CHARACTERIZATION OF SELECTED WINTER HARDINESS TRAITS IN PEA (*PISUM SATIVUM* L.)

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MASTER OF SCIENCE

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ABSTRACT

Pea (*Pisum sativum* L.) is an important crop from an agronomic and nutritional standpoint. Winter pea has further agronomic benefits for producers; however, sufficient winter hardiness to survive harsh North Dakota conditions is lacking. Winter hardiness was evaluated in the field and greenhouse using replicated trials with 267 recombinant inbred lines derived from the cross 'Medora'/'Melrose'. Similar reactions were observed between the two trials. An optimum protocol based on acclimation time and scoring method to predict winter hardy genotypes using controlled environment conditions was studied. Twelve genotypes were acclimated for 0, 1, 2, 3, and 4 weeks at 4^0C prior to being frozen at -8 or -12^0C for 1 hr. Three weeks of acclimation and scoring 21 days after freezing provided the best differentiation among genotypes. This research provided direction for development of winter pea varieties suited to the harsh winter conditions of North Dakota.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Importance of Pea

United States and North Dakota Production

Pea (*Pisum sativum* L.) is an important crop worldwide and is grown in many countries including the United States (US). In 2011 the US ranked ninth in world dry pea production with 255,150 metric tons (MT) (FAOSTAT, 2011). Dry pea is a widely grown pulse crop in the US, and in 2008 North Dakota produced 64% of the nation's dry peas (North Dakota Farm Bureau, 2009). Within the US, North Dakota ranked first in pulse production through 2010, but in 2011, due to wet conditions, North Dakota ranked third behind Montana and Washington, respectively, with 106,350 MT of pea, lentil, and chickpea produced (USA Dry Pea and Lentil Council, 2011). North Dakota produced 60,306 MT of pea, 912 MT of chickpeas (*Cicer arietinum* L.), and 45,132 MT of lentils (*Lens culinaris* Medik.) in 2011 (USA Dry Pea and Lentil Council, 2011).

Until the 1990's, peas were produced entirely in the Palouse region of Washington and Idaho (Schatz and Endres, 2009) when Minnesota, Montana, North Dakota, and South Dakota started producing pea. The main production region of pea in the United States includes the Northern Great Plains and the Pacific Northwest. The major region for pea production in North Dakota is the Northwest corner due to economics and environment. The eastern portion of North Dakota grows more corn (*Zea mays* L.), soybeans (*Glycine max* (L.) Merr.), and sugar beets (*Beta vulgaris* L.).

Pea Market Classes

Pea has many uses and predominantly is grown for human or animal consumption. Human consumption can either be immature seed or pods harvested fresh or as mature dried seed. Fresh peas are canned, frozen, or eaten fresh whereas dry pea is used in soups or animal

feed. Pea is used for both human and animal consumption because it is a rich source of nutrition for animal feed and is rich in lysine, starch, and provides essential amino acids and energy required by animals (Saskatchewan Pulse Growers, 2009). Pea can be mixed with cereal grains to increase the levels of lysine and tryptophan. Pea is a good livestock feed because of the high levels of digestible nutrients, 80-86% (Schatz and Endres, 2009). Pea provides a range of minerals including calcium, iron, potassium, phosphorus, sodium (Muehlbauer and McPhee, 1997), and selenium (Thavarajah et al., 2010).

The market classes of pea include Austrian winter, marrowfat, yellow cotyledon, and green cotyledon. Marrowfat peas are larger seeded, irregularly shaped peas and are used to produce dried, processed snacks, for example, wasabi peas. Spring dry pea, which includes the yellow and green cotyledon types, is planted in the spring and harvested in the late summer/early fall and is the most widely grown type. A third type of pea is the winter pea, also known as fall-sown pea, is planted in the fall and harvested in late summer.

Spring-sown pea is the most widely grown type of pea, partly because winter pea varieties are not adapted to harsh climates. Adapting winter pea to harsher environments is important because growers would have more options when it comes to winter crops which are beneficial for soil health. In 2011, production of Austrian winter pea was 771 MT, 3770 MT, and 177 MT in North Dakota, Montana, and Washington, respectively (Table 1.1). In comparison, those same states produced 59,534 MT, 134,343 MT, and 58,581 MT of spring-sown pea (Table 1.1).

Winter pea is generally used for pigeon feed and green manure due to pigmentation of the seeds. This pigmentation is indicative of the ‘Austrian Winter’ type. However, some newly developed varieties are more suited for human consumption markets. ‘Specter’ (McPhee and

Muehlbauer, 2007) and 'Windham' (McPhee et al., 2007) are two such varieties, although they were released as a winter feed pea. They were developed by scientists working with the USDA-ARS in Pullman, Washington, who began making crosses in the early 1990's, to combine winter hardiness with the edible seed qualities of spring types.

Table 1.1. Production of spring and winter pea in 2011 by state.

State	Green	Yellow	Total spring	Austrian winter	% of total production*
	----------MT----------				
Idaho	17746	3351	21097	3672	14.8
Washington	54769	3812	58581	177	0.3
Oregon	3402	0	3402	848	20.0
North Dakota	23814	35720	59534	771	1.3
Montana	34952	99391	134343	3770	2.7
Others	1191	2347	3538	0	0.0
Total	135874	144621	260495	9238	3.4

Source: USA Dry Pea and Lentil Council (2011)
* % of total production is the percentage of winter pea from all production

Agronomic Benefits

Pea is a cool season legume which fits well into cereal-based rotations. As a broadleaf crop, when used in rotations with cereals it can help break disease cycles of cereal pathogens, improve soil tilth, and allow control of grassy weeds. Another benefit of pea is the ability to fix nitrogen and; therefore, does not require nitrogen fertilizer which reduces input costs for the grower. Nitrogen is an extensively used input in many rotations and legumes in general reduce the need for the fossil fuel inputs required for making fertilizer (Pulse Canada, 2011). Production of legumes is beneficial for the environment by keeping nitrates out of ground water (Brewin et al., 1993). Atmospheric nitrogen fixed by legumes, including pea, are available to subsequent crops, typically a cereal crop.

Origin and Domestication of Pea Production

Pea was domesticated in the Fertile Crescent in 7000-6000 B.C. (Zohary and Hopf, 1973; Smartt 1990; Muehlbauer and McPhee, 1997). Carbonized pea seeds from this era have since been discovered; however, these remains do not provide enough information to determine whether cultivation occurred during this period (Zohary and Hopf, 1973; Smartt, 1990). Based on archeological evidence, pulse crops, such as pea, lentil, and chickpea, were domesticated along with or shortly after wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Zohary and Hopf 1973). Although crops were likely gathered before domestication, the necessity for increased and stable food supplies may have led to domestication.

One difference between wild and domesticated pea is the seed coat (Zohary and Hopf, 1973). The wild relatives of *P. sativum* have a rough seed coat, while cultivated pea has a smooth seed coat. However, wild pea is more genetically diverse which may offer breeders disease tolerance and environmental adaptations that may be lacking in current cultivated varieties.

Another difference between wild and domesticated pea is seed size (Zohary and Hopf, 1973). Carbonized pea seed was smaller than currently cultivated varieties in the 1980's. Seed dormancy and pod shatter were also a problem in wild pea (Smartt, 1990). Pod shatter of earlier maturing pods causes harvest to be earlier which can lead to immature seed of later maturing pods. Less pod shatter in cultivated varieties makes it easier for producers to harvest and have uniform yield across the field. Pod shatter causes a loss of seeds which equals lower yield and potential weed problems the subsequent year. Current cultivated varieties were bred to avoid these problems.

Pea production spread all over the world in part due to domestication and breeding efforts. Pea was historically a winter crop in the Mediterranean basin and was adapted to cooler

environments (Smartt, 1990). However, the current climates where winter peas are grown are colder than the Mediterranean basin. Breeding efforts to adapt pea to new environments is a major reason that pea production has spread. Breeding for winter hardiness promises to expand and increase pea production.

Winter Hardiness

Levitt (1956) defined winter hardiness as the survivability of plants in severe winters. During this time, research on winter hardy plants was primarily in the field; however, only some winters were severe enough to show differential survival. Winter hardiness in pea for North America's harsher climates has not been well studied, although, research in milder climates and other crops, such as winter wheat, lend valuable insight into the hardiness of pea.

Environmental conditions such as, snow cover, temperature, and acclimation are important to winter survival. Étévé (1985) studied snow cover, soil temperature, air temperature, and acclimation time. Acclimation time, a main factor of survival, varies depending on the crop and location. Acclimation is described as the increase in freezing stress resistance (tolerance) of plants when exposed to chilling (Palta and Simon, 1993; Levitt, 1980). Acclimation is witnessed in the fall when the weather gets cooler but before freezing occurs.

A study conducted in Sweden by Lööf and Andersson (1963) discovered that plant stands are impacted by environmental variations. The amount of light during acclimation and deacclimation can play a role in the hardiness of rape (*Brassica napus* L.) and turnip (*Brassica rapa* ssp. *rapa*) (Lööf and Andersson, 1963). Sugar and water content decreased when acclimated under low light conditions; however, the roots did not show this decrease. The researchers did not mention whether low light was a factor in lower hardiness.

Survival of winter crops require tolerance to other environmental factors, such as frost-heaving, water-logging, and diseases (Murray et al., 1988), drainage (Markarian and Andersen, 1966), and tillage. Spring soil drainage affects winter pea, mainly by drowning (Markarian and Andersen, 1966). In their study, either a hillside or ridge was used to facilitate drainage and avoid plant submersion. Plants may be able to survive the winter, but can be killed by a lack of respiration in the spring. They concluded that the survival of winter pea is dependent on more than winter conditions.

Winter crops have shown adaptive mechanisms, especially winter wheat. Winter wheat needs about twelve weeks of growth, including vernalization, for full winter hardiness (Fowler, 2002). Winter wheat needs to be exposed to cool, above freezing temperatures for full vernalization. Winter peas also vernalize, but it is not a requirement. Trevino and Murray (1975) noted that vernalization will reduce the time and number of nodes present before flowering. Winter pea can be planted in the spring and will flower and produce seed; however, winter lines will mature later than spring lines.

Different overwintering conditions, with respect to growth, acclimation, and environment, result in differential survivability across varieties. Pea is similar to wheat, with respect to acclimation, in the fact that both crops require some amount of acclimation for full survival. However, wheat and pea are also different. They have different acclimation and temperature requirements. Wheat is also better adapted to the cold temperatures due to breeding efforts, whereas most pea varieties do not currently have enough hardiness to survive harsh North Dakota winters. Warm fall temperatures do not induce full acclimation and may cause plant and stand death. Both pea and wheat require cool temperatures in the fall to start hardening required for survival.

Winter wheat has a higher susceptibility to injury or death under certain conditions (Fowler, 2002). In late fall, soil temperatures are warmer than air temperature but as the seasons change the soil temperature usually becomes colder. Snow cover is important to buffer soil temperatures and keep the crown alive. Winter pea and winter wheat benefit similarly from snow cover to keep the growing point alive. However, the amount of snow cover required is not known and may be based on air temperature, which indicates that colder climates, such as North Dakota, would need more snow cover.

In a study conducted by Markarian and Andersen (1966) in Michigan, it was determined that snow cover was important for survival. Most of the field had little snow cover and the stands did not survive; however, where the snow was deeper due to drifting the stands had better survivability. The authors discussed that air temperature alone cannot determine survivability if adequate snow cover is present; therefore, temperature and snow cover need to be considered together when conducting field studies.

Winter pea has greater yield potential than spring sown pea. Regrowth or branching habit in pea has the potential to increase seed production. Earlier spring growth and flower initiation enables the pea crop to avoid heat and water stress later in the summer which also favors yield potential (Chen et al., 2006). Survival may differ across a field with full survival in some locations and little or no survival in others. The difference in survival may be related to drifting snow or other overwintering conditions (Skinner and Mackey, 2009).

Even with adequate winter conditions, other conditions throughout the growing season must also be met. Fall emergence and stand establishment are critical factors for winter survival of winter wheat (*Triticum aestivum* L.), (Lindstrom et al., 1976). Sowing date and soil moisture are important to obtain satisfactory stands. Low soil moisture in the fall may reduce emergence,

but high soil moisture may cause the seed to rot. Some findings in winter wheat are applicable to pea because pea requires adequate growth for good overwintering, and sowing dates play a role in the survival of pea varieties.

Methods for Assessing Cold Tolerance

Cold or freezing tolerance can be assessed in the laboratory and field (Murray et al., 1988). Laboratory tests can predict cold and freezing tolerance, but these controlled tests do not assess other factors affecting survival in the field. Field experiments may better reflect the overwintering conditions, but the plants may be killed due to other factors, such as disease, weed pressure, and temperature or water stress. Field testing should also include a control variety to help understand the survivability level of plants across different locations in the field (Murray et al., 1988).

Laboratory tests or controlled tests can be conducted on whole plants or parts of plants (Murray et al., 1988). Standard procedures must be followed for all plants, and injury is assessed after the plants have thawed. A good screening temperature for non-hardy pea plants is -9^0C (Swensen, 1980; Auld et al, 1983). Genotypes that are winter hardy, but have varying degrees of hardiness may be harder to differentiate. Percent survival was calculated four weeks after freezing (Auld et al., 1983). It was determined that spring lines survived at low freezing temperatures, but had lower survival at colder temperatures. Differences were noticed when the temperature changed by 3^0C. Controlled freezing tests are quicker and can be replicated over time. Artificial freezing tests may be inconclusive if incorrect temperatures are used and all or none of the plants are killed when there should be differentiation between varieties with high or low levels of winter hardiness (Dexter, 1956).

Genetics of Winter Hardiness and Survival

Skinner and Mackey (2009) concluded that the genetics controlling increased freezing tolerance is complex. The authors studied wheat and determined that complementary gene action may be involved in increased freezing sensitivity. Freezing tolerance is the ability to withstand cold temperatures, and freezing sensitivity is sensitivity to cold temperatures. The study used saturated soil which may yield slightly different results than studies in dry soil due to different stresses on the plant.

Palta and Simon (1993) noted that differences in freezing protocols may have an effect on the inheritance of winter hardiness. Harsh or moderate freezing stress plays a role in the determination of winter hardiness inheritance. Inheritance of freezing resistance, the ability to resist freezing, and freezing sensitivity have both been shown to be partially dominant. These results are conflicting and not conclusive, which indicates the need for a protocol that uses both moderate and harsh freezing stress.

