ABSTRACT

BAUCOM, ANDREW LEE. Grain Yield and Quality Effects of a Late Spring Freeze on Soft Red Winter Wheat (Under the direction of Dr. Angela R. Post).

Small grains in reproductive growth stages are impacted by spring freeze events in the Southeastern United States. In addition to producing varieties for grain yield, certified seed growers must seek to examine the damage resulting from a spring freeze event on commercial wheat varieties, determine the type of damage as well as the location of damage in individual varieties, and quantify the effect of a spring freeze event on the grain quality and harvest yield for individual varieties. Certified seed producers should rely on the evaluation of field germination differences between seed harvested from a crop following a freeze event and seed harvested from a crop that did not experience a freeze event. This would allow them to begin to determine if grain yield and end use quality differences exist between these contrasting seed types when used to plant a crop in the following season. The effects of freeze damage on grain head development, grain yield, and grain quality on soft red winter wheat [*Triticum aestivum* (L.)] were investigated in Perquimans, Rowan, and Union County, North Carolina.

In 2017, Perquimans County, North Carolina did not experience a freeze event and produced higher grain yields than Rowan County (11%) and Union County (23%) which did experience a March 2017 freeze event. Grain quality differences were observed by location, variety type, and location by variety analysis. Falling number on earlier maturing varieties was affected in Union County, while Rowan County had a wider range of maturities that had lower falling numbers. Wheat harvested from Union County had a higher protein content than both Perquimans and Rowan Counties, suggesting that freezing may reduce protein content. Healthy tillers produced more grain heads and kernels within each grain head than wheat tillers that suffered stem damage or flag leaf damage at both Rowan and Union County Locations.

Rowan County tillers produced a larger number of kernels on secondary tillers, while Union County tillers produced a larger number of kernels on primary tillers. Wheat yields do appear to be affected by spring freeze events when in an advanced growth stage. While grain quality, falling number and protein content, do not seem to be as significantly affected by a spring freeze event. This information provides growers with a knowledge base of how best to manage their wheat crop following a spring freeze event.

The effects of freeze damage on percent seed germination, grain weight, and grain quality on soft red winter wheat were investigated in Rowan and Union County, North Carolina. In 2018 new, certified seed had a higher laboratory germination percentage (96.9) than seed that had experienced a freeze event the following growing season and was re-planted, 93.6% for Rowan County and 93.5% for Union County. In-field plant counts were similar across all seed types. Seed type did not affect harvested grain weight or grain quality across locations. In 2018, harvested grain weight, protein content, and falling number were all higher in Union County, NC. Syngenta Viper and Dyna-Gro 9600 had the highest falling number and protein content between varieties analyzed regardless of location or seed type. Wheat seed that had previously experienced a freeze event the previous growing season appears to not be affected in-field by loss of germination, grain weight, or grain quality, which may improve the efficiency of certified seed producers and instill certified seed is available to grain producers in the Southeast.

Grain Yield and Quality Effects of a Late Spring Freeze on Soft Red Winter Wheat

by
Andrew Lee Baucom

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Crop Science

Raleigh, North Carolina
2019

APPROVED BY:

____
Dr. Angela R. Post
Committee Chair

____
Dr. J. Paul Murphy

____
Dr. David Livingston III

DEDICATION

To the Baucom Girls: Lauren & Naomi. You have my heart, and You are my heart.

BIOGRAPHY

Andrew was born in 1984 and grew up in Lanes Creek, North Carolina. As a child he spent much of his time playing sports and climbing trees. He attended Forest Hills High School, and the friendships he forged there through mutual struggle and understanding continue to shape his life to this day. Following graduation, Andrew attended North Carolina State University for his undergraduate education. There he began to discover his passion for agriculture and the wonderous beauty of witnessing a seed begin to grow.

Andrew was fortunate enough to convince Dr. Angela Post and the rest of the Crop Science department in allowing him to work on his Masters of Science in her small grains extension program at North Carolina State University. During this time Andrew continued to gain insight from incredible N.C. State Extension agents across the state who make a positive difference in the daily lives of their communities. Andrew hopes to one day inspire the wonder of agriculture into the hearts of future generations.

ACKNOWLEDGMENTS

I would like start by thanking my advisor, Dr. Angela Post, who took a chance on me when no one else would, and who has been incredibly generous with her resources and guidance. I have learned how large the state of North Carolina actually is, and the amount of time it takes to travel from the mountains to the sea.

I would like to thank my committee members, Dr. Paul Murphy and Dr. David Livingston, for your patience, insights, and technical support throughout this process. This would not be possible without your most gracious support and dedication. I would also like to thank Ryan Heiniger for allowing me the platform to push boundaries and express my thoughts, even when it was not helpful.

A very special thank you goes to Megan Miller for not only her technical assistance, but most importantly for her friendship. You embody the value of truth, honesty, and integrity and I am extremely proud to call you my friend.

Thank you to the members of the Small Grains and Official Variety Trial team, past and present: Phil Johnson, Johnny Denton, Kaitlyn Moody, Ezekiel Overbaugh, Jeremy Davis, Ian Fleming, and Austin Gilliard. Angela and Ryan would not be able to accomplish the work they do without all of you. You guys taught me how important it is to work together and the value of enjoying the people with whom you work with. You also willingly chose to cut wheat grain heads out of the field with scissors for hours on end in the middle of July.

Finally, I would like to thank my family and friends for your patience, encouragement, and understanding throughout this process and I am grateful to have you all in my life.

TABLE OF CONTENTS

LIST OF TABLES

Chapter II: Evaluation of Damage Type and Subsequent Yield Depression from Spring Freeze Damage on Soft Red Winter Wheat

LIST OF FIGURES

Chapter I: Literature Review

CHAPTER I: Literature Review

Winter Wheat

Common hexaploid wheat (*Triticum aestivum* L.) was domesticated roughly 10,000 years ago in the Middle East and brought to North America in the 18th century (Ball, 1930). It is the most widely produced cereal grain in the world and accounts for approximately twenty percent of human caloric intake (Awika, 2011). Common wheat is grown on more land than any other food crop and production is expected to continue to expand due to the unique properties of gluten proteins. Soft red winter wheat, one of several classes of wheat grown in the United States, is most often planted east of the Mississippi River. Soft wheats have lower protein and less gluten strength which make them ideally suited for cookie and cracker production (Wang et al., 2016). In North Carolina, a significant portion of the wheat crop is utilized as animal feed due to the fact that we are a grain deficient state.

Winter wheat originated in temperate regions and can survive low temperatures during early vegetative growth stages (Gusta et al., 2013). It requires a period of vernalization and a certain increasing day length to progress from the vegetative to the reproductive growth stage (Ruelland et al., 2009). In North Carolina, winter wheat is planted from late September to early December, with an ideal planting window from mid-October to mid-November (Weisz, 2013). The most popular rotation has wheat planted behind corn (*Zea mays*) or full season soybeans (*Glycine max* (L.) Merr.). Wheat is typically harvested from late May through late June in North Carolina being influenced by planting date, variety maturity, and weather conditions. A wheat crop is typically followed by double-crop soybeans in July. Each grain head, on average, produces between 25 and 50 kernels (Lyon et al., 2007). The average wheat yield in North Carolina is 3,685 kg ha^{-1} (55bu/A), but yield contest winners, who tend to be the best growers, can average 6,700 kg ha^{-1} (100bu/A) in Union County.

Impact of Temperature on Wheat Development at Spring Green-Up

Wheat breaks dormancy following vernalization after reaching a temperature above 5^{o}C (Lendent, 1979), provided any daylength threshold has been met for that variety. The plant progresses through Zadoks growth stage 30 (Zadoks et al., 1974), whereby the apical meristem elongates above the protective soil surface level inside the stem, and enters the reproductive phase. Head development continues during stem elongation and the fully developed head emerges from the boot at Zadoks growth stage 59. Once wheat enters the reproductive stage, the mechanisms whereby freeze-tolerance protects the plant are suspended and the plant reverts to the freezing tolerance of a non-acclimated plant (Mahfoozi et al., 2001b; Single, 1964, 1985). The actual temperature at which the developing head is susceptible to damage depends on the growth stage. For example, at Zadoks growth stage 21, damaging temperatures begin at -11^{o}C, but temperatures ranging from -4^{o}C at Zadoks growth stage 30 to -1^{o}C at Zadoks growth stage 50 will damage the plant and potentially reduce grain yield (Shroyer et al., 1995). Alternating warm and freezing temperatures represent an abiotic stress that can negatively impact wheat. Warm temperatures induce rapid growth and development, which can be detrimental if followed by frost (Cannell and Smith, 1986). In addition, unusually cold temperatures, at or below 0^{o}C, after stem elongation has progressed, can result in severe yield reductions (Shroyer et.al, 1995).

Freezing temperatures cause significant damage to the plant, particularly during Zadoks growth stage 35-47 when the developing head is in the boot (Livingston et al., 2016; Livingston et al., 2018). A normal, uninjured growing point is bright white to yellow-green and turgid; freeze injury causes it to become off-white or brown and water soaked in appearance (Shroyer et al., 1995). Damage from a spring freeze event includes leaf chlorosis, white awns, spikelets trapped inside of the boot, male and female sterility and damage to lower stems (Shroyer et al., 1995). Low temperature stress during reproductive development induces flower abscission, pollen sterility, pollen tube distortion, ovule abortion and reduced fruit set, which ultimately lowers yield (Thakur et al., 2010). The reproductive phase, particularly meiosis, is more sensitive than the vegetative phase, and while low temperature causes more damage to male reproductive organs than to female organs, the entire process is susceptible to cold stress (Thakur et al., 2010). As crop sensitivity to frost is increased after awns or spikes begin to emerge at Zadoks growth stage 49 (Zadoks et al., 1974), a yield reduction may be attributed to freeze induced damage to spike development (Kang et al., 2013); impairment of photosynthesis (Thakur et al., 2010); decreases in shoot biomass, flag leaf size, and specific leaf area (Valluru et al., 2012); and altered carbohydrate metabolism (Theocharis et al., 2012; Zeng et al., 2011). Spring freeze not only affects winter habit wheat, but both spring and winter habit types suffer damage at more moderate temperatures during reproductive stages (Fuller et al., 2007; Livingston and Swinbank, 1950; Paulsen and Heyne, 1983).

Livingston and Swinbank, (1950) found that greenhouse plants grown in soil of high fertility were injured more than plants grown in soil of average fertility, and plants that were wet during freezing were injured more than those that were dry. Chilling and freezing temperatures limit the growth and development of plants and may significantly decrease crop yield and grain quality (Chinnusamy et al., 2007), so it is important for researchers to carefully inspect trials for pre-existing damage and unrelated sterility before frost events (Frederiks et al., 2011). Fuller et al. (2007) proposed that cellular frost damage does not need to be extensive but if it affects critical reproductive organs the net effect is sterility, which is catastrophic for yield. Damage to the peduncle is often the most noticeable stem damage (Woodruff et al., 1997), with damage to stems being usually observed adjacent to the nodes (Frederiks et al., 2015). Less severe damage may result in chlorotic bands (Shroyer et al., 1995) with mild stem injury not appearing to interfere with the wheat plants ability to take up nutrients from the soil and translocate them to the developing grain (Shroyer et al., 1995). Severe stem damage may sever the vascular connection between the head and the rest of the plant causing the head to die (Afanasev, 1966). According to Livingston and Swinbank (1946), serious stem injury is defined as bending of the stem at the lowest joint forming an elbow, twisting of the stem near the base followed usually by breaking and rotting, or splitting of the stem near the base followed usually by rotting. There is also great variation between varieties in the number of plants lodged as a result of stem injury (Livingston and Swinbank, 1946). Freezing in the grain head is a slow process that is characterized by florets freezing separately in compartments (Livingston et al., 2016). This suggests barriers may exist within the rachis of the head as described by Single and Marcellos (1981). If the head emerges after such a frost event, the damage often presents as a bleached section with incomplete ear structure and aborted florets (Woodruff et al., 1997).

Spring Freeze Potential

In North Carolina, Zadoks growth stage 30 typically occurs around March 10 in central North Carolina but varies about the mean by10 to 14 days from the southern to northern tiers of counties. Head emergence typically occurs between April 10th and April 30th across the state. The mean date of the last spring freeze in major wheat production regions in North Carolina ranges from March 20 in coastal southeastern counties to April 21 in the western and northern Piedmont (Figure 1.1). Nevertheless, this mean only indicates the day when the probability is 50 percent that the last spring freeze has occurred. Reality for the grower can be significantly different. For example, in Lumberton NC, the mean last spring freeze occurs on April 1st, but the 75 percent probability that the last spring freeze has occurred is April 12 and the 90 percent probability that the last spring freeze has occurred is April 22nd (Figure 1.2). Similarly, although the mean date of the last spring freeze in Elizabeth City is March 31st, a freeze can occur twice in a ten-year period up to April 11th. (Perry, 1996). Similar anomalous events during a ten-year period can occur up to April 12th in Kinston, April 20th in Salisbury, April 24th in Monroe and April 25th in Plymouth.

Widespread freeze events ranging from 1992 through 2018 show lowered overall wheat yields in North Carolina (Figure 1.3). Widespread freeze events occurred in 1998, 2001, 2007 and 2017, although the latter was not reflected in a major impact on statewide production. In 2001, a temperature of -1.1°C occurred on the morning of April 19 at Kinston (Figure 1.4). Wheat heads were coated in a thin layer of ice resulting in major losses in production from Greenville through Wilmington (J. P. Murphy personal communication). April 19th was also the heading date of Roane, the latest maturing variety in the NC Official Variety Test at that location. In 2007, a temperature of -6°C was recorded at Kinston on April 8th causing widespread damage to wheat yields in eastern NC, although the majority of the crop was still in the boot stage. In addition, during 2012 and 2017, unusually warm winter temperatures resulted in premature crop development and subsequent isolated losses due to freezing temperatures that did not occur within the low probability window. During early spring 2017, the wheat crop was in an advanced stage of development due to unusually warm temperatures in January and February. Several days of freezing weather occurred during March 15th to 17th with temperatures as low as -10°C recorded at Lumberton, NC (Livingston et al., 2018). This was an example of a “false spring” (Marino et al., 2011) causing fall-sown wheat to suffer what Fredericks et al. (2015) refer to as post-head-emergence frost (PHEF) when an unusually early break in spring results in crops maturing early.

This advanced maturity increases the susceptibility of wheat to freeze injury (Paulsen and Heyne, 1983). Spring freeze events are a phenomenon that are increasing in frequency due to climate change (Gu et al., 2008; Marino et al., 2011; Rigby and Porporato, 2008). The warmer the climate during the vegetative phase of growth, the greater the risk of injury due to a faster development of plants through the susceptible reproductive stages, and a coincidence with the shortest days when frosts are more likely (Single, 1985).

Within a production region, practices have evolved so planting date facilitates head emergence after the main frost risk period. Crop losses due to frost typically arise in two ways: firstly, crop loss due to direct frost damage; secondly, lost yield resulting from late planting to delay heading until after the main period of frost has passed (Single and Marcellos, 1974). Full sensitivity to freezing temperatures is not achieved until after head emergence, with both female and male floral structures usually equally affected following freezing damage (Frederiks et al., 2011; Livingston and Swinbank, 1950). Further analysis of yield components showed the young spikes at booting had relatively lower cold resistance than at jointing (Zhong et al., 2008) and were more easily killed by low temperature, resulting in a significant decrease in spike number per plant (Thakur et al., 2010). Low temperature events at jointing and booting stages also significantly reduced the effective tiller number per plant of wheat (Li et al., 2015). These observations again confirmed the importance of stage of development in determining a plant’s ability to respond to temperature, and restates that plant development toward flowering progressively reduces the ability of wheat to acclimate to low-temperature particularly after the main shoot meristem has advanced to the reproductive growth stage (Limin and Fowler, 2006).

