Evaluation of frost damage in seed corn (Zea mays): with special emphasis on seed composition and moisture content

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Evaluation of frost damage in seed corn (Zea mays L.): with special emphasis on seed composition and moisture content

by

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A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Majors: Crop Production and Physiology

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Chapter 3

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Chapter 1. General Introduction

Introduction

The occurrence of frost during the early fall is a serious problem for seed corn producers in the Midwest. If frost occurs early enough, seed germination and quality can be severely reduced. One reason seed corn producers intentionally harvest seed corn as early as possible is to avoid substantial revenue losses from detrimental seed quality effects of early frost. Even with this careful management practice, frost is still a concern for seed corn producers approximately once every five years (Burris and Knittle, 1985).

Genetics and seed maturity influence tolerance of the seed to frost damage (Rossman, 1949a; DeVries, 2006). It is unknown, however, whether seed composition plays a role in frost tolerance. Understanding the relationship between seed composition and frost damage could improve current harvest management practices by refining the predictions for possible frost susceptibility in seed corn.

Freezing in seed

Freezing is a process by which a solution changes from a liquid state to a solid state. Freezing in all living tissues happens in the same sequential manner (Mazur, 2004). First, the solution within and surrounding the cell supercools. Next, ice nucleation occurs and water in solution forms ice crystals releasing heat. This peak of temperature due to the release of heat is known as the freezing point temperature and depends on the amount of free water available in the seed (Vertucci, 1993), the solute
concentration (Lineberger and Steponkus, 1980), and presence of ice-nucleating agents (Wisniewski et al., 1997).

When the rate of temperature decrease is slow during the frost event, extracellular water freezes first (Mazur, 2004). As these ice crystals begin to expand, intracellular water migrates to the extracellular region of the cell, dehydrating the cell (Mazur, 2004). When this event occurs, damage from the frost mimics dehydration stress. Cells can withstand water loss levels up to 0.25 g H₂O/g dry matter (Bewley and Black, 1994). Beyond this level, bound water from the cell membrane is removed and the liquid crystalline structure of the membrane is compromised and loses its selective permeability.

When the rate of temperature decrease during the frost event is rapid, intracellular water forms ice crystals that can puncture cell walls and membranes and damage organelles (Burke et al., 1976). A punctured membrane loses its ability to regulate the diffusion of molecules, transport electrons, or prevent free radicals from spreading. The loss of seed vigor and viability is greater when the temperature is very low or the decrease is rapid rather than slow (Rossman, 1949a).

Seed damage usually increases with lower freezing temperatures and longer exposure (Black et al., 2006). However, the degree of seed injury incurred during freezing is not exacerbated by rate or length of thawing (Fick, 1989). The severity of frost injury is influenced by several other factors such as topography, species, genotype, and stage of maturity/development (DeVries, 2006; Woltz, 2003). All of these factors listed above may independently or collectively influence frost injury (Woltz, 2003).
Mechanisms to prevent freeze damage

Mature orthodox seeds that have acquired desiccation tolerance have ability to withstand some freezing (DeVries et al., 2006; Woltz et al., 2005). Mechanisms that allow the seed to tolerate desiccation also allow the seed to withstand a frost. Some seeds are not affected by freezing. Oats and barley seeds can survive low temperatures for long periods of time with little effect on seed quality (Brown and Escombe, 1898). In contrast, seed from recalcitrant species do not acquire desiccation tolerance and have very little ability to withstand frost (Black et al., 2006).

Soluble carbohydrates like raffinose and starchyose are produced in orthodox seeds near the end of seed maturation during the phase known as acquisition of desiccation tolerance. These sugars can prevent sucrose crystallization, allowing sucrose to form a glassy state (Chen and Burris, 1990; Hoekstra et al., 1997). Glass is a liquid with such extreme viscosity that it has similar properties of a solid, stabilizing cell membranes during dehydration (Vertucci and Farrant, 1995). This stabilization of cell membranes by glass formation can prevent cell collapse, harmful free radicals from spreading, and further rapid water loss. Orthodox seeds form glasses upon dehydration. Some recalcitrant species also have glassy states; however, the presence of a glassy state does not indicate freezing or desiccation tolerance in recalcitrant species (Pammenter and Berjak, 2000).

Corn husks protect seeds from freezing damage by acting as an insulator. When intact ears are exposed to freezing temperatures, the temperature differential between the air outside the husk and the seed can be as much as 4°C (Rossman 1949b). This additional protection results in higher vigor seeds produced in ears
exposed to freezing temperatures with husks intact, compared to those with husks removed from the ear (Fick, 1989).

Antioxidants such as tocopherol, sucrose, and phytate scavenge for free radicals produced during metabolism (Vertucci and Farrant, 1995). These free radicals can lead to lipid peroxidation and can attack cell DNA, frequently leading to seed deterioration (Bailly, 2004). Antioxidants serve as one mechanism for coping with products of catabolic reactions that occur during dehydration (Vertucci and Farrant, 1995).

During seed maturation and acquisition of desiccation tolerance, lipid bodies migrate to the perimeter of cells (Cordova-Tellez and Burris, 2002a; Perdomo and Burris, 1998). Cordova-Tellez and Burris (2002b) found that this event reduced damage associated with rapid drying. Lipid bodies reduce the surface area of the membrane, which can lose water and protect the plasma membrane by slowing the rate of cell dehydration (Cordova-Tellez and Burris, 2002a). Lipid bodies also act as a reserve of membrane materials that can be used when the cell rehydrates, allowing for expansion of the membrane during rehydration (Cordova-Tellez and Burris, 2002a).

**Freezing injury to seed germination and vigor**

The physiological damage of seed associated with freezing injury can negatively affect seed viability and vigor (DeVries, 2007). However, not all seeds experience the same level of damage when exposed to freezing temperatures. In some cases, freezing injury can be severe and reduce seed germination (Kiesselbach and Ratcliff, 1920) while in others, it reduces seed vigor but not seed germination (DeVries, 2007). Kiesselbach and Ratcliff (1920) showed that a single frost event reduced seed
germination in corn, while repeated frost events increased the extent of seed damage (Rossman, 1949b). But even under the severe conditions of a “killing” frost event, some seeds can survive (DeVries, 2007). Physiologically mature seeds survive frost events better than immature seeds (Woltz et al., 2006; DeVries, 2007). A frost injured seed lot can still produce crop yields comparable to those of healthy seeds provided an acceptable stand is established (Kiesselbach and Ratcliff, 1920).