Palta and Simon (1993) noted differences among above and below ground tissue in a study with carrots in Europe. In Europe, carrots are often left in the field and harvested throughout the winter and into early spring. The reasoning behind this is to allow for harvest for the fresh market during the winter. Leaving crops in the field poses some problems regarding freeze-thaw cycles. It was observed that there was a correlation with the depth of the crown and the injury (injury was indicated by cracks). It was indicated that breeding of carrots for reduced damage was possible, when factors, including temperature, are taken into account. Damage to carrot tissue left in the field over the winter is not indicative of injury to pea since the crops have different growth habits. However, this study looked at damage to the crown and crown damage is noticed in other winter crops, such as pea.

Physiology of Winter Hardiness

Understanding plant physiology is important for winter hardiness. Physiological responses to cold stress have not been studied as extensively as responses to heat or water stress. Heat and water stress are more common worldwide during a normal crop cycle. A physiological response to freezing winter crops versus spring crops is the amount of soluble sugar in the plants (Bourion et al., 2003). Spring pea has a lower accumulation of soluble sugars (glucose, sucrose, and fructose) in the leaves compared with winter pea; however, this trend was not noticed in all parts of the plant. The sugar content increases in winter pea during cold treatment. Eventually, the sugar, mainly sucrose and fructose, will stabilize, but the spring varieties will not survive long enough to show this increase. Sugar concentrations in wheat show similar trends. A study conducted in China by Zeng et al. (2011) used two varieties of wheat Dongnongdongmai 1 and Jimai 22. Dongnongdongmai 1 had more winter hardiness while Jimai 22 was not winter hardy. Above freezing, Dongnongdongmai 1 had a higher concentration of sucrose and fructose in the tillers and the sugar concentration had a slower decrease. In comparison, Jimai 22 had a lower sugar concentration.

Palta and Simon (1993) recognized two survival mechanisms in some plants for freezing stress, i.e. avoidance and tolerance. Avoidance is the plant's ability to avoid extracellular and intercellular ice formation, and tolerance is the ability to survive ice formation. Both mechanisms can be used by the same plant. Acclimation is important for survival and deacclimation, adjustment to gradual warming temperatures, is important for continued survival and recovery. Winter plants have evolved to either avoid or tolerate ice. These mechanisms tend to be seasonal in herbaceous plants.

Ice crystals in winter hardy plants are not always lethal because they can occur extracellularly in the apoplast (Nilsen and Orcutt, 1996). Ice formation in the cell harms the plant, while ice formation intercellularly is not as harmful (Dexter, 1956). The physiology of the plant allows for the reduced lethality. Ice formation and injury have been studied, and two mechanisms have been mentioned. The first mechanism is sugar concentration in the winter lines of wheat and other crops. The second mechanism is the osmotic potential and a lower freezing point inside the plant. Plants with a greater freezing tolerance and subsequent winter hardiness tend to have a higher osmotic concentration.

Sugar concentration is linked to plant protection (Dexter, 1956). The higher sugar concentration helps inhibit the formation of ice in the cell. The osmotic potential is increased which reduces the freezing point inside the plant. Sugar is not the only compound which contributes to increased protection, nitrogen compounds have been linked to increased protection and increase when exposed to low temperatures. Hydrophilic colloids inside the plant cell can bind with water and decrease the ice formation. These colloids are typically found during acclimation.

Agronomic Management

The emergence type for pea is active epicotyl which means that the cotyledons stay below ground and the plant is better able to re-grow when injured. This re-growth is also known as branching. Winter plants show damage or death of the main stem but branching would indicate survival of the plant.

Limited research has been conducted on the best agronomic practices for winter pea. Muehlbauer (1998) recommended, based on a study in the Pacific Northwest, that stubble or crop residue is necessary to capture snow and minimize plant death. Similar conclusions have been

reached elsewhere in other crops. For example, Lööf and Andersson (1963) had similar results in winter rape (*Brassica napus* L.). Based on these studies, a conclusion can be reached that crop stubble or residue is beneficial to winter survival; however, it is mentioned that proper equipment is necessary for planting into stubble which may be an issue for some growers. Standing stubble decreases tillage which in turn decreases erosion, and increases snow cover. Snow cover is necessary for survival of the pea seedling because it serves as a means of insulation and buffers soil temperatures.

Another recommendation for increased winter survival is to optimize the planting date (Muehlbauer, 1998). Planting date is essential to the survival of the crop and differs among climates. Early September is the target planting date in North Dakota and Montana, but in milder climates, such as the Pacific Northwest, late September to early October is adequate. Cooler overall temperatures, early onset of cooler temperatures, and harsher winters of North Dakota and Montana require earlier planting dates in order to provide adequate growth for establishment and survival.

Chen et al. (2006) studied planting dates in the Pacific Northwest and the Northern Great Plains for winter pea and lentil. The Northern Great Plains was determined to have a smaller planting window due to colder conditions. Reduced yield was noted at some locations in both the Pacific Northwest and Northern Great Plains when the planting date was later because there may not have been sufficient acclimation time for the plant to successfully survive the winter. However, Murray et al. (1988) noted that earlier planted pea did not have as much cold tolerance because temperature is more important than plant size during acclimation. Planting dates need to be set so the plant will have strong roots, adequate growth, temperature, and time for

acclimation. Optimizing planting date can be difficult due to annual variation in climatic conditions.

Plant stands may need to be increased in winter crops to help ensure adequate spring stands after winterkill (Murray et al., 1988). A higher plant density provides better protection against the cold and allows for adequate spring stands if a few plants are killed. However, Markarian and Andersen (1966) observed that plant densities are reduced to allow for branching. Branching in winter plants is expected since the main stem is killed in the winter. Therefore, a balance must be found when it comes to seeding density to allow for branching and optimal protection.

Studies have been conducted to determine the winter hardiness of pea in both the field and laboratory. Field tests were conducted in Bozeman, Montana and Moscow, Idaho by Auld et al. (1983) and the laboratory tests were conducted in a controlled environment with a growth chamber. Studies in Moscow, ID, had three planting dates while those in Bozeman, MT, had only one date with the exception of the 1977/1978 winter. Nineteen lines; including thirteen winter hardy lines and six spring cultivars were tested. Differences were observed between winter and spring types in all experiments. As expected, the winter types performed better with regard to winter hardiness in all experiments. Due to a lack of snow cover in the field, some cultivars had a low survival percentage. Under the same conditions, winter lines had better survival at both locations and the laboratory with up to 96% survival in some locations.

Factors involved in winter survival of pea include agronomic and cultural practices and seed quality. In areas that traditionally grow spring crops, the introduction of winter crops may take time (Murray et al., 1988). Cultural practices need to be adapted along with modifying the genetic makeup of the plants to include disease resistance or tolerance. Seed quality is important

when planting to ensure adequate growth and acclimation (Murray et al., 1988). Seed quality affects germination which affects stands and survival.

Studies concerning seed size and yield have been conducted by Murray et al. (1984). Some years showed a decrease in yield in small seeded varieties. Seed yields in some locations were also lower due to low moisture levels in the field during fall growth. It was determined that larger seeded winter pea had better spring growth under unfavorable conditions.

Pea is an important crop for many agronomic reasons. Winter pea is important from an agronomic standpoint; however, many varieties do not have sufficient hardiness to survive harsh winter conditions. Studies have been conducted on other winter crops and the results can be applied to research on pea. Many factors affect survival beyond the winter, including spring and summer conditions. Physiology and genetics of winter crops must be understood to further study winter pea.

Objectives

The objectives of this research are to:

1. Establish a screening protocol for freezing in artificial conditions by identifying an optimum temperature and optimum acclimation time and;
2. Establish an effective rating scale for winter hardiness.

CHAPTER 2. EFFECTS OF ACCLIMATION ON SURVIVAL OF WINTER PEA (*PISUM SATIVUM* L.)

Introduction

Pea is important agronomically and economically to North Dakota. Winter pea is equally important, but many winter pea lines are not adapted to North Dakota environments. Agronomically, pea is good for rotations to reduce inputs and break up cereal rotations. Winter pea production recommendations include planting into the previous crops standing stubble. Planting into stubble reduces the need for tillage. Fall planting eliminates the need for spring planting under adverse conditions. Economically, pea is important for North Dakota as it was the number one producer through 2010.

Winter pea adaptation involves screening germplasm in both field and greenhouse conditions. Screening germplasm in the field is time consuming and survival depends on the environment while using the greenhouse is faster, although, a good method has not yet been established. Greenhouse evaluations predict winter hardiness and field tests confirm the winter hardiness reaction. Winter hardiness is a complex trait and winter pea requires resistance to disease and pests, and the ability to survive unfavorable summer conditions.

Certain conditions must be met in both the greenhouse and field when testing for winter hardiness, one of which is acclimation. Acclimation is the time in which a plant is exposed to cool temperatures to help initiate the hardening process for increased survival. Acclimation in the field is simulated in a growth chamber and in the field by fall temperatures. In North Dakota, acclimation is typically observed in the field in the late fall.

The objective of this study was to help establish a screening protocol for screening winter pea germplasm in artificial conditions and help identify the optimum temperature for freezing.

Materials and Methods

Plant Materials

Twelve lines with some degree of winter hardiness were planted in a randomized complete block design with six replicates. Seven commercial varieties ('Fenn', 'Glacier', 'Lynx', 'Melrose', 'Romack', 'Specter', and 'Windham') and five breeding lines (PS0017018, PS03100635, PS03101160, PS03101269, and PS05300239) were used. The commercial varieties 'Fenn', 'Glacier', 'Romack' and 'Melrose' had purple flowers and yellow cotyledons. 'Windham' and 'Specter' had white flowers and yellow cotyledons. 'Lynx' had white flowers and green cotyledons.

Experimental Design

The experiment was conducted twice using the following procedure. Plants were grown in the greenhouse at 20^0C for two weeks before acclimation at 4^0C in Sunshine mix LC-1 soil (Sun Gro Horticulture, Saba Beach, AB, Canada) in six pack trays. Five acclimation times were used 0, 1, 2, 3, and 4 weeks. The plants were transferred to an ESPEC BTU- 433 freezing chamber (ESPEC North America Inc., Hudsonville, MI) after the appropriate acclimation period had passed. The freezing chamber began at 4^0C and the temperature was reduced at 2^0C per hour. The minimum temperature, -8^0C and -12^0C, was held for one hour before the temperature was increased back to 4^0C at a rate of 2^0C per hour (Figure 2.1). The plants were returned to the acclimation chamber for 24 hours before being moved back to the greenhouse and scored at 7, 14, 21, 28, and 35 days after freezing. Assessment of freezing tolerance was scored on a 1 to 9 scale, where 1 = full survival and 9 = plant death (Table 2.1).

The freezing chamber had two shelves. Replicates 1, 2, and 3 were placed on the bottom shelf while replicates 4, 5, and 6 were on the top shelf. A thermometer was used to record the

maximum and minimum temperatures in the chamber to ensure that all runs experienced the same temperature.

Statistical Analysis

The data was collected using a 1 to 9 scale with each plant receiving a value. Scores were taken at five separate scoring dates and analyzed using SAS® 9.3 (SAS Institute Inc., USA). One way analysis of variance was calculated using PROC MIXED. Replicates were considered random.

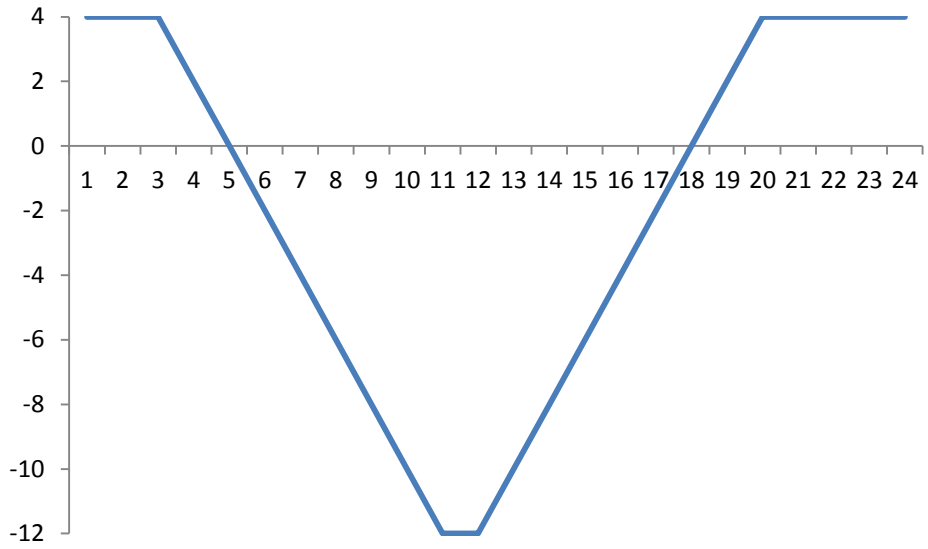

Figure 2.1. Theoretical temperature regime for freezing tests in the ESPEC BTU- 433.

Table 2.1. Descriptions of visual scores for pea plants subjected to freezing stress.

Score	Visual ID
1	Plant is completely green with or without re-growth
2	Plant has minimal freezing damage
3	Plant is at least 75% green
4	Plant has between 50-75% green tissue
5	Plant is 50% green
6	Plant as between 25-50% green tissue
7	Plant is 75% green
8	Plant is almost dead but still has some green
9	Plant is completely dead

Results

Acclimation Times

Plant survival increased with extended periods of acclimation. The control treatment was 0 weeks of acclimation and showed little to no survival (scores of 9). The control treatment at -8^{0}C had some survival initially (Figure 2.2), but the -12^{0}C control treatment had no survival by 14 days after freezing and all lines had a mean of 8 or above at 7 days after freezing (Figure 2.7).

The higher temperature, -8^{0}C, (Figures 2.2- 2.6) showed better survival than -12^{0}C (Figures 2.7- 2.11) across all lines and acclimation times. Plants were scored for 35 days after freezing; however, the trend indicates that survival begins to decrease after 21 days. Decisions on survival should be made at or before 21 days after freezing and not based on 35 days after freezing.

As acclimation increased so did survival. Survival at 7, 14, and 21 days after freezing increases for most named varieties including Melrose. This trend was not observed at four weeks of acclimation when survival decreased among most lines. Melrose, the most winter hardy line, had the best survival of all lines at -8^{0}C 0 weeks of acclimation with a mean of 4.8 at 21 days after freezing.

As acclimation time increased, trends were noticed amongst the lines overall. One week of acclimation showed survival in some lines through all scoring days, including Melrose which had a mean of 1 through 21 days after freezing. The breeding lines tended to have higher means than the commercial varieties across all scores. Two weeks of acclimation showed increased survival at both temperatures through 14 days after freezing, but -12^{0}C had complete death by 21 days after freezing while -8^{0}C still had survival in some lines. Three weeks of acclimation showed increased survival at both -8^{0}C and -12^{0}C. Survival increased through 21 days after

freezing when compared with two weeks of acclimation. The lower temperature, -12^{0}C, had complete death 28 days after freezing. Melrose had a mean of 3.5 21 days after freezing, which was the lowest of all lines, and a 9.0 28 days after freezing. Four weeks of acclimation showed decreased survival across all lines and scoring dates. It is not understood why this occurred but it may be related to the plants' physiology and a future study could be done to evaluate acclimation between 3 and 4 weeks to determine when the decline occurs.