The false spring of 2007 should not be considered an isolated event; literature has documented several similar events over the United States (Marino et al., 2011). Parts of the USA, including the Midwest and Southeast, as well as Canada, all experienced recent spring freeze events that caused drastic yield losses to wheat (Gu et al., 2008). Between 1955 and 2010 in Kansas, 41 cold stress events occurred, resulting in an annual wheat yield loss of more than 536 kg ha^{-1} (8bu/A) (Dolferus et al., 2011). Adverse weather conditions can cause severe fluctuations in grain yield and substantial yield losses from 30% to as high as 90% (Al-Issawi et al., 2013; Frederiks et al., 2015; Fuller et al., 2007; Thakur et al., 2010). The false spring event of 2007 caused damage in every state from Kansas and Oklahoma eastward to the Carolinas and Georgia, with estimates of agricultural losses of about $2 billion (Marino et al., 2011).

In North Carolina, conventional reliance on specific growth characteristics of popular varieties may need to be questioned as these weather events potentially affect grain yield and quality. Growers are adjusting their wheat production practices attempting to minimize the potential for spring freeze damage. Frost escape by manipulating heading time is the main method currently available to minimize risk suggested by Frederiks (2015), however Livingston (2016) suggests that is may be possible to develop resistant varieties. Some of these practices have included selecting a later maturing variety and delayed planting dates to avoid flowering at freeze-risk periods. Later planting dates will result in later flowering and grain fill in wheat; however, heat and drought during grain filling and ripening can also dramatically reduce crop yield potential (Frederiks et al., 2015).

Vegetative Tolerance

Freezing tolerance is the ability to tolerate extracellular ice formation within tissues (Livingston et al., 2016). Freezing tolerance often requires a period of cold acclimation and the level of tolerance achieved following acclimation varies (Gusta and Wisniewski, 2013; Wisniewski et al., 2014). While freezing tolerance can be broken down into vegetative and reproductive growth stages, tolerance to freezing damage during the vegetative stages does not appear to confer tolerance in the reproductive stages in wheat (Fuller et al., 2007; Livingston et al., 2016). There is also significant variation in winter-habit and spring-habit wheats (Fowler et al., 1999; Limin and Fowler, 2006) regarding days of exposure and survival temperatures. Some studies show that vegetative tolerance requires 7-10 days of exposure to temperatures less than 8^{o}C before significant increases in frost tolerance (Fuller et al., 2009), culminating with cold acclimated winter-habit wheat that has survival characteristics to temperatures of -21^{o}C (Fowler and Carles, 1979). The concern is that freezing tolerance develops over periods of days and months while frost damage occurs when temperatures drop to damaging levels over a period of several hours (Gusta, et al., 2003).

Reproductive Tolerance

The reproductive stage of wheat development begins when the last leaf is formed. This leaf is called the flag leaf primordia (Robertson et al., 1996). Even though there are high levels of freezing tolerance in winter-habit wheats, there is no additional protection from frost injury in the reproductive stages comparatively to spring-habit wheat (Marcellos and Single, 1984; Paulsen and Heyne, 1983). This is a contrast to the vegetative tolerance properties that winter-habit wheat has compared to spring-habit wheat. In winter-habit wheat, the transition from vegetative to reproductive growth stages is crucial to the regulation of low-temperature tolerance. Unfortunately, this means that freezing tolerance of seedlings and cold acclimated plants is a poor predictor of spring freeze tolerance (Frederiks et al., 2011; Livingston et al., 2016).

Variety Specificity

There are conflicting ideas in the literature on the premise that winter wheat can be damaged by a freeze event based on genotype differences and growth stage. Cereal scientists seem to generally accept that differences between genotypes in spring freeze tolerance are a result of differences in maturity, with early varieties being more susceptible than later ones (Frederiks et al., 2015; Livingston et al., 2016), while extension publications from the Midwest argue there is little to no difference in susceptibility between wheat varieties at the same growth stage (Shroyer et al., 1995). What can be agreed upon is that wheat in the vegetative stage is more tolerant of freeze events than wheat in the reproductive state (Thomashow et al., 2001).

Viability of Seed Following a Freeze Event

The harvested grain quality of wheat that underwent a freeze event varies. Freeze events can cause wheat heads to become fully or partially sterilized (Chatters and Schlehuber, 1953). Seed harvested from a partially sterile head is typically characterized by shriveled grain with decreased kernel weight, excessive screenings and poor test weight (Woodruff et al., 1997; Cromey et al., 1998). Kernels may be discolored, and the grain may be a mixture of kernels of different sizes and maturities (Shroyer et al., 1995). Grain with low test weight and small kernels are not desirable at baking and flour mills and can be rejected outright or purchased at discounted rates. Studies showed that test weight steadily decreased as the level of frost damage increased, kernel weights of all grains decreased with severity of injury and deficiencies in germination and emergence were reported with increased content of small kernels in frost-damaged seed lots (Foster et al., 1997).

Acclimation / Vernalization

Vegetative freezing tolerance requires a period of cold acclimation before full tolerance is expressed and is a natural phenomenon to improve winter wheat survival. Vernalization is a process where the wheat plant goes through a period of cool temperature to allow it to transition into the reproductive growth stage. This process requires roughly a 7-week period of temperatures near 4-5^{o}C depending on the maturity of the variety (Lendent, 1979). The vernalization process is known to maintain the low temperature tolerance of a genotype by limiting the plant to the early stage of phenological development (Limin and Fowler, 2006). When the vernalization requirement period has been met and the vegetative growth stage ends, winter wheat gradually loses its ability to tolerate below-freezing temperatures (Mahfoozi et al., 2001b). This suggested that genes for vernalization are a master switch for frost acclimation (Mahfoozi et al., 2001a; Prášil et al., 2004). As temperatures rise the plant loses its capability to re-acclimate (Fowler et al., 1996; Fuller et al., 2007; Mahfoozi et al., 2001b; Prášil et al., 2004). The loss of cold acclimation ability is progressive, in-step with ongoing phenological development. Cereals are known to vary in their response to major temperature cues (Mahfoozi et al., 2001b), reaffirming the importance of the vegetative/reproductive transition in low temperature acclimation.

Supercooling / Freezing

Supercooling, defined as water remaining in the liquid state in a plant at temperatures below 0^{o} C, is a freeze avoidance mechanism that normally prevents freeze-damage in plants (Wisniewski et al., 2014). Fuller et al. (2007) noted the common mistake of assuming all plants exposed to freezing temperatures will freeze and that they freeze at the same temperature. Post head-emergence wheat may supercool to temperatures below 0^{o}C and avoid damage (Single, 1964). In some situations, plants can supercool and escape frost damage (Lindow, 1995; Fuller et al., 2009; Wisniewski et al., 2009). Subedi et al. (1998) investigated the effects of freeze stress from booting to flowering on wheat yield and found that the grain number per spike was significantly reduced under freeze stress, resulting in 35 to 78 percent decreases in grain yield. Plants that froze quickly died and heads never matured. Those that super-cooled produced a head that was partially or nearly completely fertile depending on the genotype (Livingston et al., 2016).

Supercooling does not prevent the plant from suffering any damage due to a freeze event. Even though vegetative material may show no visible frost damage, plants at grain-fill can exhibit floret infertility (Fuller et al., 2009). While frost susceptibility generally increases with plant maturity (Single, 1964, 1985), wheat sensitivity to frost is increased after the awns or spikes begin to emerge from the auricle of the flag leaf at Zadoks 49 (Zadoks et al., 1974). In actual production, the impact of below freezing temperature on wheat growth and development is often affected by the combination of low temperature level and duration. Subedi et al. (1998) found plants that froze quickly died and heads never matured. Those that super-cooled produced a head that was partially or nearly completely fertile, depending on the genotype (Livingston et al., 2016).

Temperature Levels

The temperature experienced by a crop can vary widely due to the interactions of topographical, meteorological, environmental, plant physiological and canopy architectural factors (Gusta and Wisniewski, 2013; Marcellos and Single, 1975). Frederiks (2015) showed using thermocouples at a single point in the top of the crop canopy there is up to 2 degrees Celsius difference in lower parts of the crop canopy, while Livingston (2018) showed significantvariance in total canopy temperatures when IR analysis was performed. Crops with a more open canopy may allow better drainage of cold air from above the canopy near the heads, reducing the risk of damage ((Marcellos and Single, 1975: Frederiks et al., 2015). Under field frost conditions, where damage can occur at plant minimum temperatures of approximately -4^{o}C, universal severe damage is typically observed once the temperature of the wheat heads (Zadoks 50) fall lower than approximately -6^{o}C (Frederiks et al., 2011). In freezing chambers, wheat heads routinely supercool to temperatures lower than -5^{o}C without freezing (Fuller et al., 2009). The level of freezing stress varies tremendously across a field (Fowler, 1979); hence, studies on cold acclimation and freezing survival usually are conducted under controlled, artificial freezing conditions. However, Frederiks et al. (2011) notes that results from artificial frost chambers should be interpreted with caution and be validated by field experiments.

Weather Station Data / In-Field Conditions

The main factors in whether a wheat plant will freeze are duration, temperature level, and growth stage of the plant. There are varying opinions in the literature as to what extent each of those factors play. In wheat, cold temperatures can affect both phenology and grain filing, prolonging the time taken to complete the pre-flowering process (Subedi, et al., 1998). Although temperature had a greater effect on ice nucleation than the length of exposure, time was significant (Ashworth et al., 1985). Thus, it seems appropriate that both cooling rate and the length of exposure to subzero temperatures need to be considered when designing experiments to determine plant freezing temperatures (Ashworth et al., 1985). Radiant frost occurs when still, cold air, clear skies and a dry atmosphere combine, allowing rapid radiation of heat to the night sky (Hocevar and Martsolf, 1971; Willcocks and Stone, 2000). Meteorologists report ‘ground frosts’ when grass temperatures fall to less than $0^{o}C$ (Hocevar et al., 1971). Official weather stations may not reflect true in-field temperatures. It is not unusual, for instance, for wheat growers to report markedly lower temperatures than are recorded at the nearest official weather station (Shroyer et al., 1995). The Stevenson screen air temperatures most commonly quoted by meteorologists can be poorly correlated with plant temperatures during radiant frosts (Hayman et al., 2007; Frederiks et al., 2011). Heat flux from the soil as it freezes is a significant contribution to temperature fluctuations within the crop canopy (Livingston et al., 2018). Depending on exposure, wind speed, and cloud cover, the temperatures of tissues within the same plant may differ by as much as 1 to 2 degrees Celsius (Ashworth et al., 1985). Because cooling rate, exposure time, dew, and plant temperature all affect nucleation temperature, simulating frost conditions is difficult (Ashworth et al., 1985). Under field conditions the extent of freeze-injury can vary depending on the position in the field, stage of growth, variety, and air temperature (Livingston et al., 2016). Small differences in topography can cause significant temperature variation by impeding or facilitating the drainage of cold air, and soil temperatures within the crop can be several degrees warmer than air or plant temperatures (Frederiks et al., 2015).

Primary Objectives

Due to the limited availability of information on late spring freeze events in a field environment, wheat growers currently have limited resources on how to manage their crop if a freeze event occurs. With continued warmer winter weather in North Carolina, coupled with more frequent late spring freeze events, growers need practical, field-based information that can be applied real-time on their farms. There are numerous examples in the literature of greenhouse, or laboratory-based experiments regarding a freeze event at early reproductive growth stages. However, field conditions vary greatly in contrast to controlled environments, resulting in data that are not especially useful to growers. To evaluate harvest yield and grain quality effects of a late spring freeze event on advanced reproductive growth stage wheat, the following research objectives were developed:

1. Determine the percent grain yield loss and grain quality reductions in selected wheat varieties following a spring freeze event,
2. Examine the susceptibility and plant damage observed in wheat varieties following a spring freeze event,
3. Compare seed germination of wheat seed harvested from plants following a spring freeze event with germination of seed not subjected to a spring freeze event, and

4. Compare grain yield and quality in wheat seed harvested from plants following a spring freeze event with grain yield and quality in seed not subjected to a spring freeze event

Literature Cited:

Afanasev, M.M. 1966. Frost Injury in Wheat. Plant Dis. Rep. 50:929-930.

Al-Issawi, M., H.Z. Rihan, N. El-Sarkassy, and M.P. Fuller. 2013. Frost hardiness expression and characterisation in wheat at ear emergence. J. Agron. Crop Sci. 199(1):66–74.

Ashworth, E.N., G.A. Davis, and J.A. Anderson. 1985. Factors affecting ice nucleation in plant tissues. Am. J. Plant Physiol. 79(4):1033–1037.

Awika, J.M. 2011. Major cereal grains production and use around the world. In: Advances in cereal science: implications to food processing and health promotion. American Chemical Society. p. 1-13.

Ball, C.R. 1930. The history of american wheat improvement. Agricultural History. 4(2):48-71.

Chinnusamy, V., J. Zhu, and J.K. Zhu. 2007. Cold stress regulation of gene expression in plants. Trends Plant Sci. 12(10):444–451.

Dolferus, R., X. Ji, and R.A. Richards. 2011. Abiotic stress and control of grain number in cereals. Am. J. Plant Sci. 181(4):331–341.

Equiza, M.A., J.P. Miravé, and J.A. Tognetti. 2001. Morphological, anatomical and physiological responses related to differential shoot vs. root growth inhibition at low temperature in spring and winter wheat. Ann. Bot. (Oxford, U. K.). 87(1):67–76.

Foster, N.R., L.A. Burchett, and G.M. Paulsen. 1997. Planting wheat seed damaged by sprouting before harvest. Dept. Agro. Contr. 97-176.

Fowler, D.B., A.E. Limin, and J.T. Ritchie. 1999. Low-temperature tolerance in cereals: model and genetic interpretation. Crop Sci. 39(3):626–633.

Fowler, D.B., A.E. Limin, S. Wang, and R.W. Ward. 1996. Relationship between low-temperature tolerance and verndlization response in wheat and rye. Can. J. of Plant Sci. 76:37–42.

Frederiks, T.M., J.T. Christopher, S.E.H. Fletcher, A.K. Borrell. 2011. Post head-emergence frost resistance of barley genotypes in the northern grain region of Australia. Crop Pasture Sci. 62(9):736–745.

Frederiks, T.M., J.T. Christopher, M.W. Sutherland, A.K. Borrell. 2015. Post-head-emergence frost in wheat and barley: Defining the problem, assessing the damage, and identifying resistance. J. Exp. Bot. 66(12):3487–3498.

Fuller, M.P., J. Christopher, T. Fredericks. 2009. Low-temperature damage to wheat in head-matching perceptions with reality. Plant Cold Hardiness: From the Laboratory to the Field. 12–18.

Fuller, M.P., A.M. Fuller, S. Kaniouras, J. Christophers, J., T. Fredericks. 2007. The freezing characteristics of wheat at ear emergence. Eur. J. Agron. 26(4):435–441.

Gu, L., P.J. Hanson, W.M. Post, D.P. Kaiser, B. Yang, R. Nemani, R. et al. 2008. The 2007 eastern U.S. spring freeze: increased cold damage in a warming world. BioScience. 58(3): 253–262.

Gusta, L.V., and M. Wisniewski. 2013. Understanding plant cold hardiness: an opinion. Physiol. Plant. 147(1):4–14.

Hocevar, A., and J.D. Martsolf. 1971. Temperature distribution under radiation frost conditions in a central Pennsylvania valley. Agricultural Meteorology. 8:371-383.

Kang, G., G. Li, W. Yang, Q. Han, H. Ma, Y. Wang et al. 2013. Transcriptional profile of the spring freeze response in the leaves of bread wheat (*Triticum aestivum L.*). Acta Physiol. Plant. 35(2):575–587.

Lendent, J. 1979. Vernalization and anthesis in a collection of wheat cultivars (Triticum Aestivum L.) A Quantitative Study in Controlled Environment. Bull. Soc. R. Bot. De Belg. 112(2):186-192.

Li, X., H. Pu, F. Liu, Q. Zhou, J. Cai, T. Dai, T. et al. 2015. Winter wheat photosynthesis and grain yield responses to spring freeze. Agron. J. 107(3):1002–1010.

Limin, A.E., and D.B. Fowler. 2006. Low-temperature tolerance and genetic potential in wheat (*Triticum aestivum L.*): response to photoperiod, vernalization, and plant development. Planta. 224(2):360–366.

Livingston, D.P., T.D. Tuong, T.G. Isleib, and J.P. Murphy. 2016. Differences between wheat genotypes in damage from freezing temperatures during reproductive growth. Eur. J. Agron. 74:164-172.

