# Dormancy regulation in conventional and oleic sunflower lines (*Helianthus annuus L.*)

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

Major: Crop Production and Physiology

Program of Study Committee:
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Iowa State University

Ames, Iowa

2005

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Iowa State University

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# INTRODUCTION

Sunflower (*Helianthus annuus* L.), a native to North America, is currently the second most important source of vegetable oil in the world (National Sunflower Association, 2000, www.sunflowernsa.com/stats). Over 1.8 million acres of sunflowers were planted in the United States in 2004. The effect of dormancy in sunflower hybrid seed production normally does not pose an economic risk for in-season domestic production. This is due to the amount of time that elapses between harvest and shipment to the customer. Seed dormancy in sunflowers does pose a risk for off-season seed production in South America. Seed companies must hold the seed lots from shipment until they are assured that they will meet minimum germination specifications and will perform for the customer. If the seed maintains its dormant state for an extended period of time it can affect product shipment and field performance.

This investigation was undertook to examine the differences in dormancy between SF270, a traditional oil sunflower hybrid, and X8927 (SF287) a mid oleic sunflower hybrid. Past experience with these two hybrids indicated differences in dormancy.

The objectives of this research are:

a) Investigate potential differences in dormancy due to production year and genotype.
b) Evaluate the effects of storage temperature on the duration of dormancy of these genotypes.
c) Evaluate the ability of available germination tests to predict the quality of seed lots.

## LITERATURE REVIEW

In the wild, the ability for seeds to delay germination until an appropriate set of conditions is met is an important survival mechanism. Seed dormancy has been defined (Hilhorst, 1995), "as the absence of germination in a mature intact seed under conditions of light, temperature and oxygen which would normally favor germination within a specific time period."

Seeds are formed on the mother plant, and depending upon environmental factors, genotype, or a combination of effects, seeds will either be dormant or non-dormant at the time of seed dispersal (Figure 1). These seeds are subject to several possible outcomes. The non-dormant seeds will either germinate, and produce a seedling or they could encounter some environmental factor that induces a state of secondary dormancy. Dormant seeds will either continue an after-ripening process, moving to a non-dormant state or can be induced into a secondary dormancy at any point after physiological maturity.

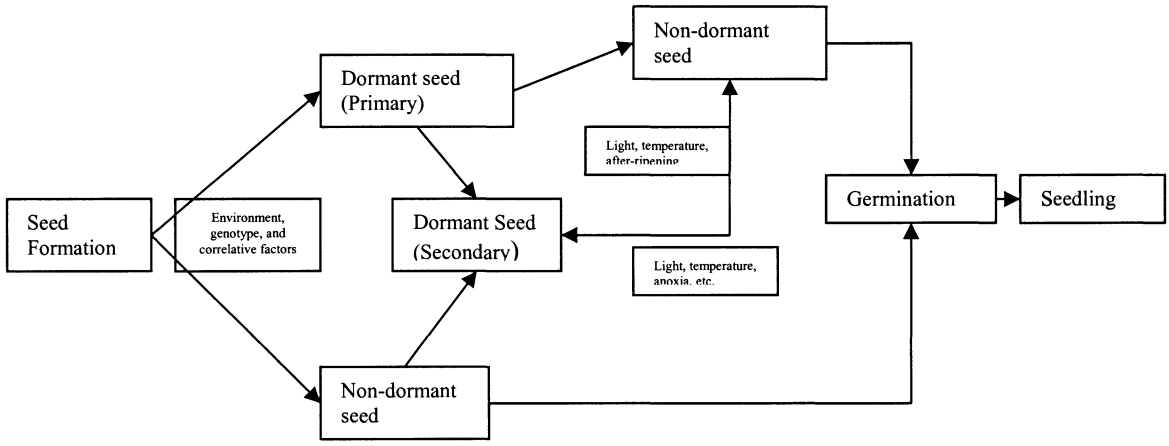



Figure 1. Dormancy and germination. Adapted from Bewely and Black (1995).

**Dormancy categories**

Bewely and Black (1994) describe several categories of dormancy: seed coat imposed or seed coat enhanced dormancy, embryo dormancy, relative dormancy, primary dormancy, secondary dormancy, and epicotyl dormancy and double dormancy. An alternate opinion of dormancy describes primary and secondary dormancy as the two categories of dormancy (McDonald and Copeland, 1995). Under this definition primary dormancy has two forms, exogenous and endogenous dormancy, each with several underlying categories of dormancy.

Seed coat imposed or seed coat enhanced dormancy is exhibited in species where the embryo (if isolated) is able to germinate but, the seed coat (tissue enclosing the embryo such as the endosperm, pericarp, testa, lemma, etc) prevents germination. According to their definition, exogenous dormancy is influenced by the physical properties of the seed coat that may prevent the seed from germinating. These physical properties can include blocking water uptake, gas exchange, and mechanical restriction. Seed coat imposed/enhanced dormancy is described by as the seed coat's physical ability to prevent germination inhibitors from exiting the embryo. Species such as *Hordeum spp.* (palea, pericarp), *Betula pubescens* (pericarp), *Phaseolus lunatus* (testa), and *Lactua sativa* (endosperm) are examples of species that exhibit seed coat imposed dormancy.

Embryo dormancy is defined as the situation where, regardless of the removal of the seed coat, the embryo will germinate. This type of dormancy is more profound than in species with seed coat imposed dormancy. Embryo dormancy often simultaneously or successively occurs with coat imposed dormancy in certain species. Examples of species exhibiting are *Hordeum spp.*, *Acer saccharum*, *Prunus persica* (Bewely and Black, 1994).

Relative dormancy describes the situation where the expression of dormancy in certain species only expresses above a certain temperature value. This has been demonstrated in certain cereals and grasses (*Hordeum spp., Avena spp., Triticum aestivum* L.*)* (Bewely and Black, 1994).

Primary dormancy is the result of natural seed development and maturation and is present at seed dispersal (Bewely and Black 1994; McDonald and Copeland, 1994; Hilhorst, 1995). Dormancy is imparted on the seed during formation. Seed germination of isolated embryos may be possible at the beginning of the seed formation period but, by the time they reach physiological maturity and/or seed dispersal, they have become dormant.

McDonald and Copeland (1994) define endogenous dormancy as a form of primary dormancy where the inherent properties of the seed prevent the seed from germinating. These properties can be rudimentary embryo dormancy and/or physiological dormancy. Rudimentary embryo dormancy occurs when seeds of certain species are shed before reaching morphological maturity. Embryos require further maturation before dormancy can be broken following dispersal, which can take anywhere from days to months depending upon the species.

Physiological dormancy is caused by an imbalance between growth inhibitors and growth promoters. This can be desirable in seeds to the extent that germination is prevented during seed development and maturation. The level of dormancy may result from the presence, absence or a combination of both inhibitors and promoters. Plant hormones such as gibberellins and cytokinins are known growth promoters in seed germination. Abscisic acid (ABA) is an inhibitor of germination and appears early in seed development. The levels of these compounds are controlled by time, temperature, environmental factors, and in certain species, light. It has been identified in different species that endogenous ABA levels decrease after a stratification period, therefore allowing the seeds to become receptive to growth promoters (McDonald and Copeland, 1994).

Secondary dormancy is the result of unfavorable environmental effect(s), which induces non-dormant seeds to become dormant and can extend or maintain or deepen the depth of dormancy in dormant seeds (Bewely and Black, 1994; McDonald and Copeland, 1994; Hilhorst, 1995). Temperature, light, and darkness are the most common factors that induce secondary dormancy in many species (McDonald and Copeland, 1994).

Epicotyl dormancy describes the situation where the epicotyl fails to grow even when the radical has emerged. This type of dormancy can be found in *Paeonia* and *Liluim spp.* (Bewely and Black, 1994).

Double dormancy has been exhibited in *Trillium spp* and *Caulophyllum thalictroides* where both the radical and epicotyl have differing levels of dormancy and require different degrees of temperature treatment to allow seeds to germinate (Bewely and Black, 1994).

Sunflower seeds dormancy at the time of harvest is a result of embryo dormancy. Sunflower seed, when seeds are exposed to temperatures higher than 20°C exhibit seed coat imposed dormancy (Corbineau, 1990).

**Sunflower Dormancy Research**

Sunflower seed reach physiological maturity about 48 days after anthesis (Kole, 1982). Seed dormancy of three traditional oil sunflower hybrid, were studied over a three year period in Romania; the reported results averaged the means of each hybrid over a three year period. One hybrid was reported to maintain dormancy for a 12-15 d period versus 37-40 d period for the other hybrids after harvest. This study also reported that physiological maturity and harvest moistures, showed an increase in the occurrence and duration of dormancy. Seeds were harvested with a moisture content of 41-62% maintained their dormant state for 42-51 d period. When seeds were allowed to reach physiological maturity and 8 and 11% moisture before harvest, the duration of dormancy was reduced to a period of 22-29 d. There was no artificial drying treatment performed on these lines and the seeds were tested immediately after they were harvested (Cseresnyes, 1979). Singh (1990) indicated that sunflower seed position in the capitulum had significantly influenced many seed quality components. Seed viability, germination, and vigor increased from the inner to outer florets. Seeds harvested between 45 and 50 d after anthesis showed higher viability and germination than seed from earlier harvests. Seeds harvested at 15 and 40 d after anthesis had a ranged from 31% to 52% non-dormant seeds respectively. Dormant seed percentages

in seeds harvested between 45 and 50 d after anthesis were 17 and 13%. Delaying the harvest date increased the laboratory germination and decreased dormant seed levels. In sunflower selections in India, an after-ripening period of 11 days was observed followed by a seed-coat imposed dormancy of 45 d (Udaya Kumar, 1975).

Environmental factors on the parent plant play an important role in defining the corresponding ability of seeds to germinate (Fenner, 1991). Certain species exhibit heteroblasty where the level of seed dormancy is related to the position on the mother plant or within the fruit. Heteroblasty can manifest in various ways for different species. In *Chenopodium album*, four seed types (color, size, and seed coat thickness) can be found on the same mother plant (Bewely and Black, 1994). The four types; black seeds with a smooth reticulate, black seeds with a rough reticulate, brown seeds with a smooth reticulate, and brown seeds with a rough reticulate. The black seeds exhibited the deepest dormancy (Bewely and Black 1994). Temperature (level, stress and timing) effects during seed maturation were positively correlated with germinability in several species (Fenner, 1991). Light can affect the mother plant in a variety of ways; in some species dormancy has increased with day length. In *Chenopodium album*, thicker, harder seed coat thickness has been linked to longer day lengths (Fenner, 1991). Drought can effect seed development in many ways. In soybean it has been observed that smaller seed size, thicker seed coats, and a larger percentage of hard seed resulted from drought stress (Fenner, 1991). In other species drought can affect the level of hormones that help maintain dormancy and the embryo sensitivity to these hormones (Fenner, 1991). Nutrient deficiency can affect the level of hormones during seed development such as ABA in certain species (Fenner, 1991). Environmental factors normally do not act independently of one another. Drought can reduce nutrient uptake in plants; and, higher temperatures, usually accompanied by high light levels, can have additive or opposite effects on seed germinability. These environmental interactions when brought together with seed production practices, plant population and

competition make it difficult to predict how the duration and depth of seed dormancy will be effected (Fenner, 1991).

In France, sunflower seeds harvested at physiological maturity and tested immediately with out drying, exhibited poor germination at an optimum germination temperature of 25°C. Exposure to germination temperatures between 25 and 40°C showed an inability to germinate due to temperature induced dormancy. Seeds from the same experiment were exposed to temperatures lower than 25°C, and showed poor germination which was associated with embryo dormancy (Corbineau, 1990).

The germination rate of sunflower seed has varied due to seed characteristics such as oil content, date after harvest, and moisture content (Cseresnyes, 1979). Cseresnyes has demonstrated several temperatures for optimal sunflower seed germination; constant 25°C, constant 30°C, and alternating 20-30°C on rolled towels (1979). Seed storage at both 3°C and 10°C inhibited seed germination and dormancy breaking (Cseresnyes, 1979).