ANOVA tables for -8^{0}C 7 days after freezing showed no significance among genotypes with 0 weeks of acclimation due to the relatively uniform lack of survival for any of the genotypes (Table 2.2). The other acclimation times showed significant differences among genotypes (Tables 2.3- 2.6). The overall means of the experiment were 7.2, 6.8, 6.1, 7.3, and 8.4 at 0, 1, 2, 3, and 4 weeks of acclimation, respectively. The coefficient of variation (CV) for 7 days after freezing was 41.1%, 75.6%, 91.3%, 71.2%, and 31.1% for 0, 1, 2, 3, and 4 weeks of acclimation, respectively.

ANOVA for data collected for the -12^{0}C temperature showed statistical significance between both genotypes and replicates for the control and 1 week of acclimation treatments (Tables 2.7 and 2.8); however, only genotype main effects were statistically significant for 2, 3, and 4 weeks of acclimation (Tables 2.9- 2.11). The overall means of the experiment were 8.9, 8.2, 7.8, 7.9, and 8.5 for 0, 1, 2, 3, and 4 weeks of acclimation, respectively. The CV for 7 days after freezing was 3.9%, 48.7%, 82.6%, 63.6%, and 29.0% for 0, 1, 2, 3, and 4 weeks of acclimation, respectively.

Table 2.2. ANOVA table for data from the -8^{0}C, 0 weeks of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	9.13	1.32	0.2412^{ns}
Rep	5	13.16	1.90	0.1104^{ns}
Residual	50	6.92	-	-

ns, not significant

Table 2.3. ANOVA table for data from the -8^{0}C, 1 week of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	27.67	3.05	0.0033**
Rep	5	11.42	1.26	0.2965^{ns}
Residual	52	9.08	-	-

ns, not significant; **, $p < 0.01$

Table 2.4. ANOVA table for data from the -8^{0}C, 2 weeks of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	27.94	3.62	0.0009***
Rep	5	4.88	0.63	0.6764^{ns}
Residual	49	7.73	-	-

ns, not significant; ***, $p < 0.001$

Table 2.5. ANOVA table for data from the -8^{0}C, 3 weeks of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	47.09	6.46	<0.0001***
Rep	5	5.03	0.69	0.6333^{ns}
Residual	55	7.29	-	-

ns, not significant; ***, $p < 0.001$

Table 2.6. ANOVA table for data from the -8^{0}C, 4 weeks of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	14.54	3.27	0.0018**
Rep	5	3.92	0.88	0.4993^{ns}
Residual	53	4.44	-	-

ns, not significant; **, $p < 0.01$

Table 2.7. ANOVA table for data from the -12^{0}C, 0 weeks of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	0.24	2.10	0.0372*
Rep	5	0.62	5.39	0.0005***
Residual	51	0.12	-	-

ns, not significant; *, $p < 0.05$, *** $p < 0.001$

Table 2.8. ANOVA table for data from the -12^{0}C, 1 week of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	27.23	3.20	0.0023**
Rep	5	38.57	4.54	0.0017**
Residual	51	8.50	-	-

**, $p < 0.01$

Table 2.9. ANOVA table for data from the -12^{0}C, 2 weeks of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	25.56	2.48	0.0143*
Rep	5	9.82	0.95	0.4562^{ns}
Residual	51	10.32	-	-

ns, not significant; *, $p < 0.05$

Table 2.10. ANOVA table for data from the -12^{0}C, 3 weeks of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	36.89	3.77	0.0005**
Rep	5	5.12	0.52	0.7579^{ns}
Residual	54	9.79	-	-

ns, not significant; **, p < 0.01

Table 2.11. ANOVA table for data from the -12^{0}C, 4 weeks of acclimation treatment scored 7 days after freezing.

Source	DF	Mean Square	F Value	Pr > F
Genotype	11	23.44	5.79	<0.0001***
Rep	5	4.76	1.17	0.3350^{ns}
Residual	49	4.05	-	-

ns, not significant; ***, p < 0.001

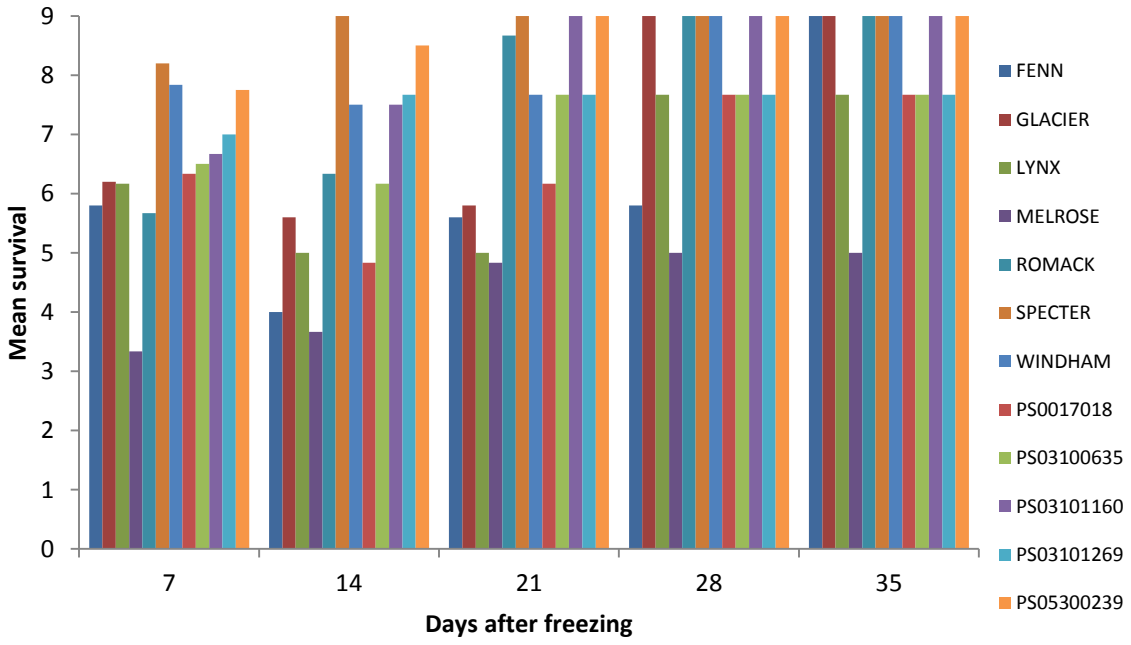

Figure 2.2. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 0 weeks at 4^{0}C.

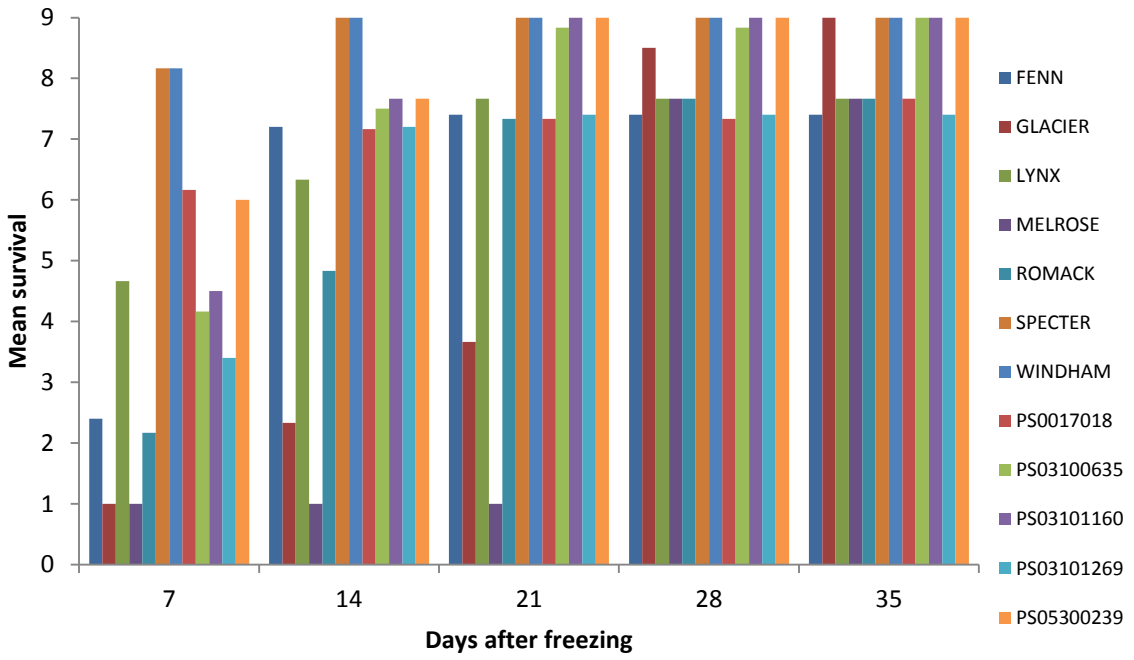

Figure 2.3. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 1 week at 4^{0}C.

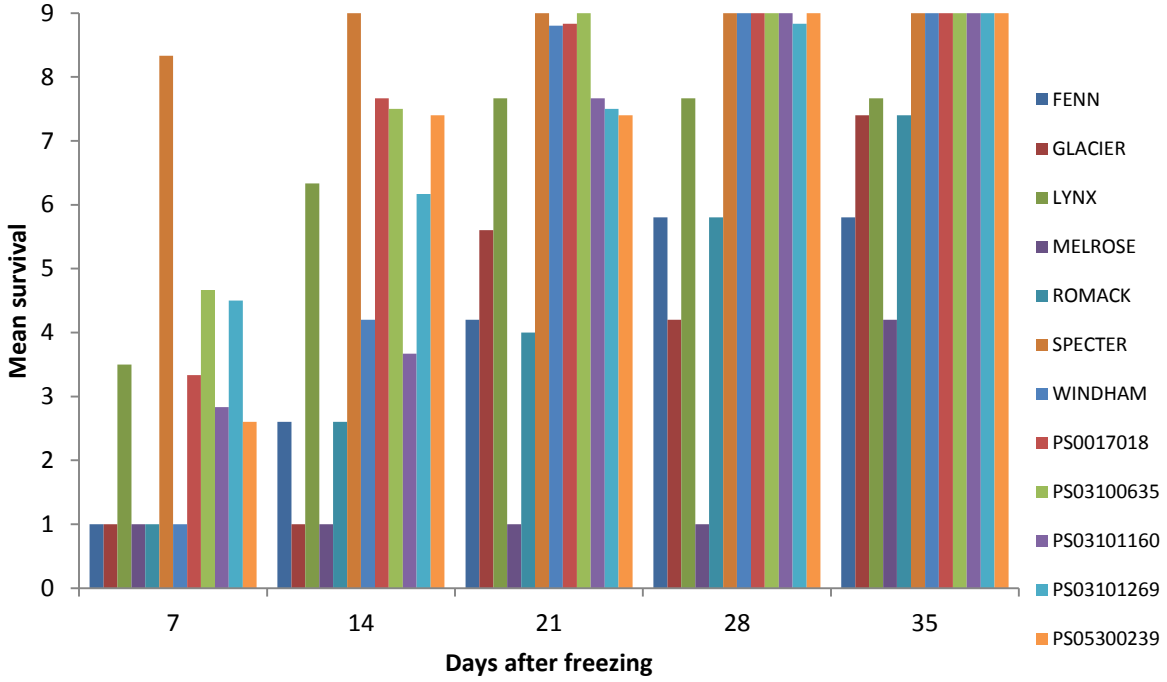

Figure 2.4. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 2 weeks at 4^{0}C.

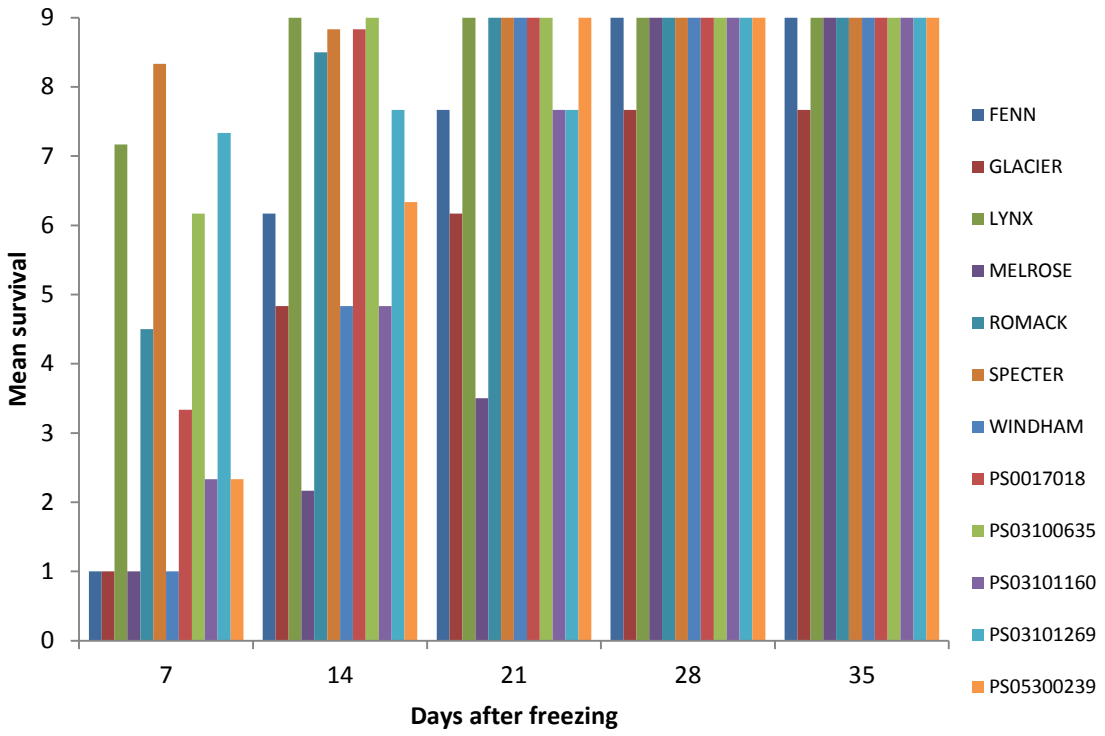

Figure 2.5. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 3 weeks at 4^{0}C.