Livingston, D.P., T.D. Tuong, T.G. Isleib, and J.P. Murphy. 2018. High-definition infrared thermography of ice nucleation and propagation in wheat under natural frost conditions and controlled freezing. Planta 247:791-806.

Livingston, J.E., and J.C. Swinbank. 1946. Two types of late spring frost injury to winter wheat. J. Am. Soc. Agron. 10:536-544.

Livingston, J.E., and J.C. Swinbank. 1950. Some factors influencing the injury to winter wheat heads by low temperatures. Agron. J. 42(3):153–157.

Lyon, D. J., E. Dryland, and C. Systems. 2007. Estimating winter wheat grain yields. G1429:1–4.

Mahfoozi, S., A.E. Limin, and D.B. Fowler. 2001a. Developmental regulation of low-temperature tolerance in winter wheat. Bot. (Oxford, U.K.). 87(6):751-757.

Mafoozi, S., A.E. Limin, and D.B. Fowler. 2001b. Influence of vernalization and photoperiod responses on cold hardiness in winter cerals. Crop Sci. 41(4):1006-1011.

Marcellos, H., and W.V. Single. 1975. Temperatures in wheat during radiation frost. Aust. J. Exp. Agric. 15(77):818-822.

Marcellos, H., and W.V. Single. 1984. Frost injury in wheat ears after ear emergence. Aust. J. Plant Physiol. 11(2): 7.

Marino, G.P., D.P. Kaiser, L. Gu, and D.M. Ricciuto. 2011. Reconstruction of false spring occurrences over the southeastern United States, 1901-2007: an increasing risk of spring freeze damage. Environ. Res. Lett. 6(2). 024015.

Paulsen, G.M., and E.G. Heyne. 1983. Grain production of winter wheat after spring freeze injury. Agron. J. 75(4):705–707.

Perry, K. 1996. Average Last Spring Frost Dates for Selected North Carolina Locations. Horticultural Information Leaflet. NC State University Extension, Raleigh, NC. HIL-705.

Prášil, I.T., P. Prášilová, and K. Pánková. 2004. Relationships among vernalization, shoot apex development and frost tolerance in wheat. Ann. Bot. (Oxford, U. K.) 94(3):413–418.

Rigby, J.R., and A. Porporato. 2008. Spring frost risk in a changing climate. Geophys. Res. Lett. 35(12):1–5.

Shroyer, J.P., Mikesell, and G.M. Paulson. 1995. Spring Freeze Injury to Kansas Wheat. Kansas State University, Manhattan, KS. C-646.

Single, W.V. 1964. Studies on frost injury to wheat. Aust. J. Agric. Res. 15: 869-875.

Single, W.V. 1985. Frost injury and the physiology of the wheat plant. J. Aust. Inst. Agric. Sci. 51:128-134.

Single, W.V., and H. Marcellos. 1974. Studies on frost injury to wheat. IV. Freezing of ears after emergence from the leaf sheath. Aust. J. Agric. Res. 25(5):679–686.

Subedi, K.D., P.J. Gregory, R.J. Summerfield, and M.J. Gooding. 1998. Cold temperatures and boron deficiency caused grain set failure in spring wheat (*Triticum aestivum L*). Field Crops Research, 57(3):277–288.

Suneson, C.A. 1937. Emasculation of wheat by chilling. Agron. J. 29(3):247-249.

Thakur, P., S. Kumar, J.A. Malik, J.D. Berger, and H. Nayyar, H. 2010. Cold stress effects on reproductive development in grain crops: an overview. Environ.Exp. Bot. 67(3):429–443.

Theocharis, A., C. Clément, and E.A. Barka. 2012. Physiological and molecular changes in plants grown at low temperatures. Planta. 235(6):1091–1105.

Thomashow, M.F., S.J. Gilmour, E.J. Stockinger, K.R. Jaglo-Ottosen, and D.G. Zarka. 2001. Role of the Arabidopsis CBF transcriptional activators in cold acclimation. Physiologia Plantarum. 112(2):171–175.

Valluru, R., J. Link, and W. Claupein. 2012. Consequences of early chilling stress in two *Triticum* species: plastic responses and adaptive significance. Plant Biology. 14(4):641–651.

Wang, N., G.G. Hou, M. Kweon, and B. Lee. 2016. Effects of particle size on the properties of whole-grain soft wheat flour and its cracker baking performance. J. Cereal Sci. 69:187–193.

Weisz, R. 2013. Small grain planting dates. Small grain production guide. North Carolina Cooperative Extension Service. North Carolina. AG-580 Revised. 14-16.

Wisniewski, M., L.V. Gusta, G. Neuner. 2014. Adaptive mechanisms of freeze avoidance in plants: a brief update. Environ. Exp. Bot. 99:133–140.

Wisniewski, M., L.V. Gusta, M.P. Fuller, and D. Karlson. 2009. Ice nucleation, propagation and deep supercooling: the lost tribes of freezing studies. Plant Cold Hardiness: From the Laboratory to the Field. 17–58.

Zadoks, J., T. Chang, and C. Konzak. 1974. A decimal growth code for the growth stages of cereals. Weed Res. 14(14):415–421.

Zeng, Y., J. Yu, J. Cang, L. Liu, Y. Mu, J. Wang et al. 2011. Detection of sugar accumulation and expression levels of correlative key enzymes in winter wheat (*Triticum aestivum*) at low temperatures. Biosci., Biotechnol., Biochem. 75(4):681–687.

Zhong, X., X. Mei, Y. Li, H. Yoshida, P. Zhao, X. Wang. 2008. Changes in frost resistance of wheat young ears with development during jointing stage. J. Agron. Crop Sci. 194(5):343–349.

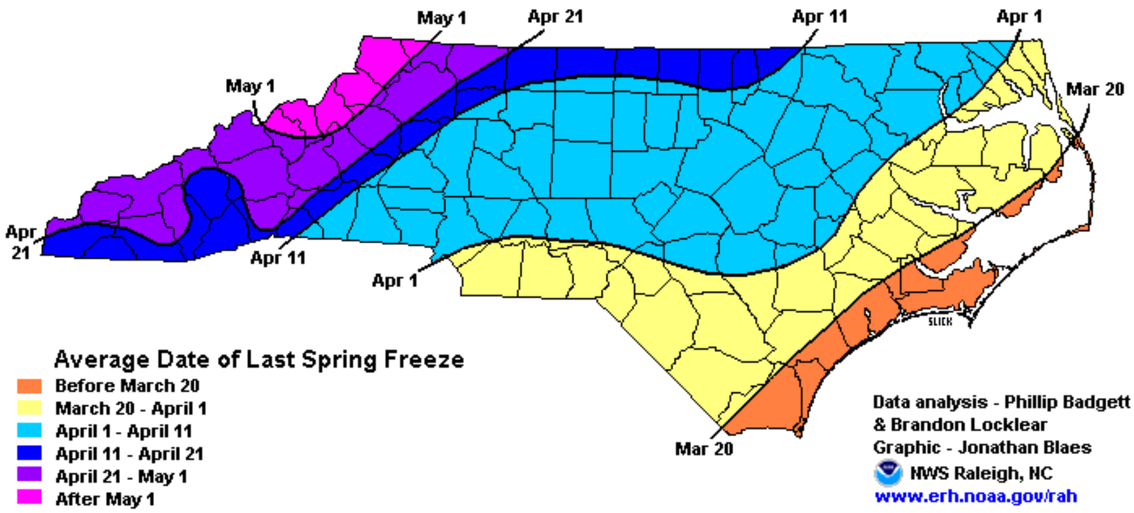

Figure 1.1. Mean date of last spring freeze in North Carolina courtesy of the National Oceanic and Atmospheric Administration website.

Probability the Last Spring Freeze has occurred, sorted by Date:

Date	Probability
Feb 10	0.1%
Feb 15	0.2%
Feb 20	0.6%
Feb 25	1.3%
Mar 1	2.8%
Mar 5	4.7%
Mar 10	8.5%
Mar 15	14.2%
Mar 20	22.1%
Mar 25	32.0%
Apr 1	48.2%
Apr 5	57.8%
Apr 10	69.1%
Apr 15	78.8%
Apr 20	86.5%
Apr 25	92.0%
May 1	96.1%
May 5	97.8%

Probability the Last Spring Freeze has occurred, sorted by Probability:

Probability	Date
1%	Feb 23
5%	Mar 5
10%	Mar 11
20%	Mar 18
25%	Mar 21
30%	Mar 24
40%	Mar 28
50%	Apr 1
60%	Apr 5
70%	Apr 10
75%	Apr 12
80%	Apr 15
90%	Apr 22
95%	Apr 29
99%	May 10

Earliest Last Spring Freeze: Feb 23
Latest Last Spring Freeze: May 10

Figure 1.2. Probability of last spring freeze occurred sorted by date and probability courtesy of Weather.Gov.

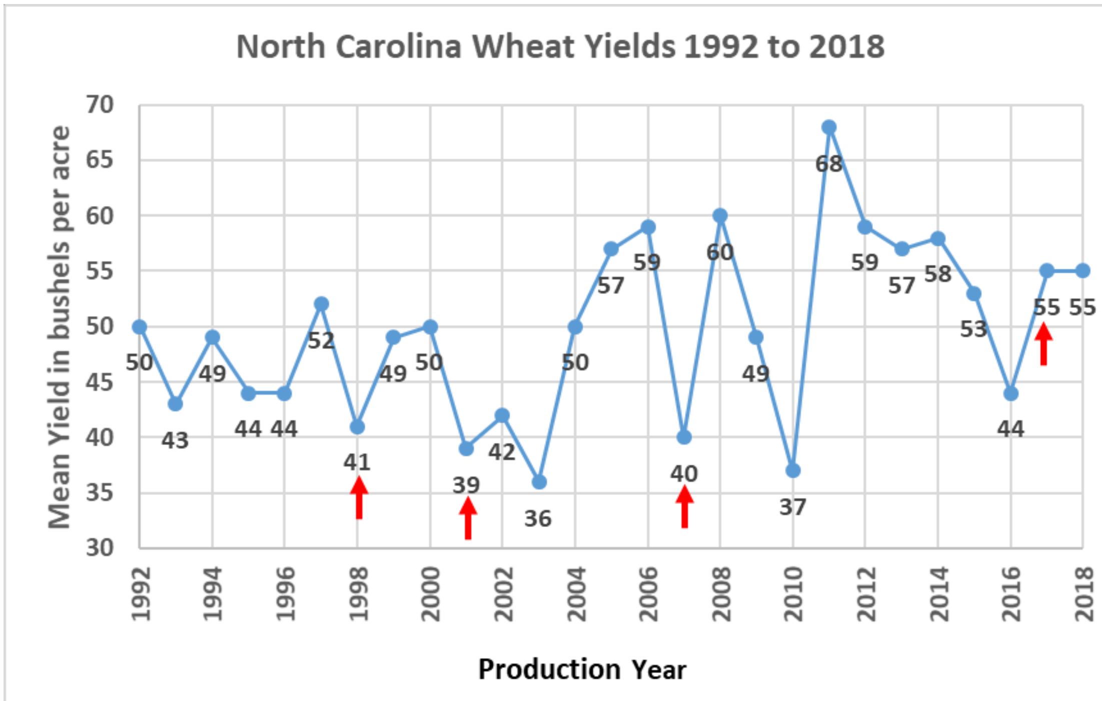

Figure 1.3. Mean North Carolina wheat grain yields in bushels per acre during 1992 through 2018. Yields marked by a red arrow were associated with a widespread spring freeze event.

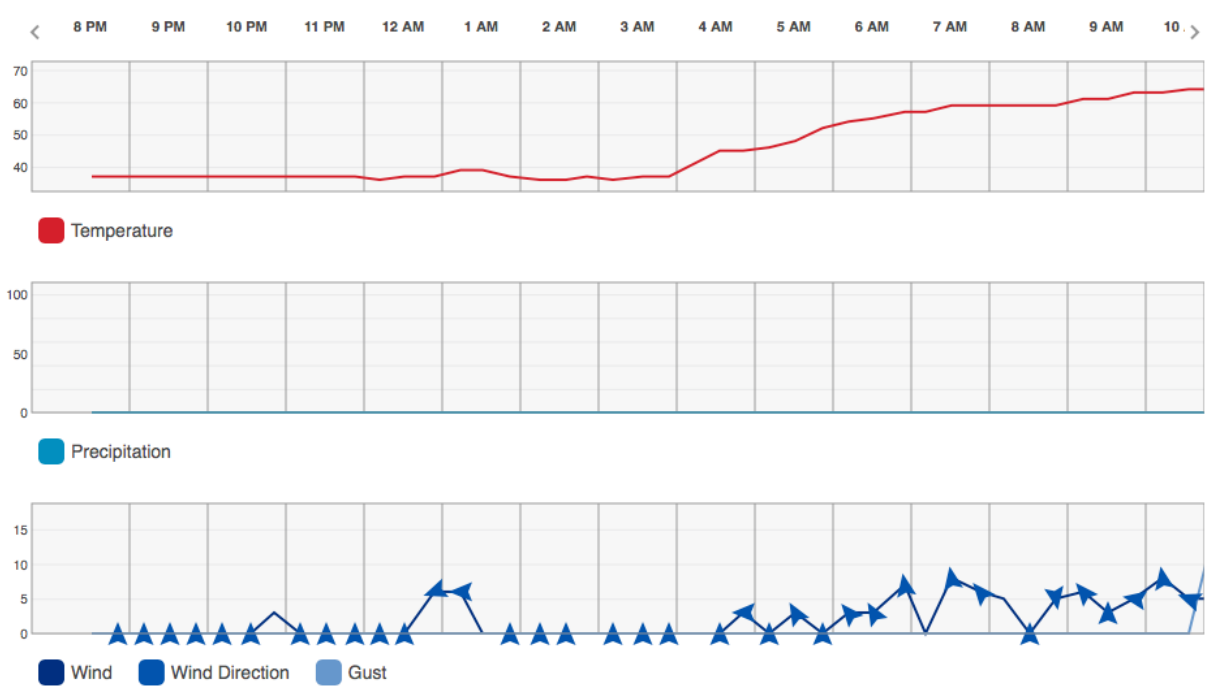

Figure 1.4. Weather for Kinston, North Carolina on April 19, 2011 courtesy of Intellicast.com.

Chapter II:

Evaluation of Damage Type and Subsequent Yield Depression from Spring Freeze Damage on Soft Red Winter Wheat

Abstract

Small grains in reproductive growth stages are impacted by spring freeze events in the Southeastern United States. This study was conducted to examine the damage resulting from a spring freeze event on 10 commercial wheat varieties, determine the type of damage as well as tissue specific damage in individual varieties, and quantify the effect of a spring freeze event on the grain quality and harvest yield for individual varieties. The effects of freeze damage on grain head development, grain yield, and grain quality on soft red winter wheat [*Triticum aestivum* (L.)] were investigated in Perquimans, Rowan, and Union County, North Carolina. In 2017, Perquimans County, North Carolina did not experience a freeze event and produced higher grain yields than Rowan County (11%) and Union County (23%) which did experience a March 2017 freeze event. Grain quality differences were observed by location, variety type, and location by variety analysis. Falling number on earlier maturing varieties was affected in Union County, while Rowan County had a wider range of maturities that had lower falling numbers. Wheat harvested from Union County had a higher protein content than both Perquimans and Rowan Counties, suggesting that freezing may reduce protein content. Healthy tillers produced more grain heads and kernels within each head than wheat tillers that suffered stem damage or flag leaf damage in both Rowan and Union Counties. Rowan County produced a larger number of kernels on secondary tillers, while Union County produced a larger number of kernels on primary tillers. While grain quality, falling number and protein content, do not seem to be as significantly affected by a spring freeze event.

This information provides growers with a knowledge base of how best to manage their wheat crop following a spring freeze event.

