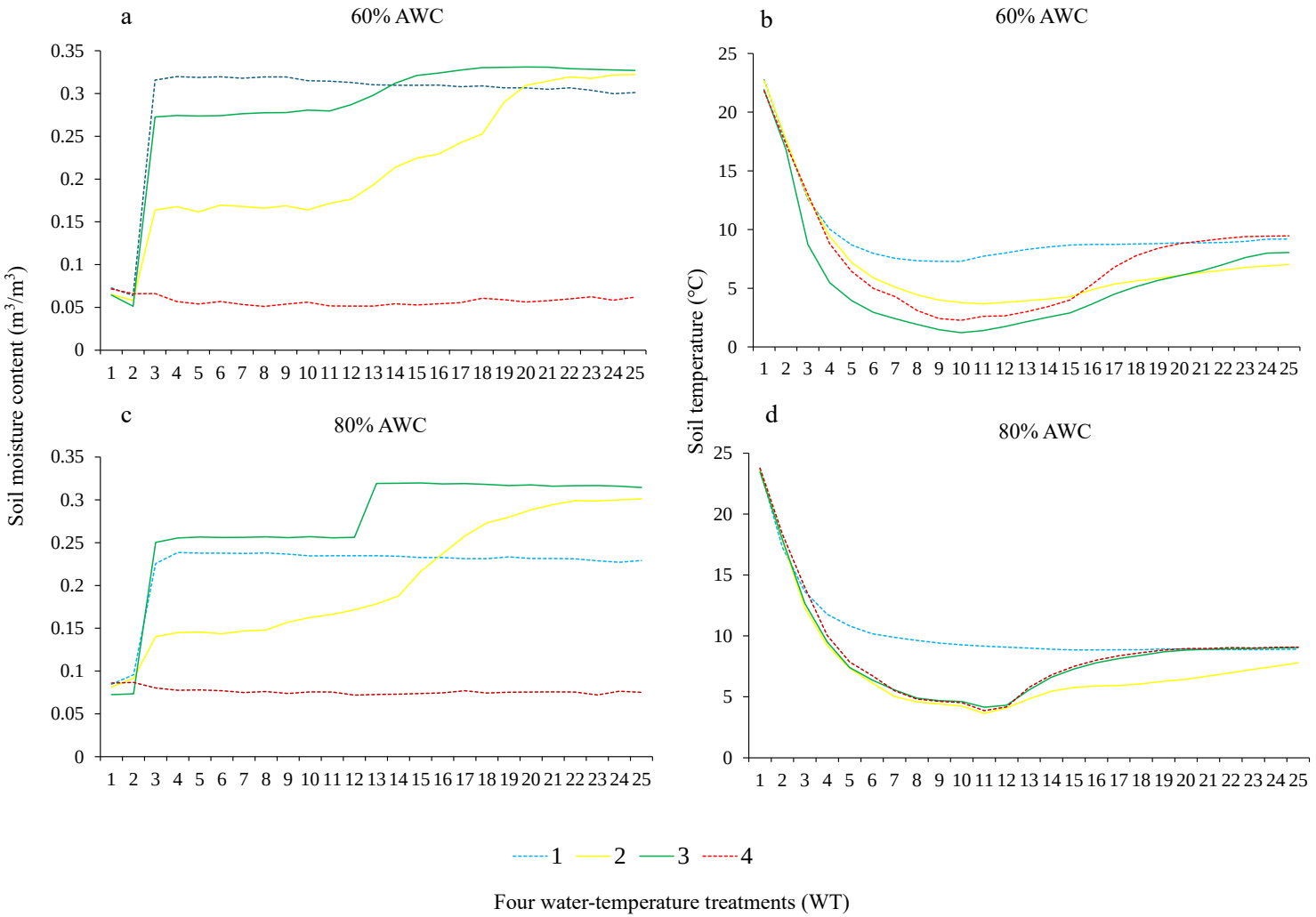


Figure 4.2. Soil moisture content (m^3 m^{-3}) (a, c) and soil temperature (°C, b,d) for the four water-temperature treatments in the 60% available water content soil (top row panels a-b) and the 80% available water content soil (bottom row panels c-d). Water-treatment numbers in the legend correspond to the treatment ID values presented in Table 4.3.

4.4.2 Inoculum impact on seedling biomass and emergence

The total emergence, and seedlings with root lesions (crown necrosis) were all affected by the inoculation of *F. graminearum*. However, the seedling biomass was unaffected by the application of the inoculum. The total emergence, which includes both normal and abnormal seedlings, was found to be significantly influenced by the interaction of the four WT treatments and inoculation treatments (Figure 4.3). For dead seeds AWC × inoculum and cultivar × inoculum interactions were significant.

Table 4.4. Analysis of variance results for biomass and emergence percentage of normal, abnormal, and total seedling biomass and percentage of dead seeds, seedlings exhibiting crown necrosis and total emergence of soybean for available water content (AWC), water-temperature treatment (WT), presence or absence of inoculum (Inoculum), cultivar, and their interactions in 11 days after initiation. P-values below 0.05 are indicated with bold.

Effect	p-value							
	Normal seedling		Abnormal seedling		Dead seed	Root lesions	Total biomass	Total emergence
	Biomass	Counts	Biomass	Counts	Counts	Counts		
AWC	0.1146	0.2873	0.8927	0.8259	0.3975	0.9720	0.2007	0.5394
WT	**0.0007**	**0.0210**	0.3709	0.5068	**0.0394**	0.7778	**0.0013**	0.0461
Inoculum	0.3373	0.2621	0.5780	0.9013	0.3046	**<0.0001**	0.3423	**0.0389**
Cultivar	**0.0176**	0.1158	**0.0207**	**0.0136**	0.8152	**0.0164**	**0.0298**	0.8769
AWC × WT	0.0769	0.5734	0.9252	0.7800	0.2997	0.9993	0.1098	0.7902
AWC × Inoculum	0.7713	0.3662	0.3178	0.3909	**0.0249**	0.7740	0.9323	0.8058
WT × Inoculum	0.4545	0.2827	0.3097	0.3717	0.7152	0.8924	0.4960	**0.0380**
AWC × Cultivar	0.3809	1.0000	0.5741	0.3906	0.1087	0.5451	0.2278	0.4406
WT × Cultivar	0.8346	0.7229	0.4403	0.6748	0.4307	0.9762	0.8372	0.8821
Inoculum × Cultivar	0.8358	0.8188	0.7733	0.6051	**0.0417**	0.0530	0.7395	0.8769
AWC × WT × Inoculum	0.6584	0.6614	0.8867	0.8462	0.0741	0.9346	0.6427	0.4647
AWC × WT × Cultivar	0.1454	0.7106	0.8035	0.9530	0.9825	0.6369	**0.0446**	0.7436
AWC × Inoculum × Cultivar	0.9369	0.6473	0.6691	0.8629	0.8152	0.9309	0.9748	0.6426
WT × Inoculum × Cultivar	0.4358	0.5423	0.1226	0.2168	0.5899	0.8594	0.4722	0.4668
AWC × WT × Inoculum × Cultivar	0.4278	0.4936	0.8194	0.6748	0.7881	0.8013	0.2530	0.7468

Total emergence of treatment 2 and 3 ([treatment 2: application of ice 1h after post-planting with 1.7℃ temperature exposure during the first phase]; [treatment 3: application of liquid water at 1.7℃ with 1.7℃ temperature exposure within the first phase]) declined with inoculum compared to treatment without inoculum (Figure 4.3). In contrast, treatments 1 and 4, which did not involve cold water treatment, exhibited similar emergence rates regardless of whether inoculum was applied or not. However, all the treatments showed more than 85% emergence in all cases.