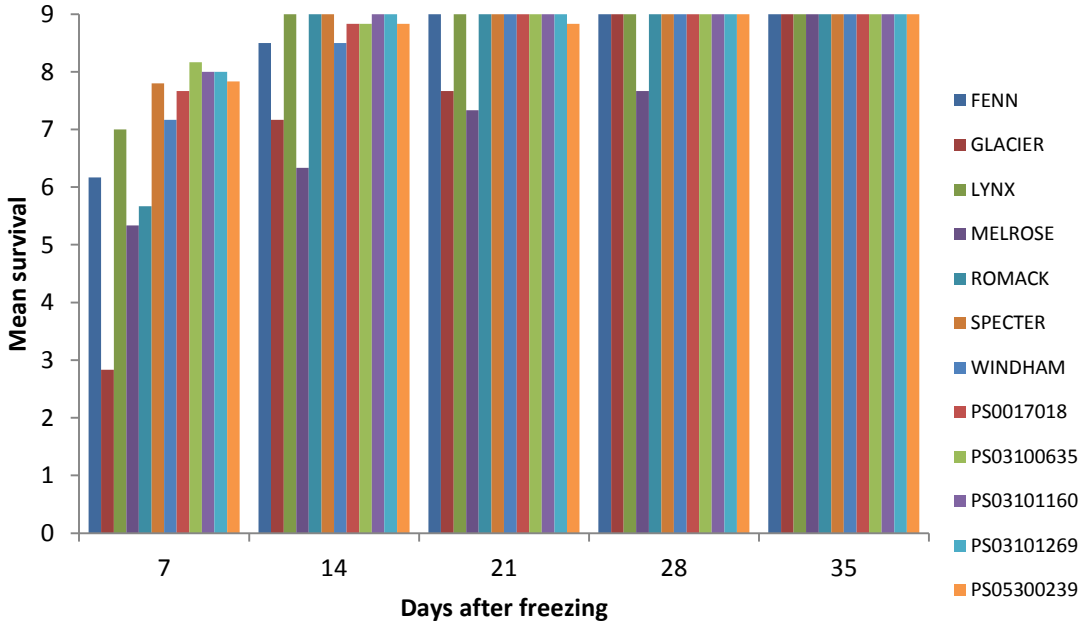

Figure 2.6. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 4 weeks at 4^{0}C

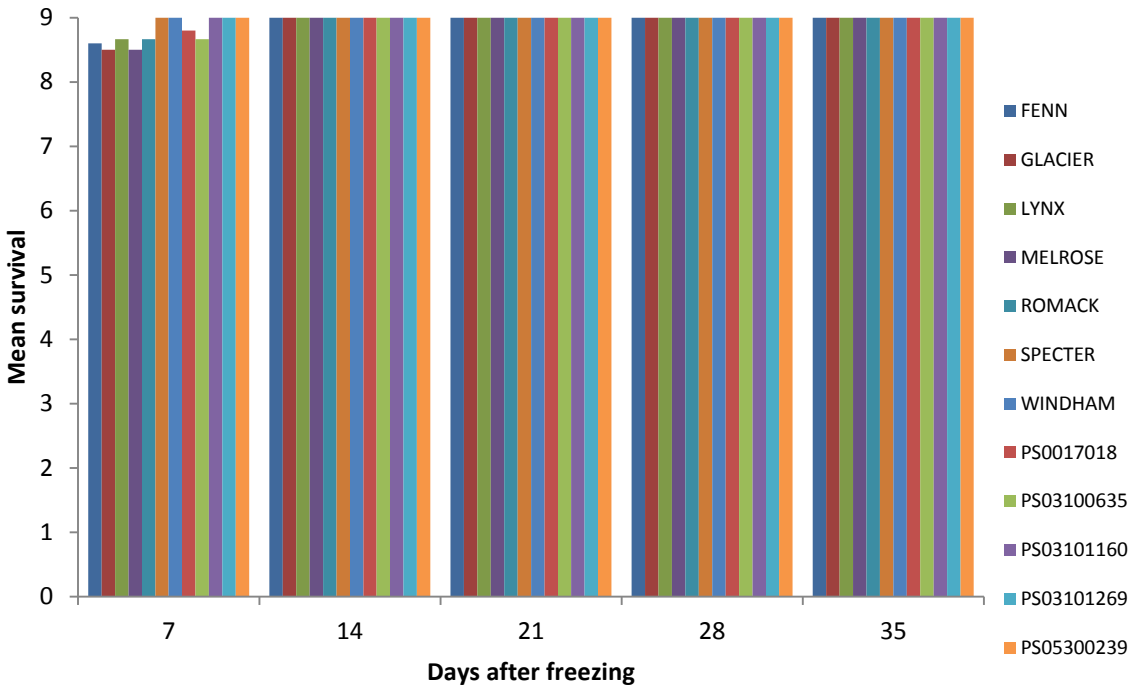

Figure 2.7. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 0 weeks at 4^{0}C.

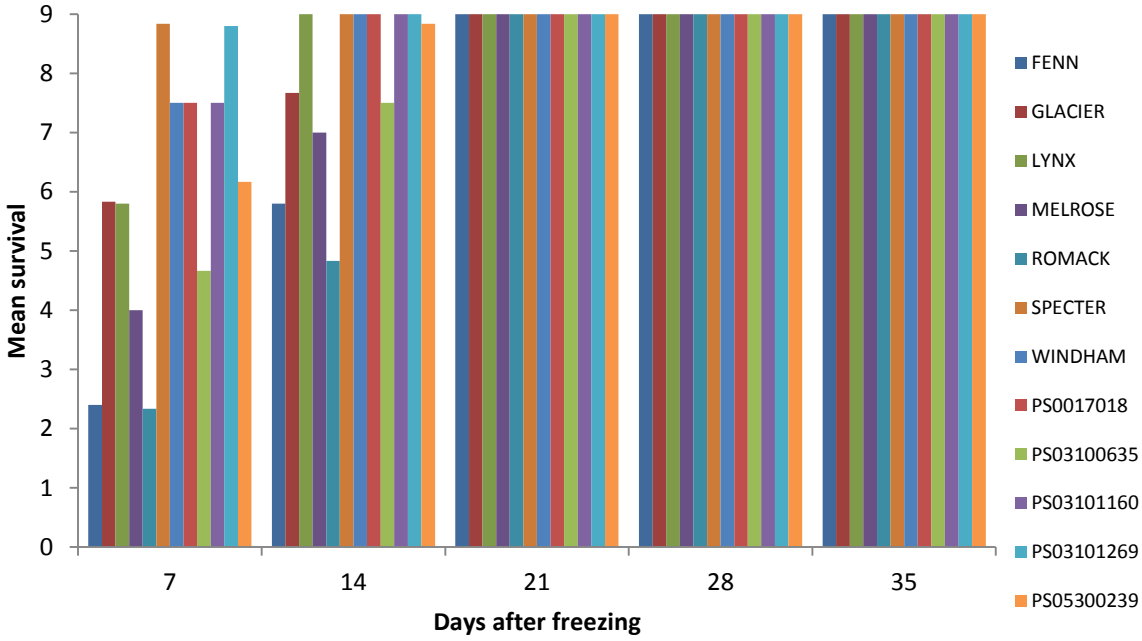

Figure 2.8. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 1 week at 4^{0}C.

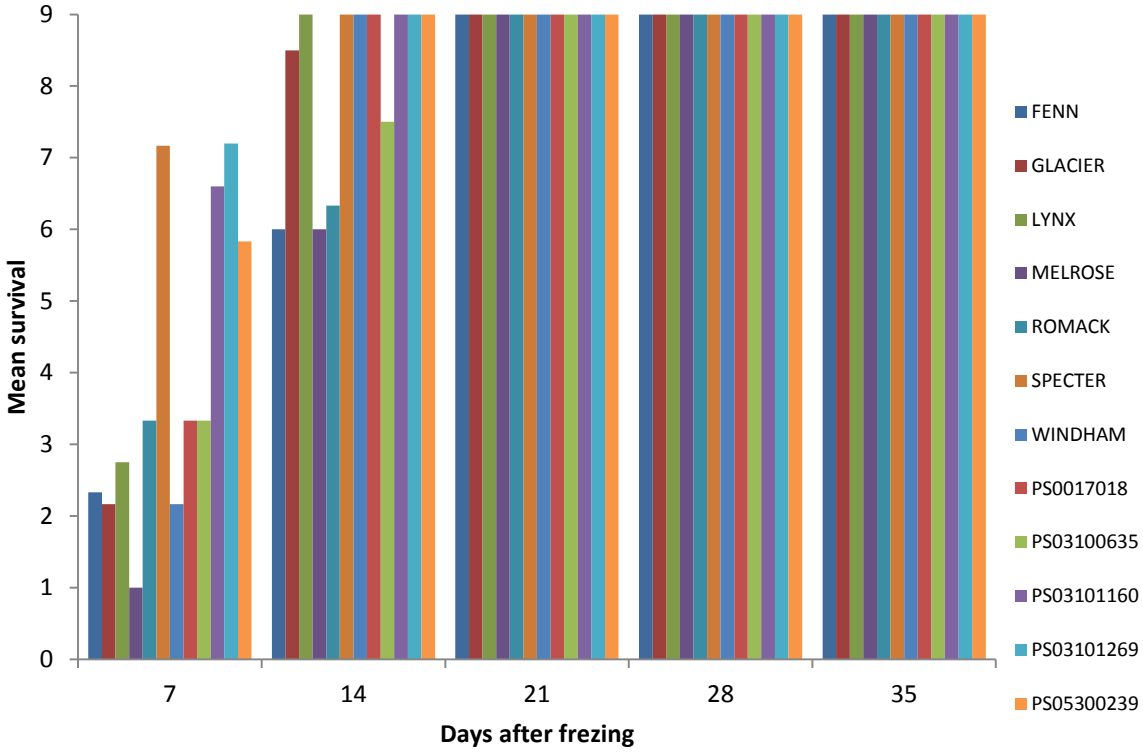

Figure 2.9. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 2 weeks at 4^{0}C.

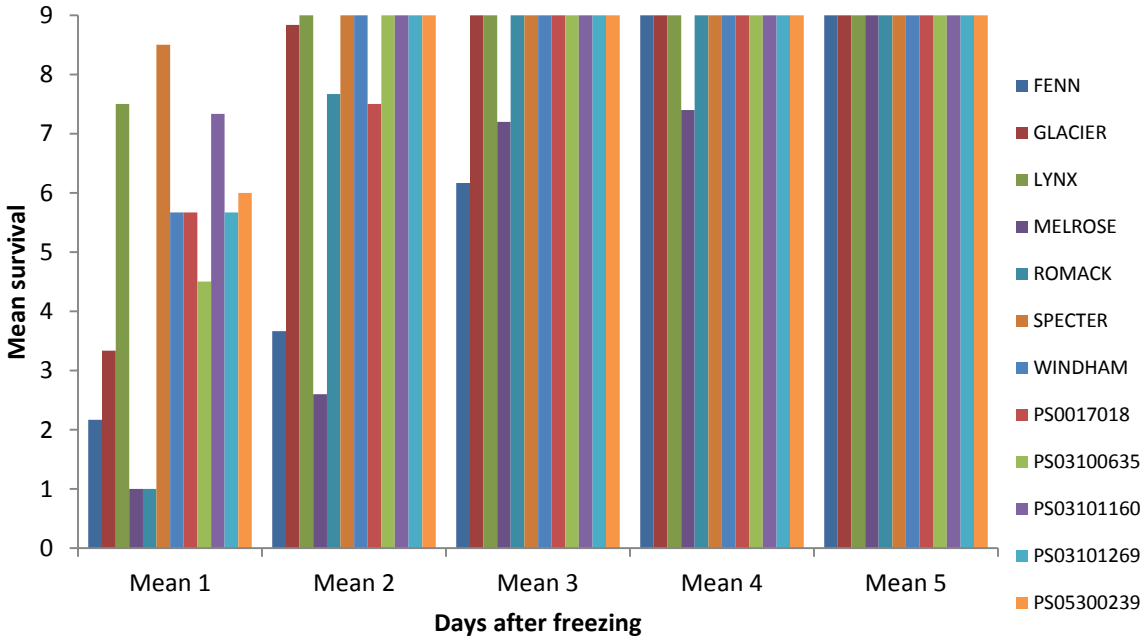

Figure 2.10. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 3 weeks at 4^{0}C.

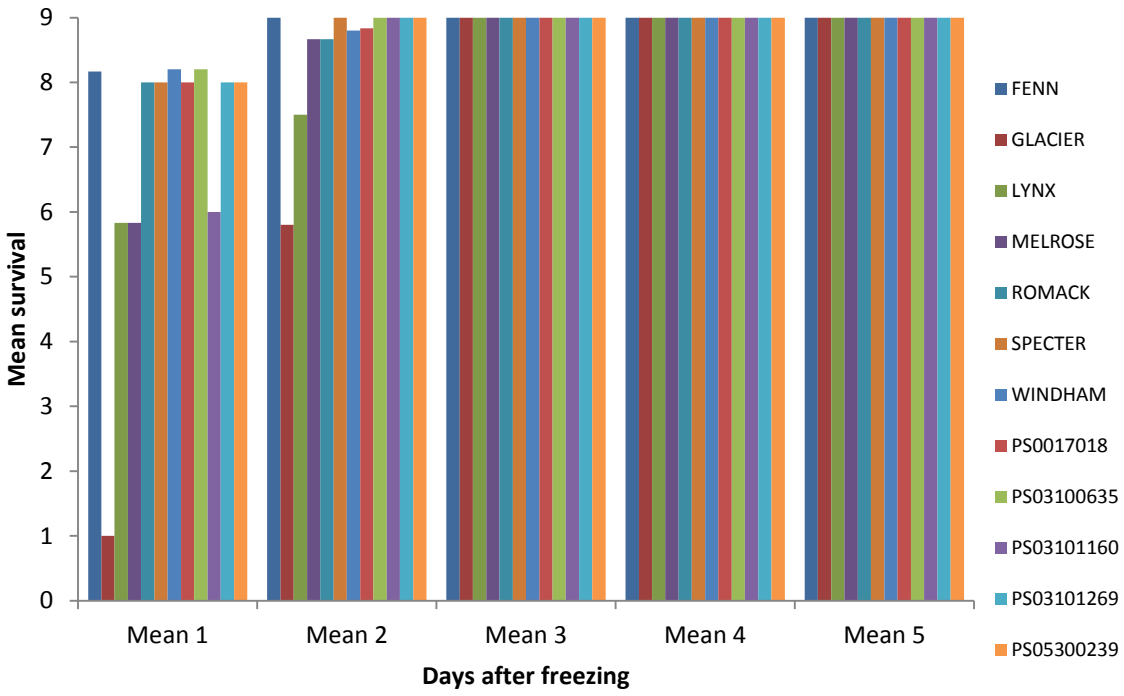

Figure 2.11. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 4 weeks at 4^{0}C.