During seed development and maturation, the depth of sunflower embryo dormancy increased from low levels in immature embryos (30% dormancy), to a peak of 100% dormancy 22 d later, dormancy levels then began to decrease as seeds were allowed to continue to desiccate on the mother plant (Corbineau, 1988 &1990). Abscisic acid (ABA) has been shown to induce embryo dormancy during the seed maturation period (Le Page-Degivry, 1989). ABA levels, as determined by radioimmunoassay, increased sharply during the first half of the seed development period, then dropped when embryo dormancy was established. However, when ABA synthesis was inhibited by fluridone, embryo dormancy did not develop (Le Page-Degivry, 1989 &1991). Bianco and Le Page-Degivry (1994) measured the involvement of GA3 and ABA on embryo development. This work indicated that the physiological state of the sunflower seed embryo correlated with the changes in sensitivity to hormones. Exposing sunflower embryos harvested 19 d after anthesis to $GA_3$ growth media showed little or no effect on breaking dormancy. After a 3 d drying treatment, the sunflower embryos exposed to the $GA_3$ broke dormancy (Bianco, 1994). This is similar

to what has been reported in *Arabidopsis*, where the responsiveness to GA decreased during the onset of dormancy and increased as dormancy was released (Derkx, 1993). On the mother plant, ABA levels were at a high at the onset to dormancy and GA levels were suppressed. As seeds went through an after-ripening period, the ABA levels declined, embryo sensitivity of GA hormone receptors is believed to have increased. In *Arabidopsis* wild type and GA deficient mutants, it was demonstrated that the release from dormancy was not dependant upon the synthesis of GA in the embryo (Karssen and Laçka, 1986). Responsiveness to GA was believed to be due to the activation of GA receptors as a result of desiccation resulting in changes to membrane structure and composition (Hilhorst and Karrsen (1992). Bianco (1994) observed that sunflower embryo responsiveness to GA increased whereas responsiveness to ABA decreased under dry storage conditions. ABA in sunflower embryos not only plays a major role in induction but, also in maintenance of dormancy. Le Page-Degivry (1990) demonstrated that at some point in sunflower embryo development, the capacity for ABA synthesis is induced. The embryo's ability to continue to synthesize ABA is related to ABA responsiveness (i.e. if the embryo is continuing to synthesize ABA, growth promoters will have little or no effect). The level of dormancy is theorized to be maintained by ABA synthesis capacity and ABA levels, even though GA levels were sufficient to promote germination. Le Page-Degivry (1990) suggests that this behavior supports the hypothesis from Karssen and Laçka (1986) that ABA and GA do not interact during seed development. ABA was found at sufficient levels to induce dormancy but in later stages, GA was at levels sufficient to stimulate germination (Karssen and Laçka 1986).

**Dormancy Breaking**

Ethylene and its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) have been shown to stimulate germination (Corbineau, 1990; Chandler, 1985; Ruud, 1976). Corbineau (1990) showed that ethylene allowed germination of dormant sunflower seeds over a range of

temperatures, rendered them less sensitive to hypoxia, and that its stimulatory effect was dependent upon the duration and timing of the application.

Corbineau (1990) exposed immature sunflower embryos to two inhibitors of ethylene biosynthesis (amino-oxyacetic acid (AOA) and carbonyl dichloride ($CoCl_2$)) and two inhibitors of ethylene action (silver thiosulfate (STS) and 2,5-nonbornadiene (NBD), to determine if endogenous ethylene regulated germination. The immature embryos were compared to embryos taken from 6-month old seed that had been under 20°C dry storage. ACC increased germination in the fresh embryos from 18% to 98% and in non-dormant embryos (98% to 100%). AOA, $CoCl_2$, STS, NBD inhibited germination of the fresh embryos. AOA inhibited germination in non-dormant embryos by 50%, $CoCl_2$ by 52%, STS by 22% and NBD inhibited germination by 100% (Corbineau 1990).

Gibberellic acid ($GA_3$) stimulated germination of dormant sunflower seeds, but not to the extent that showed laboratory germination ( Cseresnyes, 1978; Corbineau, 1987; Chandler, 1985; Seiler, 1998). Cseresnyes (1978) showed that pre-chilling the seed at 0°C for four and nine d durations alone, or in combination with $GA_3$, induced the germination of all viable seeds in the seed lots. Corbineau (1990) showed contradicting data that indicated neither cold nor $GA_3$ significantly effected broke dormancy when compared to the use of ethylene.

**Secondary Dormancy**

Secondary dormancy is the term used for seed that has broken primary dormancy, but due to unfavorable environmental conditions (water, temperature, light, nitrate, oxygen, etc.) are returned to a dormant condition. Secondary dormancy is usually associated with seeds in the soil where temperature and moisture are the predominant factors affecting the dormancy cycle; but production practices can also have an effect. Corbineau (1989) Exposed freshly harvested sunflower seeds to 45°C which induced secondary dormancy and reduced ACC conversion to ethylene. This high temperature treatment is believed to have changed the

conformation of membrane bound enzymes and membrane integrity which would explain the decrease in activity of enzymes forming ethylene (Corbineau 1989).

**Summary**

Sunflower seed dormancy depth and duration can be affected by a variety of factors; seed variety, seed maturity at harvest, environmental factors which influenced the seed while on the mother plant and at the time of harvest, condition of storage, drying rate and temperature, and hormonal levels in the embryo. Sunflower breeding programs to develop varieties with higher oil contents has led to increased dormancy in certain selections compared to the traditional oil varieties. These variations in dormancy levels can create many challenges for commercial sunflower seed production. Under normal conditions, sunflower seed dormancy will have dissipated by approximately 60 d after harvest. In these situations, the domestically produced crop in northern temperate regions has the time needed to break dormancy before the product is shipped to customers. In off-season production of hybrids in South America, the challenges increase. Timing shipments of seed to the United States and product packaging for customer shipments can be impeded greatly by lines with extended dormancy patterns. The objective of this study was to evaluate the differences in dormancy patterns of two commercial sunflower varieties, a traditional oil line and a mid-oleic line, when subjected to a variety of storage temperatures, germination tests, and across seed production years.

# MATERIALS AND METHODS

The Association of Official Seed Analyst (AOSA) standard warm germination test and two unofficial germination tests for evaluating sunflower seed quality were used to evaluate the germination and dormancy of sunflower lines. The term "seed" is used to refer to the dispersal unit of the sunflower which is the achene, which includes the seed and the pericarp.

The mention of products and or manufacturers in the following section does not constitute a recommendation. These were merely the equipment and supplies that were available for use in this research project.

## Seed source

The hybrid SF270 (a traditional oil genotype) and X8927 (a mid oleic genotype) sunflower seed used in this study were obtained from commercial seed production fields in Dumas, Texas and Slaton, Texas in 1999 and 2000 by Cargill Hybrid Seeds (CHS) and Mycogen Seeds, Indianapolis, Indiana.

Six seed lots were selected from each production year for a total of 13 seed lots used (in 1999 an additional lot of X8927 was included for analysis). All hybrid sunflower seed production for CHS and Mycogen Seeds in Texas was done utilizing irrigation as needed to supplement any lack of rainfall. The seed fields were harvested in early to mid August at 12% moisture. The bulk seed was then dried in a Farm-Hand Continuous Flow Dryer, which was modified to operate as a batch dryer, at 35°C for 3-4 hours to achieve a final moisture of 7%. The seed was shipped as bulk material. Seed produced in 1999 was tested in Aurora, IL at the Cargill Hybrid Seeds Quality Control Lab. Seed produced in 2000 was tested in Marshalltown, IA at the Mycogen Seed Quality Control Lab.

## Sample preparation

Upon arrival at the laboratory in Illinois, the seed samples were processed. For this study, a sample consisted of seed passing through a 4.763 X 19.050mm slotted upper screen but held by a 3.175 X 19.050mm slotted screen (screens were purchased from Seedburo). The resulting seed sample was left in a bulk grade, meaning, we did not attempt to create further grade size separations. The bulk seed samples were then thoroughly mixed using a Boerner Divider. To achieve working samples for each of the seed lots, samples were divided according to the AOSA rules for obtaining a working sample (2004). Each lot was first divided into three bulk sub-samples (one for each storage temperature) using a Boerner Divider. Each temperature sub-sample was further divided into 22 sub-samples with a Gamet divider. These 22 samples represent each testing date. Each testing date sub-sample was then divided into 4 sub-samples using a Gamet divider. This gave a total of 5760 samples in 1999 and 4320 samples in 2000. Samples were placed randomly into storage containers. Storage temperatures were 10 °C, 14 °C and ambient room temperature (22°C). The 10 (+/-1) °C samples were stored in climate controlled cold storage where the temperature was monitored daily. The 14 (+/-1)°C samples were stored in a Baxter Scientific Cryo-Fridge incubator that was monitored daily. Ambient room temperature seed was stored in lab warehouses to mimic seed storage conditions. Samples were planted on the same day that they were removed from storage conditions. Samples were tested on two week intervals after arrival at the lab, being planted on the first and fifteenth day of each month. The testing period started the middle of September and ended in July of the following year for a total of 22 testing periods.

**Germination tests**

Three tests used to evaluate the dormancy and germinability of seed were the AOSA standard warm germination test (constant warm 20 °C), alternating 20-30 °C warm germination test, and the Ethrel warm germination test (20 °C). All tests were performed using the rolled towel germination method. Germination towel paper (8.37 kilo) obtained

from Anchor Paper Co., St. Paul, MN, was used as the germination medium. The paper was pre-moistened by saturating sheets in distilled water. A mass of paper was weighed to obtain 5 pounds (140 sheets), which was placed in a plastic tub and 6800 ml of distilled water was allowed to infiltrate the towels and come to room temperature before being used for planting. Papers for the Ethrel tests were prepared in the same manner but 5.10 ml of Florel (Southern Agricultural Insecticide, Inc, Hendersonville, NC) was added to the 6800 ml of water before application to the towels. Seeds were placed on a single towel using a 100 seed vacuum head (Hoffman Manufacturing, Salem, OR). An additional moist towel was placed over the seeds and then rolled up. To secure the roll, a rubber band was applied to the bottom end of the roll. The rolls were placed upright in a small plastic garbage can and covered with a plastic bag. The sealed tubs were placed in two dark germinators. The constant temperature warm and ethrel tests were placed in a Federal germinator. The alternating 20-30 °C tests were placed in a Baxter Scientific Cryo-Fridge incubator.

All tests were conducted for seven days with a pre-reading at 4 days. Each replicate was planted and randomly placed in separate tubs and in separate parts of each germination chamber. Seedlings were evaluated as germinated if the radicle emerged from the pericarp without abnormalities caused from physiological sources or diseases. Seeds that produced seedlings with abnormalities were classified as abnormal according to the AOSA Seedling Evaluation Handbook (2000). The tetrazolium test was conducted on the non-germinated seeds remaining at the end of the germination tests to classify as either dormant or dead.

For this experiment, maximum laboratory germination, is defined as no further increase in germination percentage and the remaining seeds on the test were determined to be non-viable.

**Ethrel test**

The Ethrel test (Ruud, 1976) was used to break dormancy in sunflowers. This test has been used to determine seed viability. In the original development of this test, a $10^{-4}$

molar concentration of Ethrel was used. In Cargill Hybrid's Seed lab version of the ethylene mediated germination test, a commercially available product called Florel™ was substituted for Ethrel. Florel™ is the brand name for Ethephon (2 choroethyl phosphonic acid (3.9% active ingredient)). Ethephon is a ripening agent and a mutagen which can cause abnormalities in the seed lots if too much chemical is applied. For this test, seedlings were considered germinated if the radicle emerged from the pericarp.

**Tetrazolium test**

The Tetrazolium test was used to determine seed viability. This test can be used to distinguish between viable and non viable embryo tissue by measuring the dehydrogenase enzyme activity. The colorless tetrazolium salt solution is reduced by these enzymes to form a water insoluble red formazan. Live, respiring embryo tissue stains red. Dead tissue will not stain (AOSA Seed Vigor Testing Handbook 1983). Interpretation of the staining requires that the radicle and the junction between the radicle and the cotyledons stain red to be considered viable. All non-germinated seeds from the tests were checked using this method to determine viability.