Brennan et al. (2003) observed that optimal mycelial growth and inhibition of wheat seedling growth occur within a temperature range of 10-30℃. However, seeds can also germinate quickly and are less susceptible to disease within this temperature range (Ellis et al., 2011). The study conducted by Yan and Nelson (2022), a reduction in soybean emergence occurred in 10℃ in treatment with Fusarium compared to 20 and 25℃. This fungus predominantly infects under cold temperatures, which also slow seed germination. The cool and wet conditions created by the application of ice and liquid water at 1.7℃ may be the reason for the lower emergence observed in treatments 2 and 3 when inoculated.

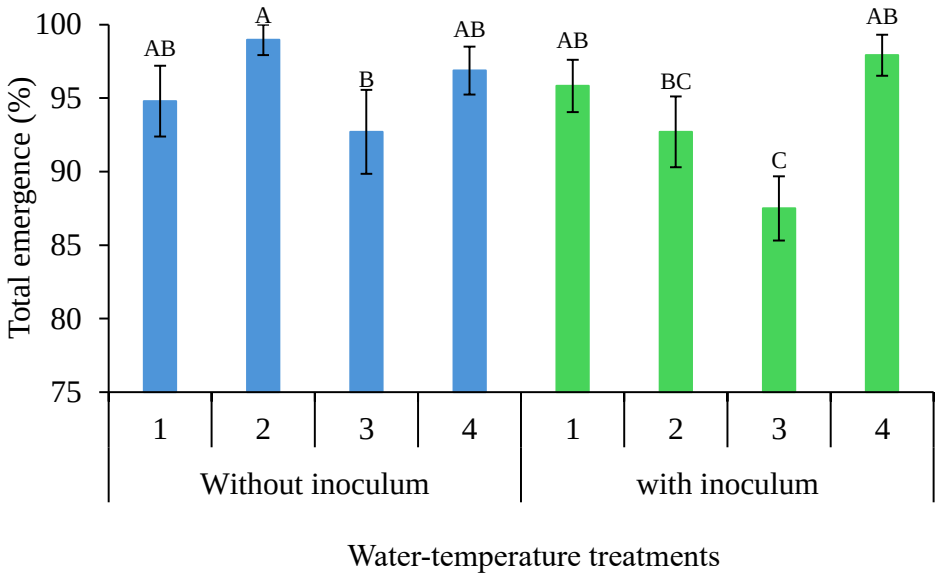


Figure 4.3. Percentage of total emergence (normal seedling + abnormal seedling) as a function of interaction between water-temperature treatment (WT) and inoculum conditions. The WT treatment ID values are described in Table 4.3. Means followed by same letters were not significantly different (P<0.05). Error bars within a series depict standard error of the mean.

The interaction of inoculum condition with cultivar on the percentage of dead seeds was significant and cultivar 3.4 RM exhibited a higher percentage of dead seeds when inoculum was applied. In contrast, the percentage of dead seeds in Cultivar 3.7 RM remained unaffected, regardless of whether inoculum was applied or not (Figure 4.4). Both cultivars were untreated. According to the company literature, both varieties exhibit some level of Phytophthora field tolerance and resistance to sudden death syndrome though no ratings for Fusarium resistance were available. According to company literature, Cultivar 3.7 RM demonstrated better resistance to Phytophthora than Cultivar 3.4 RM. Regardless, dead seeds were less than 5% of the total planted for each cultivar. Furthermore, lower dead seeds percentage was recorded under 60% AWC without inoculation compared to 80% AWC and 60% AWC with inoculation conditions (data not shown).

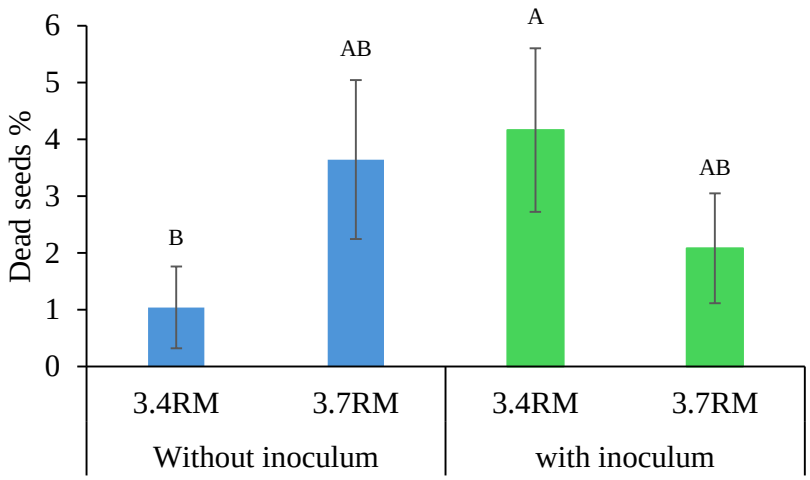


Figure 4.4. Dead seeds percentage (%) as a function of interaction between cultivar (C) and inoculum application (inoculum). Mean followed by same letters were not significantly different (P<0.05). Error bars within a series depict standard error of the mean.

Some of the seedling that were infected with inoculum, brown color lesions can be observed on root bottom (Figure 4.6). The significant effect of the inoculum ($p<0.0001$) was determined through an ANOVA result (Table 4.4) that included both inoculated and non-inoculated data. The documented lesions were not present on the seedlings that were not inoculated with *F. graminearum*. Apart from the cultivar effect, the number of plants with brown lesions on the root was not influenced by the imposed WT treatments or AWC. For Cultivar 3.4 RM, 27% of seedlings were infected with brown-colored lesions, while for 3.7 RM, only 19% were infected. These were cultured in the laboratory, and the growing *F. graminearum* conidia can be seen in Figure 4.5. When comparing the root growth patterns of soybean seedlings with and without the inoculum, no visual differences were observed (Figure 4.6a, b).