The severity of a frost event greatly affects seed vigor. A mild frost may not initially reduce germination but instead reduces seed vigor (DeVries et al., 2007). Seed vigor is further reduced with decreasing freezing temperatures, increasing seed moisture content at the time of frost or longer duration of the frost event (Neal, 1961). The extent of seed damage caused by natural frost can be determined by using common seed quality tests. Both the saturated cold test and the soak test can predict seed quality and field emergence following a frost event (DeVries et al., 2007).

**Observing freezing events**

Thermocouples are a common instrument for recording freezing events in seeds (Fick, 1989; Woltz et al., 2005; and DeVries, 2006). Thermocouples simply measure plant tissue temperatures and can be used to determine supercooling and freezing point temperatures. Supercooling is the minimum temperature reached before ice nucleation occurs (Zachariassen, 1985). When ice nucleation occurs, latent heat of fusion is released; this spike in temperature is known as an exothermic peak (DeVries, 2006). The warmest point during this spike in temperature is the freezing point (DeVries, 2006). Thermocouples cannot be used to determine the location of ice nucleation in the seed for two reasons. Ice nucleation is random and its location within
a tissue cannot be predicted (Fick, 1989). The wire of the thermocouple itself cools faster than the surrounding tissue and can act as an ice nucleator (Burke et al., 1976; Fick, 1989).

Despite these limitations, thermocouples have proven useful to study the freezing process in seeds. Corn seeds attached to the ear supercool to temperatures between -4 and -2 °C (Fick, 1989). When temperatures are lower during supercooling, seeds sustain more damage (Fick, 1989; Burke et al., 1976). This finding confirmed Rossman’s (1949a) observation that the temperature at which ice nucleation occurs determines the amount of damage to the seed.

Seed genotype also affects freezing injury susceptibility; and freezing point temperatures are dependent on the seed characteristics rather than the environment (Woltz et al., 2005). Mature and low moisture content seeds have lower freezing points than immature seeds (Fick, 1989). At low seed moisture content, no exotherms are detectable because little water is available to freeze and release measurable heat (Woltz et al., 2005). The same is true for different parts of the seeds. The endosperm generally has lower moisture content and lower supercooling and freezing point temperatures than the embryo (Woltz, 2003).

**Role of seed oil during freezing events**

Corn oil freezes at lower temperatures (-20 °C) than water (0 °C) (Lide, 2008). Seeds with more oil than water could potentially reach much cooler temperatures before ice nucleation occurs because oil would remain somewhat viscous at low temperatures.

The proportion of unsaturated fatty acids in plant and seedling oils is linked to cold tolerance. During periods of cold and freezing temperatures, the desaturase
enzyme is most active (Martz et al., 2006) causing plants in these environments to accumulate higher levels of unsaturated fatty acids (Nishida and Murata, 1996). This metabolic process also occurs during cold acclimation and allows for maintenance of membrane fluidity at freezing temperatures (0°C or less) (Nishida and Murata, 1996; Wallis and Browse, 2002; Martz et al., 2006). Similarly, the environmental temperature during seed maturation controls the ratio and accumulation of unsaturated fatty acids in sunflower seed (Harris et al., 1978) and soybean (Dornbos et al., 1989; Dornbos and Mullen, 1992). Also, progeny from Arabidopsis thaliana plants grown in cold environments have higher levels of unsaturated fatty acids and emerged faster when germinated and grown in cold temperatures (Blodner et al., 2007).

Oils in lipid bodies may also play a role in reducing seed susceptibility to freezing temperatures. When the cooling rate during a frost event is slow, water within the cell migrates to the outside of the cell (Mazur, 2004), thus mimicking dehydration stress. It is well established that during seed maturation, the lipid bodies inside the cell migrate and align on the internal perimeter of the cell protecting it from dehydration damage (Chen and Burris, 1990). It is possible that by this lipid body alignment, the cell also gains some protection against the dehydration produced by frost. Consequently, oily seeds closer to maturity may be able to survive frost better than other seeds (Leprince and Vertucci, 1995).

**Role of seed protein during freezing events**

The role of proteins in preventing freezing in plant tissues is well documented, but little is known about their role in conferring seed frost tolerance. Proteins accumulated in plants during periods of low temperature are different that those
accumulated during normal growing conditions (Marmiroli et al., 1986). In plants an accumulation of specific proteins is correlated with freezing tolerance. Orchardgrass, spinach, and mulberry trees have all been shown to accumulate high molecular weight glycoproteins during cold acclimation (Yoshida et al., 1984; Guy and Haskell, 1989; and Yoshida, 1984). This accumulation of glycoproteins is associated with freezing tolerance. Temperate trees also accumulate soluble protein in bark during cold acclimation to enhance freezing tolerance (Siminovitch and Briggs, 1949). A specific family of proteins in *Arabidopsis* sp., the CFB/DREB1, accumulates during periods of low temperature and regulates the expression of freezing tolerance genes (Liu et al., 1998). This family of proteins protects the plant against freezing stress and other stresses involving dehydration such as salinity stress (Liu et al., 1998).

Dehydrins are a specific subset of late embryogenesis abundant (LEA) proteins. Dehydrins are surfactants capable of preserving structural integrity of cell membranes in seeds. Sugars and LEA proteins combine to form an artificial glass state in membranes, preventing their collapse during dehydration (Walters, 2004). These proteins also protect and stabilize the membranes from dehydration stress and potentially from cold temperature stress during seed maturation (Koag et al., 2003; Beck et al., 2007). Dehydrins specifically accumulate in maize scutellum parenchyma cells (Close, 1997). In seedlings, high levels of dehydrin expression were associated with cold temperature tolerance (Allagulova et al., 2003).

Antifreeze proteins have been associated with freezing tolerance in both plants and fish. In fish, antifreeze proteins bind to ice and interact with membranes to reduce freezing injury (Fletcher et al., 1999). In plants, antifreeze proteins are secreted into the
apoplast of leaves and the crown. These antifreeze proteins reduce freezing injury by slowing growth and recrystallization of ice (Griffith et al., 2005). To date, these antifreeze proteins have not yet been linked to frost tolerance in seeds.

**Thesis Organization**

This thesis presents two manuscripts. Chapter 2 explores how seed oil and protein content affect the outcome of frost early in the fall during seed corn production. This paper is intended to be published in Crop Science. Chapter 3 presents a description of freezing characteristics of maize seeds with different seed oil and protein contents using thermocouple technology. These observational data will be later used to design a statistical experiment to learn more about physiological processes of seed freezing. The references are cited individually for each of the manuscripts and the general introduction.