**Experimental Design**

A split plot design was used. Genotype and production year were the main plot factors. The main plot error was the seed lot, nested within genotype and production year. Differences between genotypes and between years were compared using the lot to lot variability. Storage temperature was the split plot factor.

**Statistical Analysis**

Germination data was gathered from the two years of testing. This data was analyzed using JMP statistical software. The data analysis required several steps. The proportion of germinated seeds was calculated from the counts of normal and abnormal seedlings but

excluding dead seed from the analysis. A logistic model was used to describe the relationship between time (DAH: days after harvest) and the proportion of germinated seeds:

$$P[germ] = \frac{1}{1 + \exp(\beta_0 + \beta_1 DAH)}$$

The intercept, $\beta_0$, and slope, $\beta_1$, were estimated by logistic regression separately for each combination of genotype, production year, germination test, and storage temperature. The intercept determines the predicted germination $P[germ]$ when DAH=0. A line with a negative intercept has a higher percent germination when data collection starts. The slope determines the daily change in germination percent when the germination rate crossed the 50% germination point. Of course, a larger slope indicates a faster rate of change in germination.

Predicted germination was calculated for a range of DAH values and plotted for each combination of experimental factors. Examination of these plots suggested that data from each germination test should be analyzed separately because of interactions between germination test and other factors. The number of days until the predicted germination reaches X% was calculated as $(LN(X/(100-X))+B_0)*(-1/B_1)$ (Appendix, Table 1). This equation was used to calculate the days to 85% germination. The 85% germination target was chosen because the seed industry often uses this as the commonly accepted minimum laboratory germination for selling hybrid sunflower seeds in the United States. If a seed lot did not reach this laboratory germination target using the standard warm germination test, it would not be considered for sale. The predicted days to 85% germination was calculated for each genotype and production year (Appendix, Tables 2-5).

The intercept and slope of the logistic regression provided information about the between seed-lot variability in each parameter. A split-plot ANOVA was used to analyze the effects of genotype, production year, and storage temperature separately for each germination test. The main plot factors, genotype, year, and the interaction of genotype*year were tested against the lot –lot variability. The split plot factors, storage temperature and the interaction of storage temperature and genotype, were tested against the residual error. The intercept and slope were analyzed separately (Tables 7-13 and 14-17).

The analysis of genotype, storage temperature, and test was utilized to demonstrate how the curves were fit to the averages. These graphs demonstrate the overall differences in the interaction between genotype, storage temperature, and test (Figures 5-10, Appendix Table 6).

# RESULTS

## Analysis of Variance Fit Test

Genotype, storage temperature, test and lot data was analyzed for the predicted slope and intercept for each seed lot, production year, and germination test. The intercept data indicates the speed with which the seed broke dormancy and germinated after harvest. The slope data indicates the length of time that seed took to reach its laboratory germination (Table 1, Appendix A).

## Intercept data

For the genotype SF270, the intercepts crossed the y axis at a much higher predicted germination percentage than X8927. Upon the initial analysis, the genotype SF270 samples were less dormant than X8927.

**Table 7. Split plot analysis of intercept for the standard warm germination test.**

Tests with Random Effects

| Source | DF Num | SS | MS Num | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Genotype | 1 | 236.191 | 236.191 | 24.0601 | 0.0008 |
| Genotype*Production Year | 1 | 0.32 | 0.32 | 0.0326 | 0.8607 |
| Production Year | 1 | 28.1692 | 28.1692 | 2.8695 | 0.1245 |
| Lot[Genotype, Production Year]&Random | 9 | 88.3506 | 9.81673 | 4.3975 | 0.0022 |
| | | | | | |
| Storage Temp | 2 | 66.0244 | 33.0122 | 14.788 | <.0001 |
| Genotype*Storage Temp | 2 | 26.2894 | 13.1447 | 5.8883 | 0.009 |
| Error | 22 | 49.11189 | 2.2324 | Prob > F | |
| C. Total | 38 | 493.0471 | | <.0001 | |

Genotype, storage temperature and the genotype*storage temperature interaction for the standard warm germination test indicated significant differences (Table 7). In Table 8

(Appendix A), the difference of 2.3 between the means of the two genotypes indicates a faster response in breaking dormancy for SF270. Figure 2 (Appendix B), demonstrates the results of genotype x storage temperature. The genotype SF270 had higher initial germination than X8927. Storage at constant 14°C and constant 10°C prolonged the dormancy period for X8927. Both genotypes kept at ambient room temperature broke dormancy faster than samples kept at cooler storage temperatures.

Genotype and production years indicate significant differences for the alternating 20-30°C warm germination test (Table 9). The differences in genotype outweigh the production year differences by tenfold (Table 9).

**Table 9. Split plot analysis of intercept for the alternating 20-30°C warm germination test**

**Tests with Random Effects**

| Source | DF Num | SS | MS Num | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Genotype | 1 | 108.895 | 108.895 | 71.8157 | <.0001 |
| Genotype*Production Year | 1 | 0.47086 | 0.47086 | 0.3105 | 0.5903 |
| Production Year | 1 | 10.5306 | 10.5306 | 6.9449 | 0.0261 |
| Lot[Genotype, Production Year]&Random | 9 | 13.797 | 1.533 | 1.483 | 0.2152 |
| | | | | | |
| Storage Temp | 2 | 5.89924 | 2.94962 | 2.8534 | 0.0791 |
| Genotype*Storage Temp | 2 | 5.54254 | 2.77127 | 2.6809 | 0.0908 |
| Error | 22 | 22.74185 | 1.03372 | Prob > F | |
| C. Total | 38 | 177.21926 | | <.0001 | |

In Table 10, the least squares means indicates that SF270 was breaking dormancy faster than X8927.

**Table 10. Least squares means table for genotype, production year and genotype*production year of intercept for alternating 20-30°C warm germination test**

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| SF270 | -1.17283 | 0.29024002 | -1.1728 |
| X8297 | 2.267254 | 0.28380658 | 2.2899 |
| | | | |
| 1999 | 0.0123246 | 0.27149468 | 0.2742 |
| 2000 | 1.0820988 | 0.30178814 | 1.17878 |

Seed produced in 1999 lost dormancy significantly faster than 2000 production (Table 10). Although the difference between production years is significant, it is not as great as the differences between the genotypes (Figure 3, Appendix B). The genotype*year interaction was not significant.

Genotype and production year had significant differences for the ethrel warm germination test (Table 11). The differences in genotype outweigh the production year differences by twofold. The genotype*production year interaction was not significant.

**Table 11. Split plot analysis of intercept for the ethrel warm germination test**

| Source | DF Num | SS | MS Num | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Genotype | 1 | 79.4179 | 79.4179 | 138.0237 | <.0001 |
| Genotype*Production Year | 1 | 0.00035 | 0.00035 | 0.0006 | 0.9809 |
| Production Year | 1 | 28.8936 | 28.8936 | 50.2155 | <.0001 |
| Lot[Genotype, Production Year]&Random | 9 | 5.19628 | 0.57736 | 0.6241 | 0.7637 |
| | | | | | |
| Storage Temp | 2 | 5.62264 | 2.81132 | 3.0391 | 0.0693 |
| Genotype*Storage Temp | 2 | 6.14689 | 3.07345 | 3.3225 | 0.0558 |
| Error | 21 | 19.42589 | 0.92504 | Prob > F | |
| C. Total | 37 | 141.63354 | | <.0001 | |

SF270 lost dormancy faster than X8927 (Table 12). The differences in production year that were indicated to be significant in Table 11 can be seen in Figure 4 (Appendix B). Both genotypes were germinating at a faster rate in the 1999 production year than in the 2000 production year. This chart demonstrates parallel germination patterns between the two

genotypes and production years. However, there is a lack of a significant interaction between genotype*production year.

**Table 12. Least squares means table for genotype, production year and genotype*production year effect tests of intercept for the ethrel warm germination test.**

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| SF270 | -1.842482 | 0.1787912 | -1.8425 |
| X8297 | 1.075391 | 0.17239101 | 0.9583 |
| | | | |
| 1999 | -1.263537 | 0.17239101 | -1.1467 |
| 2000 | 0.496446 | 0.1787912 | 0.4964 |
| | | | |
| SF270,1999 | -2.725539 | 0.25284893 | |
| SF270,2000 | -0.959425 | 0.25284893 | |
| X8297,1999 | 0.198466 | 0.23439723 | |
| X8297,2000 | 1.952317 | 0.25284893 | |

## Slope data

In Table 1 and Table 6 (appendix A) the larger negative slope values indicated a longer response time to dormancy breaking. The genotype SF270, has steeper slopes than X8927, indicating a faster response time to reaching maximum laboratory germination.

The analysis of slope for the standard warm germination test indicates that differences in the storage temperatures had more of an effect on the slope response than the other factors but it does not indicate which temperature(s) contributed to these differences (Table 13).

**Table 13. Split plot analysis of slope for the standard warm germination test**

| Source | DF Num | SS | MS Num | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Genotype | 1 | 0.00726 | 0.00726 | 1.2506 | 0.2924 |
| Genotype*Production Year | 1 | 0.00106 | 0.00106 | 0.1822 | 0.6795 |
| Production Year | 1 | 0.00107 | 0.00107 | 0.1842 | 0.6779 |
| Lot[Genotype, Production Year]&Random | 9 | 0.05226 | 0.00581 | 2.8249 | 0.0226 |
| | | | | | |
| Storage Temp | 2 | 0.03285 | 0.01642 | 7.9901 | 0.0025 |
| Genotype*Storage Temp | 2 | 0.00668 | 0.00334 | 1.6257 | 0.2195 |
| Error | 22 | 0.04522359 | 0.002056 | Prob > F | |
| C. Total | 38 | 0.14996382 | | 0.0063 | |

Utilizing a multiple comparison test on the LS means in Table 14, the germination of seed stored at ambient room temperature was determined to be different than the storage temperature of constant 14°C. Seed stored at constant 10°C was determined to be different than the storage temperature of constant 14°C. There was no difference in the comparison of the means between constant 10°C and ambient room temperature. Samples of both genotypes under constant 10°C were slower to reach maximum laboratory germination. Samples maintained at ambient room temperature reached maximum laboratory germination faster than those maintained at the other storage temperatures.

**Table 14. Least squares means table for temperature effect test of slope for the standard warm germination test.**

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| Temp 1 (10C) | -0.0645813 | 0.01263232 | -0.06575 |
| Temp 2 (14C) | -0.1299997 | 0.01263232 | -0.1333 |
| Temp 3 (Ambient Temp) | -0.0727305 | 0.01263232 | -0.07387 |

The analysis of slope for the 20-30°C warm germination test indicated significant differences between the storage temperatures (Table 15).

**Table 15. Split plot analysis of slope for the alternating 20-30°C warm germination test.**

| Source | DF Num | SS | MS Num | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Genotype | 1 | 0.00186 | 0.00186 | 1.7206 | 0.2209 |
| Genotype*Production Year | 1 | 0.00004 | 0.00004 | 0.0337 | 0.8583 |
| Production Year | 1 | 0.00006 | 0.00006 | 0.0515 | 0.8254 |
| Lot[Genotype, Production Year]&Random | 9 | 0.00985 | 0.00109 | 1.7431 | 0.1383 |
| | | | | | |
| Storage Temp | 2 | 0.00943 | 0.00471 | 7.507 | 0.0033 |
| Genotype*Storage Temp | 2 | 0.0018 | 0.0009 | 1.4352 | 0.2595 |
| Error | 22 | 0.01381425 | 0.000628 | Prob > F | |
| C. Total | 38 | 0.0374491 | | 0.0318 | |

Using the multiple comparison test on the LS means in Table 16 to determine which temperature(s) contributed to this effect determined that the differences in storage temperatures were not significant for this test.

**Table 16. Least squares means table for temperature effect test of slope for the alternating 20-30°C warm germination test**

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| Temp 1 | -0.0758385 | 0.0084406 | -0.0789 |
| Temp 2 | -0.0948304 | 0.00887432 | -0.09295 |
| Temp 3 | -0.0757524 | 0.0084406 | -0.07737 |

Genotype and the genotype*year interaction was considered significant for the ethrel warm germination test analysis (Table 17).