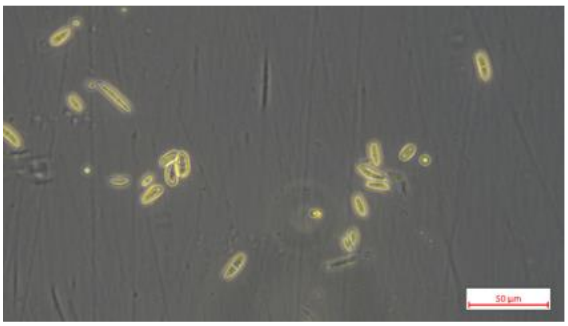


Figure 4.5. Microscopic view of spores (conidia) of *Fusarium graminearum*.

a b c

Figure 4.6. (a) Seedling with brown lesions in on the crown area, (b) seedlings infected with *F.graminearum*, (c) Seedlings grown without inoculum

The biomass of normal seedlings, abnormal seedlings, and total seedlings was not influenced by the application of inoculum. There is limited research available that investigates the activity of this fungus under cold temperature conditions. It is also possible the termination of the experiment 11 days after planting was not sufficient time to fully quantify biomass reductions and potential death resulting from *F. graminareum* infection.

The normal seedling biomass was significantly impacted by WT treatments, irrespective of whether an inoculum was applied. Among all the water-temperature treatments, Treatment 3 (which involved the application of liquid water at 1.7℃ with a temperature exposure of 1.7℃

during the first phase) resulted in the lowest normal seedling biomass (as shown in Figure 4.7). Upon examining the temperature fluctuations depicted in Figure 4.3, it was observed that Treatment 3 experienced a greater decline compared to the other treatments. This greater fluctuation in temperature could potentially be a contributing factor to the observed results. In the experiment outlined in Chapter 3, Figure 3.4, it was observed that the application of liquid water at 1.7℃ and 10℃, or ice, resulted in a lower normal seedling biomass compared to the treatment where no water or ice was applied. However, in this study, treatments involving the application of ice or liquid water at 10℃ reported higher biomasses than the treatment with liquid water application at 1.7℃. This discrepancy may be attributed to differences in the media used and its characteristics, which can significantly impact seedling emergence.

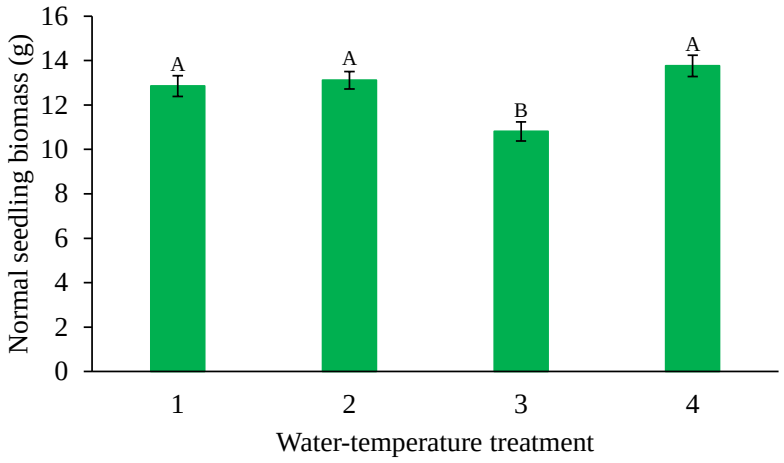


Figure 4.7. Normal seedling biomass (g) of soybean as a function of four water-temperature treatments. The WT treatment ID values are described in Table 4.3. Means followed by same letters were not significantly different ($P<0.05$). Error bars within a series depict standard error of the mean.

4.4.3 Daily emergence pattern

The T_{10}, T_{50}, and T_{90} values were not significantly affected by any imposed factors. Even though, treatment 2 and 3 showed a greater declining of total emergence with WT, time to emergence was not affected by imposed WT (Table 4.5).

Table 4.5. Analysis of variance results for time in days spent to achieve 10, 50, 90 % emergence and difference between 10 to 90 % emergence (T_{10}, T_{50}, T_{90}, $T_{10\text{-}90}$) of soybean for available water content (AWC), water-temperature treatment (WT), inoculum application (inoculum), cultivar, and their interactions.

	p-value			
Effect	T_{10}	T_{50}	T_{90}	$T_{10\text{-}90}$
AWC	0.4221	0.5645	0.5192	0.4755
WT	0.4267	0.3182	0.1947	0.1489
Inoculum	0.3321	0.9360	0.8285	0.7131
Cultivar	0.3245	0.5947	0.5088	0.4926
AWC × WT	0.4264	0.3359	0.2743	0.3190
AWC × Inoculum	0.3321	0.7503	0.6749	0.5385
WT × Inoculum	0.4178	0.7426	0.7372	0.6225
AWC × Cultivar	0.3244	0.1921	0.2886	0.3603
WT × Cultivar	0.3249	0.6276	0.7592	0.7963
Inoculum × Cultivar	0.3244	0.3117	0.4625	0.5463
AWC × WT × Inoculum	0.4184	0.2549	0.4057	0.498
AWC × WT × Cultivar	0.4052	0.6262	0.6731	0.7265
AWC × Inoculum × Cultivar	0.3246	0.441	0.3854	0.3731
WT × Inoculum × Cultivar	0.4059	0.7744	0.7941	0.7500
AWC × WT × Inoculum × Cultivar	0.4057	0.8331	0.7831	0.7345

4.5 Conclusion and recommendation

Cold water or ice reduced soybean emergence under *F. graminearum* inoculated media by 6% compared to non-inoculated media. Inoculated media produced more dead seeds under 60% moisture content compared to 80% moisture content. However, the presence of inoculum did not

affect the total biomass accumulation in soybean seedlings. The study suggests that the activity of *F. graminearum* had some effect on seedling emergence under cold precipitation, though future research should be conducted to expand upon these results.

References

Association of Official Seed Analysis (AOSA) (1983) Seed Vigor Testing Handbook, Contribution No. 32 to the handbook on Seed Testing.

Association of Official Seed Analysts (AOSA). (2018). Rules for testing seeds. Association of Official Seed Analysts.

Broders, K. D., Lipps, P. E., Paul, P. A., & Dorrance, A. E. (2007). Evaluation of Fusarium graminearum associated with corn and soybean seed and seedling disease in Ohio. *Plant disease*, *91*(9), 1155-1160. https://doi.org/10.1094/PDIS-91-9-1155

Characteristic rating of Pioneer® band soybean products for 2023-24. Pioneer Delivers 80 New Corn Products and Soybean Varieties | Pioneer® Seeds. (accessed on May 30 2024).

Ellis, M. L., Broders, K. D., Paul, P. A., & Dorrance, A. E. (2011). Infection of soybean seed by Fusarium graminearum and effect of seed treatments on disease under controlled conditions. *Plant disease*, *95*(4), 401-407. https://doi.org/10.1094/PDIS-05-10-0317.

Koenning, S. R., & Wrather, J. A. (2010). Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. *Plant Health Progress*, *11*(1), 5. https://doi.org/10.1094/PHP-2010-1122-01-RS.

Leslie, J. F., & Summerell, B. A. (2008). *The Fusarium laboratory manual*. John Wiley & Sons.

Marburger, D. A., Smith, D. L., & Conley, S. P. (2017). Impact of Fusarium graminearum on early-season soybean growth and seed yield under field conditions. *Canadian journal of plant pathology*, *39*(4), 464-474. https://doi.org/10.1080/07060661.2017.1378727.