Introduction

The winter wheat crop in the Midwest and Southeast United States, as well as parts of Canada, have experienced spring freeze events between 2004 and 2007 that caused drastic yield losses (Gu et al., 2008). After the end of winter dormancy, wheat yield can be significantly reduced by freezing stress during reproductive growth. Freezing stress at jointing, Zadoks GS 31 to GS 39, can reduce grain producing tillers, while cold stress from early boot stage to flowering, Zadoks GS 45 to GS 58, can reduce the numbers of grains per spike (Christopher et al., 2015). In addition, alternating warm and freezing weather patterns can cause abiotic stress that endangers wheat vegetation, as warm conditions induce rapid plant growth and development (Cannell and Smith 1986). A "false spring" resulting in abnormal, early crop development can cause damage as unexpected low temperatures in a normal developing crop (Marino et al., 2011).

Stress caused by chilling and freezing can affect both plant development and grain yield (Kasuga et al., 1999). When the reproductive stage begins and crop sensitivity to frost is increased, a yield reduction may be attributed to freeze-induced damage to spike development (Kang et al., 2013); impairment of photosynthesis (Thakur et al., 2010); decreases in shoot biomass, flag leaf size, and specific leaf area (Valluru et al., 2012). Chilling, or supercooling can also cause damage. Vegetative material may not show immediate frost damage; however, floret infertility can occur, reducing yield (Fuller et al., 2009). Plants that die as a result of freezing will not produce mature heads. Plants that supercool will produce a head that is partially or nearly completely fertile, depending on the genotype (Livingston et al., 2016). Damage to the plant may also include leaf chlorosis, white awns, and damage to the lower stems (Shroyer et al., 1995). While damage to the peduncle is often the most noticeable form of stem damage (Woodruff et al., 1997), severe damage will result in bending of the stem at the lowest joint, twisting of the stem near the base, or breaking, splitting and rotting of the stem near the base or closest node (Livingston & Swinbank, 1946). Less severe damage may result in chlorotic bands around the stem. This type of damage is typically cosmetic and does not interfere with the plant's ability to take up nutrients from the soil and translocate them to the developing grain head (Shroyer et al., 1995).

While it is true that maturity is related to freeze tolerance, there are also genotype differences (Livingston et al., 2016), position in the field and air temperature also play a role in the extent of freeze injury. As the level of freezing stress varies across a field, studies on cold acclimation and freezing survival are typically conducted under controlled environments. Livingston et al. (2017) noted that experiments conducted under controlled environments should be interpreted with caution and validated by field experiments.

With spring freeze events increasing in frequency due to climate change (Marino et al., 2008), it is crucial to evaluate the severity of a freeze event on wheat varieties and the effects on overall grain quality and yield. The objectives of this study were to: (1) examine the damage resulting from a spring freeze event on 10 commercial wheat varieties, (2) determine the type of damage as well as the location of damage on the plant in individual varieties, and (3) quantify the effect of a spring freeze event on the grain quality and harvest yield for individual varieties.

Materials & Methods

Combine harvested seed experiment

Fifty-seven commercial varieties were planted in a five replicate, randomized complete block design in Perquimans County on October 21st, 2016, Rowan County on October 19th 2016, and Union County on October 26th 2016 (Table 2.01). Plots were 1.5 meters wide and 8.5 meters long, seeded at a rate of 3.705 million seed per ha^{-1} with an Almaco brand cone plot planter (Almaco Company, Boone, Iowa). Each trial had 272.55 L ha^{-1} of 30 percent liquid nitrogen applied with flat fan nozzles pre-plant and 181.7 L ha^{-1} applied with flat fan nozzles at Zadoks growth stage 30. The Union County location had 1.81 tonnes of poultry manure with an analysis of 15.8 kg/ton^{-1} of available nitrogen applied with a manure spreader at burndown in addition to the liquid nitrogen. All sites received herbicide and fungicide as necessary using commercially available pesticides. Locations were harvested with a Wintersteiger Delta small plot combine (Wintersteiger, Salt Lake, Utah). Harvest moisture, test weight, and grain weight were collected per plot using a HarvestMaster Classic (Juniper Systems, Logan, Utah). The fan speed for harvest was 2800rpm, resulting in the potential elimination in the field of the lighter, damaged seed. A 400-500g harvest sample was taken from individual plots at each location for grain quality analysis that included standardized moisture, protein content and falling number. Kernals used to measure protein were dried to 12% moisture and measured using a PerTen® DA 7250 NIR analyzer. Samples were milled using a benchtop cyclone sample Udy® mill and falling number was calculated using a PerTen® Falling Number 1700 (USDA, 2013). Data were analyzed in SAS 9.4 using PROC MIXED at $p<0.05$.

Hand harvested tiller experiment

Five categories of tillers were identified in ten commercial varieties (Table 2.02) in the first replicate of the Rowan and Union County official variety trails. The five tiller categories were: (1) randomly selected primary tillers, (2) randomly selected secondary tillers, (3) healthy tillers: primary or secondary, but displaying no evidence of freeze damage, (4) visually stem-damaged primary tillers and, (5) tillers with a damaged flag leaf: displaying visible leaf damage and the flag leaf was visibly emerged from the boot at the time of the freeze event. Ten tillers in each category were tagged with different colored zip ties one week following the freeze event in each of the ten lines at both locations for a total of 50 tillers per line per location. Tagged tillers were manually harvested on May 23rd 2017 in Union County and May 25th 2017 in Rowan County. Tillers were harvested at maturity and examined for the presence of a grain head and individual kernel number. Tillers with grain heads were individually threshed by hand and kernels were stored by tiller type for analysis.

Results

Grain Yield

Variation in variety trial grain yield between the 57 varieties was significant at all three locations (Table 2.03; Table 2.04; Table 2.05). The mean grain yield across variety types was 6,110kg ha^{-1} (91.2bu/A) at the Perquimans County location. AgriMAXX 473 had the highest grain yield at 7,839kg ha^{-1} (117.5bu/A), similar to one other variety. Progeny P357 had the lowest grain yield at 4,013kg ha^{-1} (59.9bu/A). The mean grain yield across variety types was 5,474kg ha^{-1} (81.7bu/A) at Rowan County. Croplan SRW 9606 had the highest grain yield at 6,707kg ha^{-1} (100.1bu/A), while Seedway SW59SR had the lowest grain yield at 4,301kg ha^{-1} (64.2bu/A). The mean grain yield across variety types at Union County was 4,757kg ha^{-1} (71bu/A) (Table 2.01). Harvey's AP 1882 had the highest grain yield at 7,196kg ha^{-1} (107.4bu/A), and AgriMAXX 464 had a significantly lower grain yield at 2,915kg ha^{-1} (43.5bu/A). There was significance for overall grain yield between locations, with Perquimans County having a 11 percent advantage in overall grain yield in comparison to Rowan County, and a 23 percent advantage in overall grain yield in comparison to Union County.

Grain Quality Analysis

Significant variation was observed by location, variety, and location by variety for falling number (Table 2.06). Out of the 10 varieties selected for analysis, Syngenta SY VIPER in Union County had the highest falling number at 428 in a combined location analysis, and was similar to 8 other varieties. Harvey's AP 1882 in Union County had a significantly lower falling number at 288, and was similar to 6 other varieties. The mean falling number across locations and variety types was 356 (Table 2.07).

There were significant differences in mean falling number between varieties over locations. AgriMAXX 464 in Rowan County had a 391 falling number and was eight percent above the total mean, while AgriMAXX 464 in Union County had a 291 falling number and was 19 percent below the total mean. Nine of out the ten total varieties tested in Rowan County had a higher mean falling number than the total mean, with Pioneer 26R10 having a mean that was 14 percent below the total mean. Union County only had 4 varieties: SY VIPER, SY OAKES, AGS 2024, and DG 9600 above the total mean, with the rest falling from 6 percent to 19 percent below the total mean. Only SY VIPER and AGS 2024, both earlier maturing varieties, did not have significantly different falling numbers by location.

Significant variation was observed for location and variety for protein content; however, location by variety interaction was not significant (Table 2.08). Perquimans County and Rowan County had identical mean protein content values (11.5%) and both had significantly lower protein content than Union County (12.1%) (Table 2.09). AG South Genetics 2024 had the highest protein at 12.28%, similar to three other varieties. Uni-South Genetics 3316 had the lowest protein content at 11.12% with three other varieties having similar significance. The mean protein content for all nine varieties was 11.68 (Table 2.10). There were significant differences found between variety types, however there was no pattern based on maturity. The four varieties with protein content below the mean ranged from early maturing to late maturing lines.

Hand Harvested Tillers

Damage: grain head presence

Results from the analysis for head damage on ten varieties that evaluated location, variety type, damage type, and location by variety showed significant differences between variety by damage type (Table 2.11). Seven out of the ten varieties with healthy tillers were statistically similar for grain head presence by variety, with only AgriMAXX 464, Syngenta SY VIPER, and Syngenta SY OAKES being statistically different for grain head presence. All varieties by damage type with the highest percent grain head presence were represented by a healthy tiller designation. Statistically similar damage types for the lowest percent grain head varied between flag leaf and stem damage (Table 2.12). Whether flag leaf or stem damage resulted in more grain head production depended upon which variety was planted.

There were statistical differences in mean grain head presence between the three damage types. Flag leaf damage had a mean head presence of only 10%. Grain head percent between stem damage was 24%, while healthy tillers had a head percent of 64%. Healthy tillers of AgriMAXX 415 in a combined location analysis had a mean grain head percent of 85%, 63% above the total mean for all varieties by damage type. Flag leaf damage types for both Harvey’s AP 1882 and AgriMAXX 464 was 0% for grain head presence.

Damage: kernel number

Significant differences were observed between variety, damage, and variety by damage for mean kernel number; however, there was no significant difference for location or the interaction of location by damage (Table 2.13). Dyna-Gro 9600 with a healthy tiller designation had the highest mean kernel number at 21.6 in a combined location analysis and it was statistically similar to four other varieties by damage type. Both Harvey's AP 1882 and AgriMAXX 464 with flag leaf tiller damage recorded a 0 mean kernel number and were statistically similar to 15 other varieties by damage type. Healthy tiller designations made up nine out of the top ten mean kernel numbers with Harvey's AP 1882 with stem damage having the only other damage type in that top grouping (Table 2.14).

Mean kernels number were affected greatly by tiller damage type. Dyna-Gro 9600 with a healthy tiller type had a mean of 21.6 kernels per grain head, stem damaged tillers had 3.70 mean kernels per head, and lastly flag leaf damaged tillers for Dyna-Gro 9600 had a mean kernel number of 1.35. Similarly, Harvey's AP 1882 had a mean of 19.3 kernels on healthy tillers, 10.30 kernels on stem damaged tillers, and 0.00 kernels on flag leaf damaged tillers.

Primary/Secondary Tillers: grain head presence

Significant differences were found for grain head presence between variety by tiller category but not for location (Table 2.15). P-values for all sources of variation were highly significant. Uni-South Genetics 3316 on a primary tiller had the highest percentage of mean grain heads with kernels present at 95 percent, and there were six other varieties by tiller type with similar results. Dyna-Gro 9600 on a primary tiller had the lowest percentage of mean grain heads with kernels present at 5 percent, and there were three other varieties by tiller type that had similar results (Table 2.16). Primary tillers across all varieties and locations had a mean of 57 percent for grain head presence, while secondary tillers had a mean of 61.5 percent. Total percent grain head presence with kernels present was 57 percent.

Significant differences in kernel number were found in primary and secondary tillers kernel number, location, variety, location by tiller, variety by tiller, and location by variety by tiller

(Table 2.17). Uni-South Genetics 3316 on a primary tiller in Union County had the highest kernel mean at 34.5, with 10 other varieties by tiller type by location having similar results. Dyna-Gro 9600 on a primary tiller in Rowan County had the lowest mean kernel number at 0.0, with 16 other varieties by tiller type by location having significantly similar results (Table 2.18). Mean kernel numbers were 15.32 for location by variety by tiller type. Primary tiller types (15.89) had a 8 percent higher kernel number than secondary tiller types (14.75), and a 4 percent higher number than the total mean. Union County had the highest kernel number mean at 16.13, 11 percent higher than Rowan County (14.51) and 6 percent higher than the total mean.

There were significant differences in mean kernel number between the tillers selected. Uni-South Genetics 3316 had the two highest kernel numbers overall at 34.5 (Union) and 30.8 (Rowan) on primary tillers, while secondary tillers had kernel numbers of 11 (Union) and 7.2 (Rowan). In contrast, Dyna-Gro 9600 had 26.7 kernels on secondary tillers in Rowan County, 11.1 kernels on secondary tillers in Union County, and 3 (Union) and 0 (Rowan) kernels on primary tiller types.

Discussion

A severe freeze event in the spring of 2017 when the majority of wheat was between Zadoks growth stage 31 and 45 provided an opportunity for a detailed study of the consequences of late season freezing, stress, and subsequent plant and seed development. This has particular relevance to producers making decisions on whether or not to take a crop to harvest following a freeze event. In addition, interruption of high-quality seed supplies can have negative consequences extending over several production seasons for certified seed availability. The objectives of this study were to: (1) examine the damage resulting from a spring freeze event on 10 commercial wheat varieties, (2) determine the type of damage as well as the location of damage in individual varieties, and (3) quantify the effect of a spring freeze event on the grain quality and harvest yield for individual varieties.

We had locations in two counties that experienced a freeze event, Rowan and Union County, which exhibited poorer grain yields from the 57 varieties harvested compared to Perquimans County, which did not experience a freeze event. This suggests that wheat in an advanced growth stage that undergoes a spring freeze event is less likely to have as high of yields as a location with similar growth stages that does not undergo such event (Table 2.01). The mean yield for Perquimans County was 6,110kg ha^{-1} (91.2bu/A), 5,474kg ha^{-1} (81.7bu/A) at Rowan County, while Union County had a mean of 4,757kg ha^{-1} (71bu/A). The mean yield between locations for all 57 varieties evaluated was 5,454kg ha^{-1} (81.4bu/A) (Table 2.01). AgriMAXX 464 had the lowest grain yields at each freeze location with 4,328kg ha^{-1} (64.6bu/A) in Rowan County and 2,914.5kg ha^{-1} (43.5bu/A) in Union County. In Perquimans County AgriMAXX 464 yielded 6,298kg ha^{-1} (94bu/A). This underscores the sensitivity of wheat following vernalization and the influence of freezing temperature, longevity of freezing temperature, and growth stage at the time of the freeze event can have on grain yields at harvest.

The fact that five out the ten highest yielding varieties at the Rowan and Union County locations were the same, suggests that climatic patterns affect varieties in a similar way regarding yield regardless of location. In this study, locations in the Piedmont region of North Carolina that experienced a March 2017 freeze event, while wheat varieties were in an advanced growth stage, had significantly lower yields than a location in the Tidewater region of North Carolina that did not experience a March 2017 freeze event. These results are valuable for grain producers and certified seed producers and suggest that freeze damage to a conventionally managed crop could disrupt seed supplies and filling harvest contracts.

Grain quality analysis for falling number showed significant differences between location, variety type, and location by variety. Syngenta SY VIPER from Union County had the highest falling number at 428.25, 17 percent higher than the total mean. Syngenta SY VIPER in Rowan County had a similar significance to the Union County location with a mean of 374.74, a 5 percent increase from the total mean. AgriMAXX 464 in Rowan County had a mean of 391, a 9 percent increase of the total mean, while AgriMAXX 464 in Union County had a mean of 291.25, a 19 percent reduction from the total mean (Table 2.09). These results indicated that Union County had higher falling number from earlier maturing lines and lower results from later maturing varieties compared to Rowan County where a larger range of maturities yielded similar high falling number.

Two locations, Perquimans County and Rowan County, had significantly poorer protein content than did Union County, indicating that protein content is not necessarily related to falling number, nor grain yields (Table 2.09). AG South 2024, Dyna-Gro 9600, Syngenta SY OAKES, and Harvey's AP 1882 had the highest protein content, were statistically similar and have a wide range of maturities (Table 2.10). The combined location analysis underscores the importance of climatic conditions at the time of grain fill and harvest, and the lesser of importance on climatic events immediately following vernalization.