**Table 17. Split plot analysis of slope for the ethrel warm germination test**

| Source | DF Num | SS | MS Num | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Genotype | 1 | 0.01257 | 0.01257 | 24.6908 | 0.0008 |
| Genotype*Production Year | 1 | 0.00797 | 0.00797 | 15.662 | 0.0035 |
| Production Year | 1 | 0.00019 | 0.00019 | 0.3767 | 0.5549 |
| Lot[Genotype, Production Year]&Random | 9 | 0.0046 | 0.00051 | 0.5572 | 0.8161 |
| | | | | | |
| Storage Temp | 2 | 0.0029 | 0.00145 | 1.5795 | 0.2296 |
| Genotype*Storage Temp | 2 | 0.00397 | 0.00199 | 2.165 | 0.1397 |
| Error | 21 | 0.01926882 | 0.000918 | Prob > F | |
| C. Total | 37 | 0.04940516 | | 0.0616 | |

The sharper slope of genotype SF270 suggests that it was reaching its maximum laboratory germination at a faster rate than that of the other genotype (Table 18).

**Table 18. Least squares means table for genotype and genotype*production year effect tests of slope for the ethrel warm germination test**

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| SF270 | -0.0689599 | 0.00774204 | -0.06896 |
| X8297 | -0.0831637 | 0.00757043 | -0.08248 |
| | | | |
| SF270,1999 | -0.0514387 | 0.00752002 | |
| SF270,2000 | -0.076138 | 0.00752002 | |
| X8297,1999 | -0.1173757 | 0.00697125 | |
| X8297,2000 | -0.0836093 | 0.00752002 | |

The interaction between genotype*year indicates that the differences between production years for the genotype SF270 was much smaller than that for X8927. In 1999, the genotype X8927 demonstrated broader slopes or longer response times to dormancy breaking with the ethrel test than in 2000 over all storage temps (Figures 9 and 10).

## Genotype, storage temperature, and test (HTT) data

Figures 5 through 10 are the result of fitting the line to the curve from the calculation, X number of days to germination= $(LN(X/(1-X))+B_0)*(-1/B_1)$ where $B_0$ is the intercept and

$B_1$ is the slope and X is any possible value using the HTT data sets. These curves were used to demonstrate how the slope and intercept are interacting for each genotype, storage temperature and test.

Figure 5 and 6 (Appendix B) demonstrate the warm germination test response to genotype SF270, where the intercepts are crossing the y axis at a much higher predicted germination percentage than X8927. The SF270 samples had broken initial dormancy before testing began. Both genotypes responded to ambient room temperature storage with higher initial intercepts indicating these samples had a higher initial laboratory germination than the samples stored at lower temperatures. Storage at constant 10°C prolonged the longevity of the dormancy period for X8927.

Figure 7 and 8 (Appendix B) demonstrate the alternating 20-30C warm germination test response to the differences in initial germination values between genotype and year in dormancy for the alternating 20-30C warm germination test. SF270 had higher germination values at the time of initial analysis, the corresponding slope for storage temperatures and tests were shorter and steeper, indicating a faster response to dormancy breaking. In X8927, for all three tests, the slopes were similar across both production years for each test and temperature response. The lower intercept values for the X8927, at the time of initial analysis, corresponded to broader more gradual dormancy breaking slopes.

Figure 9 and 10 (Appendix B) demonstrate the ethrel warm germination test differences between genotype and year, where the initial data collection began. The ethrel test demonstrated the ability to break dormancy in both genotypes better than among the other tests. SF270 demonstrated higher initial laboratory germination and a shorter response time to dormancy breaking than the X8927 genotype for both production years. In 1999 both genotypes demonstrated broader slopes or longer response times to dormancy breaking with the ethrel test than in 2000 over all storage temps.

**85% laboratory germination prediction for genotype, storage temperature, and test (HTT) data 1999**

In 1999, the data for SF270 indicates that seed samples at all storage temperatures and tests (with the exception of constant 10°C) broke dormancy and reached the target 85% germination goal before the initial testing began on all tests and storage temperatures (Table 2). This is indicated by a negative value for the "X days to 85% germination." The larger the negative value indicated the speed with which the sample broke dormancy post harvest. Positive values indicated the number of days past the initial analysis that the sample required to reach the germination target. The larger the positive value, the longer it took for the sample to break dormancy after harvest.

Hybrid X8927 took 29 days for room temperature, 33 days for constant 14°C, and 88 days for constant 10°C to break dormancy and reach the 85% germination target for the warm germination test. The ethrel test averages were 1 day for room temperature, 16 days for constant 14°C, and 24 days for constant 10°C to break dormancy and reach the 85% germination goal (Table 3).

**85% laboratory germination prediction for genotype, storage temperature, and test (HTT) data 2000**

Genotype SF270 took an average of 15 days for room temperature storage, 20 days for constant 14°C, and 28 days for constant 10°C to break dormancy and reach the 85% germination goal on the warm germination test. For the alternating 20-30 °C warm germination test, it took an average of 14 days for room temperature storage, 15 days for constant 14°C, and 19 days for constant 10°C to break dormancy and reach the 85% germination goal. The ethrel test averages were 10 days for room temperature storage, 18 days for constant 14°C, and -2 days for constant 10°C to break dormancy and reach the 85% germination goal (Table 4). The negative data indicated that the seed was able to break

dormancy and reached a higher germination rate than the germination goal before the initial testing began.

Genotype X8927 took an average of 41 days for room temperature storage, 60 days for constant 14°C, and 116 days for constant 10°C to break dormancy and reach the 85% germination goal on the warm germination test. For the alternating 20-30 °C warm germination test, it took an average of 41 days for room temperature storage, 49 days for constant 14°C, and 105 days for constant 10°C to break dormancy and reach the 85% germination goal. The ethrel test averages were 37 days for room temperature storage, 45 days for constant 14°C, and 53 days for constant 10°C to break dormancy and reach the 85% germination goal (Table 4).

Over both years, the pattern for dormancy breaking was similar between the two hybrids. The 2000 data indicates that both genotypes took longer to break dormancy than in 1999, but displayed a similar pattern of dormancy breaking. The data also indicated that, under room temperature storage, dormancy was broken at a faster rate than under the other storage temperatures for both hybrids over both years (Table 5).

# DISCUSSION

The primary objective of this research was to investigate the potential differences in dormancy between genotypes. From the data reported above, the conclusion can be made that the genotype SF270, a traditional oil sunflower hybrid and X8927 (SF287), a mid oleic sunflower genotype are different in their response to dormancy breaking. SF270 was able to break dormancy and reach its maximum laboratory germination at a faster rate and a shorter time delay than X8927 (Figures 5-10). The number of days to reach the target 85% germination goal for SF270 over both production years indicated that this target was reached soon after harvest and well before any testing measurements were made. Germination slope values for the two production years were similar, which indicated a negligible year effect on the longevity of dormancy in both genotypes.

Ambient room/warehouse temperature reduced the dormancy longevity period faster than a constant 14°C or 10°C. Across both genotypes and production years, storage of seed at 10°C maintained the depth of dormancy and thus increased the longevity of dormancy in the genotypes. Seed storage at constant 14°C affected the longevity of the dormancy period but, was not as much as storage at constant 10°C. Ambient room temperature storage and the ethrel tests broke dormancy in the two genotypes earlier than the other combination of tests and temperatures.

The standard warm germination test compared to the ethrel and alternating 20-30°C germination tests took the longest period of time to reach the target germination goal of 85%. This difference in test was observed in both genotypes and production years. The ethrel test was most successful in breaking dormancy at a faster rate in the samples of each genotype to indicate the potential laboratory germination value. The alternating 20-30 °C test was less effective than the ethrel test but, more successful than the standard warm germination test for a quick response in breaking dormancy in the samples studied. From the data, the ethrel test was most successful in its ability to predict the laboratory germination by the use of chemical

means to break dormancy. The dormancy breaking response in the samples to the tests was also dependant upon the storage temperature of the samples.

# CONCLUSIONS

## Practical Considerations

This work sheds light on the timing of the natural dormancy breaking process for genotypes SF270 and X8927 as affected by germination test methodology and storage temperature. It cannot be assumed that these two genotypes were representative of all possible genotypes. The genotype SF270 broke dormancy soon after harvest, often before initial laboratory germination analysis could be completed. Genotype X8297 took longer to break dormancy than the traditional oil genotype. Both genotypes broke dormancy in a consistent pattern across the two production years. The increase in the oil profile of the mid and high oleic varieties through breeding programs could explain these differences. In increasing the oil content, other components of the seed could have been affected. Higher oil content could have changed membrane properties and/or ABA synthesis, activity, and response in these seeds. All of these physical and chemical properties play a role in the dormancy phenomenon.

This study has produced two very useful tools; identification of the average number of days after harvest to reach the target 85% germination goal, and the depth of dormancy or longevity of dormancy for a particular genotype. If a seed producer had this information for all possible genotypes in their production program, it could be used as a risk assessment tool to plan seed production placement, shipping, and anticipate product release dates. Storage temperature was demonstrated to play a role in the longevity of dormancy in both genotypes. Typical storage procedures for a seed producer are to place high value seed in cold storage (constant 10°C) to ensure that quality and germination is maintained. This may be appropriate for carryover product but, depending upon the genotype, this temperature could prolong the dormancy longevity in new crop seed lots. Storage at ambient room temperature allowed the samples to continue to after-ripen in a way that facilitated the breaking of

dormancy. Storing samples at a constant 10°C and 14°C appeared to have slowed the after-ripening process allowing the seed samples to maintain their dormancy for a longer period.

Although the ethrel test was indicated to be the best predictor of laboratory germination,n it is not recognized as an "official" test for measuring sunflower seed germination. This is also the case for the alternating 20-30°C warm germination test. The standard warm germination test is the only germination test recognized by the AOSA. For a seed company, the ethrel and alternating 20-30°C warm germination test are viable tools for predicting germination. The ethrel test can and is used to evaluate bulk seed lots by measuring the laboratory germination percentage. This germination and viability information can be used by gravity table operator to better determine equipment setting in the conditioning process, resulting in a higher quality product. The alternating 20-30°C warm germination can be used in a similar manner as the ethrel test. However, this test has the ability to better evaluate the potential for physiological abnormalities in the seed lot sample. The alternating temperatures on bulk seed can give a more accurate prediction of laboratory germination over that of the standard warm test on bulk seed.

Shipping packaged, finished seed to customers poses several challenges. All sunflower hybrid seed falls under the statute of the Federal Seed Act Truth in Labeling Laws (USDA-AMS 1998). Each unit of seed is marked with a tag indicating crop kind, hybrid and/or variety, lot, germination, date of test, origin, pure seed, inert matter, weed seed, noxious weed seed, other crop seed, seed count, and treatment. State and federal inspectors have the ability to inspect seed lots for commercial sale and pull samples at various points (company warehouses, distributors, customers, etc.). Subsequent germination and purity inspections are conducted to verify that the seed being offered for sale meets (or in the case of germination meets or exceeds represented values) the information provided on the seed bags to the consumer. This testing is performed according the AOSA rules for testing seed. The standard warm germination test is the final indicator if a seed lot can be released for sale. If the state findings are significantly different than that of the company's data the seed can be

blocked from sale. Situations like this have not only cost implications to a seed company trying to meet shipping and customer deadlines. If the dormancy is broken in the sample, it may not be indicative of the entire seed lot. Use of this information could cause the early release of product to the customer that could result in uneven field emergence and uneven maturation at harvest. This type of error can cost a seed company not only in monetary considerations to reimburse the customer but, also the non-measurable costs of customer confidence and future sales. The alternating 20-30°C and ethrel warm germination tests, until recognized and used by state and federal labs, can be tools for a seed company to assist in identifying the potential of a seed lot but, should not be relied on as the final decision making tool for product release.