Mengistu, A., Arelli, P. A., Bond, J. P., Shannon, G. J., Wrather, A. J., Rupe, J. B., ... & Pantalone, V. R. (2011). Evaluation of soybean genotypes for resistance to charcoal rot. *Plant Health Progress*, *12*(1), 6. https://doi.org/10.1094/PHP-2010-0926-01-RS.

Yan, H., & Nelson Jr, B. (2022). Effects of soil type, temperature, and moisture on development of Fusarium root rot of soybean by Fusarium solani (FSSC 11) and Fusarium tricinctum. *Plant Disease*, *106*(11), 2974-2983. https://doi.org/10.1094/PDIS-12-21-2738-RE.

Chapter 5. Overall Summary and Recommendation

5.1 Summary

The research presented within this thesis primarily investigated the effects of cold temperatures and water on soybean germination and emergence. The research was structured around three main objectives, each corresponding to the second, third, and fourth chapters of the thesis. The second chapter quantified the impact of the duration of exposure to cold conditions during the imbibition and early germination phases on seed germination in two soybean cultivars. It concluded that the cold temperature during the imbibition phase had a minimal effect on soybean germination, resulting in an 83% or higher germination rate for all durations of cold exposure. However, it was found that initial exposure to cold temperatures for 12 to 24 hours was the most detrimental to soybean seeds. This resulted in a 6-7% lower germination rate compared to the untreated control after 10 days. Though germination assays were conducted using rolled towel methods in controlled temperature conditions, these results do suggest cold temperatures alone may not fully explain stand reductions in field environments.

Farmers often encounter not only cold temperatures but also cold precipitation or snowfall shortly after the early planting of soybeans. In field conditions, factors such as temperature, soil moisture, planting depth, and variety selection - all part of farmer management practices - can contribute to stand reduction associated with early planting. The third chapter reported on the impact of soil moisture content at planting, planting depth, temperature, and form of precipitation affect soybean emergence and early growth. It concluded that cold precipitation within first 24 hours after planting reduced soybean emergence and seedling biomass substantially, though

exposure to cold temperatures (above freezing) within the first 11 hours, without any water application within the first 24 hours, did not reduce soybean germination and emergence. Planting at a deeper depth in drier soil under cold temperatures resulted in better biomass and emergence as compared to shallower depth under drier soil conditions.

The extended period of seed germination and emergence, often associated with early planting and cold temperature conditions, can expose soybean seeds to infections from seed or soil-borne pathogens. This can lead to a reduction in stand due to pre-emergence or post-emergence damping off, as well as seed decay caused by these pathogens. In fourth chapter, cold water/ice precipitation increased *F. graminearum* activity on soybean emergence. . However, the presence of inoculum did not affect the total biomass accumulation in soybean seedlings.

5.2 Recommendations and Suggestions

These studies helped emphasize that the first 24 hours after planting are crucial for soybean germination. Therefore, it is important for farmers to closely monitor weather forecasts within the next 24 hours. If there is a chance of cold precipitation within the first 24 hours, it is advisable to postpone planting on (at least some) hectares. Additionally, deeper planting is recommended if soil moisture is low (20% AWC) during early planting. Given the observed impact on seedling emergence under cold precipitation due to *F. graminearum* activity, seed treatment is likely necessary to combat early-season soil or seed-borne diseases when exposed to cold temperatures and moisture.

5.3 Limitations and Comparisons with existing literature

One limitation of second and third study were the practice of uprooting seedlings within 11 days. In studies where seeds were planted directly into soil or media, emergence percentages were as low as 20% in some instances. Some literature suggest that germination under cold soil conditions can extend up to 14 to 16 days. Therefore, future studies might consider extending the observation period. This may have also helped increase emergence totals beyond the values reported here.

Compared to most previous literature, this study recorded a higher final germination rate (83% or greater) using the rolled paper towel method. In contrast to past studies where soybean seeds were held at 25℃ before exposure to cold temperatures, this study placed seeds between 10 to 15.5℃ prior to cold exposure. This approach may have prevented the seeds from experiencing a sudden cold shock, which is often the case in past studies where seeds were directly placed at 25℃ before being exposed to cold temperatures. In this study, seeds were exposed to relatively lower temperatures before the cold temperature exposure, which better acclimatized the seeds in preparation for the cold.

5.4 Future research needs

While this study is primarily based on greenhouse and laboratory conditions, the impact of these setups on field conditions needs to be further explored. Future trials are necessary to provide more conclusive recommendations for farmers. One key finding from the second study is that cold water is more detrimental than cold temperature in terms of soybean germination and emergence. Therefore, it is crucial to understand how soil moisture content affects seed germination, seed moisture content, and soil crusting/compaction. Planting deeper in drier soil emerged as a

significant conclusion from this study. However, how this practice varies in field conditions also needs to be investigated. Another component to be considered when studying ultra-early planting could be to adjust seeding rates, so even if 60% attrition of seeds is experienced a critical stand may still be achievable to fully leverage the early planting date yield gains.

Although we concluded in the fourth chapter that seed treatment is necessary to combat disease pressure with early planting, both seed types used in this study were untreated. Therefore, further studies should be conducted to understand how *F. graminearum* activities interact with treated and untreated seeds. These recommendations for future research will help to bridge the gap between laboratory findings and practical field applications, ultimately benefiting farmer applications.

Bibliography

Abendroth, L. J., Miguez, F. E., Castellano, M. J., & Hatfield, J. L. (2019). Climate warming trends in the US Midwest using four thermal models. *Agronomy Journal*, *111*(6), 3230-3243. https://doi.org/10.2134/agronj2019.02.0118

Alsajri, F. A. (2018). *Temperature Effects on Soybean Growth, Development, Physiology, Yield and Seed Quality*. Mississippi State University.

Anderson, M., Vittetoe, R., Licht, M. A., & Rieck-Hinz, A. (n.d.). *Stand Assessments—Soybean | Integrated Crop Management*. Retrieved February 23, 2024, from https://crops.extension.iastate.edu/encyclopedia/stand-assessments-soybean

Andrade, F. H., Otegui, M. E., & Vega, C. (2000). Intercepted Radiation at Flowering and Kernel Number in Maize. *Agronomy Journal*, *92*(1), 92–97. https://doi.org/10.2134/agronj2000.92192x

Association of Official Seed Analysis (AOSA) (1983) Seed Vigor Testing Handbook, Contribution No. 32 to the handbook on Seed Testing.

Association of Official Seed Analysts (AOSA). (2018). Rules for testing seeds. Association of Official Seed Analysts

Bandara, A.Y., Weerasooriya, D.K., Bell, T.H. and Esker, P.D. 2021. Prospects of alleviating early planting-associated cold susceptibility of soybean using microbes: New insights from microbiome analysis. Journal of Agronomy and Crop Science, 207(2), pp.171-185.