Three varieties with flag leaf damaged tillers, AgriMAXX 415, Harvey's AP 1882, and AgriMAXX 464 had the lowest percentage of grain heads and the lowest kernel number across all varieties by tiller types, indicating that fewer grain heads and fewer kernel numbers are directly related to flag leaf damaged tillers for these varieties. Conversely, healthy tiller types for Harvey's AP 1882, Uni-South Genetics 3316, AG South Genetics 2024, Dyna-Gro 9600, and Croplan SRW 9415 had the highest grain head presence and mean kernel number with similar significance across all varieties by tiller types (Table 2.12; Table 2.14). This suggests that more grain heads and more kernel numbers per grain head are directly related to healthy tiller types for these selected variety types.

The fact that healthy tillers lead to more grain heads and more kernels, and that flag leaf damaged tillers will produce the fewest grain heads and kernel numbers is a confirmation of past research. However, Croplan SRW 9415 with a stem damaged tiller type, out performed a flag leaf damaged tiller type of the same variety in both grain head presence and kernel number (Table 2.12; Table 2.14). These results suggest that either secondary tiller production off of the primary tiller occurred, or that in some varieties, stem damage may have a larger effect on grain head and kernel production.

Primary tillers from Uni-South Genetics 3316 had the highest percentage of tillers with a grain head with kernels present in a combined location analysis. This variety with primary tillers in both Rowan and Union County also had the highest kernel number mean at 34.5 (Union) and 30.8 (Rowan) (Table 2.16; 2.18). This should be expected that primary tillers would have the largest percentage of tillers with a grain head present as well as the highest mean kernel numbers. However, Dyna-Gro 9600 had more grain heads on secondary tillers at 55 percent, with primary tillers only having 5 percent grain head presence. DG 9600 also reported its largest kernel number from secondary tillers in Rowan County at 26.7, with Union reporting secondary tillers kernel number at 11.1. Primary tillers from both counties had considerably lower kernel numbers at 3 (Union) and 0 (Rowan) (Table 2.16; Table 2.18). These results reiterate the damage that a spring freeze event can do to wheat varieties at an advanced growth stage at the time of the freeze event. Dyna-Gro 9600 primary tillers experienced damaging effects from the March freeze event at both

Rowan and Union County locations, and secondary tillers produced the majority of the grain heads and kernels within those grain heads for that variety.

The spring freeze event of 2017 resulted in loss of yield and grain quality for producers in North Carolina. The opportunity that was provided for a detailed study in the consequences of late season cold stress and subsequent plant and seed development has great relevance to North Carolina wheat producers and Certified Seed growers. The objectives of this study were to: (1) examine the damage resulting from a spring freeze event on 10 commercial wheat varieties, (2) determine the type of damage as well as the location of freeze damage in individual varieties, and (3) quantify the effect of a spring freeze event on the grain quality and harvest yield for individual varieties. Results from this study show that harvest yield was impacted at both freeze locations when compared to a non-freeze location. Perquimans County, which did not experience a freeze event, had higher yield averages per variety than did Rowan and Union Counties. Falling number and protein content was not affected by the freeze event in this study. Grain quality analyses identified that Union County had the highest falling number and protein content numbers out of the three locations studied. Varieties studied at each freeze location showed significant effects of freeze damage to the stem and flag leaf of the plant. Healthy tillers produced more grain heads with kernels present and kernel numbers per tiller than any damage type. Stem and flag leaf damage to grain head numbers and kernel number varied on the variety selected. Primary tillers also produced more grain heads per tiller and a larger number of kernels than secondary tillers in the majority of varieties in this study. Location did not seem to matter in regards to amount of grain heads produced, while kernel number was affected by not only location but variety type and type of tiller (primary or secondary). The results of this study are encouraging to North Carolina wheat producers and suggest that crop yield may suffer freeze damage, however, grain quality in a conventionally managed crop may not necessarily be affected.

Literature Cited:

Al-Issawi, M., H.Z. Rihan, N. El-Sarkassy, and M.P. Fuller. 2013. Frost hardiness expression and characterisation in wheat at ear emergence. J. Agron. Crop Sci. 199:66-74.

Cannell, M.G.R., and R.I. Smith. 2010. Climatic warming, spring budburst and forest damage on trees. J. Appl. Ecol. 23:177-191.

Elhani, S., V. Martos, Y. Rharrabti, C. Royo, and L.F. García del Moral. 2007. Contribution of main stem and tillers to durum wheat (Triticum triduum L. var. durum) grain yield and its components grown in Mediterranean environments. Field Crops Res. 103:25-35.

Fischer, R. 1985b. Number of kernels in wheat crops and the influence of solar radiation and temperature. J. Agri. Sci. 105:447-461.

Frederiks, T.M., J.T. Christopher, S.E.H. Fletcher, and A.K. Borrell. 2011. Post head-emergence frost resistance of barley genotypes in the northern grain region of Australia. Crop Pasture Sci. 62: 736–745.

Frederiks, T.M., J.T. Christopher, M.W. Sutherland, M. W., and A.K. Borrell. 2015. Post-head-emergence frost in wheat and barley: Defining the problem, assessing the damage, and identifying resistance. J. Exp. Bot. 66:3487–3498.

Fuller, M. P., J. Christopher, and T. Fredericks. 2009. Low-temperature damage to wheat in head-matching perceptions with reality. Plant Cold Hardiness: From the Laboratory to the Field. 12–18.

Fuller, M.P., A.M. Fuller, S. Kaniouras, J. Christophers, and T. Fredericks. 2007. The freezing characteristics of wheat at ear emergence. Eur. J. Agron. 26:435–441.

Gonzalez-Navarro, O.E., S. Griffiths, G. Molero, M.P. Reynolds, and G.A. Slafer. 2016. Variation in developmental patterns among elite wheat lines and relationships with yield, yield components and spike fertility. Field Crops Res. 196:294–304.

Gooding M.J., G. Smith, W.P. Davies, and P.S. Kettlewell. 1997. J Agric Sci 128:135–142.

Gu, L., P.J. Hanson, E.L. Post, W. Mac, D.P. Kaiser, B. Yang, B. et al. 2008. The 2007 Eastern US Spring Freeze: Increased cold damage in a warming world? BioScience, 58:253–262.

Hagberg S. 1961. Note on a simplified rapid method for determining alpha-amylase activity. Cereal Chemistry 38:202-203.

Hellevang, K.J. 1995. Grain moisture content effects and management. Fargo, ND: North Dakota State University, Agriculture and University Extension.

Johansson E. 2002. Euphytica 12:6143–6149.

Kang, G., G. Li, W. Yang, Q. Han, H. Ma, Y. Wang et al. 2013. Transcriptional profile of the spring freeze response in the leaves of bread wheat (*Triticum aestivum L.*). Acta Physiol. Plant. 35:575–587

Kasuga, M., Q. Liu, S. Miura, K. Yamaguchi-shinozaki, and K. Shinozaki. 1999. Trancription factor--stress tolarance transformation study. 287–291.

Kettlewell P.S. 1997. Ann. Appl. Biol. 130:569–580.

Li, X., H. Pu, F. Liu, Q. Zhou, J. Cai, T. Dai. 2015. Winter wheat photosynthesis and grain yield responses to spring freeze. Agron. J. 107:1002–1010.

Livingston, D.P., T.D. Tuong, T.G. Isleib, and J.P. Murphy. 2016. Differences between wheat genotypes in damage from freezing temperatures during reproductive growth. Eur. J. Agron. 74:164-172.

Livingston, J.E., and J.C. Swinbank. 1946. Two types of late spring frost injury to winter wheat. J. Soc. Am. Agron.

Mahfoozi, S., A.E. Limin, and D.B. Fowler. 2001b. Influence of vernalization and photoperiod responses on cold hardiness in winter cereals. Crop Sci. 41:1006–1011.

Marino, G.P., D.P.Kaiser, L. Gu, and D.M. Ricciuto. 2011. Reconstruction of false spring occurrences over the southeastern United States, 1901-2007: An increasing risk of spring freeze damage? Environ. Res. Lett. 6:1-8.

McMaster G.S., D.E. Smika. 1988. Estimation and evaluation of winter wheat phenology in the central Great Plains. Agri. Meteorol. 43:1–18.

Oklahoma Cooperative Extension Service. PSS-2149-2.

Rigby, J.R., and A. Porporato. 2008. Spring frost risk in a changing climate. Geophys. Res. Lett. 35:1–5.

Single, W.V. 1985. Frost injury and the physiology of the wheat plant. J. Aust. Inst. Agric. Sci. 51:128-134.

Shroyer, J.P., Mikesell, and G.M. Paulson. 1995. Spring Freeze Injury to Kansas Wheat. Kansas State University Cooperative Extension Service. C-646.

Slafer, G.A., H.M. Rawson. 1994. Sensitivity of wheat phasic development to major environmental factors: a re-Examination of some assumptions made by physiologists and modelers. Funct. Plant Bio. 21:393-426.

Smika, D.E., and B.W. Greb. 1973. Protein content of winter wheat grain as related to soil and climatic factors in the semiarid central great plains. Agron. J. 65:433.

Subedi, K.D., P.J. Gregory, R.J. Summerfield, and M.J. Gooding. 1998. Cold temperatures and boron deficiency caused grain set failure in spring wheat (Triticum aestivum L). Field Crops Res. 57:277-288.

Thakur, P., S. Kumar, J.A. Malik, J.D. Berger, and H. Nayyar. 2010. Cold stress effects on reproductive development in grain crops: An overview. Environ. Exp. Bot. 67:429–443.

Thakur, P., and H. Nayyar. 2013. Facing the cold stress by plants in changing environment: sensing, signaling, and defending mechanisms. In: N. Tuteja and S.S. Gill, editors, Plant acclimation to environmental Stress. Springer Science+Business Media., New York. P. 29-69.

United States Department of Agriculture. 2013. Federal Grain Inspection Service, 2013. Directive 9180.38.

Valluru, R., J. Link, and W. Claupein. 2012. Consequences of early chilling stress in two Triticum species: plastic responses and adaptive significance. Plant Bio. 14:641–651.

Wang, J., E. Pawelzik, J. Weinert, Q. Zhao, and G.A. Wolf. 2008. Factors influencing falling number in winter wheat. Eur. Food Res. Tech. 226:1365–1371.

Woodruff, D., N. Douglas, and V. French. 1997. Frost damage in winter crops, Crop Link. Brisbane: The State of Queensland, Department of Primary Industries.

Table 2.01: Mean grain yields and test weights for variety selections in Perquimans, Rowan, and Union Counties, North Carolina during the 2016-17 season. Yields are reported in bushels per acre. Test weight is reported in pounds per bushel. Mean Julian date is combined locations.

Location:		Perquimans County		Rowan County		Union County	
Variety:	Mean Julian Date	Yield bu/A	Test Weight lbs/A	Yield bu/A	Test Weight lbs/A	Yield bu/A	Test Weight lbs/A
Harvey's AP 1947	100	96.7	55.9	75.7	57.3	61.1	54
Syngenta SY Viper	100	91.9	56.2	91.9	57.5	74.2	54.7
Ag South Genetics 2033	101	88.1	55.6	81	57.3	48.5	52.5
Croplan SRW 8530	101	92.1	53.9	68	52.7	75.5	52.3
NC State NC09-20986	101	82.9	57.1	74.2	59	55.4	55.3
AgriMAXX 463	102	79.7	52.5	75.4	53.7	64.4	51.5
Dyna-Gro 9600	102	97.9	54.1	71.2	52.8	69.6	52.8
Dyna-Gro 9772	102	87.6	53.4	64.8	52.3	50.7	51.9
Featherstone 73	102	89.1	56.5	81.5	58.6	67.7	54
Featherstone VA-258	102	86.7	53.9	78.3	55.9	67	52.4
Hilliard	102	102.7	55.6	89.4	57.4	89.1	54.3
AG South Genetics 2024	103	74.4	55.1	79.4	56.8	65.6	53
Dyna-Gro 9750	103	81.1	52.6	73.4	54	66.7	52.3
MAS #67	103	80.9	52.3	73.1	53.6	56.5	51.8
Progeny Mayhem	103	110	55.7	91.2	56.6	92.5	53.5
Seedway SW49SR	103	83.5	52.5	73.1	53.5	67	51.7
AG South Genetics 2055	104	82.3	54.1	78.7	55.1	63.9	52.5
AgriMAXX 415	104	102	56.6	81.4	56.8	79	54.9
AgriMAXX 464	104	94	53.9	64.6	52.4	43.5	52.4
AgriMAXX 474	104	101.1	54.5	93.3	55.4	82.5	51.8
Croplan SRW 9606	104	105.7	54.7	101.4	56.6	88	53
MAS #6	104	88.7	53.8	77.7	54.5	72.8	52
Pioneer 26R59	104	98.5	54	84.9	56.3	71.4	52.1
Progeny P243	104	89.5	55.2	83.5	56.8	71.4	52.6
Seedway SW59SR	104	89.9	53.6	65.2	52	53.4	51.1
Southern Harvest SH 4300	104	87.9	52.2	91.6	55	83.9	51.7
Southern Harvest SH 7200	104	85	55.6	91.2	58.2	63.9	53.9
Syngenta SY Harrison	104	83.8	52.2	85.6	55.7	59.3	51.6
Syngenta SY Oakes	104	85.6	57.6	82.6	58.4	57.1	54.5
Uni-South Genetics 3197	104	93.9	53.9	66.2	52.3	50.8	51.1
Uni-South Genetics 3228	104	78.3	52.3	74.2	54	62	51.7
AG South Genetics 2038	105	62.2	54.6	70.4	57.2	60.7	52.4
AgriMAXX 444	105	93.7	54.4	91.2	56.6	85.7	54.4
AgriMAXX 473	105	117.6	56.2	88.8	56.5	93.2	53.5
Croplan SRW 8550	105	109.4	55.9	90.9	56.7	97.4	53.9

Table 2.01: Mean grain yields and test weights for variety selections in Perquimans, Rowan, and Union Counties, North Carolina during the 2016-17 season. Yields are reported in bushels per acre. Test weight is reported in pounds per bushel. Mean Julian date is combined locations. (Continued)

Dya-Gro 9701	105	110.5	55.6	91.4	56.6	92.8	54.2
Gerard 557	105	109	57.6	90	59.5	72.8	55.8
LCS Panther	105	88.8	55.6	73.4	55.2	67.4	54.4
MAS #7	105	81.6	54.4	84.5	55.3	86.9	53.8
Pioneer 26R41	105	106.4	55.8	83.8	55.4	73.1	52.3
Progeny BOSS	105	90.1	53.5	77.2	54.2	77.2	52.4
Seedway SW550	105	93.7	54.4	90.1	56.4	76.2	53.1
Uni-South Genetics 3201	105	95.5	56.1	84.1	57.1	82.4	54
Uni-South Genetics 3316	105	73.2	52.3	84.2	56.7	67.5	53.1
Uni-South Genetics 3895	105	100	54.7	90.1	56.6	76.3	52.6
Pioneer 26R36	106	105.6	56.1	93.5	56.7	66.8	53.8
Pioneer 26R53	106	82.9	55.2	76.5	57	52.5	53.1
Pioneer 26R10	106	90.8	53.3	89.9	55.3	64.3	52.4
Progeny P357	106	59.9	50.1	74.8	52.5	59.8	49.8
Seedway SW63SR	106	73.3	52.6	82.7	56.4	62.1	53.2
Southern Harvest SH 4400	106	87.9	55.2	93.1	55.9	78.6	54.8
Croplan SRW 9415	107	90.1	54.7	77.3	55.8	61.2	53.3
Harvey's AP 1882	107	102.2	55.9	86.5	56.4	107.4	56.2
Uni-South Genetics 3404	107	89	54.5	92.4	56.9	81.3	54.2
AgriMAXX 446	108	89.7	54.5	79.4	55.7	67.1	53
Harvey's AP 1871	109	87.5	54.3	80.2	55.8	61.1	53.7
Uni-South Genetics 3536	109	113.8	56.2	94.4	57	99.3	54.5
Mean:	104	91.2	54.6	82	55.8	71	53.1
CV (%)		6.2		4.9		10.9	
avg SEM		2.5		1.8		3.4	
avg LSD (p=0.10)		5.7		3.7		7.2	
df LSD		224		223		169	

Table 2.02: Ten commercial varieties selected from 2016/2017 variety trails in Rowan and Union County, North Carolina for damage type analysis. Growing degree units (GDU) are calculated for 10 varieties. Yields are represented in bu/A for 2016-2018 growing seasons.