**Literature Cited**


Chapter 2. Late-season Frost Influences Seed Vigor of Corn (Zea mays L.) Hybrids with Different Seed Oil and Protein Content

A paper to be submitted for publication in Crop Science

ADDITIONAL INDEX WORDS. seed maturity, seed viability, seed storage

ABSTRACT. Seed corn (Zea mays L.) producers in the Midwest region of the United States are affected by an early fall frost approximately every five years. The negative effects of frost on seed quality vary according to genotype and thus possibly seed composition. Our objective was to determine the effect of seed composition (oil and protein) and seed maturity on seed viability and vigor of artificially frost-damaged seed. Hybrid seed was produced by using a B73 inbred derivative with varying seed oil and protein content from the USDA GEM (United States Department of Agriculture Germplasm Enhancement of Maize) project as the female parent and IRF 311 as the male parent. Field plots were planted in a randomized complete-block design with two replications in one and two locations in 2008 and 2009, respectively. Ears were harvested at 300 to 350, 400 to 450, and 500 to 550 g H$_2$O kg$^{-1}$ moisture content. One-half of the harvested ears in each range were placed in an artificial frost cycle that mimicked a severe killing frost, and the remainder of the ears served as controls. Seeds were dried, threshed, and stored for 42 or 84 days before evaluating seed viability using the standard germination test and seed vigor using the saturated cold test and soak test. The artificial frost treatment decreased the percentage of normal...
seedlings in the standard germination, saturated cold, and soak tests except for the high oil content hybrid at 300 to 350 g H$_2$O kg$^{-1}$. Low moisture content seed or mature seeds survived the frost event better than immature seeds, regardless of seed oil and protein contents. These findings suggest that seed producers should harvest less mature genotypes first when faced with the possibility of an early frost. Conversely, more mature and high-oil genotypes could be harvested later as they are unlikely to suffer the detrimental effects from an early frost.

**Introduction**

Early fall frost is a serious concern for seed corn (*Zea mays* L.) producers in the Upper Midwest of the United States. In this region, early fall frosts affect seed production once every four to five years by reducing germination and vigor (Burris and Knittle, 1985). The extent of frost injury is influenced by several factors, such as topography, genotype, and developmental stage of the crop. Seed vigor tests, such as the saturated cold test and the soak test, can be used for evaluating the extent of frost damage (DeVries et al., 2007).

Freezing temperatures during a frost event damages seeds by extracellular and/or intracellular ice formation. If the rate of cooling is slow, extracellular water freezes first (Mazur, 2004). As these ice crystals begin to expand, intracellular water migrates to the extracellular region outside the cell, mimicking dehydration stress (Mazur, 2004). If the cooling rate is rapid, ice crystals form in intracellular water which causes direct damage to cell membranes and organelles (Burke et al., 1976). Loss of
seed vigor and viability is greater when the temperature rapidly decreases rather than slowly decreases (Rossman, 1949).

Plants have adapted mechanisms that provide some frost injury protection to the seeds. Corn husks can reduce the extent of frost injury to corn seed when compared with ears without husks (Fick, 1989; Rossman, 1949). Intracellular water within plant tissues can also supercool, preventing ice formation (Mazur, 1969). Intracellular water in some plant tissues can reach -10 °C before intracellular ice is formed (Mazur, 1969).

Seed oil content may also influence the seeds’ resistance to frost injury. Corn oil freezes at a lower temperature (-20 °C) than water (Lide, 2008). The capacity of oil to remain “somewhat” viscous at low temperatures may prevent intracellular water from freezing. Also, the migration of lipid bodies to the perimeter of the cell supports the plasma membrane during seed maturation (Cordova-Tellez and Burris, 2002), which could possibly mitigate the impact of freezing-induced dehydration stress.

Proteins synthesized when plants are grown under “ideal” conditions are different than those produced when plant tissues are exposed to low-temperatures stress (Marmiroli et al., 1986). However, this change in protein synthesis does not necessarily increase a plant’s ability to tolerate frost (Guy, 1990). Only specific proteins accumulated are correlated to a plant’s ability to withstand frost (Guy, 1989). For example, the accumulation of specific soluble proteins in tree bark during periods of low temperature has been correlated to frost tolerance (Siminovitch and Briggs, 1949).

Antifreeze proteins are also effective against frost damage because they slow growth and recrystalization of ice (Griffith, 2005). Our objective was to determine the influence
of seed composition (oil and protein) and seed maturity on seed viability and vigor of artificially frost-damaged from hybrid seed corn resulting from the cross between five B73-derivative female lines selected for their seed composition and IRF 311 as the common male parent.

Materials and Methods

Seed production

Hybrid seed corn was produced following ICIA certified seed production practices (Iowa Crop Improvement Association, 2007) in isolation blocks at Iowa State University’s Agronomy Research Farm, Ames, IA (U.S. Department of Agriculture, 2008). One location was planted in 2007 and two locations were planted in 2008. Four rows of male parent IRF311 were planted for every six rows of females. Based on prior research (DeVries et al., 2006), five female parents were selected from B73-derivative lines according to seed-composition. The seed composition characteristics of the female were a high oil inbred, low oil inbred, high protein inbred, low protein inbred, and high oil combined with high protein inbred when compared to a standard line. Seeds of the female lines were obtained from the Germplasm Enhancement of Maize Project (GEM) (Pollak, 2002). Male seed was obtained from the Iowa State University Research Foundation, Inc. (ISURF). All plots were planted by using a three-point mounted Almaco twin-row planter (Almaco, Nevada, IA). Plots were planted in a 6:4 pattern and seeded at 61,750 seeds/hectare with a final stand between 49,000 and 54,000 plants/hectare. All six female rows and two male rows were planted on the same day. Additionally, two male rows were planted 10 to 15 days later to ensure a longer
pollination period and blooming synchrony. Planting dates, growing conditions and
harvest periods for each seed production environment are shown in Table 1.

Twenty ears per female plot were harvested by hand at moisture levels of 300 to
350, 400 to 450, and 500 to 550 g H₂O kg⁻¹ fresh weight for all environments. All ears
were harvested with husks and shank intact. Fifty seeds were removed from the center
of three ears for all plots. These seeds were weighed and placed in a drying oven at
100 °C for 48 h. Seeds were weighed again and moisture content was calculated on
fresh weight basis. Ears used to determine moisture content before the frost treatments
were discarded because the husks were no longer intact.