Baule, W., Allred, B., Frankenberger, J., Gamble, D., Andresen, J., Gunn, K.M., & Brown, L. (2017). Northwest Ohio crop yield benefits of water capture and subirrigation based on future climate change projections. *Agricultural Water Management, 180*, 87-97. http://dx.doi.org/10.1016/j.agwat.2017.04.019.

Baum, M. E., Licht, M. A., Huber, I., & Archontoulis, S. V. (2020). Impacts of climate change on the optimum planting date of differentmaize cultivars in the central US corn belt. European Journal ofAgronomy, 119, 126101. https://doi.org/10.1016/j.eja.2020.126101

Bohner, H. 2003. Do soil temperatures at planting impact soybean yield? Ministry of Agriculture, Food and Rural Affairs. Available at; https://cropwatch.unl.edu/2019-CW-News/2019-images/soybean/Do-soil-temperatures-at-planting-impact-soybean-yield-Horst Bohner.pdf (accessed May 2023).

Bramlage, W.J., Leopold, A.C. and Parrish, D.J. 1978. Chilling stress to soybeans during imbibition. *Plant Physiology*, 61(4), pp.525-529. https://doi.org/10.1104/pp.61.4.525

Broders, K. D., Lipps, P. E., Paul, P. A., & Dorrance, A. E. (2007). Evaluation of Fusarium graminearum associated with corn and soybean seed and seedling disease in Ohio. Plant disease, 91(9), 1155-1160. https://doi.org/10.1094/PDIS-91-9-1155.

Characteristic rating of Pioneer® band soybean products for 2023-24. Pioneer Delivers 80 New Corn Products and Soybean Varieties | Pioneer® Seeds. (accessed on May 30 2024).

Cheng, L., Gao, X., Li, S., Shi, M., Javeed, H., Jing, X., ... & He, G. (2010). Proteomic analysis of soybean [Glycine max (L.) Meer.] seeds during imbibition at chilling temperature. Molecular Breeding, 26, 1-17. https://doi.org/10.1007/s11032-009-9371-y

Conley S., Holshouser D., Inman M., Lee C., Lindsey L., Licht M., Kandel H., Kleinjan J., Knott C., Naeve S., Nafziger E., Ross J., Singh M., Specht J. & Vann R., (n.d.). The best soybean planting date, The best soybean management practices by Extension researchers from across the United States, Science for Success, University of Michigan State. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.canr.msu.edu/agronomy/Science%20for%20Success-%20Planting%20Date.pdf.

Cruz, D. R., Leandro, L. F. S., Mayfield, D. A., Meng, Y., & Munkvold, G. P. (2020). Effects of soil conditions on root rot of soybean caused by Fusarium graminearum. *Phytopathology*, *110*(10), 1693-1703. https://doi.org/10.1094/PHYTO-02-20-0052-R

De Bruin, J. L., & Pedersen, P. (2008). Soybean seed yield response to planting date and seeding rate in the Upper Midwest. *Agronomy Journal*, *100*(3), 696-703. https://doi.org/10.2134/agronj2007.0115

Dhaliwal, L. K., & Angeles-Shim, R. B. (2022). Cell membrane features as potential breeding targets to improve cold germination ability of seeds. *Plants*, *11*(23), 3400.

Dogras, C. C., Dilley, D. R., & Herner, R. C. (1977). Phospholipid biosynthesis and fatty acid content in relation to chilling injury during germination of seeds. *Plant Physiology*, *60*(6), 897-902.

Duke, S. H., Kakefuda, G., & Harvey, T. M. (1983). Differential leakage of intracellular substances from imbibing soybean seeds. *Plant Physiology*, *72*(4), 919-924. https://doi.org/10.1104/pp.72.4.919.

Egli, D. B. and Cornelius, P. L. 2009. A regional analysis of the response of soybean yield to planting date. *Agronomy Journal*, 101(2), pp. 330-335. https://doi.org/10.2134/agronj2008.0148

Ellis, M. L., Broders, K. D., Paul, P. A., & Dorrance, A. E. (2011). Infection of soybean seed by Fusarium graminearum and effect of seed treatments on disease under controlled conditions. Plant disease, 95(4), 401-407. https://doi.org/10.1094/PDIS-05-10-0317.

El-Maarouf-Bouteau, H. (2022). The seed and the metabolism regulation. Biology, 11(2), 168.

Ernst, M. (2024). Seeding: Back to the Basics. *Crops & Soils*, *57*(3), 12-17. https://doi.org/10.1002/crso.20357

Fernandez, M. R., and Fernandes, J. M. C. 1990. Survival of wheat pathogens in wheat and soybean residues under conservation tillage systems in southern Brazil Can. J. Plant Pathol.-Rev. Can. Phytopathol. 12:289-294.

Gaspar, A. P., & Conley, S. P. (2015). Responses of canopy reflectance, light interception, and soybean seed yield to replanting suboptimal stands. *Crop Science*, *55*(1), 377-385. https://doi.org/10.2135/cropsci2014.03.0200

Grady, K. A., Chen, L., & Ford, T. W. (2021). Projected changes to spring and summer precipitation in the Midwestern United States. *Frontiers in Water*, *3*, 780333. https://doi.org/10.3389/frwa.2021.780333

Greaves, J. A. (1996). Improving suboptimal temperature tolerance in maize- the search for variation. Journal of Experimental Botany, 47(3), 307–323. https://doi.org/10.1093/jxb/47.3.307

Haidar, S., Lackey, S., Charette, M., Yoosefzadeh-Najafabadi, M., Belzile, F., Rajcan, I., ... & Samanfar, B. (2023). Genome-wide analysis of cold imbibition stress in soybean, Glycine max. Frontiers in Plant Science, 14, 1221644.

Hatfield, J.L. and Egli, D.B. 1974. Effect of temperature on the rate of soybean hypocotyl elongation and field emergence 1. *Crop Science*, 14(3), pp.423-426. https://doi.org/10.2135/cropsci1974.0011183X001400030025x

Hankinson, M. W., Lindsey, L. E., & Culman, S. W. 2015. Effect of planting date and starter fertilizer on soybean grain yield. Crop, Forage & Turfgrass Management, 1(1), 1-6. https://doi.org/10.2134/cftm2015.0178

Herner, R. C. (1986). Germination under cold soil conditions. *HortScience*, *21*(5), 1118-1122.

Herner, R. C. (1990). The effects of chilling temperatures during seed germination and early seedling growth. *Chilling injury of horticultural crops*, 51-69.