The second replicate run of the experiment produced slightly different results. Increased duration of acclimation did not increase survival as markedly as the first run (Figures 2.12- 2.21). The two experiments were similar in that four weeks of acclimation resulted in decreased survival compared to three weeks of acclimation. The apparent optimum time to score the plants is 21 days after freezing.

The disparity of means was evident in Melrose, the most winter hardy variety. With 3 weeks of acclimation and frozen at -8^{0}C Melrose had means of 1.0, 2.2, 3.5, 9.0, and 9.0 in the first run. The second run under the same conditions 1.0, 3.7, 6.2, 9.0, and 9.0. The -12^{0}C treatment with 3 weeks of acclimation showed the same trend in Melrose. The means from the first run are 1.0, 2.6, 7.2, 7.4, and 9.0 and the means from the second run are 2.2, 6.2, 9.0, 9.0, and 9.0. Melrose and most other lines tended to have higher scores in the second run compared

with the first run. However, the overall results the determine acclimation time and scoring date were the same.

Four lines, Fenn, Glacier, Melrose, and Windham had a mean of 1.0 7 days after freezing with 3 weeks of acclimation at -8^{0}C during the first run. In the second run only two lines had a mean of 1.0, Fenn and Melrose.

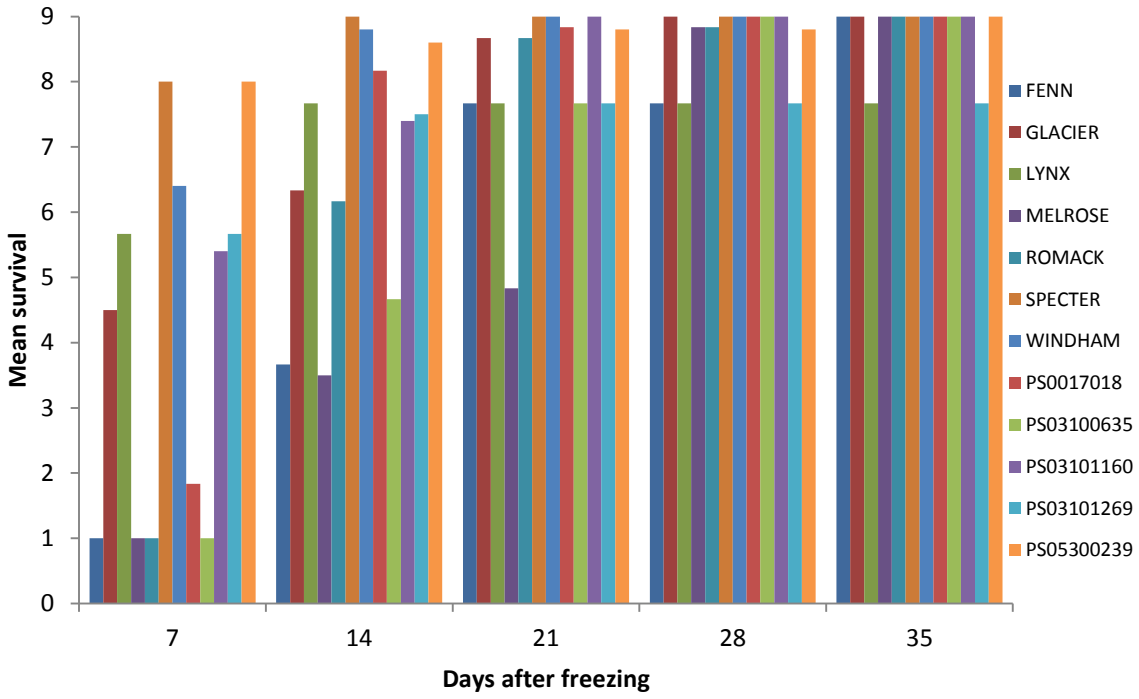

Figure 2.12. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 0 weeks at 4^{0}C.

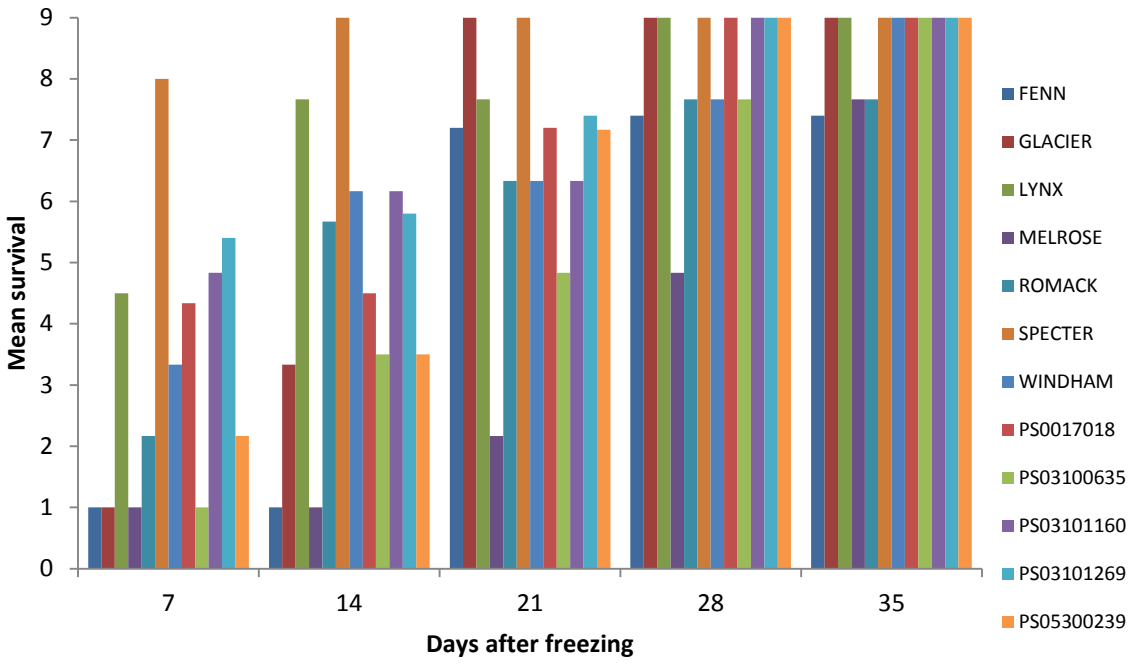

Figure 2.13. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 1 week at 4^{0}C.

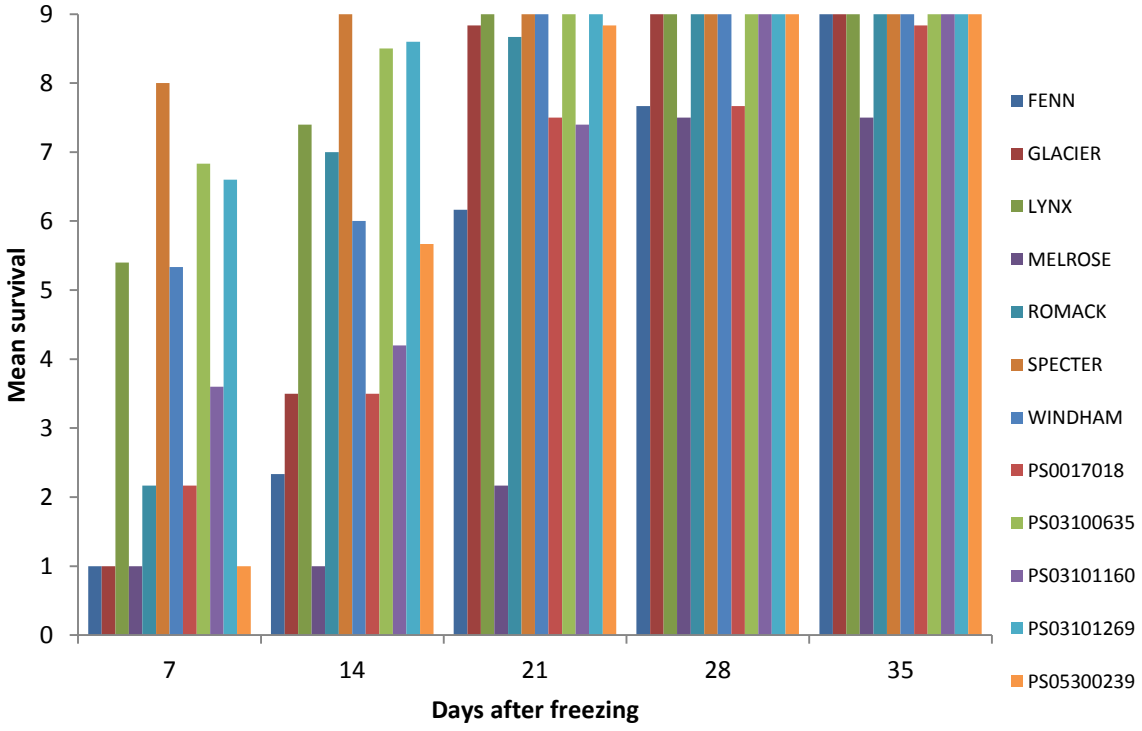

Figure 2.14. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 2 weeks at 4^{0}C.

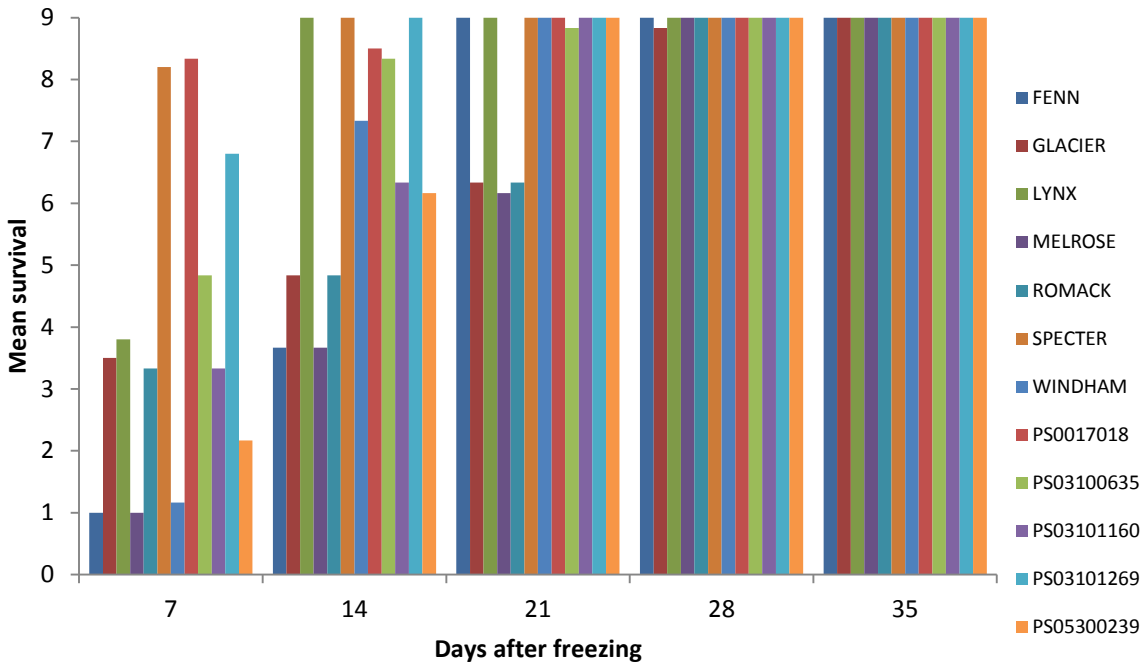

Figure 2.15. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 3 weeks at 4^{0}C.

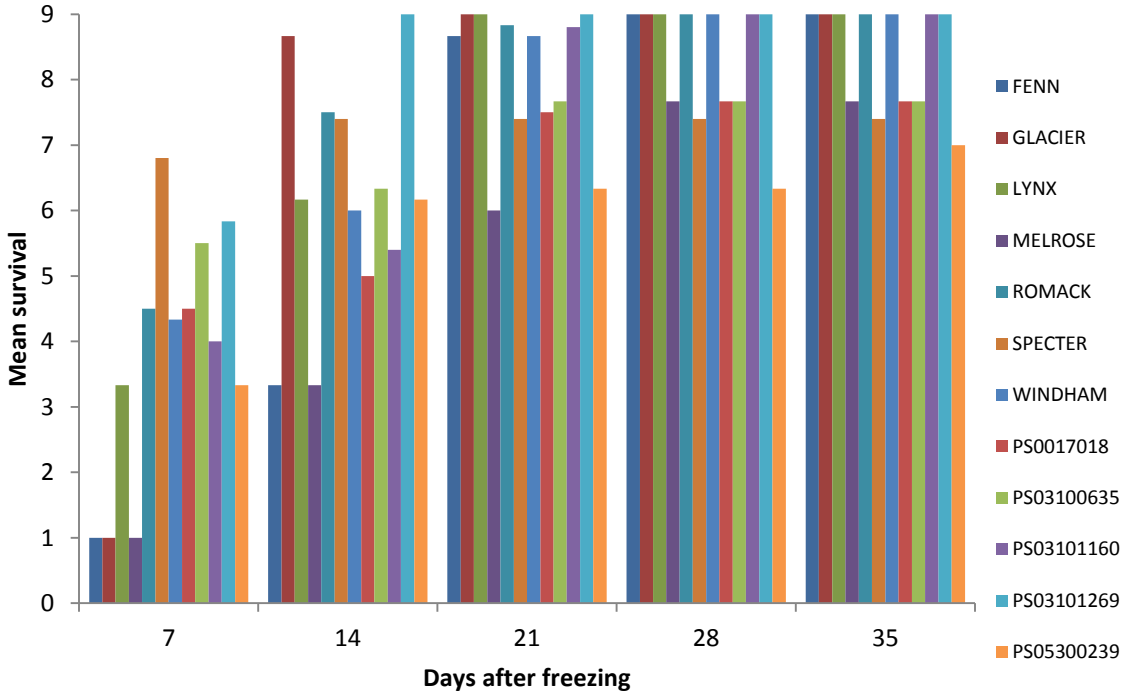

Figure 2.16. Mean survival rating of 12 pea genotypes tested at -8^{0}C and acclimated for 4 weeks at 4^{0}C.