		2016			2017			2018		
GDU	Variety	Perquimans bu/A	Rowan bu/A	Union bu/A	Perquimans bu/A	Rowan bu/A	Union bu/A	Perquimans bu/A	Rowan bu/A	Union bu/A
1790	Syngenta SY Viper	49.1	.	58.4	91.9	91.9	74.2	91.6	122.5	120.3
1800	AG South Genetics AGS 2024	42.0	.	57.8	74.4	79.4	65.6	82.7	101.6	102.7
1800	Dyna-Gro 9600	63.6	.	50.8	97.9	71.2	69.6	87.5	108.6	114.0
1850	AgriMAXX 464	.	.	.	94.0	64.6	43.5	.	.	.
1860	Syngenta SY Oakes	48.4	.	44.9	85.6	82.6	57.1	.	.	.
1890	AgriMAXX 415	55.8	.	48.3	102.0	81.4	79.0	81.4	102.5	108.5
1980	Uni-South Genetics USG 3316	40.7	.	35.3	73.2	84.2	67.5	.	.	.
2020	Pioneer 26R10	45.7	.	49.5	90.8	89.9	64.3	88.1	110.1	110.5
2040	Croplan SRW 9415	.	.	.	90.1	77.3	61.2	83.2	108.9	110.9
2120	Harvey's AP 1882	57.8	.	63.4	102.2	86.5	107.4	.	.	.

Varieties without a yield recorded were lost during harvest.

Table 2.03: Analysis of Variance of 2017 variety trial in Perquimans County, North Carolina.

Source	Degrees of Freedom	Type III SS	Mean Square	F Value	Pr > F
Variety	56	40196.75663	717.79923	16.90	<.0001
Block	4	463.81555	115.95389	2.73	0.0300

R-Square: 0.81
Coeff. of Variation: 7.16
Mean Square Error: 6.52
LSD: 1.97

Table 2.04: Analysis of Variance of 2017 variety trial in Rowan County, North Carolina.

Source	Degrees of Freedom	Type III SS	Mean Square	F Value	Pr > F
Variety	56	20275.70074	362.06608	18.50	<.0001
Block	4	3364.93446	841.23362	42.99	<.0001

R-Square: 0.85
Coeff. of Variation: 5.36
Mean Square Error: 4.42
LSD: 1.97

Table 2.05: Analysis of Variance of 2017 variety trial in Union County, North Carolina.

Source	Degrees of Freedom	Type III SS	Mean Square	F Value	Pr > F
Variety	56	38325.06535	684.37617	11.60	<.0001
Block	3	5085.47474	1695.15825	28.72	<.0001

R-Square: 0.81
Coeff. of Variation: 10.79
Mean Square Error: 7.68
LSD: 1.98

Table 2.06: Analysis of Variance of 2017 grain quality-falling number test of ten selected varieties from Rowan and Union County, North Carolina.

Source	Degrees of Freedom	Type III SS	Mean Squares	F Value	Pr > F
Location	1	11905.21	11905.2058	7.94	0.0067
Variety	9	50945.53	5660.61483	3.78	0.001
Location*Variety	9	49074.24	5452.69372	3.64	0.0013

R-Square: 0.61
Coeff of Variation: 10.91
Mean Square Error: 38.72
LSD: 3.79

Table 2.07: 2017 individual location grain quality-falling number test by variety for Rowan and Union County, North Carolina. (α = 0.05) Falling number is represented in seconds.

GDU	Variety	Rowan County FN (seconds)	Union County FN (seconds)
1790	Syngenta SY Viper	374.74	428.25
1800	AG South Genetics AGS 2024	375.75	394.64
1800	Dyna-Gro 9600	375.25	358.25
1850	AgriMAXX 464	391	291.25
1860	Syngenta Oakes	373.25	400.64
1890	AgriMAXX 415	366	303
1980	Uni-South Genetics USG 3316	385	336.75
2020	Pioneer 26R10	308.25	336
2040	CROPLAN SRW 9415	373.5	302
2120	Harvey's AP 1882	367.5	288.75
	Mean:	369.02	343.95

Table 2.08: 2017 Analysis of Variance of 10 varieties for grain quality-protein content for Rowan and Union County, North Carolina.

Source	Degrees of Freedom	Type III SS	Mean Square	F Value	Pr > F
Location	2	5.6009505	2.8004752	9.32	0.0004
Variety	8	9.5344824	1.1918103	3.97	0.0012
Location*Variety	16	6.4879652	0.4054978	1.35	0.2086

R-Square: 0.65

Coeff of Variation: 4.69

Mean Square Error: 0.55

LSD: 0.31

Table 2.09: 2017 combined variety grain quality-protein content test by location for Perquimans, Rowan, and Union County, North Carolina. (α = 0.05)

Location	Mean Protein %
Perquimans County	11.50
Rowan County	11.50
Union County	12.05
Mean:	11.64

Mean Square Error: 0.30

LSD: 0.54

Table 2.10: 2017 Combined location grain quality-protein content test by variety for Perquimans, Rowan, and Union County, North Carolina. (α = 0.05)

GDU	Variety	Protein Content %
1790	Syngenta SY Viper	11.63
1800	AG South Genetics AGS 2024	12.28
1800	Dyna-Gro 9600	12.17
1850	AgriMAXX 464	11.29
1860	Syngenta Oakes	11.82
1890	AgriMAXX 415	11.71
1980	Uni-South Genetics USG 3316	11.12
2020	Pioneer 26R10	11.36
2120	Harvey's AP 1882	11.79
	Mean:	11.69

Table 2.11: 2017 Analysis of Variance of 10 varieties for damage type-grain head presence test for Rowan and Union County, North Carolina. (α = 0.05)

Source	Degrees of Freedom	Type III SS	Mean Square	F Value	Pr > F
Location	1	0.04013826	0.04013826	0.25	0.6174
Variety	9	2.76406733	0.30711859	1.91	0.048
Location*Variety	9	2.92760955	0.32528995	2.02	0.0347
Damage Type	2	30.62055695	15.31027847	95.31	<.0001
Location*Damage Type	2	0.37896497	0.18948248	1.18	0.3082
Variety*Damage Type	18	5.06783713	0.28154651	1.75	0.0279
Location*Variety*Damage Type	18	3.19114394	0.17728577	1.1	0.3444

R-Square: 0.34

Coeff of Variation: 123.32

Mean Square Error: 0.40

LSD: 4.74

Table 2.12: 2017 Combined location damage type-grain head presence test (%) by variety for Rowan and Union County, North Carolina. (α = 0.05)

GDU	Variety	Flag Leaf Damage %	Stem Damage %	Healthy Tiller %
1790	Syngenta SY Viper	3	3	45
1800	AG South Genetics AGS 2024	19	1	7
1800	Dyna-Gro 9600	5	15	65
1850	AgriMAXX 464	0	5	55
1860	Syngenta SY Oakes	2	15	35
1890	AgriMAXX 415	4	25	85
1980	Uni-South Genetics USG 3316	1	3	75
2020	Pioneer P26R10	15	35	65
2040	Croplan SRW 9415	1	1	65
2120	Harvey's AP 1882	0	45	75
	Mean:	11	22	64

Table 2.13: 2017 Analysis of Variance of 10 varieties for damage type-kernel number test for Rowan and Union County, North Carolina. (α = 0.05)

Source	Degree of Freedom	Type III SS	Mean Square	F Value	Pr > F
Location	1	31.63512	31.63512	0.29	0.5896
Variety	9	2121.716	235.7462	2.17	0.0226
Location*Variety	9	1385.672	153.9636	1.42	0.1769
Damage Type	2	21415.43	10707.71	98.62	<.0001
Location*Damage Type	2	57.11685	28.55843	0.26	0.7688
Variety*Damage Type	18	3481.308	193.406	1.78	0.0245
Location*Variety*Damage	18	2820.407	156.6893	1.44	0.1058

R-Square: 0.35
Coeff of Variation: 137.68
Mean Square Error: 10.42
LSD: 4.89

Table 2.14: 2017 Combined location damage type-number of kernels in heads of plants with 3 types of damage test by variety for Rowan and Union County, North Carolina. (α = 0.05)

GDU	Variety	Flag Leaf Damage %	Stem Damage %	Healthy Tiller %
1790	Syngenta SY Viper	3.3	6.4	12.15
1800	AG South Genetics AGS 2024	3.39	4.7	16.5
1800	Dyna-Gro 9600	1.35	3.7	21.6
1850	AgriMAXX 464	0	1.75	9.45
1860	Syngenta SY Oakes	2.7	3.5	9.55
1890	AgriMAXX 415	0.68	4.6	13.75
1980	Uni-South Genetics USG 3316	1.15	8.55	20.45
2020	Pioneer P26R10	2.25	7.7	14.35
2040	Croplan SRW 9415	2	1.25	20.5
2120	Harvey's AP 1882	0	10.3	19.3
	Mean:	1.68	5.25	15.76

Table 2.15: 2017 Analysis of Variance of 10 varieties for primary/secondary tiller-grain head presence test for Rowan and Union County, North Carolina. (α = 0.05)

Source	Degree of Freedom	Type III SS	Mean Square	F Value	Pr > F
Location	1	0	0	0	1
Variety	9	16.34	1.815556	10.02	<.0001
Location*Variety	9	1.6	0.177778	0.98	0.4548
Damage Type	1	0.81	0.81	4.47	0.0351
Location*Damage Type	1	1.69	1.69	9.33	0.0024
Variety*Damage Type	9	8.29	0.921111	5.09	<.0001
Location*Variety*Damage Type	9	4.11	0.456667	2.52	0.0082

R-Square: 0.33

Coeff of Variation: 74.66

Mean Square Error: 0.43

LSD: 4.65

Table 2.16: 2017 Combined location primary/secondary tiller-grain head presence test by variety for Rowan and Union County, North Carolina. (α = 0.05)

GDU	Variety	Primary Tiller (count)	Secondary Tiller (count)
1790	Syngenta SY Viper	25	75
1800	AG South Genetics AGS 2024	25	50
1800	Dyna-Gro 9600	5	55
1850	AgriMAXX 464	45	50
1860	Syngenta SY Oakes	45	55
1890	AgriMAXX 415	90	85
1980	Uni-South Genetics USG 3316	95	45
2020	Pioneer P26R10	65	90
2040	Croplan SRW 9415	40	30
2120	Harvey's AP 1882	90	80
	Mean:	52.5	61.5

Table 2.17: 2017 Analysis of Variance of 10 varieties for primary/secondary tiller-kernel number test for Rowan and Union County, North Carolina. (α = 0.05)

Source	Degree of Freedom	Type III SS	Mean Square	F Value	Pr > F
Location	1	1214.523	1214.523	7.01	0.0084
Variety	9	9165.603	1018.4	5.88	<.0001
Location*Variety	9	1205.303	133.9225	0.77	0.6412
Tiller Type	1	131.1025	131.1025	0.76	0.3848
Location*Tiller Type	1	995.4025	995.4025	5.75	0.017
Variety*Tiller Type	9	13699.32	1522.147	8.79	<.0001
Location*Variety*Tiller Type	9	6224.123	691.5692	3.99	<.0001

R-Square: 0.34

Coeff of Variation: 85.91

Mean Square Error: 13.16

LSD: 4.83

Table 2.18: 2017 Combined location primary and secondary tiller-kernel number test by variety for Rowan and Union County, North Carolina. ($\alpha = 0.05$)

	Location:	Rowan County (count)		Union County (count)	
GDU	Variety:	Primary	Secondary	Primary	Secondary
1790	Syngenta SY Viper	6.6	18.1	3.1	17.5
1800	AG South Genetics AGS 2024	5.9	6.8	5.1	23.4
1800	Dyna-Gro 9600	0	11.1	3	26.7
1850	AgriMAXX 464	7.4	16.5	17.5	10.4
1860	Syngenta SY Oakes	8.3	18	23.7	10.7
1890	AgriMAXX 415	24.8	16.7	30.2	17.4
1980	Uni-South Genetics USG 3316	30.8	11	34.5	7.2
2020	Pioneer P26R10	11	20.9	24.5	13.7
2040	Croplan SRW 9415	1.2	12.5	24.5	3.2
2120	Harvey's AP 1882	29.7	14.2	26	18.9
	Mean:	12.57	14.58	19.21	14.91

Chapter III:

Evaluation of Freeze Damaged Seed in Relation to Non-Freeze Damaged Seed Planted the Following Growing Season for Grain Weight and Quality on Soft Red Winter Wheat

Abstract

Small grains in reproductive growth stages are impacted by spring freeze events in the United States. This study was conducted for certified seed producers to evaluate field germination differences between seed harvested from a crop following a freeze event and seed harvested from a crop that did not experience a freeze event, and to determine if grain yield and end use quality differences exist between these contrasting seed types when used to plant a crop in the following season. The effects of freeze damage on percent seed germination, test weight, and grain quality on soft red winter wheat [*Triticum aestivum* (L.)] were investigated in Rowan and Union County, North Carolina. In 2018 new, certified seed had a higher percent germination (96.9) than seed that had experienced a freeze event the following growing season and was re-planted, 93.6% for Rowan County and 93.5% for Union County. In-field plant counts were similar across all seed types. Seed type did not affect test weight or grain quality across locations. In 2018, test weight, protein content, and falling number were all higher in Union County, NC. Syngenta Viper and Dyna-Gro 9600 had the highest falling number and protein content regardless of location or seed type. Wheat seed that experienced a freeze event the previous growing season was not affected by loss of germination, test weight, or grain quality, which may improve the efficiency of certified seed producers and instill certified seed is available to grain producers in the Southeast.

Introduction

High quality wheat seed is characterized by high germination rates, high test weight, genetic purity and lack of contaminants (Foster et al., 1997). The absence of any of these attributes can cause poor emergence of seedlings and low grain yields. Seed Certification is a quality control process whereby certifying agencies inspect and document that seed produced by specific growers is genetically pure, true to type and has a minimum level of quality. Certified seed is a vital component of US wheat commercial seed supply, and any disruption of the seed supply can have multi-year, negative ripple effects (Copeland and McDonald, 1999).

Freezing temperatures during spring affects phenology and delays grain filling (Subedi et al., 1998). Early flowering varieties are particularly sensitive to freezing temperatures during the reproductive phase of growth. Freeze events result in fully or partially sterilized wheat heads (Chatters and Schlehuber, 1953), and chilling stress, ranging in temperature from -3 to -5°C (Single and Marcellos, 1981), may affect the kernel growth by altering cell division and differentiation and rate and duration of grain filling (Thakur et al., 2010).

Seed harvested following a freeze event may have lower test weight, kernels may be shriveled or discolored, and they may be a mixture of sizes and maturities (Shroyer et al., 1995). Test weight steadily decreases as the level of frost increases (Foster et al., 1997). Kernel weights decrease with severity of injury (Marcellos and Single, 1984), and deficiencies in germination and emergence may result from increased content of small kernels in frost-damaged seed lots (Foster et al., 1997). Frost during grain filling may result in a blistered appearance on the back of the grain (Newton and McCalla, 1935). The shriveled grain, if harvested, can be down-graded at the mill due to excessive screenings (Cromey et al., 1998). Shriveled kernels should not be used as seed because field emergence is poor, even if germination percentage is high (Foster et al., 1997). In addition, shriveled seeds produce less vigorous seedlings and usually yield less grain than seedlings from good quality seed (Klein, 2017). In comparison, non-freeze damaged seed lots have a higher percentage of large kernels and few small kernels, whereas severely damaged seed lots have fewer large kernels and more small kernels (Foster et al., 1997).

The suitability of grain harvested from a freeze-damaged crop for use as seed the following season might be improved by conditioning. Conditioning is a cleaning process which uses air and sieving through a gravity separator (Tibola et al., 2016). Conditioning removes small shriveled, or immature kernels, leaving the largest kernels and increasing the test weight of the lot to acceptable levels (Shroyer et al., 1995). Frost damage is selective, affecting some kernels and leaving others without damage (Al-Issawi et al., 2013). Large kernels from seed lots that were exposed to frost still have high germination, excellent seedling emergence, and good storability (Foster et al., 1997).