Postharvest handling and storage

After each harvest, ears were prechilled in open plastic tubs at 10 °C for 2 to 4 h
to ensure that ears began the frost cycle at a similar temperature. After the prechill
period, ears from control plots were immediately husked and placed in small-scale
laboratory dryers (Navratil and Burris, 1982) and dried with ambient, forced air for 48 h
followed by heated air (< 35 °C) until the seed reached 120 g H₂O kg⁻¹ fresh weight (fw).
Ears from frost-treatment plots were placed in a Conviron growth chamber (Controlled
Environment Ltd., Winnipeg, MB, Canada) programmed with a 24-h freezing cycle
(DeVries et al., 2007). The cycle was designed to mimic an autumn severe killing frost
in the Midwest. This cycle started at 10 °C and the chamber temperature declined to 0
°C at a rate of -1.4 °C h⁻¹. Ears spent a total of 9 h below 0 °C with 2 h of this time at -5
°C. Afterward, the chamber temperature was raised to 10 °C at a rate of 4.7 °C h⁻¹.
Following the frost cycle, ears were husked and placed in small-scale laboratory dryers.
Ears were dried by following the same procedure described for ears from the control
plots. When seeds reached 120g H$_2$O kg$^{-1}$ fresh weight, ears were removed from the dryers and placed in storage at 10 ℃ and 50% relative humidity until shelling and conditioning. All dried ears were shelled by using a Custom Seed Sheller (Custom Seed Engineering & Equipment, Altoona, IA) and conditioned using a Westrup LA-LS air screen cleaner (Westrup A/S, Slagelse, Denmark). After shelling and conditioning, seeds were stored at 10 ℃ and 50% relative humidity until seed testing. Oil and protein concentration of the seed was determined using a FOSS 1241 NIT wholegrain composition analytical machine (FOSS NIRSystems, Inc, Laurel, MD). Results from seed oil and protein analyses are shown in Table 2.

**Seed testing**

Seed germination and vigor were tested after 42 or 84 d of storage (± 10 days to complete all tests). Standard germination tests were conducted following the Rules for Testing Seeds (Association of Official Seed Analysts, 2004) using crepe cellulose paper as the medium (Kimberly Clark, Neenah, WI) under constant 25 ℃ with 12 h of light daily for a 7 d period. Light photon flux density (400 to 700 nm) inside the empty germination carts placed against the room’s fluorescent light walls was 25 μmol s$^{-1}$ m$^2$. Light measurements were made with a LI-1400 data logger with a LI-190SA spectral response point quantum sensor (LI-COR, Lincoln, NE). One-hundred seeds of each seed lot, two field replications, and treatment were tested. The saturated cold tests were conducted according to Martin et al. (1988) with one exception: 100 seeds were tested for each seed lot. Soak tests were conducted according to Martin et al. (1991) with one modification: after immersing the seeds for 24 h at 20 ℃, seeds were germinated on crepe cellulose covered by ~ 1.5 cm of sand to inhibit fungal growth.
Seedlings from all tests were evaluated according to the Association of Official Seed Analysts (2002).

Field emergence data were obtained from seed lots of all combinations of frost, hybrid, and seed moisture treatments in a randomized complete block with 3 replications. Plantings were made on 18 May 2008 using seed produced in 2007 and seedling emergence was evaluated 14 d after planting. A seedling was considered emerged when the first leaf emerged and was visible from the coleoptile. The planting site was at the Agronomy and Agricultural Engineering Research Center located near Ames, IA. The soil was predominately a Webster loam (fine-loamy, mixed, mesic Typic Haplaquoll) and contained some Nicollet loam (fine-loamy, mixed, mesic Aquic Hapludoll). The site received above average rainfall during the evaluation period and averaged 35.6 cm of rain from May 16 to June 5.

**Statistical design**

This experiment was a split-plot design. Whole plots were a randomized complete block design with a five-by-two factorial treatment structure of female inbreds and frost treatments. Compositon and frost treatment were considered fixed effects. Plots for seed production were planted in three environments, Location 1 in 2007 and location 2 and 3 in 2008. The combination of year and location was treated as a single factor(environment), and was considered fixed effects. Within each environment, 2 replications were used and considered random. Three levels of moisture content were modeled as the split-plot factor. Initial statistical analyses indicated no significant difference for storage duration; therefore, test results were averaged across storage duration. Treatment means were analyzed and corrected for multiple comparisons using
the Tukey-Kramer Adjustment and considered significant at the 0.05 level.

Homoscedacity and normality assumption of the data were assessed by plotting studentized residuals against predicted values, and using a quantile-quantile plot of the residuals.

**Results**

The analysis of the entire data set revealed a significant three-way interaction among hybrid seed composition, moisture, and frost treatments for only the saturated cold test (Table 3). Results of the standard germination test indicated significant two-way interactions between composition by frost and moisture by frost treatments. In the soak test the only significant two-way interactions was for the composition by frost treatments (Table 3). Storage duration and treatment interactions with storage duration were not significant in all three tests (data not shown).

Frost injury lowered standard germination values and the difference in injury level varied by seed composition and seed moisture (Table 4). Greatest frost injury was observed for low oil, high protein, and high oil-high protein seeds with injury levels averaging 27% higher than frost injury levels for high oil and low protein seeds (over all seed moistures). Frost injury levels were higher in seeds with greater moisture content. The average percentage point difference value for injury level in seeds was 54 (averaged across all hybrids) for seeds at 300-350 g H₂O kg⁻¹ but the average percentage point difference values for seeds at 400-450 and 500-550 g H₂O kg⁻¹ were 67 and 66, respectively.
Germination results obtained from the saturated cold test generally followed similar trends to the standard germination test (Table 5). Frost injury difference values were greater for higher moisture seeds and for low oil, high protein, and high oil-high protein seeds. Frost injury was lowest for high oil seeds at 300-350 g H₂O kg⁻¹ averaging only an 8% percentage point difference in injury level compared with non-frosted seeds, which was not significant. All other combinations of seed composition and seed moisture had significant frost injury cold test differences of 24% percentage points or greater.