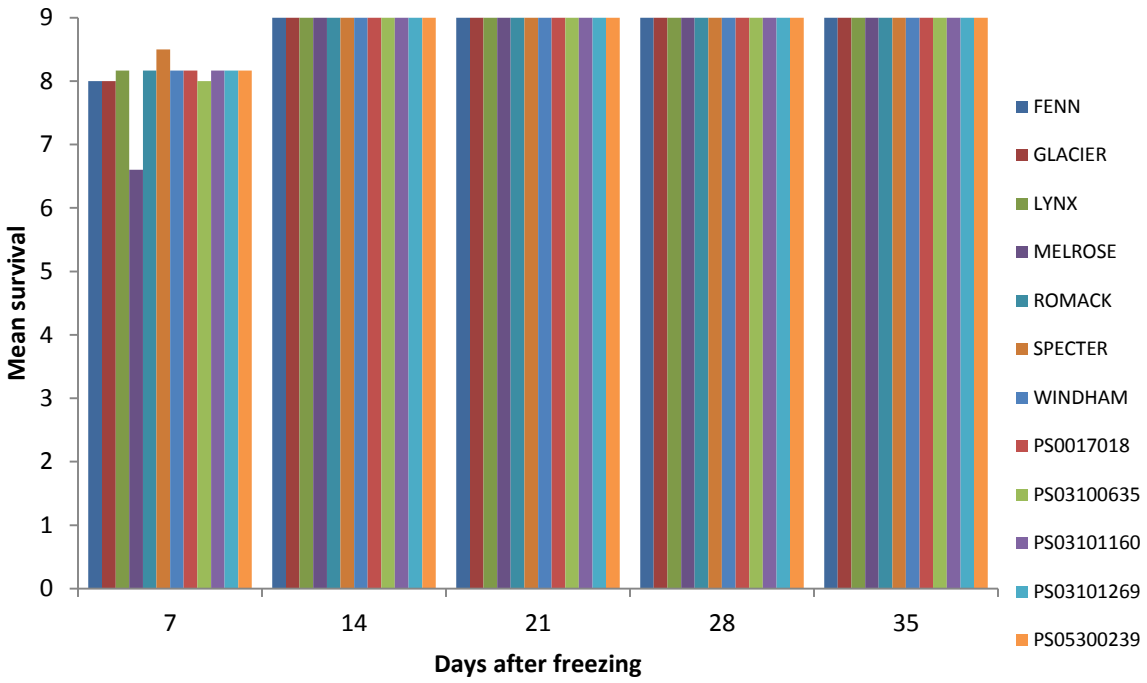

Figure 2.17. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 0 weeks at 4^{0}C.

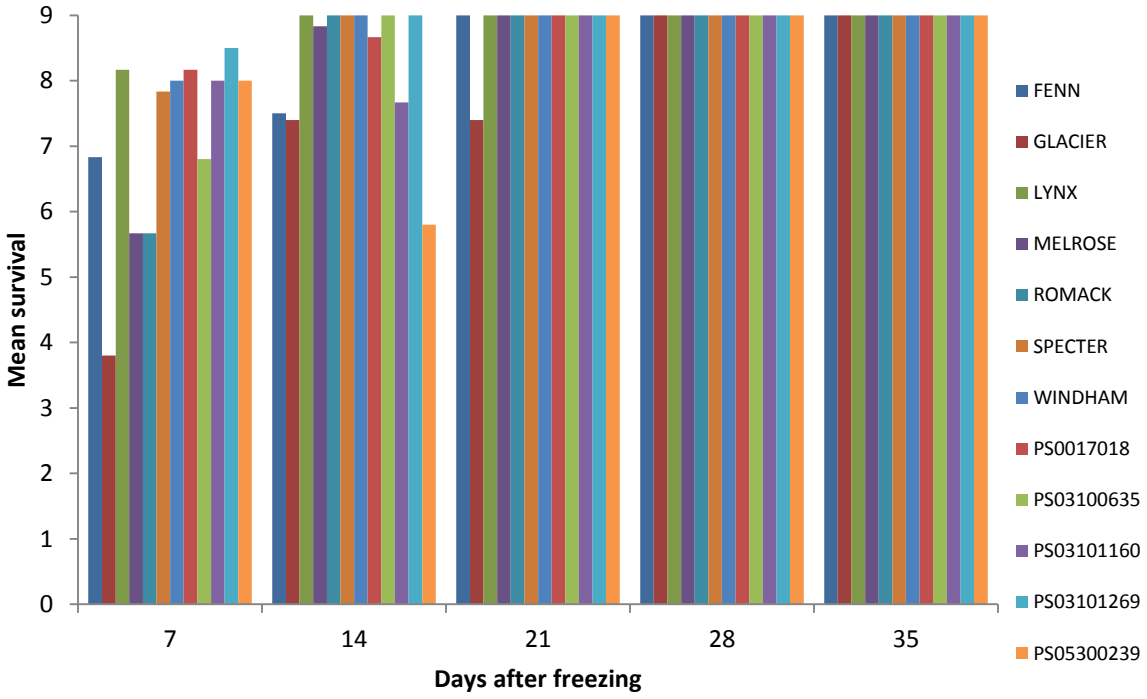

Figure 2.18. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 1 week at 4^{0}C.

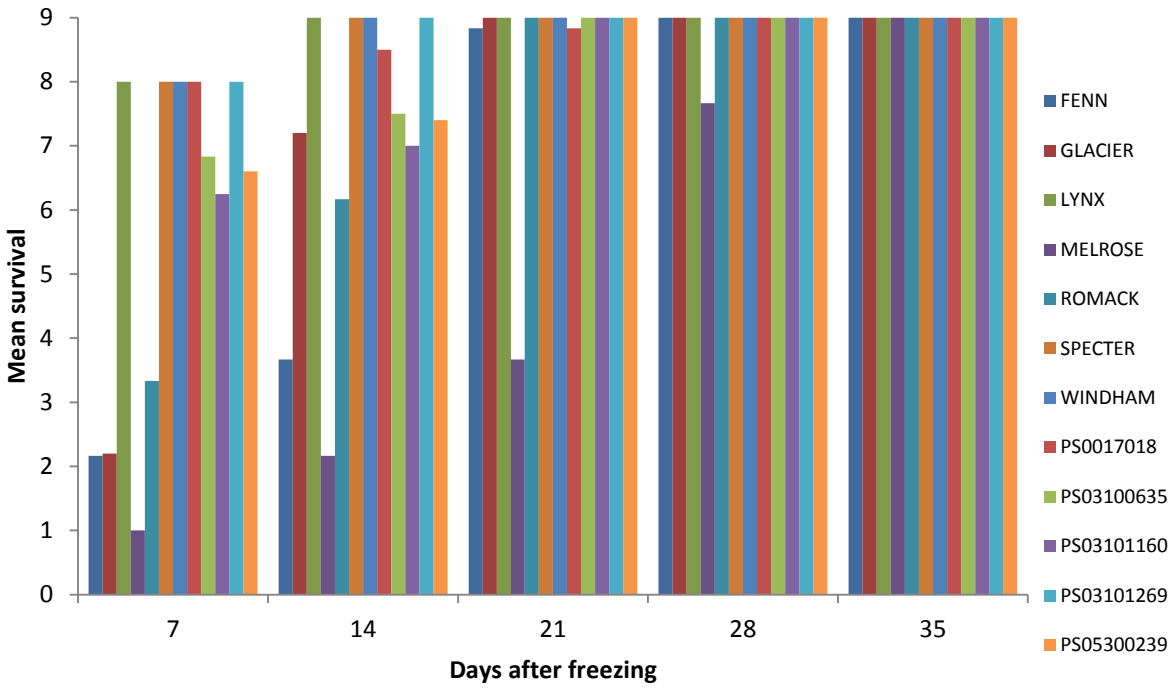

Figure 2.19. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 2 weeks at 4^{0}C.

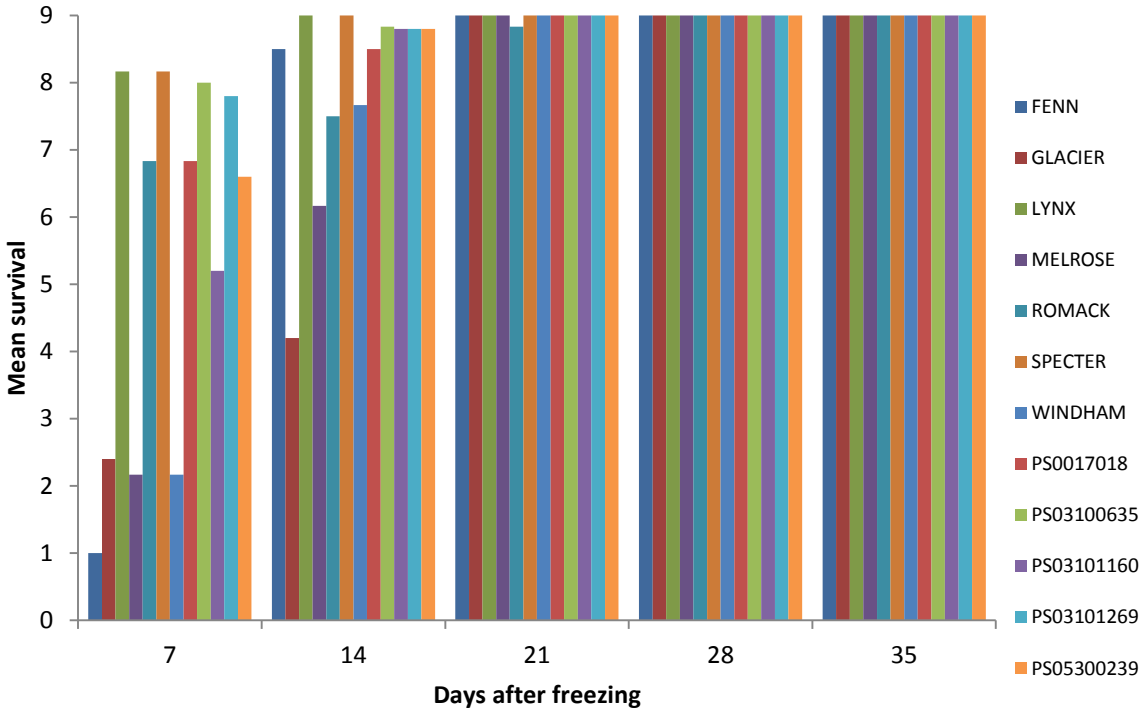

Figure 2.20. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 3 weeks at 4^{0}C.

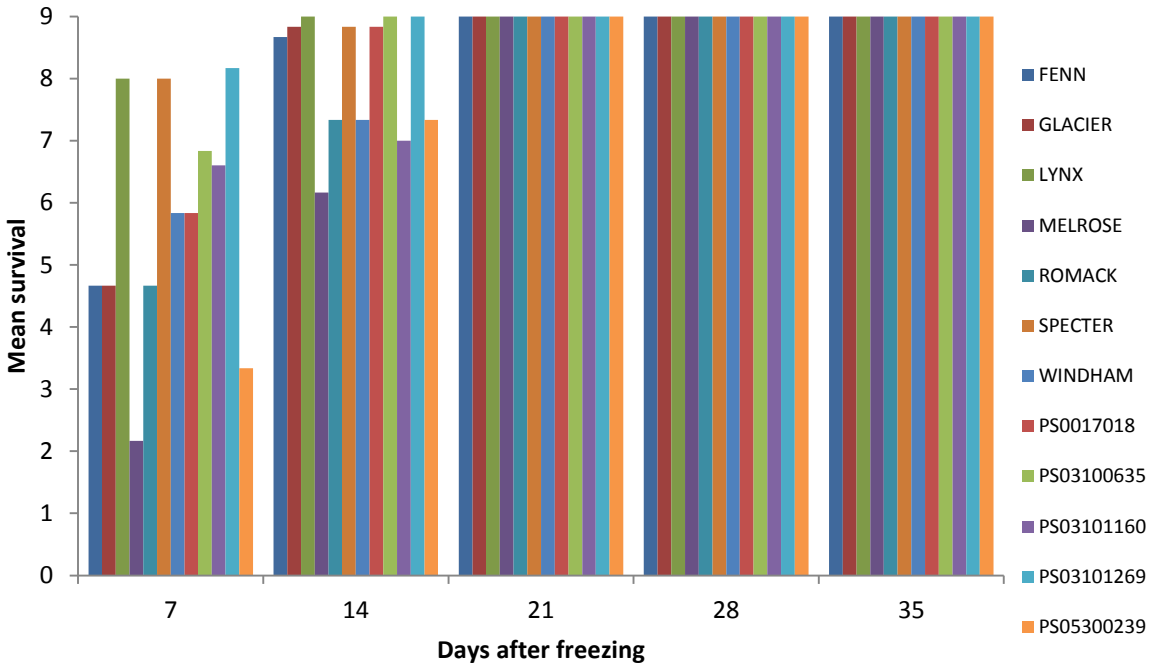

Figure 2.21. Mean survival rating of 12 pea genotypes tested at -12^{0}C and acclimated for 4 weeks at 4^{0}C.

Discussion

Winter hardiness is an important factor for survival of winter pea and screening can be difficult. Field screening requires the adequate snow cover and other factors for screening and greenhouse evaluations are only predictions of winter hardiness. Greenhouse conditions do not fully address all stresses that affect plants grown in the field. Greenhouse conditions are controlled while field conditions can vary. Establishing a screening protocol for artificial conditions would speed up the screening process and improve the prediction of winter hardiness. Protocols used for other winter crops can be used as a guideline for establishing a winter pea screening protocol.

The objective of the study was to establish a protocol for screening winter peas in artificial conditions. This was accomplished by determining the amount of acclimation and

scoring necessary for differential survival. Three weeks of acclimation had the best survival across most lines and a decrease in survival was seen with longer acclimation. Three weeks of acclimation reduces the amount of time required for screening, thus speeding up the screening process.

Two temperatures were tested during this study. The lower temperature (-12^{0}C) was too harsh as evidenced by near complete plant death and higher mean injury scores across all lines, including Melrose, the most winter hardy entry. Freezing to -8^{0}C showed good differential survival and was determined to be a good test temperature. Other temperatures could be tested, such as -10^{0}C, to establish a lower limit for freezing.

The recommendation based on these results is to acclimate plants for three weeks, score for 21 days after freezing, and use -8^{0}C as a good temperature to gauge survival. The original protocol called for scoring for 35 days after freezing but increased death was noted at this point, so 21 days after freezing was determined to be the best date to score. Scoring 21 days after freezing instead of 35 days after freezing decreased the testing time by an additional two weeks. This research can be used in future studies to further optimize the protocol which would allow for a better predictor of winter hardiness.

The future for winter hardy crops looks promising and having good screening protocols for artificial conditions will increase the ability of winter pea to spread. Using the best screening protocol will decrease the amount of field testing required because non-hardy lines can be eliminated and only lines that appear promising would be advanced. Field testing takes longer and correct environmental conditions must be met.