Hobbs, P.R. and Obendorf, R.L. 1972. Interaction of Initial Seed Moisture and Imbibitional Temperature on Germination and Productivity of Soybean. *Crop Science*, 12(5), pp.664-667. https://doi.org/10.2135/cropsci1972.0011183X001200050033x

Hu, M., & Wiatrak, P. 2012. Effect of planting date on soybean growth, yield, and grain quality. *Agronomy Journal*, 104(3), 785-790. https://doi.org/10.2134/agronj2011.0382

Iowa State University. (n.d.). Accelerated Aging. *ISU Seed Lab*. Retrieved February 23, 2024, from https://seedlab.iastate.edu/portfolio-items/accelerated-aging/

Kannberg, S., Lindsey, A. J., Chiavegato, M. B., & Lindsey, L. E. (2024). Effect of ultra-early, early, and normal soybean planting dates and rye cover crop on soybean grain yield. *Agronomy Journal*. https://doi.org/10.1002/agj2.21550

Knott, C., Herbek, J., & James, J. (2019). Early Planting dates maximize soybean yield in Kentucky. *Crop, Forage & Turfgrass Management*, *5*, 180085. https://doi.org/10.2134/cftm2018.10.0085

Koenning, S. R., & Wrather, J. A. (2010). Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. *Plant Health Progress*, *11*(1), 5. https://doi.org/10.1094/PHP-2010-1122-01-RS.

Kucharik, C. J. (2006). A multidecadal trend of earlier corn planting in the central USA. Agronomy Journal, 98(6), 1544-1550. https://doi.org/10.2134/agronj2006.0156

Kunkel, K. E., L. E. Stevens, S. E. Stevens, L. Sun, E. Janssen, D. Wuebbles, S. D. Hilberg, M. S. Timlin, L. Stoecker, N. E. Westcott, and J. G. Dobson. 2013: Regional Climate Trends and Scenarios for the U.S. National Climate Assessment: Part 3. Climate of the Midwest U.S. NOAA Technical Report NESDIS 142-3. 103 pp., National Oceanic and Atmospheric Administration, National Environmental Satellite, Data, and Information Service, Washington, D.C

Lee, C. D., Egli, D. B., & TeKrony, D. M. (2008). Soybean response to plant population at early and late planting dates in the Mid-South. *Agronomy Journal*, *100*(4), 971-976. https://doi.org/10.2134/agronj2007.0210

Leopold, A. C. (1980). Temperature effects on soybean imbibition and leakage. Plant Physiology, 65(6), 1096-1098. https://doi.org/10.1104/pp.65.6.1096

Leopold, A. C., & Musgrave, M. E. (1979). Respiratory changes with chilling injury of soybeans. *Plant physiology*, *64*(5), 702-705. https://doi.org/10.1104/pp.64.5.702

Leslie, J. F., & Summerell, B. A. (2008). *The Fusarium laboratory manual*. John Wiley & Sons.

Licht M., Integrated crop management, Extension and Outreach. Iowa state university. Imbibitional chilling or cold injury | Integrated Crop Management (iastate.edu)

Licht M. (2021). Corn, and Soybean Planting Date Considerations. Integrated crop management, Extension and Outreach. Iowa state university. Available at https://crops.extension.iastate.edu/blog/mark-licht-zachary-clemens/corn-and-soybean-planting-date-considerations. (accessed 24 February 2024).

Lindsey, L. E., Tilmon, K., Michel, A., & Dorrance, A. (2017). Soybean production. In L. E. Lindsey & P. R. Thomison (Eds.), Ohio agronomy guide (15th ed., Bulletin no. 472, pp. 56–68). The Ohio State University Extension.

Lindsey, A.J., Ortez, O.A., Thomison, P.R., Carter, P.R., Coulter, J.A., Roth, G.W., Carrijo, D.R., Quinn, D.J. & Licht, M.A. (2024). Severe storm damage and short-term weather stresses on corn: A review. *Crop Science*. https://doi.org/10.1002/csc2.21212

Loeffler, N. L., Meier, J. L., & Burris, J. S. (1985). Comparison of two cold test procedures for use in maize drying studies. Seed Science and Technology, 13(3), 653-658.

MacDonald, J. M., Korb, P., & Hoppe, R. A. (2013). Farm size and the organization of US crop farming. Available at https://ageconsearch.umn.edu/record/262221.

McDonald Jr, M.B., Vertucci, C.W. and Roos, E.E.1988. Soybean seed imbibition: water absorption by seed parts. Crop Science, 28(6), pp.993-997. https://doi.org/10.2135/cropsci1988.0011183X002800060026x

McDonald, M. B. (1994). Seed germination and seedling establishment. *Physiology and determination of crop yield*, 37-60. https://doi.org/10.2134/1994.physiologyanddetermination.c3.

Mengistu, A., Arelli, P. A., Bond, J. P., Shannon, G. J., Wrather, A. J., Rupe, J. B., ... & Pantalone, V. R. (2011). Evaluation of soybean genotypes for resistance to charcoal rot. *Plant Health Progress*, *12*(1), 6. https://doi.org/10.1094/PHP-2010-0926-01-RS.

Mourtzinis, S., Specht, J.E. and Conley, S.P. 2019. Defining optimal soybean sowing dates across the US. *Scientific Reports*, 9(1), pp.2800. https://doi.org/10.1038/s41598-019-38971-3

Nielsen, R. L. (2020, April 6). When Should Maize Planting Begin? (Purdue University). https://www.agry.purdue.edu/ext/Maize/news/articles_20/PltDateStart_0406.html

Nonogaki, H., Bassel, G. W., & Bewley, J. D. (2010). Germination—still a mystery. Plant Science, 179(6), 574-581. https://doi.org/10.1016/j.plantsci.2010.02.010

Obendorf, R. L., & Hobbs, P. R. (1970). Effect of Seed Moisture on Temperature Sensitivity During Imbibition of Soybean 1. *Crop science*, 10(5), 563-566. https://doi.org/10.2135/cropsci1970.0011183X001000050034x

Ohio soybean council, fact sheet, 2024. Available at chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.soyohio.org/wp-content/uploads/2013/07/Ohio-Soybean-Council-Fact-Sheet-UPDATED.pdf. (accessed 20 March 2024).

O'Neal, M.R., Nearing, M.A., Vining, R.C., Southworth, J., & Pfeifer, R. A. (2005). Climate change impacts on soil erosion in Midwest United States with changes in crop management. Catena, 61, 165-184. https://dx.doi.org/10.1016/j.catena.2005.03.003

Parrish, D. J., & Leopold, A. C. (1977). Transient changes during soybean imbibition. *Plant Physiology*, *59*(6), 1111-1115. https://doi.org/10.1104/pp.59.6.1111

Pollock, B., & Toole, V. K. (1966). Imbibition period as the critical temperature sensitive stage in germination of lima bean seeds. Plant physiology, 41(2), 221-229. https://doi.org/10.1104/pp.41.2.221

Pryor, S. C., Barthelmie, R. J., & Schoof, J. T. (2013). High-resolution projections of climate-related risks for the Midwestern USA. *Climate research*, *56*(1), 61-79.