Results of the soak test indicated a significant composition by frost treatment interaction (Table 3). Seed moisture and treatment interactions with seed moisture were not significant (Table 3). The lack of statistical significance for seed moisture was surprising since frost injury difference values averaged 8-10% points greater for the higher moisture seeds (Table 6). Frost injury was greater for low oil, high protein, and high oil-high protein seeds with injury levels averaging 30 percentage points higher than frost injury levels for high oil and low protein seeds (averaged across all seed moistures). Both the saturated cold and soak tests predicted field emergence for the frost treated seed (R>0.9) (data not shown). Standard germination test did not accurately predict field emergence of frost treated seed with a correlation value of 0.3.

Discussion

Artificial-frost treatment reduced seed viability and vigor in all hybrids. Seed maturity was an important seed quality factor. In addition, the protein and oil content of hybrid seed impacted seed response to frost, and seed composition influenced the
effect of seed maturity on seed damage. Both saturated cold and soak tests equally predicted field emergence of the frost treated seed lots.

Artificial-frost treatment used in our experiments reduced the percentage of normal seedlings for all tests. Both viability and vigor were reduced by frost, which was also observed by Burris and Knittle (1985). Water freezing inside the seed can affect cell integrity in two ways, by the formation of extracellular ice crystals or intracellular ice formation. Injury could occur when extracellular ice crystals form and expand; intracellular water migrates to the extracellular region and freezes, causing cellular dehydration (Mazur, 2004). Rapid dehydration causes membranes to lose their selective permeability and will therefore leak or potentially collapse (Bewely and Black, 1994). If intracellular ice crystals form, the integrity of the membranes is compromised, ultimately causing cellular destruction (Mazur, 2004). Cellular destruction leads to abnormal seedlings and dead seeds, accounting for the reduction in the percentage of germination (DeVries et al., 2007). Also DeVries et al. (2007) found that seeds exposed to frost emerged poorly in field conditions.

Viability and vigor of artificially frost treated seed were affected differently according to seed composition. The high-oil hybrid at 300 to 350 g H₂O kg⁻¹ was not significantly affected by frost when compared to other hybrids at the same moisture content. These results were interesting since high-protein content in corn seed has been associated with high seed germination and vigor, while high-oil content seeds have poor seed quality (Munamava et al., 2004). However, seeds with high-oil had better seed quality than high-protein seeds after exposure to artificial frost. These results could be partially explained by the differential thermal properties of lipids
This author highlights that lipid transitions occur within a wide range of cooling temperatures, and that they often mask the effects of water freezing. Also, the author pointed out that the negative effects of freezing temperatures in seeds represented the interaction between water and all other cellular components, rather than the result of water freezing alone. Thus, cell lipids in high-oil seeds may prevent or reduce ice formation and cell damage.

High oil content in seed may provide some additional protection from frost at physiological maturity, which could be associated with the phenomena of migration of the lipid (oil) bodies towards cell membranes. During maturation, lipid bodies migrate to the periphery of the cell and protect the cell from dehydration damage (Perdomo and Burris, 1998). Rapid dehydration of cells can disrupt lipid migration to the cell membrane (Cordova-Tellez and Burris, 2002). Therefore, rapid cell dehydration originating from freezing could also disrupt this migration. However, lipid migration is completed in all mature seeds but any benefit of high oil seed content in mitigating frost injury would occur if the high oil content seed potentially contained larger lipid bodies or more lipid bodies. Oil has the ability to stay viscous at lower temperatures than water (Lide, 2008). Potentially, seeds with more oil could reach cooler temperatures before ice nucleation occurs.

The low-oil and high-protein hybrids were the most severely affected by the artificial frost treatment. Even though specific proteins are important during cold tolerance in plants (Siminovitch and Briggs, 1949; Griffith, 2005), protein did not protect the seed from frost damage. One possible explanation is that immature seeds were detached from the plants before exposure to the artificial frost treatment, thus having
insufficient time to accumulate antifreeze proteins and to acquire cold tolerance.
Another possibility is that the genetic lines used in this study did not contain specific antifreeze proteins in the seeds that would protect tissues against frost damage.
Further research should be conducted to decipher some of these questions.

Saturated cold and soak test are the best vigor tests available to predict field emergence of frost damaged seed lots. This result confirms previous vigor test and frost damage research from DeVries et al. (2007). The standard germination test is inaccurate for predicting field emergence of frost damaged seed lots (Woltz et al., 2006; and DeVries et al., 2007). To our knowledge, however, these experiments are the first attempt to relate seed composition and tolerance or susceptibility to frost damage and seed quality in corn seed.

Seed corn producers in the Midwest have two concepts to consider when making management decisions in areas prone to early fall frost. Producers should consider seed composition and maturity. Seeds with high oil content and more mature seeds will not be as affected by frost as seeds with high protein content or more immaturity. In case of suspecting seed frost damage, producers should use the saturated cold test and soak test to provide the best assessment of future field performance of the seed.

**Literature Cited**


Table 1. Agroclimatic conditions for the seed production environments near Ames, Iowa in 2007 and 2008.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Planting date of female parent</th>
<th>Growing degree days</th>
<th>Precipitation mm</th>
<th>Stress days</th>
<th>Harvest Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>16 May 2008</td>
<td>1247</td>
<td>696</td>
<td>10</td>
<td>4 Sept -26 Sept 2008</td>
</tr>
</tbody>
</table>

† Data obtained for Iowa Environmental Mesonet, Iowa State Ag Climate Network at the Ames location. Growing degree days base 10°C, stress days have a high temperature >30°C.
<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Oil</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Oil Female</td>
<td>12.5</td>
<td>4.4</td>
<td>68.9</td>
</tr>
<tr>
<td>High Oil Female</td>
<td>12.8</td>
<td>4.8</td>
<td>68.6</td>
</tr>
<tr>
<td>Low Protein Female</td>
<td>12.4</td>
<td>5.2</td>
<td>68.5</td>
</tr>
<tr>
<td>High Protein Female</td>
<td>12.6</td>
<td>5.2</td>
<td>68.2</td>
</tr>
<tr>
<td>High Oil and Protein Female</td>
<td>13.3</td>
<td>5.2</td>
<td>67.8</td>
</tr>
<tr>
<td>Check</td>
<td>10.0</td>
<td>3.5</td>
<td>59.4</td>
</tr>
</tbody>
</table>