**Continued research considerations**

As stated earlier, this type of study should be considered for all genotypes that a company is looking to produce. This information can then be utilized to assist in the production planning stage of the business to reduce the risk of off season seed production not breaking dormancy in time for shipment to the customer.

This study or type of study does not take into consideration physiological or biochemical differences (if any) between the traditional oils and mid and high oleic sunflower hybrids. To truly understand what is happening, further study comparing ABA levels, water storage placement in the seeds, chemical make up of the embryo, embryo development and maturation differences are several areas where information might be found to indicate the differences in dormancy breaking potential between these sunflower genotypes. More traditional production research type studies into the effects of drying temperature, harvest moisture, storage temperatures, and dormancy breaking on dormancy longevity could provide practical information to improve seed handling for these genetics.

# APPENDIX A. ADDITIONAL TABLES

Table 1. Genotype, storage temperature, test, lot (HTTL) data

| Year | Hybrid | Temp | Test | Lot | Intercept | Slope | Calculated days to 85% germ |
|---|---|---|---|---|---|---|---|
| 1999 | SF270 | Temp 1 | 20-30 C | B505B | -3.1091282 | -0.0317127 | -43.34311316 |
| 1999 | SF270 | Temp 1 | ETH | B505B | -1.9716038 | -0.0652255 | -3.633590308 |
| 1999 | SF270 | Temp 1 | warm | B505B | -1.9034293 | -0.0545301 | -3.096056024 |
| 1999 | SF270 | Temp 2 | 20-30 C | B505B | -3.0422804 | -0.037657 | -34.72606274 |
| 1999 | SF270 | Temp 2 | ETH | B505B | -2.7368782 | -0.0459778 | -21.79915404 |
| 1999 | SF270 | Temp 2 | warm | B505B | -2.2748647 | -0.0678488 | -7.962759026 |
| 1999 | SF270 | Temp 3 | 20-30 C | B505B | -2.4303862 | -0.0533347 | -13.04563717 |
| 1999 | SF270 | Temp 3 | ETH | B505B | -2.8015125 | -0.0455845 | -23.4051365 |
| 1999 | SF270 | Temp 3 | warm | B505B | -3.2708982 | -0.0362696 | -42.35770851 |
| 1999 | SF270 | Temp 1 | 20-30 C | B506B | -0.342467 | -0.0813704 | 17.10860528 |
| 1999 | SF270 | Temp 1 | ETH | B506B | -2.7350509 | -0.047533 | -21.04747953 |
| 1999 | SF270 | Temp 1 | warm | B506B | -0.908248 | -0.0852214 | 9.696544006 |
| 1999 | SF270 | Temp 2 | 20-30 C | B506B | -1.4341205 | -0.0617684 | 4.864632326 |
| 1999 | SF270 | Temp 2 | ETH | B506B | -2.9861447 | -0.05165 | -24.23124191 |
| 1999 | SF270 | Temp 2 | warm | B506B | -1.9729353 | -0.0975065 | -2.444290838 |
| 1999 | SF270 | Temp 3 | 20-30 C | B506B | -1.7131842 | -0.1298726 | 0.16490665 |
| 1999 | SF270 | Temp 3 | ETH | B506B | -3.0229646 | -0.0482941 | -26.67745221 |
| 1999 | SF270 | Temp 3 | warm | B506B | -2.6053453 | -0.0729548 | -11.93539348 |
| 1999 | SF270 | Temp 1 | 20-30 C | B705B | -1.4392429 | -0.0768029 | 3.845664101 |
| 1999 | SF270 | Temp 1 | ETH | B705B | -2.6005295 | -0.055172 | -15.69507077 |
| 1999 | SF270 | Temp 1 | warm | B705B | -0.8498554 | -0.0739417 | 11.9654492 |
| 1999 | SF270 | Temp 2 | 20-30 C | B705B | -1.69167 | -0.0754106 | 0.569297359 |
| 1999 | SF270 | Temp 2 | ETH | B705B | -2.9012947 | -0.0427263 | -27.30621759 |
| 1999 | SF270 | Temp 2 | warm | B705B | -1.9641091 | -0.1071643 | -2.141646468 |
| 1999 | SF270 | Temp 3 | 20-30 C | B705B | -1.1849182 | -0.0927075 | 5.92921668 |
| 1999 | SF270 | Temp 3 | ETH | B705B | -2.7738744 | -0.060785 | -17.09752973 |
| 1999 | SF270 | Temp 3 | warm | B705B | -3.0405655 | -0.08299 | -15.73640733 |
| 1999 | X8297 | Temp 1 | 20-30 C | B515B | 2.74066628 | -0.0528634 | 84.65719828 |
| 1999 | X8297 | Temp 1 | ETH | B515B | 1.14609215 | -0.1494723 | 19.27242175 |
| 1999 | X8297 | Temp 1 | warm | B515B | 4.06727975 | -0.0620598 | 93.48855145 |
| 1999 | X8297 | Temp 2 | 20-30 C | B515B | 2.58031631 | -0.1113094 | 38.76507613 |
| 1999 | X8297 | Temp 2 | ETH | B515B | 12.3335821 | -0.7333556 | 19.18330365 |
| 1999 | X8297 | Temp 2 | warm | B515B | 3.99097326 | -0.2075982 | 27.58007688 |
| 1999 | X8297 | Temp 3 | 20-30 C | B515B | 1.33793909 | -0.1230685 | 24.9660973 |
| 1999 | X8297 | Temp 3 | ETH | B515B | -1.0125898 | -0.107984 | 6.686279962 |
| 1999 | X8297 | Temp 3 | warm | B515B | -2.5011698 | -0.0333859 | -22.96085307 |
| 1999 | X8297 | Temp 1 | 20-30 C | B516B | 2.76639151 | -0.0548099 | 82.12006527 |
| 1999 | X8297 | Temp 1 | ETH | B516B | 1.26064193 | -0.1126745 | 26.58314868 |
| 1999 | X8297 | Temp 1 | warm | B516B | 3.73120214 | -0.0538359 | 101.5271073 |
| 1999 | X8297 | Temp 2 | 20-30 C | B516B | 0.86776676 | -0.0765852 | 33.98003551 |
| 1999 | X8297 | Temp 2 | ETH | B516B | 1.25594907 | -0.1646826 | 18.15947845 |
| 1999 | X8297 | Temp 2 | warm | B516B | 0.18829871 | -0.0704528 | 27.29344704 |
| 1999 | X8297 | Temp 3 | 20-30 C | B516B | -0.1969479 | -0.0797145 | 19.28950386 |

| Table 1. Genotype, storage temperature, test, lot (HTTL) data continued | | | | | | | |
|---|---|---|---|---|---|---|---|
| Year | Hybrid | Temp | Test | Lot | Intercept | Slope | Calculated days to 85% germ |
| 1999 | X8297 | Temp 3 | ETH | B516B | -2.251763 | -0.0663344 | -7.796285858 |
| 1999 | X8297 | Temp 3 | warm | B516B | -2.0376955 | -0.0346119 | -8.756943266 |
| 1999 | X8297 | Temp 1 | 20-30 C | B519B | 3.79883511 | -0.0578279 | 95.68800121 |
| 1999 | X8297 | Temp 1 | ETH | B519B | 1.51858921 | -0.1249329 | 26.03950013 |
| 1999 | X8297 | Temp 1 | warm | B519B | 6.73444763 | -0.1002996 | 84.43751207 |
| 1999 | X8297 | Temp 2 | 20-30 C | B519B | 0.54711546 | -0.0829094 | 27.52060099 |
| 1999 | X8297 | Temp 2 | ETH | B519B | 1.84885465 | -0.2055072 | 17.43712972 |
| 1999 | X8297 | Temp 2 | warm | B519B | 10.5579779 | -0.3974856 | 30.92584726 |
| 1999 | X8297 | Temp 3 | 20-30 C | B519B | 0.9813582 | -0.136925 | 19.8353789 |
| 1999 | X8297 | Temp 3 | ETH | B519B | -2.7590497 | -0.0369963 | -27.69057026 |
| 1999 | X8297 | Temp 3 | warm | B519B | 2.78907506 | -0.1412854 | 32.01800126 |
| 1999 | X8297 | Temp 1 | 20-30 C | B521B | 2.79309294 | -0.0517676 | 87.4619259 |
| 1999 | X8297 | Temp 1 | ETH | B521B | 1.33002722 | -0.1149054 | 26.67088122 |
| 1999 | X8297 | Temp 1 | warm | B521B | 4.94413716 | -0.0629901 | 106.0283793 |
| 1999 | X8297 | Temp 2 | 20-30 C | B521B | 2.44607746 | -0.1008396 | 41.45869793 |
| 1999 | X8297 | Temp 2 | ETH | B521B | -0.2261847 | -0.0884985 | 17.04454149 |
| 1999 | X8297 | Temp 2 | warm | B521B | 2.20048761 | -0.0993518 | 39.60762327 |
| 1999 | X8297 | Temp 3 | 20-30 C | B521B | 1.48304169 | -0.0721556 | 44.59311191 |
| 1999 | X8297 | Temp 3 | ETH | B521B | -0.5144862 | -0.0741741 | 16.44933818 |
| 1999 | X8297 | Temp 3 | warm | B521B | 1.9942351 | -0.0972428 | 38.34562719 |
| 2000 | SF270 | Temp 1 | 20-30 C | PL02562902 | -0.7499512 | -0.0540178 | 18.22824801 |
| 2000 | SF270 | Temp 1 | ETH | PL02562902 | -0.7386454 | -0.0655424 | 15.19559332 |
| 2000 | SF270 | Temp 1 | warm | PL02562902 | -0.4501568 | -0.0478751 | 26.82906679 |
| 2000 | SF270 | Temp 2 | 20-30 C | PL02562902 | -0.004663 | -0.07861 | 22.0065902 |
| 2000 | SF270 | Temp 2 | ETH | PL02562902 | 0.59388236 | -0.1033826 | 22.5229721 |
| 2000 | SF270 | Temp 2 | warm | PL02562902 | 1.00603232 | -0.1343343 | 20.40159048 |
| 2000 | SF270 | Temp 3 | 20-30 C | PL02562902 | 0.21422885 | -0.1134193 | 17.18252454 |
| 2000 | SF270 | Temp 3 | ETH | PL02562902 | -0.3043639 | -0.1038123 | 13.77714544 |
| 2000 | SF270 | Temp 3 | warm | PL02562902 | -1.1135622 | -0.0736214 | 8.435575191 |
| 2000 | SF270 | Temp 1 | 20-30 C | PL025629022 | -0.7668994 | -0.0389521 | 24.84337572 |
| 2000 | SF270 | Temp 1 | ETH | PL025629022 | -2.4851177 | -0.027655 | -27.1385516 |
| 2000 | SF270 | Temp 1 | warm | PL025629022 | -0.2871106 | -0.055425 | 26.11620127 |
| 2000 | SF270 | Temp 2 | 20-30 C | PL025629022 | -2.9771478 | -0.0232884 | -53.35474934 |
| 2000 | SF270 | Temp 2 | ETH | PL025629022 | -1.7491596 | -0.0794524 | -0.183236058 |
| 2000 | SF270 | Temp 2 | warm | PL025629022 | -0.0938528 | -0.0828956 | 19.79294746 |
| 2000 | SF270 | Temp 3 | 20-30 C | PL025629022 | -0.8445349 | -0.062635 | 14.2103641 |
| 2000 | SF270 | Temp 3 | ETH | PL025629022 | -0.9317895 | -0.0941022 | 8.531272971 |
| 2000 | SF270 | Temp 3 | warm | PL025629022 | -0.8048079 | -0.0690495 | 13.46560302 |
| 2000 | SF270 | Temp 1 | 20-30 C | PL02562904 | -0.584889 | -0.0550158 | 20.89785217 |
| 2000 | SF270 | Temp 1 | ETH | PL02562904 | -1.9456999 | -0.0567086 | -3.722519064 |
| 2000 | SF270 | Temp 1 | warm | PL02562904 | -0.2998601 | -0.0422793 | 33.9348323 |
| 2000 | SF270 | Temp 2 | 20-30 C | PL02562904 | 2.25171163 | -0.1225184 | 32.53644094 |
| 2000 | SF270 | Temp 2 | ETH | PL02562904 | 0.14335831 | -0.0773468 | 24.27972929 |