Rattalino Edreira, J.I., Mourzinis, S., Conley, S.P., Roth, A.C., Ciampitti, I.A., Licht, M.A., Kandel, H., Kyveryga, P.M., Lindsey, L.E., Mueller, D.S., Naeve, S.L., Nafziger, E., Specht, J.E., Stanley, J., Staton, M.J., and Grassini, P. (2017). Assessing causes of yield gaps in agricultural areas with diversity in climate and soils. Agricultural and Forest Meteorology. 247:170-180. https://doi.org/10.1016/j.agrformet.2017.07.010

Rees, J., and Specht, J. 2021. Demonstrating critical imbibitional time for soybean. University of Nebraska-Lincoln Cropwatch Newsletter. https://cropwatch.unl.edu/2021/demonstrating-critical-imbibitional-time-soybean (accessed November 2023).

Robinson, A. P., Conley, S. P., Volenec, J. J., & Santini, J. B. (2009). Analysis of high yielding, early-planted soybean in Indiana. *Agronomy Journal*, *101*(1), 131-139. https://doi.org/10.2134/agronj2008.0014x

Roskruge, C.L. and Smith, M.T. 1997. Peroxidative changes associated with chilling injury in soybean seeds during imbibition. *Journal of Plant Physiology*, 15*1*(5), pp.620-626. https://doi.org/10.1016/S0176-1617(97)80239-8.

Rowntree, S.C., Suhre, J.J., Weidenbenner, N.H., Wilson, E.W., Davis, V.M., Naeve, S.L., Casteel, S.N., Diers, B.W., Esker, P.D., Specht, J.E. and Conley, S.P. 2013. Genetic gain× management interactions in soybean: I. Planting date. *Crop Science*, 53(3), pp.1128-1138. https://doi.org/10.2135/cropsci2012.03.0157.

Scholthof, K. B. G. (2007). The disease triangle: pathogens, the environment and society. *Nature Reviews Microbiology*, *5*(2), 152-156. https://doi.org/10.1038/nrmicro1596.

Schoof, J. T., Pryor, S. C., & Surprenant, J. (2010). Development of daily precipitation projections for the United States based on probabilistic downscaling. *Journal of Geophysical Research: Atmospheres*, *115*(D13). https://doi.org/10.1029/2009JD013030

Schrage S., 2018. 115 years of data reveal longer U.S. growing season, temp trends, University communication and marketing, University of Nebraska, Lincoln. Available at https://news.unl.edu/newsrooms/today/article/115-years-of-data-reveal-longer-us-growing-season-temp-trends. (accessed 3 April 2024).

Shaun Casteel, 2021. Soybean stands:Emergence?Replant?. Extension Entomology, Purdue University. https://extension.entm.purdue.edu/newsletters/pestandcrop/article/soybean-stands-emergence-replant/. (accessed 06 June 2024).

Simon, E. (1974). Phospholipids and plant membrane permeability. *New Phytologist*, *73*(3), 377-420. https://doi.org/10.1111/j.1469-8137.1974.tb02118.x

Sodak labs inc. Accelerated Aging (AA) test for soybean seed V.72021. Retrieved March 6, 2024, from https://www.sodaklabs.com/media/1576/22-accelerated-aging-aa-test-for-soybean-seed-ver-72021.pdf

Sonnenberg D, 2020. Ohio soybean production and research in 2020. Ohio Ag Net, Ohio's Country Journal. Available at https://ocj.com/2020/11/ohio-soybean-production-and-research-in-2020/. (accessed 21 March 2024).

Sonnenberg D, 2023. Early soybean planting and stand evaluation. Ohio Ag Net, Ohio's Country Journal. Available at; https://ocj.com/2023/05/early-soybean-planting-and-stand-evaluation/ (accessed 1 April 2024).

Southworth, J., Pfeifer, R. A., Habeck, M., Randolph, J. C., Doering, O. C., Johnston, J. J., & Rao, D. G. (2002). Changes in soybean yields in the Midwestern United States as a result of future changes in climate, climate variability, and CO 2 fertilization. *Climatic Change*, *53*, 447-475. https://doi.org/10.1023/A:1015266425630

Specht, James E., Jennifer M. Rees, Gary L. Zoubek, Keith L. Glewen, Brandy S. VanDeWalle, James W. Schneider, David L. Varner, and Allan R. Vyhnalek. (2012). Soybean planting date: When and Why? Nebraska Extension EC145. http://extensionpubs.unl.edu/publication/9000016368318/soybean-planting-date-when-and-why/

Specht J., Rees J., Glewen K., Grassini P. 2014. Soybean planting depth. Institute of Agriculture and Natural Resources, cropwatch, University of Nebraska. https://cropwatch.unl.edu/soybean-planting-depth-consider-planting-deeper. (accessed 2 June 2024).

Specht, James E. and Thomas Hoegemeyer. 2017. Nebraska Soybean and Corn Pocket Field Guide, 2017 Edition.

Specht, Jim, Jenny Rees, Roger Elmore, Nathan Mueller, Keith Glewen. Considerations when Planting Soybean Early, University of Nebraska Extension Cropwatch, April 25, 2019. Considerations when Planting Soybean Early | CropWatch | University of Nebraska–Lincoln (unl.edu). (accessed 04 May 2024).

Staton M, 2018. Michigan State University Extension, 2018. https://www.canr.msu.edu/news/things_to_consider_when_planting_soybeans_early (accessed April 2023)

Staton M., 2023. Soybean planting depth considerations when planting into dry soil conditions. Michigan State University Extension. Michigon State University. Available at; https://www.canr.msu.edu/news/soybean-planting-depth-considerations-when-planting-into-dry-soil-conditions (accessed 09 April, 2024).

Suo, R., Sandhu, K., Wang, M., You, F., Conner, R., Cober, E., & Hou, A. (2022). Soybean (Glycine max L.) seed germination in response to waterlogging and cold climate: a review on the genetics and molecular mechanisms of resistance to the abiotic stress. Canadian Journal of Plant Science, 103(1), 13-28. https://doi.org/10.1139/cjps-2022-0111

Szczerba, A., Płażek, A., Pastuszak, J., Kopeć, P., Hornyák, M., & Dubert, F. (2021). Effect of low temperature on germination, growth, and seed yield of four soybean (Glycine max L.) cultivars. Agronomy, 11(4), 800.

Tully, R.E., Musgrave, M.E. and Leopold, A.C. 1981. The seed coat as a control of imbibitional chilling injury. *Crop Science*, 21(2), pp.312-317. https://doi.org/10.2135/cropsci1981.0011183X002100020026x.

USDA coexistence fact sheets soybeans, (2015). United State Department of Agriculture. Available at chrome extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.usda.gov/sites/default/files/documents/coexistence-soybeans-factsheet.pdf. (accessed 22 March 2024).

USDA-ERS, (202).). Economic Research Service. U.S. Department of Agriculture. Soybean-Overview. Available at, https://www.ers.usda.gov/topics/crops/soybeans-and-oil-crops/. (accessed 19 August 2023)

USDA-ERS, (2024).). Economic Research Service. U.S. Department of Agriculture. Soybean-Overview. Available at, https://www.ers.usda.gov/topics/crops/soybeans-and-oil-crops/. (accessed 10 March 2024)

USDA-FAS, (2024). Foreign Agricultural Service, U.S. Department of Agriculture. U.S. Soybean exports in 2023. Available at https://fas.usda.gov/data/commodities/soybeans. (accessed 21 March 2024).