Widespread freeze events occurred in North Carolina in 1998, 2001, 2007, and 2017, although the latter was not reflected in a major impact on statewide production (https://www.usclimatedata.com). Freeze events impact certified seed by reducing seed size, seed germination, seed weight, and seedling vigor (Foster et al., 1997). This negatively impacts wheat producers in North Carolina by potentially limiting certified seed quantities available for purchase the following growing season. Certified seed growers need to know how a freeze event will impact the quality of their wheat crop in order to make the best decisions on marketing certified seed to producers the following growing season. The objectives of this study were to: (1) evaluate field germination differences between seed harvested from a crop following a freeze event and seed harvested from a crop that did not experience a freeze event, (2) determine if grain yield and end use quality differences exist between these contrasting seed types when used to plant a crop in the following season.

Materials & Methods

The 2016-17 Season

Combine harvested seed experiment

Twenty-two commercial varieties (Table 3.01) were planted in a five replicate, randomized complete block design on October 19th 2016 in Rowan County and October 26th 2016 in Union County, North Carolina. Certified seed produced in the 2015-16 season was utilized and remnant seed of each variety was stored at 21^{o} Celsius, with 45% humidity and no seed treatment. Plots were 1.5 meters wide and 8.5 meters long, seeded at a rate of 3.71 million seed per ha^{-1} with an Almaco brand cone plot planter. Each trial had 272.6 L ha^{-1} of 30% liquid nitrogen applied with flat fan nozzles pre-plant and 181.7 L ha^{-1} applied with flat fan nozzles at Zadoks growth stage 30. The Union County location had 1.81 tonnes of poultry manure with an analysis of 15.8 kg/ton^{-1} of available nitrogen applied with a manure spreader at burndown in addition to the liquid nitrogen. All sites received herbicide and fungicide as necessary using commercially available pesticides. Each location was monitored for 50 percent heading three days a week beginning in April. Individual plots were assigned a Julian Date when the plot was at 50 percent heading for its entirety. Individual plots were then averaged by variety type and location to generate the mean. Locations were harvested with a Wintersteiger Delta small plot combine (Wintersteiger, Salt Lake, Utah). The fan speed for harvest was 2800rpm, resulting in the potential elimination in the field of the lighter, damaged seed. Harvest moisture, test weight, and grain weight were collected per plot using a HarvestMaster Classic (Juniper Systems, Logan, Utah). A 400-500g grain sample was collected from each plot at each location for further grain quality analysis that included moisture, protein content, and falling number.

The 2017-18 Season

Manually harvested seed experiment

Field evaluation of seed type. Four categories of seed for the 22 varieties were planted in Rowan and Union Counties, North Carolina on November 17th, 2017. A four replicate randomized split-plot design was utilized at each location. Main plots were varieties and split-plots were the four categories of seed: (1) certified seed of each variety produced in the 2016-17 season at a location that did not suffer freeze damage, (2) remnant certified seed produced during the 2015-16 season, a portion of which was utilized to plant the yield trials that suffered freeze damage during the 2016-17 season. This seed was stored at 21° Celsius, with 45% humidity and no seed treatment. (3) Seed harvested by combine from Rowan County that experienced a freeze event during the 2016-17 season, and (4) seed harvested by combine from Union County that experienced a freeze event during the 2016-17 season.

A Hege 90 Head-Row planter was used to plant both locations. Each split-plot was a single row 1.2 meters long with 0.3 meters between rows. The four categories of seed of each variety were planted in four adjacent rows which formed the main plots. Fifty seeds were planted in each row. 272.6L ha^{-1} of 30% liquid nitrogen was applied pre-plant and 181.7 L ha^{-1} applied at Zadoks 30 growth stage with Teejet® StreamJet SJ3 fertilizer nozzles at each location. The Union County location had 1.8 tonnes of poultry manure with an analysis of 20.4 kg/ton^{-1} of available nitrogen applied with a manure spreader at burndown in addition to the liquid nitrogen. Both locations received herbicide and fungicide treatments as necessary using commercially available pesticides.

Stand counts were recorded at both locations 90 days after planting when plants were at the 2 to 3 tiller growth stage. The entire row for each split-plot was counted. Trials were hand harvested at maturity. The entire row for each split-plot was harvested 15 cm below the peduncle and heads were placed into a paper bag for storage in a dryer using ambient air. Three weeks following harvest, each row was threshed using a Vogel Stationary Bundle thresher, and cleaned using an Almaco Air Blast seed cleaner. Plot grain weights were recorded and samples were sent to Kansas State University's Wheat Quality Laboratory for analysis. Grain moisture and protein content were adjusted to 12 percent moisture for all samples and measured using a PerTen® DA 7250 NIR analyzer. Samples were milled using a benchtop cyclone sample Udy® mill and falling number was calculated using a PerTen® Falling Number 1700 (USDA, 2013). Data were analyzed in SAS 9.4 using PROC MIXED at $p<0.05$.

Germination testing of seed utilized in 2017-18 field evaluations.

Percent germination was determined on remnant seed of all four seed categories by variety planted in fall 2017. A four replicate completely randomized design was utilized. An experimental unit was a 100mm x 15mm petri dish. A 38# circular Anchor® brand germination paper was placed on the bottom of a 100mm petri dish and sprayed with water until saturated with a hand spray bottle. Fifty seed were placed onto the germination paper and the petri dish was closed with the lid. Individual petri dishes were labeled with a marker and randomly placed onto standard size 1020 greenhouse flats (Johnny's Select Seeds®, Fairfield, Maine, USA). Greenhouse flats were stacked on top of each other to keep light from reaching the petri dishes and then stored on Johnny's Select Seeds® seedling light carts (Johnny's Select Seeds® Fairfield, Maine, USA). No artificial light was used for this experiment. After 7 days, the percent germinated seed was estimated. Seed was sorted and counted using tweezers to determine total and percentage germination count. Following analysis using a SAS ANOVA, results were tabulated.

Percent seed germination was also counted in-field at each location. The split-plot design of the initial planting was utilized. Percent germinated seed was recorded on February 12th for both Union and Rowan locations by counting germinated plants per row. Plant emergence was determined based on the number of germinated seeds in an entire row, divided by 50, the total seeds planted. Data were analyzed using SAS 9.4 and subject to ANOVA using Fishers protected LSD at α=0.05.

Results

Germination testing of seed utilized in 2017-18 field evaluations.

Results from the 2018 laboratory germination test that evaluated variation in varieties and seed categories showed highly significant differences between varieties, seed categories, and variety by seed category (Table 3.03). AG South Genetics 2033 and NC09-20986 had significantly poorer germination than other varieties for seed harvested from freeze damaged tests. AG South Genetics 2033 had significantly poorer germination for seed harvested at both Union and Rowan counties, whereas NC 09-20986 had significantly poorer germination for seed harvested at Rowan County only. The remaining varieties displayed high germination for seed harvested from Rowan County with a range of 94.5 to 98.5 percent. Similar results were observed for seed harvested from Union County with most varieties ranged between 92.0 and 98.0 percent. Two exceptions at Union County were Featherstone 73 (88.5 percent) and Southern Harvest 7200 (89.5 percent).

There were significant differences in germination between the four seed categories. Remnant, Certified Seed from 2016/2017 had a germination of only 21.7 percent. Germination percentage was uniformly poor between the varieties, with values ranging between 15.5 and 24 percent. Certified Seed produced during the 2017-18 season had a mean germination percent of 96.9 and all but one variety (Southern Harvest 4400) displayed germination in the mid to high ninety percent range. Mean germination of seed harvested at the Rowan and Union County sites were 93.6 and 93.5 percent respectively.

Plant Emergence in 2017-18 field evaluations

No significant differences were found for plant emergence between varieties, categories of seed or the variety by category interaction in field tests in Rowan (Table 3.05) and Union Counties (Table 3.06). The mean plant emergence across varieties was 41 percent at Rowan County and 32 percent at Union County (Table 3.07). The mean plant emergence across seed categories only ranged from 40 to 42 percent at Rowan County and 31 to 33 percent at Union County (Table 3.08). There were significant differences for plant emergence between locations, with Rowan County having a nine percent greater emergence than Union County (Table 3.09).

Grain Yield

Differences between the 22 varieties for grain yield was highly significant at Rowan County, but differences between seed categories and the interaction of varieties and seed categories were not significant at this location (Table 3.10). None of these sources of variation were significant at the Union County location (Table 3.11). At the Rowan County location, NC09-20986 had the highest grain weight at 232.3 grams, similar to 10 other varieties. Southern Harvest 4400 had the lowest grain weight at 162.2 grams, similar to seven other varieties (Table 3.12). Grain yield across seed categories only ranged from 199.5 grams to 202.9 grams at Rowan County. The range at Union County was greater at 266.1 grams to 323.7 grams. There was significance for overall grain yield between locations, with Union County having a 43.7 percent advantage in overall grain yield in comparison to Rowan County (Table 3.13).

Grain Quality Analysis

Significant differences were observed between locations and varieties for falling number, but there were no significant differences between seed categories of their interaction (Table 3.15). Syngenta SY VIPER had the highest falling number at 434.4 in a combined location analysis and it was significantly superior to the other three varieties (Table 3.16). There were no significant differences between seed categories and the range over categories averaged across varieties was only 11 units (Table 3.17). Union County had a significantly higher overall falling number (411.6) compared to Rowan County (388.2) (Table 3.18).

Significant differences were observed between locations and varieties for protein content, however, there was no significance between seed categories (Table 3.19). Dyna-Gro 9600 had the highest protein content at 10.89% in a combined location analysis and it was significantly higher than the three other varieties (Table 3.20). There was no significant difference between seed categories, and the range of differences was only 0.10% between the four variety types (Table 3.21). Union County had a significantly higher overall protein content (10.70) compared to Rowan County (10.12) (Table 3.22).

Discussion

A severe freeze event in the spring of 2017 when the majority of wheat was between Zadoks growth stage 31 and 45 provided an opportunity for a detailed study into the consequences of late season cold stress on subsequent plant and seed development. In addition, it provided an opportunity to study the impact on the following season wheat crop if established with seed harvested from the freeze damaged crop. This has particular relevance to Certified Seed availability, because interruption of high-quality seed supplies can have negative consequences extending over production seasons. The objectives of this study were to: (1) determine if there were field germination differences between seed harvested from a crop following a freeze event and seed harvested from a crop that did not experience a freeze event, and (2) determine if grain yield and end use quality differences existed between these contrasting seed types when used to plant a crop in the following season.

Two early maturing varieties, AG South Genetics 2033 and NC08-20986 exhibited the poorest laboratory germination for seed harvested from the Rowan and Union County locations indicating they were in the most vulnerable growth stage when the freeze event occurred (Table 3.01). However, while AG South Genetics 2033 exhibited poor germination for seed harvested from both locations, NC09-20986 germination was only impacted by the freeze event in Rowan County. This underscored the fickle nature of freeze damage which is influenced by the degree of freezing temperature, the longevity of freezing temperature, and the growth stage of the wheat plants at the time of the freeze event. The mean heading dates for these varieties were 97 Julian Days for AG South Genetics 2033 and 98 Julian Days for NC09-20986. The mean heading date between locations for all 22 varieties evaluated was 102 Julian Days (Table 3.01).

The fact that the laboratory germination of the remaining 20 varieties, within a seed type, were quite similar indicated that seed harvested from a cold damaged crop, in general, can be utilized as a seed source the following season without an impact on germination. If an impact is observed it is likely to be seen in varieties that were in a narrow growth stage when the freeze event occurred. In this study, it appears that conventional combining of the crop led to the elimination in the field of the lighter, damaged seed that might have lowered overall germination. These results are encouraging for Certified Seed producers and suggest that freeze damage to a conventionally managed crop may not necessarily disrupt Certified Seed supplies.

Laboratory germination of the four different seed types showed significant reduction in seed germination for the 2016-2017 Certified Seed category compared to the other three categories (Table 3.14). The mean of 21.7 percent was approximately a 75 percent reduction relative to the other categories. These results indicated that the germination potential of seed that has been stored at 21^{o} Celsius, with 45% humidity and no seed treatment can be considerably reduced relative to the new Certified Seed, or even seed harvested from freeze damaged crops. It appears that the storage of seed more than one growing season from the initial harvest, and in this case Certified Seed, will reduce the germination percentages to a level that is below production standards.

In-field studies of seed emergence across all four seed categories were only 41 percent at Rowan County and 32 percent at Union County (Tables 3.07. 3.08. 3.09). There were notable differences between field emergence and laboratory germination data. Several climatic variables likely influenced the low plant emergence. Extremely dry planting conditions occurred at the time of planting in November 2017 at both locations. In October and November of 2017, only two inches of rainfall fell at both the Rowan and Union County locations (https://www.usclimatedata.com). Rowan County also experienced their first frost on November 11th (https://www.usclimatedata.com). The Rowan County location had tillage applied to the test plot prior to planting, resulting in a clean, debris free planting site, but with drier planting conditions. The Union County location had no tillage applied to the planting site, and there was significant corn residue at the location. Both of the planting site conditions did not favor high germination results. The resulting planting conditions shed some light as to why the Rowan County location had better germination rates in comparison with the Union County germination rates.

Two later maturing varieties, Southern Harvest 4400 and Uni-South Genetics 3201 had two of the lowest harvested grain weights from the Rowan County location, while two earlier maturing lines, NC09-20986 and AG South Genetics 2033, had two of the highest harvested grain weights from Rowan County. This indicates that in-field conditions during grain fill were more detrimental to mean harvested grain weight than new Certified Seed or harvested seed that had experienced a freeze event (Table 3.12). However, harvested grain weight was not significant between the 22 varieties planted in Union County. These results reiterate the importance of proper production practices throughout the growing season, and the reliance that producers have on climatic patterns for yield production. The mean grain weights for NC09-20986 and AGS 2033 were 11 and 14 percent higher than the overall mean of the other 20 varieties. SH 4400 and USG 3201 were 11 and 19 percent lower than the overall mean of the other 20 varieties.

Neither the Rowan nor Union County location allowed for high yielding environments due to climatic patterns (https://www.usclimatedata.com). Harvested grain weights from the Union County location did not show any significance with regard to variety. It appears that the conditions at grain fill for this location were more uniform than at the Rowan County location. However, a combined location analysis was significantly different between locations for grain weight. Mean grain weights for the 22 selected varieties at the Union County location were 31 percent higher than at the Rowan County location (Table 3.13).

Out of the four varieties analyzed for grain quality, the earliest maturing variety, Syngenta SY VIPER, had the largest falling number. The other three varieties, varying across middle and later maturity groups, exhibited poorer falling number qualities (Table 3.16), identifying the importance of nutrient and climatic conditions at the time from grain fill to harvest. The mean falling number for SY VIPER was 434.35 in comparison to 399.49, the overall mean for all 4 varieties. Falling numbers were significant between locations, with Rowan County 6 percent below Union County and 3 percent below the mean of the combined locations (Table 3.18).

These results indicate a difference in climatic and harvest conditions across locations, and less of a result in the quality of seed that was planted at the beginning of the growing season.

Protein content between the four varieties was significant with Dyna-Gro 9600 having the highest content at 10.89. Both Syngenta SY VIPER and Croplan SRW 9415 had the lowest protein content for variety type (Table 3.20). Across the four varieties, there was no correlation between maturities with regard to protein content. As with falling number, and the lack of significance to seed type, climatic conditions and production practices dictate protein content more so than the quality of the seed that was planted. The mean protein content across all varieties for each location yielded significance with Union County having a protein content of 10.70, and Rowan County having a protein content of 10.12. The mean protein content for Rowan County was 6 percent lower than Union County and 3 percent below the mean of the combined locations.

The spring freeze of 2017 resulted in loss of yield and grain quality for producers in North Carolina. The opportunity that it provided to study the impact on the following season wheat crop if established with seed harvested from a freeze damaged crop has great relevance to certified seed producers. This study suggests that seed germination was impacted in a laboratory setting based on seed type. Seed that has been stored for over one season after it was initially harvested proved to have a 75 percent reduction in germination when compared to certified seed that was harvested the previous season. Other results from the laboratory germination test show that varieties outside of the narrow growth stage impacted when a freeze event occurs can be used as a viable seed source the following growing season. In-field germination tests did not show any significant differences between seed types despite being planted across multiple locations. The study concludes that harvested grain weight and grain quality, specifically falling number and protein content, also yielded no significance with regard to seed type. Significance was shown in regards to the location of the trial and the variety type. Results of this study are encouraging for certified seed producers and suggest that freeze damage to a conventionally managed crop may not necessarily disrupt certified seed supplies.