Table 1. Genotype, storage temperature, test, lot (HTTL) data continued

| Year | Hybrid | Temp | Test | Lot | Intercept | Slope | Calculated days to 85% germ |
|---|---|---|---|---|---|---|---|
| 2000 | SF270 | Temp 2 | warm | PL02562904 | 0.42701742 | -0.0963389 | 22.43764954 |
| 2000 | SF270 | Temp 3 | 20-30 C | PL02562904 | -1.2614042 | -0.0521851 | 9.067662137 |
| 2000 | SF270 | Temp 3 | ETH | PL02562904 | -1.2172894 | -0.0772401 | 6.697449322 |
| 2000 | SF270 | Temp 3 | warm | PL02562904 | -0.1139788 | -0.0760878 | 21.29937067 |
| 2000 | X8297 | Temp 1 | 20-30 C | East 1 | 2.95515588 | -0.0454348 | 103.2194911 |
| 2000 | X8297 | Temp 1 | ETH | East 1 | 1.92568304 | -0.0634548 | 57.68332885 |
| 2000 | SF270 | Temp 2 | warm | PL02562904 | 0.42701742 | -0.0963389 | 22.43764954 |
| 2000 | SF270 | Temp 3 | 20-30 C | PL02562904 | -1.2614042 | -0.0521851 | 9.067662137 |
| 2000 | SF270 | Temp 3 | ETH | PL02562904 | -1.2172894 | -0.0772401 | 6.697449322 |
| 2000 | SF270 | Temp 3 | warm | PL02562904 | -0.1139788 | -0.0760878 | 21.29937067 |
| 2000 | X8297 | Temp 1 | 20-30 C | East 1 | 2.95515588 | -0.0454348 | 103.2194911 |
| 2000 | X8297 | Temp 1 | ETH | East 1 | 1.92568304 | -0.0634548 | 57.68332885 |
| 2000 | X8297 | Temp 1 | warm | East 1 | 4.61231811 | -0.0521719 | 121.6539778 |
| 2000 | X8297 | Temp 2 | 20-30 C | East 1 | 2.03818884 | -0.0789168 | 47.80718295 |
| 2000 | X8297 | Temp 2 | 20-30 C | East 1 | 2.17997563 | -0.0854086 | 45.83351894 |
| 2000 | X8297 | Temp 2 | ETH | East 1 | 2.00451159 | -0.0778782 | 48.01231468 |
| 2000 | X8297 | Temp 2 | warm | East 1 | 1.60504275 | -0.0594881 | 56.13969526 |
| 2000 | X8297 | Temp 3 | ETH | East 1 | 1.27730145 | -0.0897447 | 33.56078415 |
| 2000 | X8297 | Temp 3 | warm | East 1 | 1.39609824 | -0.0710887 | 44.03933811 |
| 2000 | X8297 | Temp 1 | 20-30 C | West 1 | 2.8793487 | -0.0423125 | 109.0446028 |
| 2000 | X8297 | Temp 1 | ETH | West 1 | 1.69798427 | -0.0760158 | 45.15620865 |
| 2000 | X8297 | Temp 1 | warm | West 1 | 5.79521779 | -0.0635725 | 118.4445923 |
| 2000 | X8297 | Temp 2 | 20-30 C | West 1 | 2.5438057 | -0.0826488 | 51.76610859 |
| 2000 | X8297 | Temp 2 | 20-30 C | West 1 | 4.94778604 | -0.1665945 | 40.11169093 |
| 2000 | X8297 | Temp 2 | ETH | West 1 | 2.8011261 | -0.1003504 | 45.19889463 |
| 2000 | X8297 | Temp 2 | warm | West 1 | 5.60816038 | -0.1171624 | 62.67165435 |
| 2000 | X8297 | Temp 3 | ETH | West 1 | 2.87700889 | -0.1123074 | 41.06238721 |
| 2000 | X8297 | Temp 3 | warm | West 1 | 2.23595794 | -0.0925685 | 42.89319796 |
| 2000 | X8297 | Temp 1 | 20-30 C | West 2 | 4.68910065 | -0.0623008 | 103.1078526 |
| 2000 | X8297 | Temp 1 | ETH | West 2 | 1.94209195 | -0.0663574 | 55.40743015 |
| 2000 | X8297 | Temp 1 | warm | West 2 | 8.90683705 | -0.1005846 | 105.7958982 |
| 2000 | X8297 | Temp 2 | 20-30 C | West 2 | 1.87002329 | -0.0738555 | 48.80644428 |
| 2000 | X8297 | Temp 2 | ETH | West 2 | 1.53526759 | -0.0779532 | 41.94656083 |
| 2000 | X8297 | Temp 2 | warm | West 2 | 9.89444622 | -0.195215 | 59.57045962 |
| 2000 | X8297 | Temp 3 | 20-30 C | West 2 | 1.83824206 | -0.0939325 | 38.0362826 |
| 2000 | X8297 | Temp 3 | ETH | West 2 | 1.50987419 | -0.0884217 | 36.69320139 |
| 2000 | X8297 | Temp 3 | warm | West 2 | 1.21399076 | -0.0791023 | 37.27567739 |

| Table 2. 1999 SF270 HTT data for 85% germination target | | | | |
|---|---|---|---|---|
| Year | Hybrid | Temp | Test | Calculated days to 85% germ |
| 1999 | SF270 | Temp 1 | warm | 7.237699352 |
| 1999 | SF270 | Temp 1 | ETH | -12.57735592 |
| 1999 | SF270 | Temp 1 | 20-30 C | 3.514253066 |
| 1999 | SF270 | Temp 2 | warm | -6.197425254 |
| 1999 | SF270 | Temp 2 | ETH | -25.04070041 |
| 1999 | SF270 | Temp 2 | 20-30 C | -5.750823821 |
| 1999 | SF270 | Temp 3 | warm | -32.96609004 |
| 1999 | SF270 | Temp 3 | ETH | -23.4196418 |
| 1999 | SF270 | Temp 3 | 20-30 C | -4.816112497 |

| Table 3. 1999 X8927 HTT data for 85% germination target | | | | |
|---|---|---|---|---|
| Year | Hybrid | Temp | Test | Calculated days to 85% germ |
| 1999 | X8297 | Temp 1 | 20-30 C | 88.00417871 |
| 1999 | X8297 | Temp 1 | ETH | 24.82505253 |
| 1999 | X8297 | Temp 1 | warm | 97.34921185 |
| 1999 | X8297 | Temp 2 | 20-30 C | 36.58304307 |
| 1999 | X8297 | Temp 2 | ETH | 16.7220833 |
| 1999 | X8297 | Temp 2 | warm | 33.57309741 |
| 1999 | X8297 | Temp 3 | 20-30 C | 28.82941649 |
| 1999 | X8297 | Temp 3 | ETH | 1.343185115 |
| 1999 | X8297 | Temp 3 | warm | 29.03501213 |

| Table 4. 2000 SF270 HTT data for 85% germination target | | | | |
|---|---|---|---|---|
| Year | Hybrid | Temp | Test | Calculated days to 85% germ |
| 2000 | SF270 | Temp 1 | 20-30 C | 19.92001534 |
| 2000 | SF270 | Temp 1 | ETH | -2.464205814 |
| 2000 | SF270 | Temp 1 | warm | 28.82759033 |
| 2000 | SF270 | Temp 2 | 20-30 C | 15.54304162 |
| 2000 | SF270 | Temp 2 | ETH | 18.97645671 |
| 2000 | SF270 | Temp 2 | warm | 20.82965943 |
| 2000 | SF270 | Temp 3 | 20-30 C | 14.03119814 |
| 2000 | SF270 | Temp 3 | ETH | 10.02120476 |
| 2000 | SF270 | Temp 3 | warm | 15.3377929 |

| Table 5. 2000 X8927 HTT data for 85% germination target | | | | |
|---|---|---|---|---|
| Year | Hybrid | Temp | Test | Calculated days to 85% germ |
| 2000 | X8297 | Temp 1 | 20-30 C | 105.515411 |
| 2000 | X8297 | Temp 1 | ETH | 53.15256995 |
| 2000 | X8297 | Temp 1 | warm | 116.0424522 |
| 2000 | X8297 | Temp 2 | 20-30 C | 49.54187401 |
| 2000 | X8297 | Temp 2 | ETH | 45.31334276 |
| 2000 | X8297 | Temp 2 | warm | 60.81303522 |
| 2000 | X8297 | Temp 3 | 20-30 C | 41.9248309 |
| 2000 | X8297 | Temp 3 | ETH | 37.60159651 |
| 2000 | X8297 | Temp 3 | warm | 41.69646158 |

| **Table 8. Least squares means table for genotype, storage** | | | |
|---|---|---|---|
| Level | Least Sq Mean | Std Error | Mean |
| SF270 | -1.140029 | 0.7384943 | -1.14 |
| X8297 | 3.820139 | 0.69079816 | 3.7108 |
| | | | |
| Temp 1 (10C) | 2.433919 | 0.41628799 | 2.6225 |
| Temp 2 (14C) | 2.080411 | 0.41628799 | 2.2441 |
| Temp 3 (Ambient Temp) | -0.494166 | 0.41628799 | -0.4507 |
| | | | |
| SF270,Temp 1 | -0.78311 | 0.60996702 | |
| SF270,Temp 2 | -0.812119 | 0.60996702 | |
| SF270,Temp 3 | -1.82486 | 0.60996702 | |
| X8297,Temp 1 | 5.650949 | 0.56667717 | |
| X8297,Temp 2 | 4.972941 | 0.56667717 | |
| X8297,Temp 3 | 0.836528 | 0.56667717 | |