**Table 2.** Mean oil and protein composition for each seed corn hybrid produced. Values are averages of 6 replications, two replications in 2007 and four replications in 2008.
**Table 3.** Analysis of variance (P values) for standard germination, saturated cold, and soak test.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Level of Significance</th>
<th>Standard germination</th>
<th>Saturated cold test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>NS</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Storage duration</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composition</td>
<td>**</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Moisture</td>
<td>*</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Composition x Moisture</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frost</td>
<td>***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composition x Frost</td>
<td>***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture x Frost</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composition x Moisture x Frost</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frost</td>
<td>NS</td>
<td></td>
<td>***</td>
</tr>
</tbody>
</table>

**Explanation:**
- **NS** indicates non-significant at the 0.05 level.
- ***** indicates significant at the 0.05 level.
- **** indicates very significant at the 0.01 level.
- ******* indicates extremely significant at the 0.001 level.
<table>
<thead>
<tr>
<th>Soak test</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>**</td>
</tr>
<tr>
<td>Storage duration</td>
<td>NS</td>
</tr>
<tr>
<td>Composition</td>
<td>***</td>
</tr>
<tr>
<td>Moisture</td>
<td>NS</td>
</tr>
<tr>
<td>Composition x Moisture</td>
<td>NS</td>
</tr>
<tr>
<td>Frost</td>
<td>***</td>
</tr>
<tr>
<td>Composition x Frost</td>
<td>***</td>
</tr>
<tr>
<td>Moisture x Frost</td>
<td>NS</td>
</tr>
<tr>
<td>Composition x Moisture x Frost</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant at the .05 probability level.

*Significant at the 0.05 probability level

**Significant at the 0.01 probability level

*** Significant at the 0.001 probability level
Table 4. Percentage of normal seedlings in the standard germination test for control and artificially frost-treated seed lots of five hybrids of B73-derivatives x IRF311 with different contents of seed protein and oil. Seed lots were harvested at three moisture contents and averaged across testing conducted at 42 and 84 d of storage.

<table>
<thead>
<tr>
<th>Moisture content (g H₂O kg⁻¹)</th>
<th>High oil</th>
<th>Low oil</th>
<th>High protein</th>
<th>Low protein</th>
<th>High oil and high protein</th>
<th>Average Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Frost</td>
<td>Difference</td>
<td>Control</td>
<td>Frost</td>
<td>Difference</td>
<td>Control</td>
</tr>
<tr>
<td>300-350</td>
<td>93</td>
<td>49</td>
<td>44</td>
<td>94</td>
<td>31</td>
<td>63</td>
</tr>
<tr>
<td>400-450</td>
<td>93</td>
<td>49</td>
<td>44</td>
<td>94</td>
<td>14</td>
<td>80</td>
</tr>
<tr>
<td>500-550</td>
<td>92</td>
<td>36</td>
<td>57</td>
<td>88</td>
<td>27</td>
<td>60</td>
</tr>
<tr>
<td>X of Diff.‡</td>
<td>48 b</td>
<td>68 ab</td>
<td>68 ab</td>
<td>56 ab</td>
<td>73 a</td>
<td></td>
</tr>
</tbody>
</table>

‡300-350 g H₂O kg⁻¹ fresh weight was considered physiological maturity.

‡Mean of the difference between control and frost treated samples among seed composition levels.

§Mean of the difference between control and frost treated samples within moisture content levels.
**Table 5:** Interaction means percentage of normal seedlings in the saturated cold test for control and artificially frost-treated seed lots of five hybrids of B73-derivatives x IRF311 with different concentration of seed protein and oil. Seed lots were harvested at three moisture contents and averaged across testing conducted at 42 and 84 d of storage.

<table>
<thead>
<tr>
<th>Moisture content (g H₂O kg⁻¹)</th>
<th>Normal Seedlings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>High oil</td>
<td></td>
</tr>
<tr>
<td>High oil and high protein</td>
<td></td>
</tr>
<tr>
<td>300-350⁺</td>
<td>88 abc</td>
</tr>
<tr>
<td>400-450</td>
<td>85 abc</td>
</tr>
<tr>
<td>500-550</td>
<td>76 bc</td>
</tr>
</tbody>
</table>

⁺300-350 g H₂O kg⁻¹ fresh weight was considered physiological maturity.

‡Means of seed lots followed by the same letter are not significantly different at the 0.05 probability level, according to unadjusted t-test of least square means.
Table 6: Percentage of normal seedlings in the soak test for control and artificially frost-treated seed lots of five hybrids of B73-derivatives x IRF311 with different concentrations of seed protein and oil. Seed lots were harvested at three moisture contents and averaged across testing conducted at 42 and 84 d of storage.

<table>
<thead>
<tr>
<th>Moisture content</th>
<th>High oil</th>
<th>Low oil</th>
<th>High protein</th>
<th>Low protein</th>
<th>High oil and high protein</th>
<th>Average Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>g H₂O kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300-350</td>
<td>86</td>
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<td>37</td>
<td>92</td>
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<td>89</td>
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<td></td>
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<td></td>
<td>91</td>
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<td>28</td>
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<td>20</td>
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<td></td>
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<td>91</td>
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<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X$ of Diff. ‡</td>
<td>46 b</td>
<td>73 a</td>
<td>67 ab</td>
<td>59 ab</td>
<td></td>
<td>67 ab</td>
</tr>
</tbody>
</table>

†300-350 g H₂O kg⁻¹ fresh weight was considered physiological maturity.

‡Mean of the difference between control and frost treated samples among seed composition levels.

§Mean of the difference between control and frost treated samples within moisture content levels.
Chapter 3. Exploratory study of freezing characteristics of maize seed with different seed moisture content and composition

A paper to be published in Seed Technology Journal

ADDITIONAL INDEX WORDS: Maize, exotherm, surpercooling, freezing point, frost, thermocouple

ABSTRACT

Seed exotherms, supercooling, and freezing point temperatures help identify seed and physiological processes leading to seed injury during the freezing event. Thermocouples can be used to characterize the physical events in seeds during freezing. The objectives of this study were to utilize thermocouple technology to determine how oil, protein, and moisture content within a seed change freezing patterns. Five hybrids with high oil, low oil, high protein, low protein, and high oil and protein seed compositions were produced in isolation. Ears were harvested at 500 to 550 and 400 to 450 H₂O kg⁻¹ fresh weight with husks and shank intact and placed in a growth chamber programmed with a 24 h artificial frost cycle. The artificial frost cycle mimicked a severe natural frost beginning and ending at 10°C with a total of 9 hours below 0°C and two hours of this time at -5°C. Internal seed temperatures were recorded using a data acquisition device with hypodermic thermocouples inserted into one seed in the center of each ear during the cycle. For all seed compositions, seeds at
500 to 550 H₂O kg⁻¹ fresh weight had larger exotherms and more exotherms during the cycle. The high oil hybrid had fewer exotherms and remained at higher seed temperatures than all other seed compositions. Freezing characteristics were different at each moisture content level. Seed oil and protein contents influenced the internal caryopsis temperature, the supercooling point temperatures, and the exotherm magnitude.