Previous research in winter pea indicates that winter pea can be adapted to harsher climates. Some lines showed promising results and testing will be continued on those lines in the

hope that they can survive harsh North Dakota conditions on a consistent basis. The protocol identified can be used for future screenings and can speed up the testing of possible winter hardy lines.

Conclusion

Results from this study showed that pea responds to acclimation time; however, additional research is needed to optimize the protocol. This research provides a baseline from which additional improvements can be made. The main conclusion from this data is that three weeks of acclimation is optimal for increased survival among winter hardy lines. Increased acclimation time did not increase survival. A decrease was indicated with four weeks of acclimation, although, this is not understood.

Using artificial conditions is a good way of screening material in a fast and efficient way to help predict winter hardy lines before field testing. Controlled environment studies take eight weeks to determine if a line has the potential to survive while a field study takes months. All lines identified in artificial conditions must be field tested to ensure winter hardiness and tolerance to other stresses not testable in the controlled environment.

Controlled environment testing is faster and requires less space. Accurate simulation in the greenhouse is important to ensure that winter hardy lines are not discarded before being field tested. Accurate simulation also reduces the number of lines to be field tested which decreased field maintenance and space.

CHAPTER 3. SURVIVAL OF PEA RECOMBINANT INBRED LINES IN FIELD AND ARTIFICIAL CONDITIONS

Introduction

Pea is beneficial to growers for many agronomic reasons and to consumers for its nutritional composition. Pea is a legume which fixes nitrogen and helps control grassy weeds and cereal pathogens when used in rotations with cereals. Winter pea has all the benefits of spring sown pea with some additional benefits such as no-till for that season and earlier harvest. Benefits of having pea in rotations include nitrogen fixation and control of grassy weeds. Winter pea varieties do not currently possess sufficient winter hardiness to withstand the harsh winters of North Dakota, but can be grown in the milder climate of Washington State.

Winter pea survival is variable depending on environmental conditions including soil and air temperatures and snow cover. Fall temperatures include acclimation which is the exposure to cooler temperatures to initiate the hardening process. Winter pea also needs to have disease resistance, including powdery mildew and root rots, to be able to survive the summer because winter hardiness alone is not enough to have a successful yield.

Winter hardiness can be predicted in artificial conditions, but field testing is necessary to validate the results. Testing for winter hardiness in the greenhouse offers an opportunity to increase genetic gain by evaluating more lines in a shorter time. The objective of this study was to evaluate recombinant inbred lines (RILs) in natural (field) and artificial (greenhouse) settings for winter hardiness.

Materials and Methods

Field Experiment

Plant Materials

Two hundred sixty-seven F_7 derived recombinant inbred lines (RILs) derived from the cross 'Medora'/'Melrose' were evaluated in artificial and field conditions. Melrose is a winter pea with purple flowers and Medora is a spring pea with white flowers. Melrose has pigmented seed, indicative of the Austrian winter type.

Experimental Design

The field was planted 8 September 2011 at Prosper, North Dakota. Prosper, ND is at 47.002°N/-97.115°W with an elevation of 284 meters (NDAWN, 2011). The soil is a silty clay loam (NRCS, 2012). Trials were direct sown in standing spring wheat stubble which was 4- 12 cm tall. Plots were comprised of three rows 2.1 m long spaced 17 cm apart and the sowing density was 140 plants m^{-2}. The seeds were sown at 1.9 to 3.2 cm deep with a Wintersteiger plot seeder fit with double disk openers. Both parents and 251 RILs were sown in a randomized complete block design (RCBD) with two replicates. Only two replicates were used due to space and seed limitations. Stand counts were taken in the fall based on a 1 m seed row from the outside rows.

The field conditions over the winter were not typical of a North Dakota winter. Temperatures in the fall were warmer than average (Table 3.1) (NDAWN, 2011). However, temperatures below freezing were observed in November and December while the plants were exposed. The first snow fall was on 14 November 2011 (personal observation, 2011); however, it was minimal measuring only a few mm and did not cover the entire plant. No measurable snow

was received until February and on 8 March 2012 the snow cover measured 14 to 17 cm (personal observation, 2012).

Table 3.1. Monthly Average Prosper air temperatures from September 2011 to April 2012.

		Prosper			
Year	Month	Avg. Air Temp (^{0}C)	Normal Avg. Air Temp (^{0}C)	Departure from Norm. Avg. Air Temp (^{0}C)	Departure from 5-yr. Avg. Air Temp (^{0}C)
2011	9	15.0E	15.0	-17.2E	-17.2
2011	10	11.1	7.2	-14.4	-13.9
2011	11	0.6	-1.7	-15.6	-17.2
2011	12	-4.4	-10.0	-12.2	-10.0
2012	1	-7.8	-13.3	-12.2	-10.0
2012	2	-6.1	-10.0	-13.9	-10.6
2012	3	3.9	-2.8	-11.1	-10.0
2012	4	8.3E	6.1	-15.6E	-15.6
Avg.		2.2E	-1.1	-13.9E	-13.3
Max.		15.0E	15.0	-	-
Min.		-7.8E	-13.3	-	-
Std. Dev.		7.9E	9.3	-	-

Source: NDAWN, 2011
E = estimated value

Plants were scored in the fall to determine freezing tolerance. Scoring was conducted using a 1 to 9 scale where, 1 = no freezing damage and 9 = 100% damage. Plants were also scored in the spring to rate winter survival. These scores were taken using a 1 to 9 scale (Table 3.2) where, 1 = completely green or having re-growth and 9 = complete death. A 5 would be 50% dead and 50% green.

Table 3.2. Visual ID descriptions of spring survival field scores.

Score	Visual ID
1	Stand is completely green with no damage
2	Some death but most plants survived
3	Stand is 25% dead
4	Stand is between 25 and 50 % dead
5	Stand is 50% dead
6	Stand is between 50 and 75% dead
7	Stand is 75% dead
8	Stand is mostly dead with only a few plants surviving
9	Stand is completely dead

Greenhouse Experiment

Plant Materials

All seed for the RILs used in this study were derived from the cross 'Medora'/'Melrose'- as previously described. All 267 RILs were included in the test. Some of the RILs did not grow and were not able to be scored. More RILs were included in the greenhouse study because less seed was needed and the amount of seed available is a limitation.

Experimental Design

Experiments were conducted in the greenhouse with an ESPEC BTU- 433 freezing chamber (ESPEC North America Inc., Hudsonville, MI). The plants were grown for two weeks in the greenhouse at 20^0C. The seeds were planted in six-pack trays using Sunshine mix LC-1 soil (Sun Gro Horticulture, Saba Beach, AB, Canada). Space limitations in the freezing chamber required that the RILs be divided into 12 sets of 22 RILs each; with the last set having less RILs. Each set plus the parents were treated as a separate experiment and arranged in a randomized complete block design (RCBD) with three replicates.

The plants were grown in the greenhouse for 2 weeks prior to being moved to the vernalization chamber (4^0C) for four weeks of acclimation. The plants were transferred to the

freezing chamber for the treatment period, returned to the vernalization chamber for one day, and then returned to the greenhouse for scoring. All RILs were tested at -4, -8, and -12^{0}C. The temperature was reduced at a constant rate of 2^{0}C hr^{-1} beginning at 4^{0}C and the minimum temperature was held for one hour before the temperature was increased back to 4^{0}C at a rate of 2^{0}C hr^{-1} (Figure 3.1).

The plants were scored at 7, 14, 21, 28, and 35 days after freezing. The scale used for scoring freezing tolerance was a 1 to 9 scale (Table 3.3) where 1 = full survival and 9 = plant death. The plants were scored individually on each of the five scoring dates.

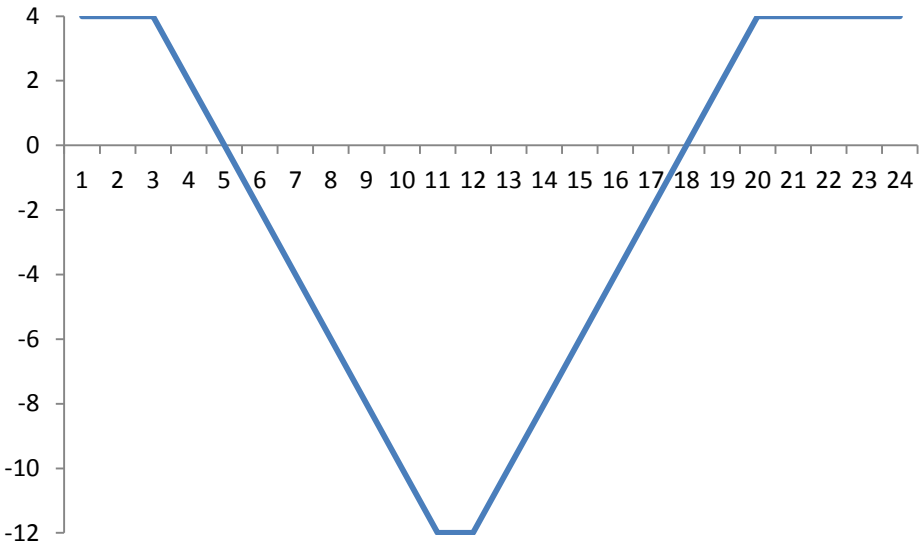

Figure 3.1. Theoretical temperature regime for freezing tests in the ESPEC BTU- 433.

A second and third replicate run of the experiments included only the first 110 RILs that had enough seed. Five sets of 22 RILs each plus the two parents were tested using the same experimental design and protocol as previously described for Run 1. The exception being that only -8 and -12^{0}C were used.

Table 3.3. Descriptions of visual scores for pea plants subjected to freezing stress.

Score	Visual ID
1	Plant is completely green with or without re-growth
2	Plant has minimal freezing damage
3	Plant is at least 75% green
4	Plant has between 50-75% green tissue
5	Plant is 50% green
6	Plant as between 25-50% green tissue
7	Plant is 75% green
8	Plant is almost dead but still has some green
9	Plant is completely dead

Statistical Analysis

A one-way analysis of variance was calculated using PROC MIXED in SAS® 9.3 (SAS Institute Inc., USA). Replicates were considered random and parents were used as checks across all runs.

Results

Field Experiment

Stand Establishment

The stand counts among the RILs varied greatly with a minimum of 0, maximum of 24, and a mean of 7.4. Emergence was low due to dry conditions in the field after planting. Rainfall in September 2011 was 6.1 cm and 9.4 cm in October (NDAWN, 2011).

Fall freezing scores ranged between 3 and 6 for the RILs indicating moderate tolerance to freezing (Figure 3.2 a and b). Melrose, the most winter hardy variety, had a mean score of 1.8 and Medora had a mean score of 6. Spring survival scores showed significantly greater loss than was expected based on the fall freezing scores. Melrose had a mean of 5 and Medora had a mean of 9. RILs that had a lower freezing score did not always have a lower survival score and over 200 RILs had a mean of 8 or 9 (Table A1) while seven RILs had mean scores better or equal to

Melrose (Table A1). PRIL-2-230 performed well based on fall and spring scores with a mean fall freezing score of 2 and a mean spring survival score of 3. The survival in the field was low due to unfavorable conditions and the field was abandoned after recording the initial survival score.

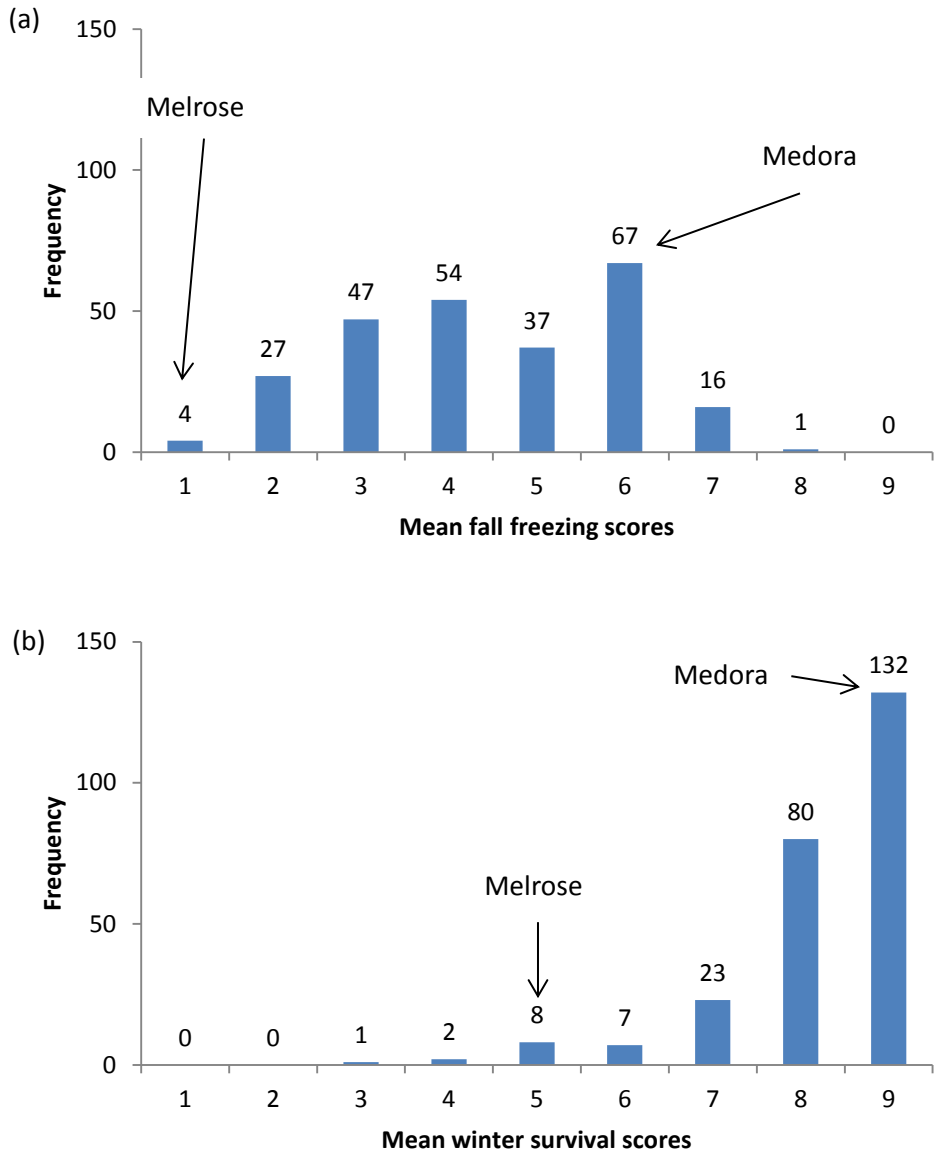

Figure 3.2. Mean fall freezing (a) and winter survival (b) scores for 251 RILs from the Medora/Melrose cross grown at Prosper, ND in 2011-2012.