## APPENDIX B. FIGURES

**Figure 2. Genotype vs. Storage Temperature (standard warm germination test)**

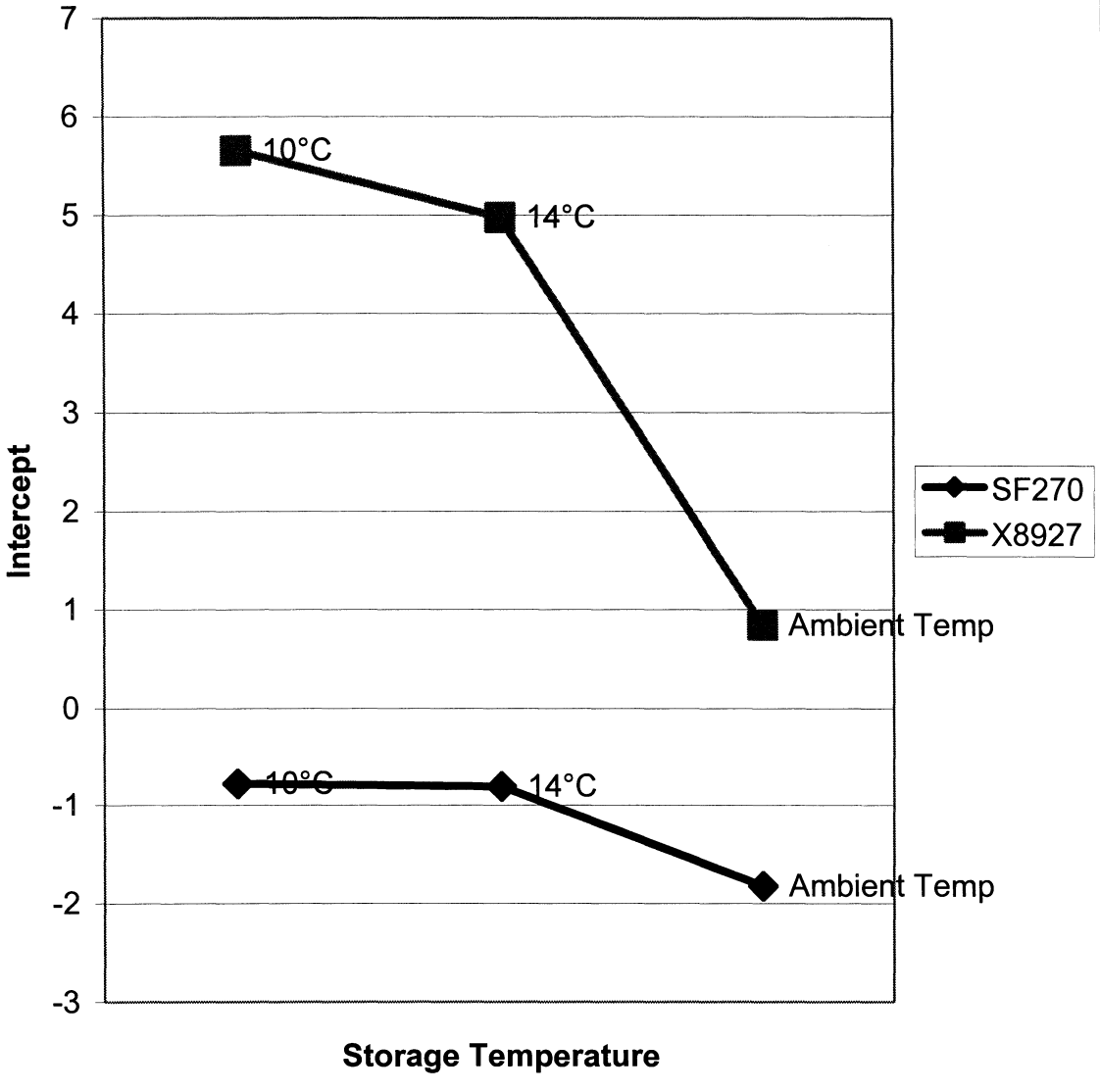


**Figure 3. Genotype vs Production Year**
**(alternating 20-30C warm germinaiton test)**

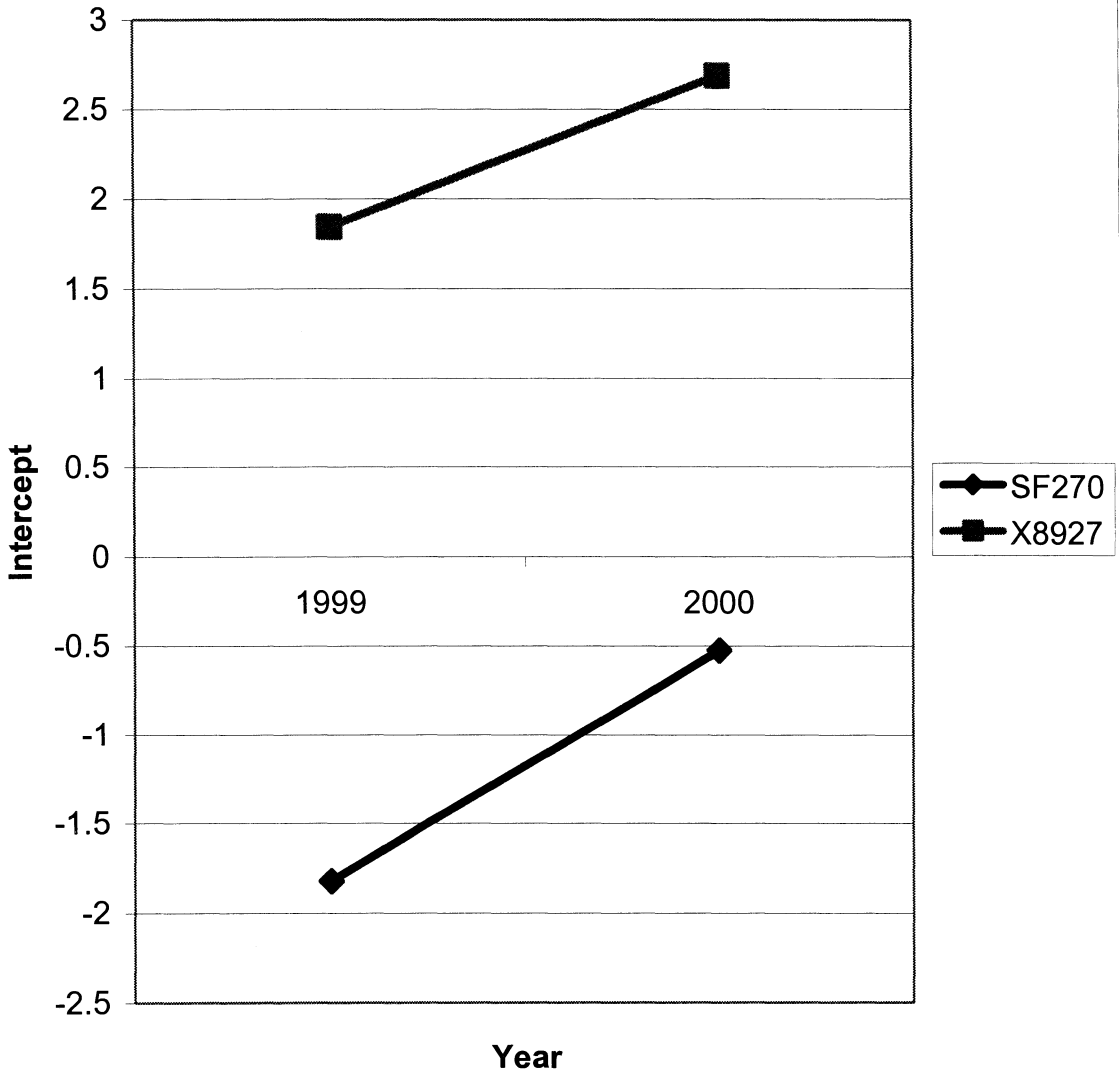


**Figure 4. Genotype vs Production Year (ethrel warm germination test)**

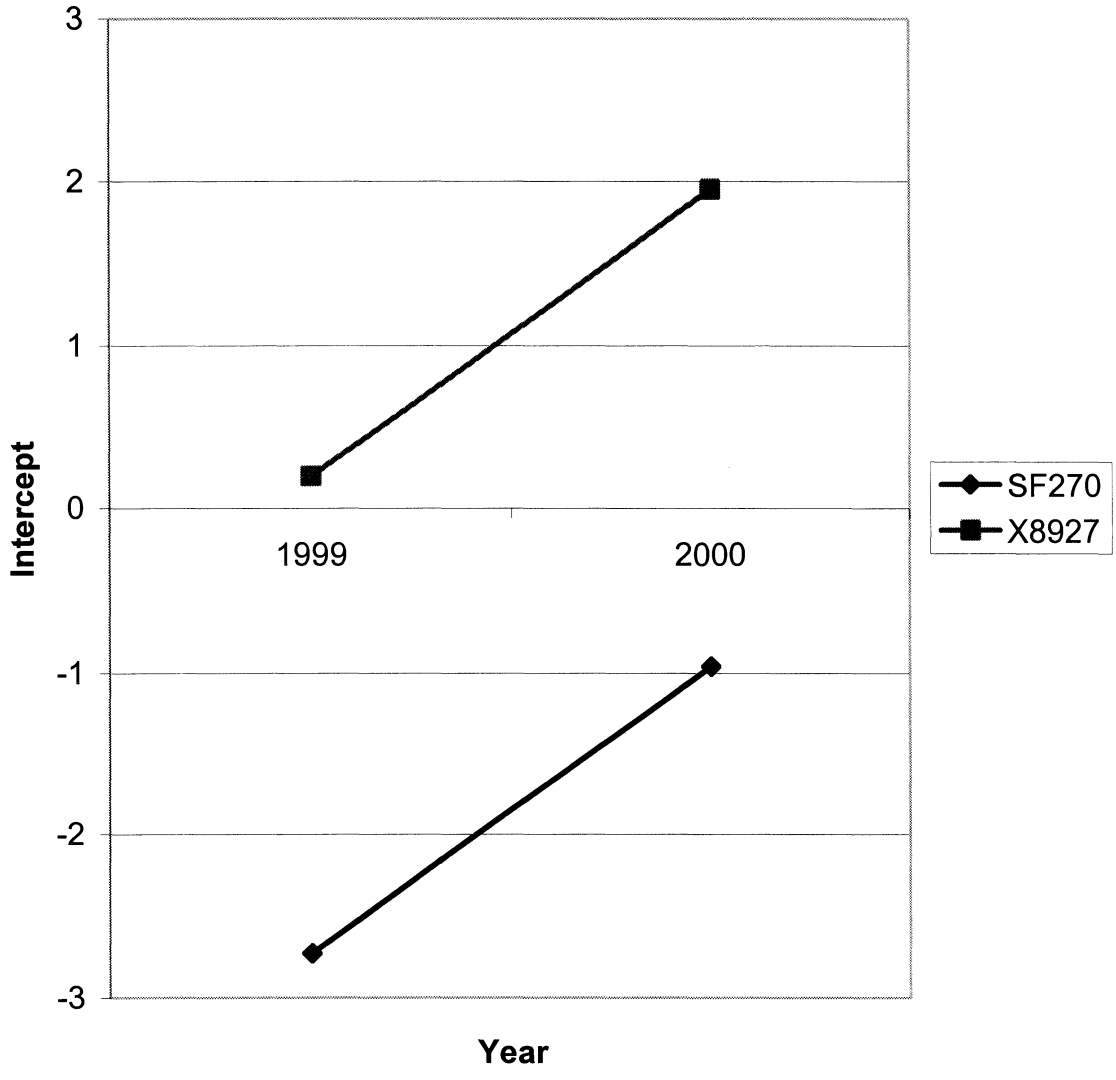


Figure 5: SF270 Standard warm germination test comparison

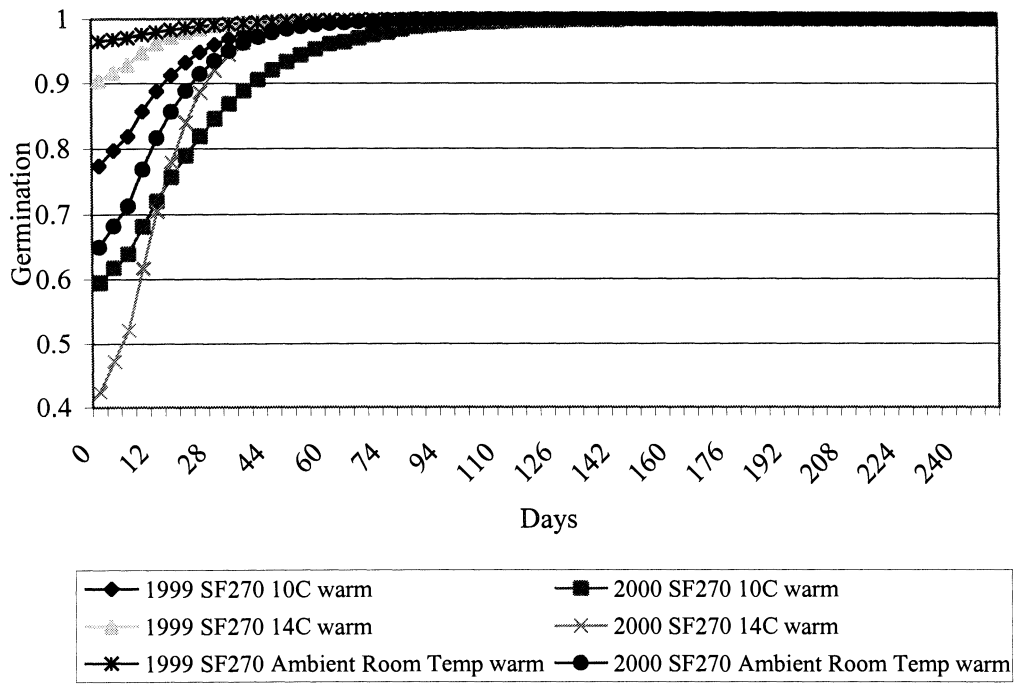



Figure 6: X8927 Standard warm germination test comparison

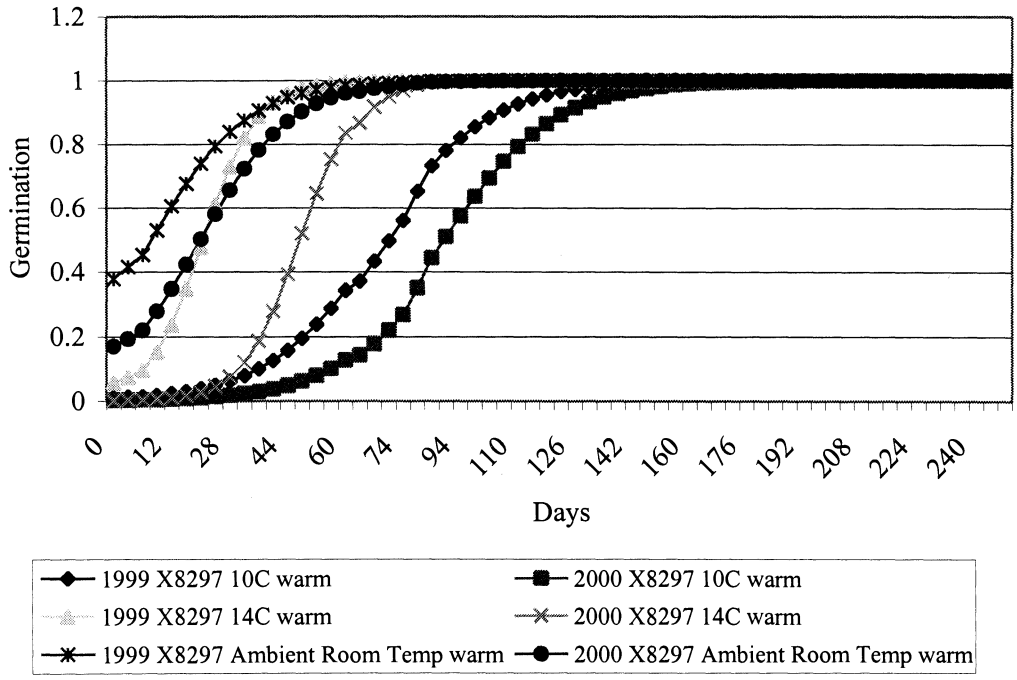


Figure 7: SF270 Alternating 20-30C warm germination test comparison

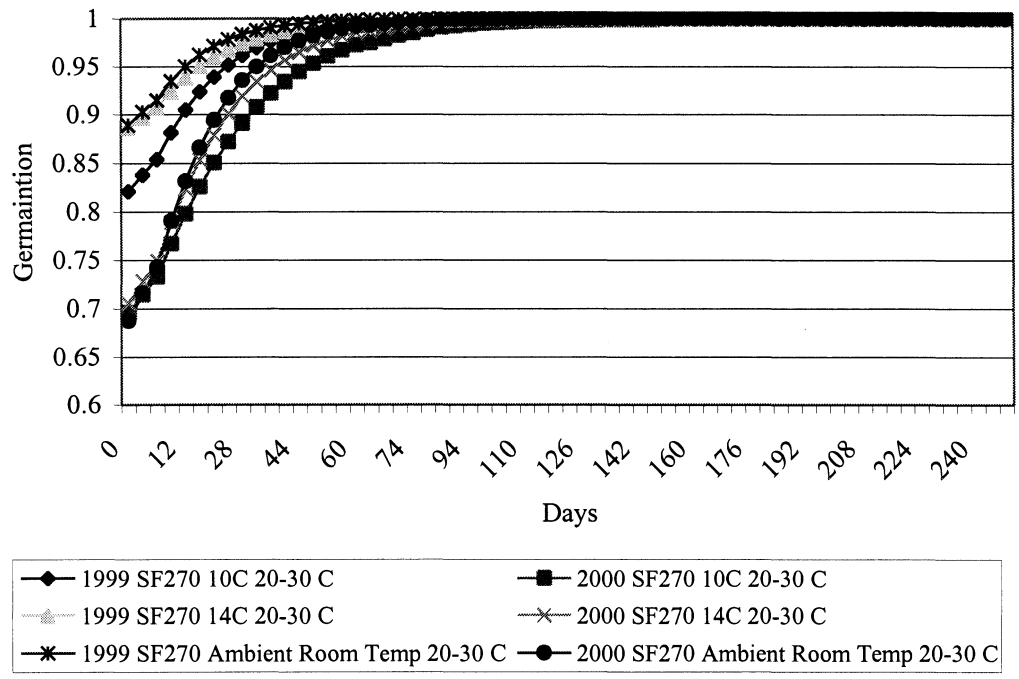



Figure 8: X8927 Alternating 20-30C warm germination test comparisons

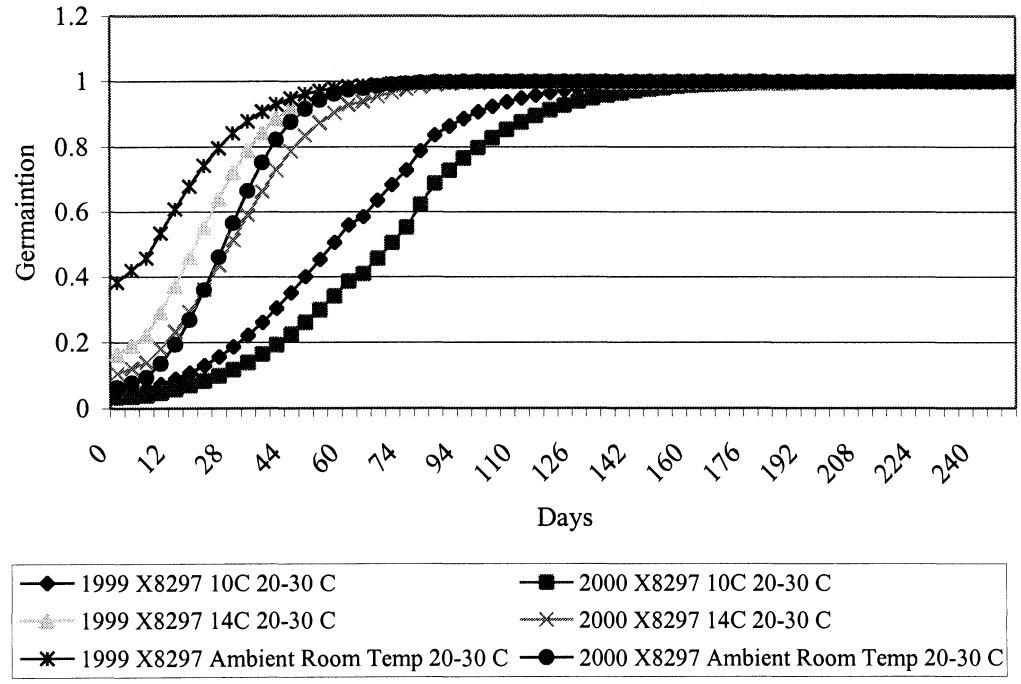


Table 9: SF270 Ethrel warm germinaiton test comparison

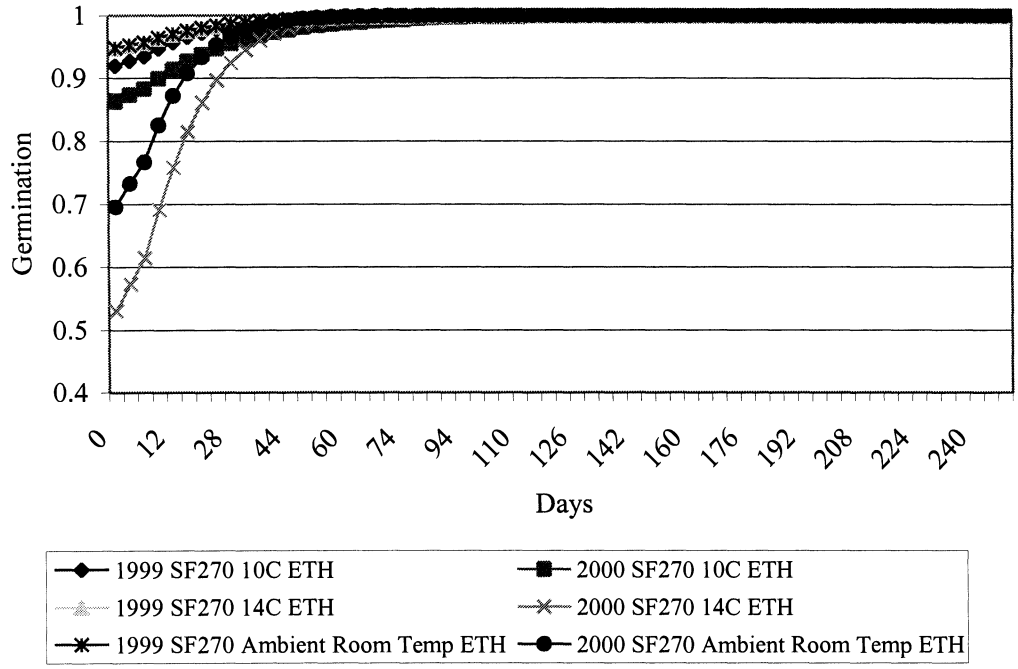
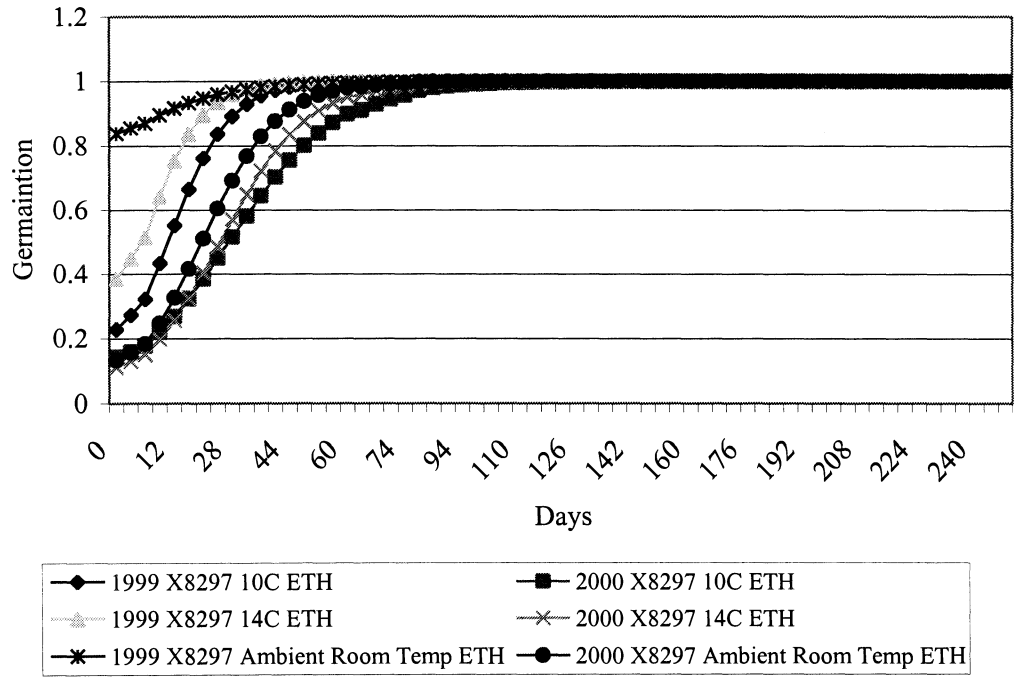



Figure 10: X8927 Ethrel warm germination test comparison



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## ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. Allen Knapp for his encouragement, advice, guidance and extreme patience through these long years of research and preparation of this manuscript.

I also thank: Cargill Hybrid Seeds Sunflower Production Group for the materials necessary for this research, The Cargill Hybrid Seeds Quality Control Lab and the Mycogen Seeds Quality Control Lab for their assistance in preparing and testing the samples in this study, Cargill Hybrid Seeds and Dow AgroSciences for funding my research, Dr. Russell Mullen for his advice and participation on my graduate committee, Dr. Philip Dixon for his assistance and guidance in analyzing and interpreting the data in this study.

Special thanks is extended to Megan Reschly for her consistent and persistent encouragement during the time that it took to complete this.