**Introduction**

Freezing of water inside the seed can damage seed tissues and decrease seed quality. The use of thermocouples has allowed monitoring and studying the temperature changes in seeds during a frost event. A better understanding of freezing physiology may soon lead to better methods of preventing freezing damage in seeds.

Thermocouples have been used to detect supercooling and freezing points. Supercooling is the lowest temperature reached before ice nucleation occurs. An exotherm is a peak in temperature due to a release of latent heat of fusion. In this study, a release of heat that measured 0.3°C or greater was considered an exotherm. The highest temperature during this exotherm peak is the freezing point temperature. Evaluation of the temperatures at which water inside the seed supercools, produces an exotherm, and the freezing point can give insight about the physiology of freezing in seeds.

Freezing characteristics of seeds may change according to seed moisture content. Thermodynamic properties of water within the seed change according to the seed total moisture content (Vertucci, 1990). Water in the seed from 0 to 250 g H₂O kg⁻¹
fresh weight is very tightly bound to the molecules within the seed and is unfreezable. This water also has the unique ability to form glass and stabilize membranes (Vertucci and Farrant, 1995). Exotherms are not detectable at this level because such little water is available to freeze (Woltz et al., 2005). At higher seed moisture contents, solute concentration in the water decreases and solute concentration decreases the thermodynamic properties of this water. Thus, water at lower solute concentration becomes more like pure water (Vertucci, 1990).

Freezing characteristics within seeds may also change according to seed composition. Seed oil and protein characteristics within plants can help tissues withstand freezing. Oil freezes at a much lower temperature than water (-20°C) (Lide, 2008). Large amounts of oil within the seed may prevent water within the seed from freezing. Oil also plays a large role in membrane stabilization. During periods of cold temperatures, the desaturase enzyme is most active (Martz et al., 2006). These unsaturated fatty acids allow membranes to maintain fluidity during periods of low temperature (Martz et al., 2006). Specific antifreeze proteins are very important to freezing tolerance in some plant tissues. Antifreeze proteins can react with ice, slowing growth and recrystalization (Griffith et al., 2005). Evidence in the literature suggests that maize seed may differ in frost injury susceptibility due to moisture, protein, and oil contents.

The objectives of this exploratory study were to graph the internal caryopsis temperature data obtained from seed at different moisture contents and with different seed compositions during a freezing event.
Materials and Methods

Seed Production and Harvest

Five B73 derivative female parents were selected based on seed composition. Seed composition characteristics were high oil, low oil, high protein, low protein, and high oil-high protein. Female seed was obtained from the Gemplasm Enhancement of Maize Project (GEM) (Pollak, 2002). A common male parent IRF 311 was obtained from the Iowa State University Research Foundation, Inc. (ISURF). Hybrid seed corn was produced in isolation blocks following Iowa Crop Improvement Seed Certification Standards. Trials were replicated in three locations; one location was planted in 2007 and two locations in 2008 at Iowa State University’s Agronomy Research Farm, Ames, IA. Plots were planted with 4 rows of male for every 6 rows of female using a three-point mounted Almaco twin-row planter (Almaco, Nevada, IA). All female rows and two male rows were planted on the same day. Additionally two male rows were planted 10 to 15 days later to ensure a longer pollination period and niche.

Twenty ears from each plot were harvested at 500 to 550 and 400 to 450 H$_2$O kg$^{-1}$ fresh weight. All ears were harvested with husks and shank intact. Following each harvest all ears were placed at 10°C for 2-4 hours to pre-chill. This pre-chill period ensured that all ears began the frost cycle at similar temperatures.

Moisture content was determined using the oven-drying method. Fifty seeds were shelled from the center of three ears harvested from each plot. Seeds were weighed and placed in a drying oven at 100°C for 48 h. Seeds were weighed again and moisture content was calculated in H$_2$O kg$^{-1}$ on a fresh weight basis. Oil and protein
content of the seed lots (Table 1) was determined by using a FOSS 1241 NIT wholegrain composition analytical machine (FOSS NIRSystems, Inc, Laurel, MD).

**Frost Cycle and Thermocouple Data**

Ears were placed in a Conviron growth chamber (Controlled Environment Ltd., Winnipeg, MB, Canada) programmed with a 24-h freezing cycle (DeVries et al., 2007). The cycle was designed to mimic an autumn killing frost in the Midwest. This cycle started at 10°C and the chamber temperature declined to 0°C at a rate of -1.4°C h⁻¹. Ears spent a total of 9 h below 0°C with 2 h of this time at -5°C. Afterward, the chamber temperature was raised to 10°C at a rate of 4.7 °C h⁻¹.

During the frost cycle 30 gauge Type T (copper-Constantan) thermocouples with hypodermic needle sheaths (Omega Engineering, Inc., Stamford, CT) were used to measure internal caryopsis temperature. These needles were inserted into a caryopsis directly through the husks at the center of the ear. Each thermocouple was connected to a personal data acquisition system (Omega Engineering, Inc., Stamford, CT) recording caryopsis temperature approximately every 1.5 seconds. Temperature and relative humidity in the center of the chamber were recorded using a CS500 temperature and relative humidity probe (Campbell Scientific, Inc., Logan, UT).

For this exploratory study, data were plotted using the open source statistical software R (R Core Development Team, 2005). During the frost cycle the chamber had coil thawing periods to prevent water from freezing in the pipes. Data were graphed in two sections, the first graphed period represents the time interval below 0°C as the chamber cools to -5°C and the second period represents the two-hour time interval beginning when the seed and chamber temperatures had stabilized at -5°C and ending
with the thawing cycle. Data interpretations are drawn from data graphics but data were not statistically analyzed.