Literature Cited:

Abdul-Baki, A.A., and J.D. Anderson. 1971. Glucose metabolism of embryos and endosperms from deteriorating barley and wheat seeds. Plant Physiol. 48: 270-272

Al-Issawi, M., H.Z. Rihan, N. El-Sarkassy and M.P. Fuller. 2013. Frost hardiness expression and characterization in wheat at ear emergence. J. Agron. Crop Sci. 199:66-74.

Chatters, R.M., and A.M. Schlehuber. 1953. A study of "frost-damaged" wheat in Oklahoma. Agron. J. 45:510.

Ching, T.M., and I. Schoolcraft. 1968. Physiological and chemical differences in aged seeds. Crop Sci. 8:407-409.

Copeland, L.O, and M.B. McDonald. 1999. Seed Certification. In: Principles of Seed Science and Technology. Springer, Boston. pp. 277-295.

Cromey, M.G., D.S.C Wright, and H.J. Boddington. 1998. Effects of frost during grain filling on wheat yield and grain structure. N. Z. J. Crop Hortic. Sci. 26:279-290.

Foster, N.R., L.A. Burchett, and G.M. Paulsen. 1997. Planting wheat seed damaged by sprouting. Kansas State University Cooperative Extension Service. SRL-114.

Klein, R.N. 2017. Freeze injury to Nebraska wheat. University of Nebraska-Lincoln Extension. EC-132.

MacKay, D.B. 1972. The measurement of viability. In: Roberts, E. H., Viability of Seeds. Chapman and Hall, London. pp. 172-208.

Marcellos, H., W.V. Single. 1984. Forst injury in wheat ears after ear emergence. Aust. J. Plant Physiol. 11: 7-15.

Newton, R., A.G. McCalla. 1935. Effect of frost on wheat at progressive stages of maturity. III. Milling and baking quality. Can. J. Res. Sect. C. 13:263-282.

Shroyer, J.P., Mikesell, and G.M. Paulson. 1995. Spring Freeze Injury to Kansas Wheat. Kansas State University Cooperative Extension Service. C-646.

Single, W., H. Marcellos. 1981. Ice formulation and freezing injury in actively growing cereals. In: Olien, C., M. Smith (Eds). Analysis and Improvement of Plant Cold Hardiness. CRC Press. Boca Raton, FL. pp: 17-33.

Subedi, K.D., P.J. Gregory, R.J. Summerfield, and M.J. Goodiing. 1998. Cold temperatures and boron deficiency caused grain set failure in spring wheat *(Triticum aestivum* L.). Field Crops Res. 57:277-288.

Tibola, C.S., J.M.C. Fernandes, and E.M. Guarienti. 2016. Effect of cleaning, sorting and milling processes in wheat mycotoxin content. Food Control. 60:174-179.

Thakur, P., S. Kumar, J.A. Malik, J.D. Berger, and H. Nayyar. 2010. Cold stress effects on reproductive development in grain crops: an overview. Environ. Exp. Bot. 67:429-443.

United States Department of Agriculture. 2013. Federal Grain Inspection Service, 2013. Directive 9180.38.

Table 3.01: Variety selections harvested following freeze events in Union and Rowan Counties during the 2016-17 season. 50% heading date is number of calculated Julian Days.

Variety	Union County Julian Date	Rowan County Julian Date
Ag South Genetics 2033	98	96
AgriMAXX 415	101	101
AgriMAXX 473	100	107
AgriMAXX 474	101	103
CROPLAN SRW 9415	104	109
CROPLAN SRW 9606	102	103
Dyna-Gro 9600	101	102
Dyna-Gro 9701	102	103
Dyna-Gro 9772	97	100
Featherstone 73	100	99
GERARD 557	100	103
Harvey's AP 1947	105	102
NC State University NC 09-20986	97	99
Pioneer 26R10	103	108
Pioneer 26R41	103	109
Southern Harvest 4300	102	100
Southern Harvest 4400	104	104
Southern Harvest 7200	100	105
Syngenta SY Viper	97	101
Uni-South Genetics 3201	103	103
Uni-South Genetics 3404	103	103
Uni-South Genetics 3536	103	104
Mean:	101	103

Table 3.02: Variety selections manually harvested for damage type following freeze events in Union and Rowan Counties during the 2016 – 2017 season.

Variety
AgriMAXX 415
AgriMAXX 464
AG South Genetics 2024
CROPLAN SRW 9415
Dyna-Gro 9600
Harvey's AP 1882
Pioneer P26R10
Syngenta SY Oakes
Syngenta SY Viper
Uni-South Genetics 3316

Table 3.03: Analysis of Variance of 2018 laboratory germination test of four seed categories.

Source	degrees of freedom	Type III SS	Mean Squares	F Value	Pr > F
Variety	21	5266.93	250.81	4.35	<.0001
Seed Category	3	352730.35	117576.78	2041.51	<.0001
Variety*Seed Category	63	6952.56	110.36	1.92	0.0002

Table 3.04: Mean germination percentage of 22 varieties, each represented by four seed categories, evaluated in a laboratory test in 2018.

	Seed Category			
Variety:	Rowan County Harvested Seed	Union County Harvested Seed	2016/2017 Certified Seed	2017/2018 Certified Seed
	Percent Germination			
AG South Genetics 2033	69.0	65.0	21.5	94.0
AgriMAXX 415	96.0	95.0	24.0	96.0
AgriMAXX 473	97.0	94.5	24.0	98.5
AgriMAXX 474	97.5	94.5	19.5	99.0
CROPLAN SRW 9415	98.0	98.0	23.5	98.5
CROPLAN SRW 9606	96.0	98.0	24.0	99.0
Dyna-Gro 9600	96.5	97.0	23.0	98.5
Dyna-Gro 9701	94.5	97.5	23.5	95.5
Dyna-Gro 9772	98.0	94.0	24.0	97.5
Featherstone 73	96.5	88.5	16.5	99.0
GERARD 557	96.0	94.5	24.0	99.5
Harvey's AP 1947	96.0	96.5	19.5	93.5
NC State NC09-20986	65.0	92.0	21.5	97.5
Pioneer 26R10	97.5	96.0	22.5	98.5
Pioneer 26R41	97.5	95.0	24.0	99.5
Southern Harvest 4300	98.5	95.0	24.0	94.5
Southern Harvest 4400	95.0	95.5	19.5	86.5
Southern Harvest 7200	95.5	89.5	23.0	96.0
Syngenta SY Viper	97.0	93.5	24.0	97.5
Uni-South Genetics 3201	94.5	97.5	15.5	97.5
Uni-South Genetics 3404	96.5	97.0	23.0	99.0
Uni-South Genetics 3536	96.0	94.0	22.0	96.0
Mean	93.6	93.5	21.7	96.9

R-Square: 0.96
Coeff. of Variation: 9.93
Mean Square Error: 7.59
LSD: 2.83

Table 3.05: Analysis of Variance of seedling emergence 90 days following planting for 22 varieties each with four categories of seed at Rowan County during the 2017-18 season.

Effect	Numerator degrees of freedom	Denominator degrees of freedom	F Value	Pr > F
Variety	21	63	0.89	0.5999
Seed Category	3	195	0.28	0.8400
Variety*Seed Category	63	195	0.60	0.9906

Table 3.06: Analysis of Variance of seedling emergence 90 days following planting for 22 varieties each with four categories of seed at Union County during the 2017-18 season

Type 3 Tests of Fixed Effects				
Effect	Numerator degrees of freedom	Denominator degrees of freedom	F Value	Pr > F
Variety	21	63	0.81	0.7014
Seed Category	3	198	0.50	0.6806
Variety*Seed Category	63	198	0.57	0.9947

Table 3.07: Mean percent emergence by variety at Rowan and Union Counties, North Carolina evaluated 90 days after planting during the 2017-18 season. No significant difference was found between varieties ($\alpha = 0.05$) at either location or across locations.

Variety:	Rowan County (%)	Union County (%)
AG South Genetics 2033	37	33
AgriMAXX 415	40	41
AgriMAXX 473	41	34
AgriMAXX 474	43	35
CROPLAN SRW 9415	41	29
CROPLAN SRW 9606	48	38
Dyna-Gro 9600	40	30
Dyna-Gro 9701	37	29
Dyna-Gro 9772	38	34
Featherstone 73	40	30
GERARD 557	46	27
Harvey's AP 1947	40	31
NC State NC09-20986	40	31
Pioneer 26R10	39	31
Pioneer 26R41	43	28
Southern Harvest 4300	40	35
Southern Harvest 4400	43	31
Southern Harvest 7200	40	39
Syngenta SY Viper	39	32
Uni-South Genetics 3201	42	28
Uni-South Genetics 3404	43	29
Uni-South Genetics 3536	42	35
Mean:	41	32

Table 3.08: Mean percent emergence by seed category at Rowan and Union Counties, North Carolina evaluated 90 days after planting during the 2017-18 season. No significant differences were found between seed categories ($\alpha = 0.05$) at either location or across locations.

Seed Category:	Rowan County (%)	Union County (%)
2016/2017 Certified Seed	41	33
2017/2018 Certified Seed	42	31
Rowan County Harvested Seed	40	32
Union County Harvested Seed	41	33
Mean:	41	32

Table 3.09: Mean percent emergence combined over seed categories at Rowan and Union Counties, North Carolina evaluated 90 days after planting during the 2017-18 season.

Location:	Emergence (%)
Rowan County	40.9
Union County	32.2
Mean:	36.6
R-Square: 0.68	
Coeff. of Variation: 22.66	
Mean Square Error: 0.01	
LSD: 4.93	

Table 3.10: Analysis of Variance of head row grain weight of 22 varieties each with four categories of seed evaluated in Rowan County during the 2017-18 season.

Type 3 Tests of Fixed Effects				
Effect	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F Value	Pr > F
Variety	21	63	2.66	0.0014
Category of seed	3	187	0.10	0.9582
Variety*Category of seed	63	187	0.78	0.8711

Table 3.11: Analysis of Variance of head row grain weight of 22 varieties each with four categories of seed evaluated at Union County during the 2017-18 season.

Type 3 Tests of Fixed Effects				
Effect	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F Value	Pr > F
Variety	21	59	1.22	0.2709
Category of seed	3	165	1.37	0.2546
Variety*Category of seed	63	165	0.99	0.5149

Table 3.12: Mean head row grain weight of 22 varieties with 4 replications averaged across seed categories evaluated at Rowan and Union Counties, North Carolina during the 2017-18 season.

Variety:	Rowan County (g/split plot)	Union County (g/split plot)
AG South Genetics 2033	224.9	304.0
AgriMAXX 415	195.1	278.2
AgriMAXX 473	193.7	274.9
AgriMAXX 474	212.4	272.1
CROPLAN SRW 9415	186.7	255.6
CROPLAN SRW 9606	220.1	262.5
Dyna-Gro 9600	198.3	271.2
Dyna-Gro 9701	180.2	304.5
Dyna-Gro 9772	206.1	327.3
Featherstone 73	204.6	285.0
GERARD 557	180.9	177.1
Harvey's AP 1947	203.9	207.1
NC State NC09-20986	232.3	262.8
Pioneer 26R10	202.0	313.2
Pioneer 26R41	222.6	292.1
Southern Harvest 4300	173.0	300.2
Southern Harvest 4400	162.6	232.3
Southern Harvest 7200	230.3	328.4
Syngenta SY Viper	213.4	278.6
Uni-South Genetics 3201	179.7	286.1
Uni-South Genetics 3404	206.4	286.6
Uni-South Genetics 3536	189.5	263.21
Mean:	200.8	275.6

R-Square: 0.52
Coeff. of Variation: 59.25
Mean Square Error: 143.88
LSD: 7.48

Table 3.13: Mean head row grain weight by location averaged over 22 varieties evaluated at Rowan and Union Counties, North Carolina during the 2017-18 season. ($\alpha = 0.05$)

Location:	Grain Weight (g)
Rowan County	200.7
Union County	288.4

Mean: 244.55

R-Square: 0.52

Coeff. of Variation: 59.25

Mean Square Error: 29702.41

LSD: 22.11

Table 3.14: Mean head row grain yield by seed category averaged over 22 varieties evaluated at Rowan and Union Counties, North Carolina during the 2017-18 season. No significance was found between seed categories ($\alpha = 0.05$) at either location or across locations.

Seed Type:	Rowan County (g)	Union County (g)
2016/2017 Certified Seed	199.5	266.1
2017/2018 Certified Seed	201.2	323.7
Rowan County Harvested Seed	202.9	282.6
Union County Harvested Seed	199.7	266.3
Mean:	200.8	284.7

Table 3.15: Analysis of Variance of 4 varieties evaluated for grain quality-falling number for Rowan and Union County North Carolina during the 2017-2018 growing season.

Source	degrees of freedom	Type III SS	Mean Squares	F Value	Pr > F
Variety	3	55285.80	18428.6	16.31	<.0001
Location	1	15844.86	15844.86	8.06	0.0296
Seed Type	3	2332.32	777.44	0.79	0.502
Variey*Location	3	5471.55	1823.85	1.61	0.2212

Table 3.16: 2018 combined location head-rows falling number test by variety for Rowan and Union County North Carolina. (α = 0.05)

Variety	Falling Number (Seconds)
Syngenta SY Viper	434.36
Dyna-Gro 9600	398.29
AgriMAXX 415	384.37
CROPLAN SRW 9415	380.94

Mean: 399.49

R-Square: 0.66

Coeff. of Variation: 7.83

Mean Square Error: 31.31

LSD: 15.93

Table 3.17: 2018 combined location head-rows falling number test by seed type for Rowan and Union County North Carolina. No significance was found between seed type (α = 0.05).

Seed Type:	Falling Number (Seconds)
2016/2017 Certified Seed	399.30
2017/2018 Certified Seed	403.81
Rowan County Harvested Seed	402.43
Union County Harvested Seed	392.94

Mean: 399.62

Table 3.18: 2018 head-rows falling number test by location for Rowan and Union County North Carolina. (α = 0.05).

Location	Falling Number (Seconds)
Rowan County	388.19
Union County	411.60

Mean: 399.90

R-Square: 0.66

Coeff. of Variation: 7.83

Mean Square Error: 31.31

LSD: 11.27

Table 3.19: 2018 ANOVA table for protein content percentage analysis performed as a PROC-GLM procedure for Rowan County and Union County North Carolina.

Source	degrees of freedom	Type III SS	Mean Squares	F Value	Pr > F
Variety	3	13.37	4.46	9.86	0.0005
Location	1	11.12	11.12	8.43	0.0272
Seed Type	3	0.36	0.12	0.63	0.5956
Variety*Location	3	1.02	0.34	0.75	0.53

Table 3.20: 2018 combined location head-rows protein content test by variety for Rowan and Union County North Carolina. ($\alpha = 0.05$)

Variety:	Protein Content (%)
Dyna-Gro 9600	10.89
AgriMAXX 415	10.55
Syngenta SY VIPER	10.15
Croplan SRW 9415	10.03

Mean: 10.41
R-Square: 0.80
Coeff. of Variation: 4.19
Mean Square Error: 0.44
LSD: 0.22

Table 3.21: 2018 combined location head-rows protein content test by seed type for Rowan and Union County North Carolina. No significance was found between seed type ($\alpha = 0.05$).

Seed Type:	Protein Content (%)
2016/2017 Certified Seed	10.34
2017/2018 Certified Seed	10.36
Rowan County Harvested Seed	10.47
Union County Harvested Seed	10.44

Mean: 10.40

Table 3.22: 2018 combined location head-rows protein content test by location for Rowan and Union County North Carolina. (α = 0.05).

Location:	Protein Content (%)
Rowan County	10.12
Union County	10.70

Mean: 10.41

R-Square: 0.80

Coeff. of Variation: 4.19

Mean Square Error: 0.44

LSD: 0.157