USDA NASS. (2024). USDA National Agricultural Statistics Service. Quick Stats. Available at https://quickstats.nass.usda.gov/. (accessed 20 February 2024).

United Soybean Board, (2024). Global market. Available at https://www.unitedsoybean.org/issue-briefs/global-markets/. (accessed 22 March 2024).

United States Environmental Protection Agency, (2024). Available at https://www.epa.gov/climate-indicators/climate-change-indicators-length-growing-season. (accessed 20 March 2024).

Vann R., and Stokes D., (2020). Soil crusting, Nebraska State University. Soybean Crusting | NC State Extension Publications (ncsu.edu)

Wang, X., Li, X., Zhou, Q., Song, S., & Dong, S. (2023). Comparison and Evaluation of Low-Temperature Tolerance of Different Soybean Cultivars during the Early-Growth Stage. *Agronomy*, *13*(7), 1716.

Webster, B. D., & Leopold, A. C. (1977). The ultrastructure of dry and imbibed cotyledons of soybean. *American Journal of Botany*, *64*(10), 1286-1293. https://doi.org/10.1002/j.1537-2197.1977.tb10822.x.

Woodstock, L. W. (1988). Seed imbibition: a critical period for successful germination. Journal of Seed Technology, 1-15.

Wuebker, E.F., Mullen, R.E. and Koehler, K. 2001. Flooding and temperature effects soybean germination. *Crop Science*, 41(6), pp.1857-1861. https://doi.org/10.2135/cropsci2001.1857

Xue, A. G., Cober, E., Voldeng, H. D., Voldeng, H. D., Babcock, C., & Clear, R. M. (2007). Evaluation of the pathogenicity of Fusarium graminearum and Fusarium pseudograminearum on soybean seedlings under controlled conditions. *Canadian journal of plant pathology*, *29*(1), 35-40. https://doi.org/10.1080/07060660709507435.

Yan, H., & Nelson Jr, B. (2022). Effects of soil type, temperature, and moisture on development of Fusarium root rot of soybean by Fusarium solani (FSSC 11) and Fusarium tricinctum. *Plant Disease*, *106*(11), 2974-2983. https://doi.org/10.1094/PDIS-12-21-2738-RE.

Yin, G., Sun, H., Xin, X., Qin, G., Liang, Z. and Jing, X. 2009. Mitochondrial damage in the soybean seed axis during imbibition at chilling temperatures. Plant and Cell Physiology, 50(7), pp.1305-131. https://doi.org/10.1093/pcp/pcp074

Yu XiaoMei, Y. X., Li AiHua, L. A., & Li WeiQi, L. W. (2015). How membranes organize during seed germination: three patterns of dynamic lipid remodelling define chilling resistance and affect plastid biogenesis. doi: 10.1111/pce.12494

Appendix

SAS code for germination percentage from Chapter 2

```
data germination;
input cultivar$ trt$ run ltemp ger abnor dead;
gerper = ger*2;
abnorper = abnor*2;
deadper = dead*2;
cards;
P34A65PR    0000  1     7.2   46    2     2
P34A65PR    0000  2     7.2   46    2     2
P34A65PR    0003  1     7.2   46    2     2
.
.
;
proc print;
run;
proc sort; by ltemp; run;
ods graphics on;
'Temperature comparisons';
proc glimmix nobound plots=(residualpanel);
class ltemp cultivar trt run;
model gerper = ltemp|cultivar|trt;
random run run*ltemp;
lsmeans ltemp|cultivar|trt/lines;
   run;
```

R code for generating Surface Map for variation of normal seedling counts with imposed duration treatments- Chapter 2

Response surface methodology (RSM)
#This package helps create surface maps
library(rms)#install this package to create the surface maps
library(rsm)#install this package to create the surface maps
library(fields)#install this package to add a legend to the surface maps.
setwd("C:/Users/anune/OneDrive - The Ohio State University/Desktop/generating heat map/data sheet arrange for R code/datasheet arrangement for seperate cultivar")#my working directory
rsmdata<-read.csv("pr34.csv", header = TRUE)#my data file
View(rsmdata)
rsm.mod_germ10=rsm(germ10d ~ SO(duration,entry), data=rsmdata)#run rsm model#SO is second order derivative

a<-contour(rsm.mod_germ10, ~duration+entry, image=TRUE, img.col=terrain.colors(100, rev=TRUE), main="Germination Percent at 10 days")#create surface map only, without legend and save the figure.
#a<-contour(rsm.mod_germ10, ~duration+entry, image=TRUE, main="Germination percent at 10 days") warnings() #run this code

a+image.plot(zlim = c(87.3,94.6), legend.only = TRUE, side = 4, col = terrain.colors(100, rev=TRUE),
axis.args = list(at = NULL, labels = NULL, cex.axis = 1.0),
legend.width = 0.6, legend.shrink = 1, legend.mar = 6, legend.lab = "Germination Percentage",
horizontal = FALSE)#this code will create legend only(with specified limits (85-100%). save the legend.group the legend and surface map in power point.

SAS code for biomass data for Chapter 3 and 4

```
data;
input moisture trt depth cultivar$ run norcount norbiomass abnorcount
abnorbiomass dead;
tbiomass = 100*((norbiomass+abnorbiomass)/8);
pnemerge = 100*(norcount/8);
paemerge = 100*(abnorcount/8);
pdead = 100*(dead/8);
temerge= 100*(norcount/8)+100*(abnorcount/8);
cards;
60    1     1.5   3.4RM 1     4     4.37  1     0.97  3
60    1     1.5   3.7RM 1     3     3.54  3     2.1   2
60    1     1     3.4RM 1     6     5.93  1     0.64  1
60    1     1     3.7RM 1     4     3.04  0     0     4
.
.
;
proc print;
run;
proc sort; by moisture; run;
ods graphics on;
proc glimmix nobound plots=(residualpanel);
class moisture trt depth cultivar run;
model norbiomass = moisture|trt|depth|cultivar;
random run moisture(run) trt*moisture(run) depth*trt*moisture(run);
lsmeans moisture|trt|depth|cultivar/lines;
run;
```

SAS code for sigmoid model from NLIN procedure for Chapter 3 and 4

```
data fgerm;
input awc wtr depth var$ rep ID day count pgerm;
y= (pgerm/100)+0.0001;
x=day+0.001;
datalines;
20    1     1     3.4RM 1     1     0     0     0
20    1     1     3.4RM 2     2     0     0     0
20    1     1     3.4RM 3     3     0     0     0
.
.
;
proc sort; by ID;
proc print;
run;
proc nlin best=40 method=newton;
parms
      a=0.0 to 1.1 by 0.05
      b=-100 to 10 by 2
      c=0 to 2 by 0.1
      d=-0.05 to 0.15 by 0.05;
model y=(a/(1 + (EXP(b-(c*x)))))+d;
by ID;
run;
```