Results

The graphed data indicated variation in freezing patterns among seed moisture content, oil, and protein. The internal seed temperature of seeds at 500 to 550 g H$_2$O kg$^{-1}$ fresh weight was warmer than seeds at 400 to 450 g H$_2$O kg$^{-1}$ fresh weight (Figure 1 and 2). This freezing pattern was consistent for both years and both periods graphed for each genotype, thus only graphed data from 2008 is presented in this study. Also, the exotherms in seeds at 500 to 550 g H$_2$O kg$^{-1}$ fresh weight were more frequent and of larger magnitude than those at 400 to 450 g H$_2$O kg$^{-1}$ fresh weight (Figure 1 and 2). For example, data from seed of the low oil hybrid at 500 to 550 g H$_2$O kg$^{-1}$ fresh weight showed three exotherms, whereas the same hybrid at 400 to 450 g H$_2$O kg$^{-1}$ fresh weight showed only one exotherm.

Data graphs of seed composition also indicated variation in seed freezing patterns. The internal seed temperature of the high oil hybrid remained warmer than all other females for both periods graphed, and both years (data not shown). The high oil hybrid internal seed temperature remained 1°C warmer and had fewer exotherms than the low oil hybrid (Figure 3). The exotherms from the high protein hybrid at 500 to 550 g H$_2$O kg$^{-1}$ fresh weight had the largest temperature variation, some as large as 0.5°C (Figure 4). Because this variation is so small, hypodermic thermocouples are important to increase accuracy.
Discussion

Based on the exploratory data-graphs, seed moisture content, oil, and protein influenced internal seed freezing patterns. The freezing characteristics of the seed varied at both moisture content levels. In our experiment seeds at 500 to 550 g H₂O kg⁻¹ fresh weight had a different freezing pattern than those at 400 to 450 g H₂O kg⁻¹ fresh weight. Seeds at 500 to 550 g H₂O kg⁻¹ fresh weight were warmer and showed more exotherms of larger magnitude than seeds at 400 to 450 g H₂O kg⁻¹ fresh weight. These observations agreed with previously published studies. Vertucci et al. (1990) showed that thermal properties of water were different for each of five seed moisture levels. As moisture content decreases, less water is available in seeds to freeze. Water present in seeds at 120 to 250 g H₂O kg⁻¹ fresh weight is unfreezable when water molecules in the cell membranes form a glass (Vertucci, 1990). The freezing of tightly bound water molecules could be undetectable by thermocouples (Wolitz et al., 2005). These authors concluded that at seed moisture contents of 250 g H₂O kg⁻¹ fresh weight, the exothermic peaks were undetectable because there was little water in the seed available to freeze and release a measurable amount of heat when using thermocouples to record temperature changes; findings from DeVries, 2006 corroborate these results.

High oil seeds remained at higher temperatures and had a lower frequency of exotherms, possibly reducing the damaging effects from frost. Researchers reported that lower supercooling and freezing point temperatures resulted in an increase in seed damage (Burke et al., 1976 and Rossman, 1949). In our experiment oily seeds held internal caryopsis temperatures as much as one degree higher than seeds with different seed compositions. Oil could potentially maintain the seed at warmer temperatures for
two reasons; it takes lower temperatures to freeze corn oil (-20°C) than water (0°C) (Lide, 2008); and oil is located mainly in the embryo, thus maintaining warmer temperatures preventing exotherm formations. Cell membranes are also primarily made of fatty acids (oils). During periods of cold temperatures, these fatty acids become unsaturated, allowing membranes to maintain fluidity during freezing (Martz, 2006).

Seeds with high protein content on the other hand, had a greater number of exotherms and reached lower internal seed temperatures. An increase in the number of times and places where water froze within the seed could potentially lead to more seed damage. If water outside the cell froze, water within the cell would migrate outside and cause dehydration stress in the seed (Mazur, 2004). If water froze inside the cell, the ice crystals formed could puncture cell membranes and completely destroy cells (Burke et al., 1976). Seeds that experience more exotherms have a greater probability of having fatal damages. In plants, specific antifreeze proteins play a role in freezing prevention and cold tolerance. Antifreeze proteins have been found to slow growth and recrystallization of ice (Griffith et al., 2005). Seeds may not contain these specific antifreeze proteins, or the ability to produce them. Future research should consider increasing these plant antifreeze proteins in seeds as a means for increasing tolerance to frost.

Thermocouple technology is effective in determining variation in freezing characteristics of maize seeds differing in moisture content and seed composition. Higher moisture seeds exhibited more exotherms of larger magnitude than lower moisture seeds. High oil seeds maintained warmer temperatures than the other seed compositions. These observational data will be used in designing future experiments
using a statistical model to identify supercooling amount, time between exotherms, and other freezing patterns in hybrids with different protein and oil contents in seeds.

**Literature Cited**


Table 1. Final oil and protein composition for each seed corn hybrid produced.†

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Oil</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Oil Female</td>
<td>12.5</td>
<td>4.4</td>
<td>68.9</td>
</tr>
<tr>
<td>High Oil Female</td>
<td>12.8</td>
<td>4.8</td>
<td>68.6</td>
</tr>
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<td>Low Protein Female</td>
<td>12.4</td>
<td>5.2</td>
<td>68.5</td>
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</tr>
<tr>
<td>Check</td>
<td>10.0</td>
<td>3.5</td>
<td>59.4</td>
</tr>
</tbody>
</table>

† Values expressed in averages.
**Figure 1.** Internal caryopsis temperature of high protein hybrid at both 500 to 550 g H$_2$O kg$^{-1}$ fresh weight (50%) and 400 to 450 g H$_2$O kg$^{-1}$ fresh weight (40%) during the 2 h period when chamber temperature was -5°C. Graph represents data from 2008.
Figure 2. Internal caryopsis temperature of low oil hybrid at both 500 to 550 g H₂O kg⁻¹ fresh weight (50%) and 400 to 450 g H₂O kg⁻¹ fresh weight (40%) during the 2 h period when chamber temperature was -5°C. Graph represents data from 2008.
**Figure 3.** Internal caryopsis temperature of high oil (HO) and low oil (LO) hybrids at 500 to 550 g H₂O kg⁻¹ fresh weight (50%) during the 2 h period when chamber temperature was -5°C. Graph represents data from 2008.
Figure 4. Internal caryopsis temperature of high protein (HP) and low protein (LP) hybrids at 500 to 550 g H$_2$O kg$^{-1}$ fresh weight (50%) during the 2 h period when chamber temperature was -5°C.
Acknowledgements

I would like to take this opportunity to sincerely thank those who helped me accomplish this master’s degree project, without whom this large task would have been insurmountable.

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