Greenhouse Experiment

Plant materials were frozen at three temperatures, -4, -8, and -12^{0}C. Freezing at -4^{0}C did not show any differential killing and all lines survived (Figure 3.3 a-e) showing that the temperature was too mild for differential selection. Scoring of the first five experiments at 14 days after freezing was based on a nutrient deficiency and not freezing damage. The full data histogram is presented in Figure A1. Data analysis for the -4^{0}C treatment at 35 days after freezing detected significant differences between genotypes and replicates (Table 3.4) which may be due to the high number of genotypes tested. The overall mean for the experiment was 1.3 and the coefficient of variation (CV) was 40.9% 21 days after freezing.

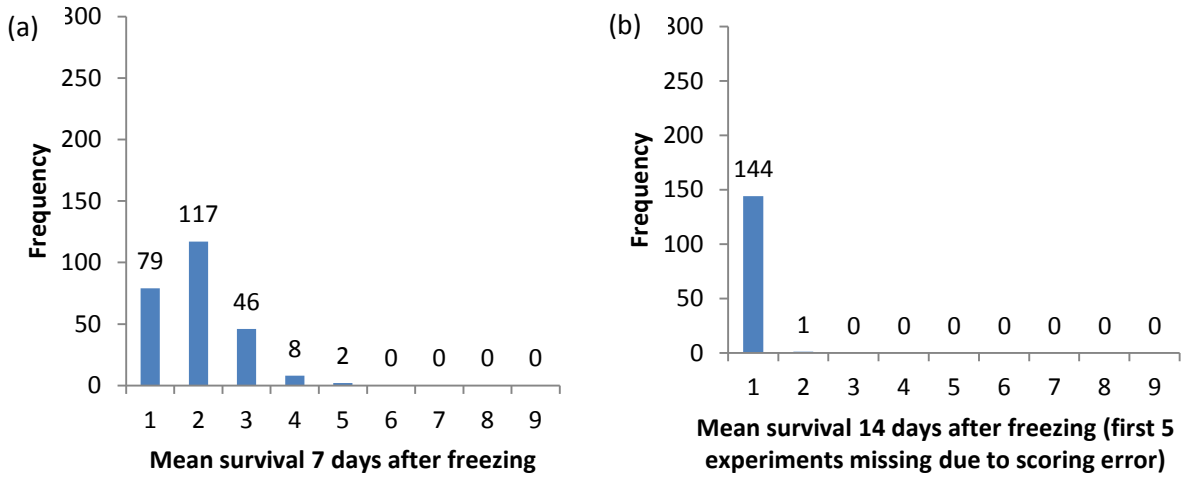

Figure 3.3. Mean survival scores for RILs frozen at -4^{0}C and scored 7 days after freezing (a), 14 days after freezing with 5 experiments missing (b), 21 days after freezing (c), 28 days after freezing (d), and 35 days after freezing (e).

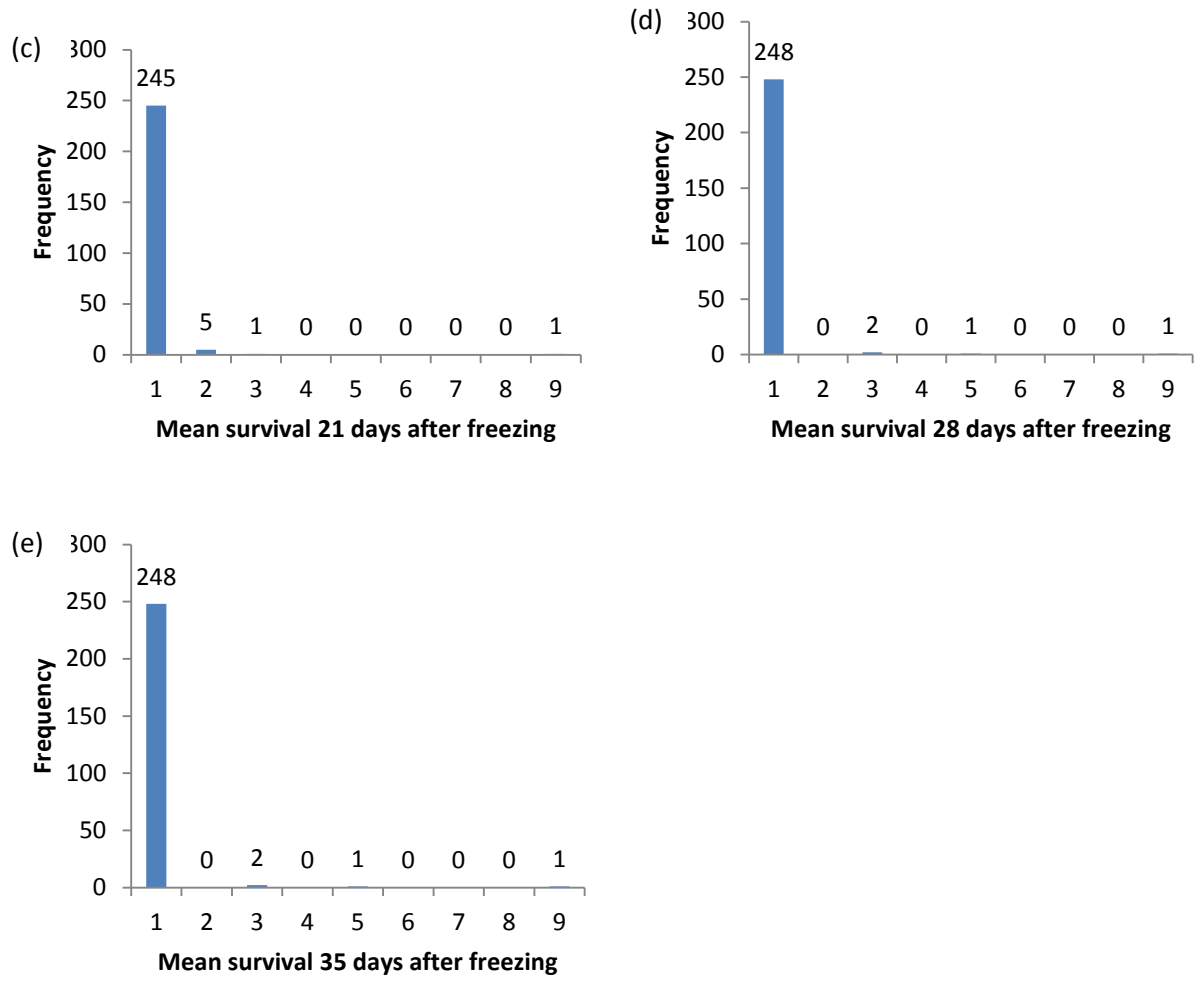

Figure 3.3. Mean survival scores for RILs frozen at -4^{0}C and scored 7 days after freezing (a), 14 days after freezing with 5 experiments missing (b), 21 days after freezing (c), 28 days after freezing (d), and 35 days after freezing (e) (continued).

Table 3.4. ANOVA for 252 RILs tested at -4^{0}C and scored 21 days after freezing.

Source	DF	Mean Square	Error DF	F Value	Pr > F
Genotype	251	0.41	441	2.82	<0.0001***
Rep	2	0.53	441	3.67	0.0264*
Residual	441	0.15	-	-	-

*, $p < 0.5$; *** $p < 0.001$

Freezing at -8^{0}C showed differential survival during the first 21 days of scoring (Figure 3.4 a - e). At 7 days after freezing 5 RILs had a mean of 9.0, 14 days after freezing 21 RILs had a mean of 9.0, 21 days of freezing 94 RILs had a mean of 9.0, 28 days after freezing 154 RILs had a mean of 9.0, and 35 days after freezing 182 RILs had a mean of 9.0. As expected, Melrose had greater initial survival than Medora. Melrose and seven RILs; PRIL-2-107, PRIL-2-146, PRIL-2-180, PRIL-2-184, PRIL-2-194, PRIL-2-225, and PRIL-2-230, performed well in both the field and greenhouse (Table 3.5). PRIL-2-230 which had the best mean survival score in the field and also performed well in the freezing chamber with a mean of 3.3 21 days after freezing (Table 3.5). Data analysis for the -8^{0}C treatment 21 days after freezing showed significance between genotypes and within replicates (Table 3.6) which may be due to the variability within the freezing chamber. The significance between genotypes was expected since one of the parents was a spring type and not expected to survive. The overall experiment mean was 6.6 and the CV was 39.9% for the data collected 21 days after freezing.

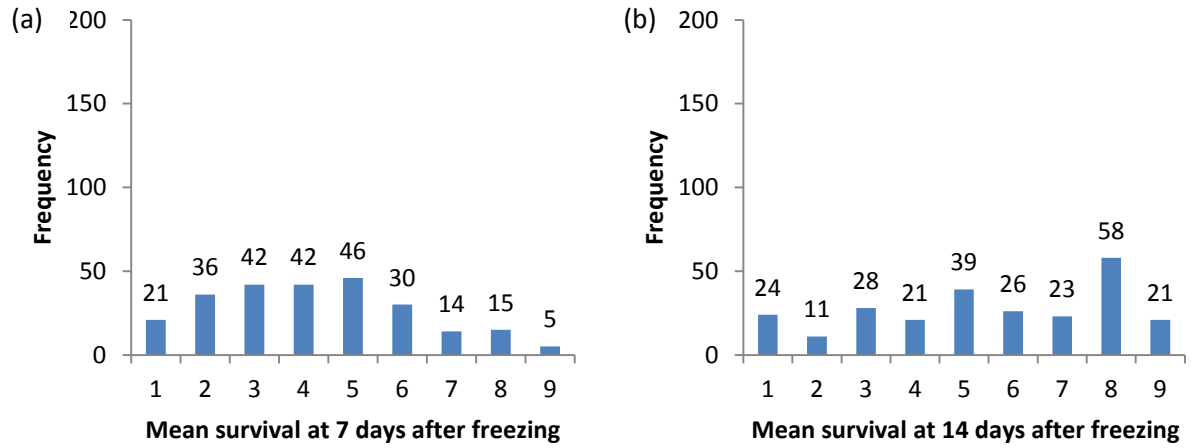

Figure 3.4. Mean survival scores for RILs frozen at -8^{0}C and scored 7 days after freezing (a), 14 days after freezing (b), 21 days after freezing (c), 28 days after freezing (d), and 35 days after freezing (e).

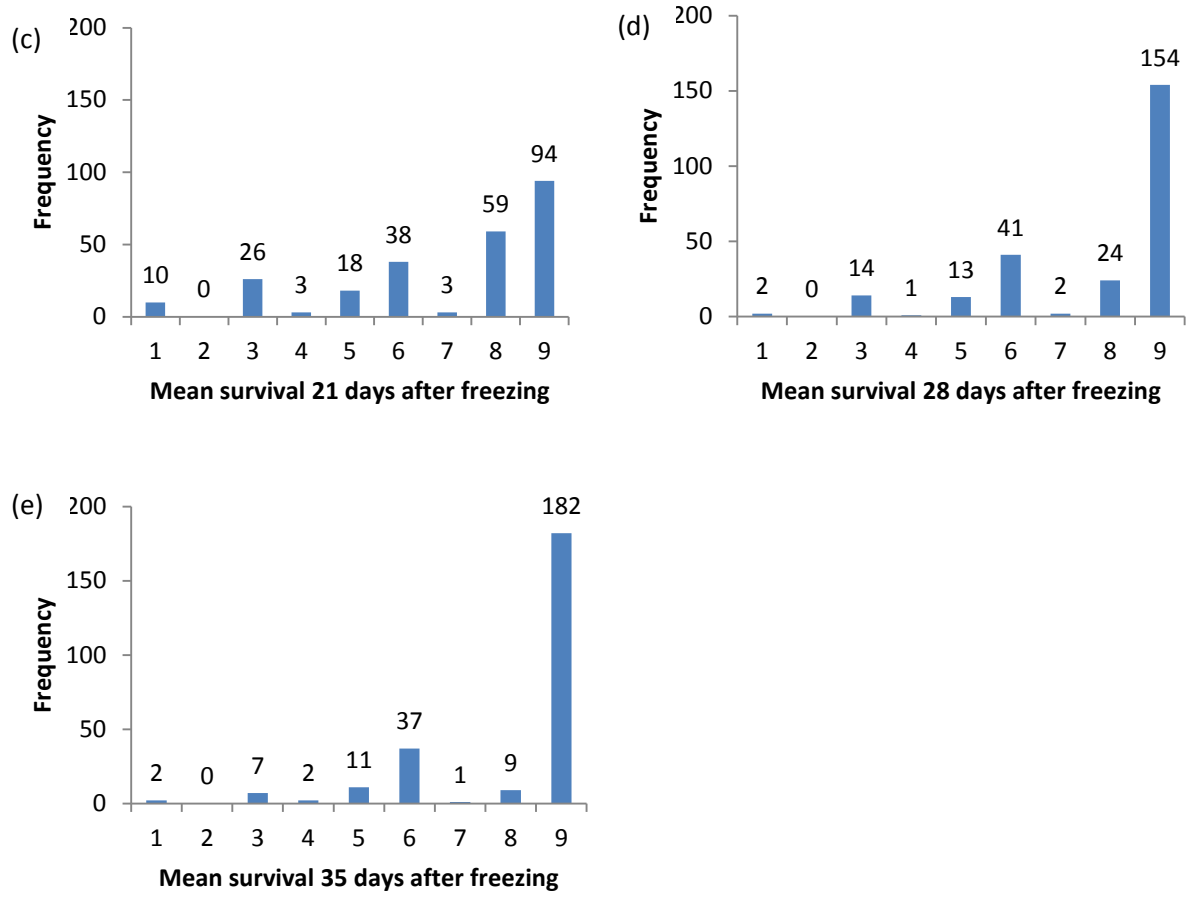

Figure 3.4. Mean survival scores for RILs frozen at -8^{0}C and scored 7 days after freezing (a), 14 days after freezing (b), 21 days after freezing (c), 28 days after freezing (d), and 35 days after freezing (e) (continued).

Table 3.5. Mean spring survival scores and mean score 35 days after freezing for the best performing lines in the field compared with the greenhouse.

Name	Mean spring survival score	Mean score 35 days after freezing
MELROSE	5.0	3.6
PRIL-2-107	4.0	4.3
PRIL-2-146	5.0	1.0
PRIL-2-180	5.0	1.0
PRIL-2-184	5.0	3.3
PRIL-2-194	4.0	3.7
PRIL-2-225	5.0	3.3
PRIL-2-230	3.0	3.3

Table 3.6. ANOVA for 251 RILs tested at -8^{0}C at scored 21 days after freezing.

Source	DF	Mean Square	Error DF	F Value	Pr > F
Genotype	250	14.12	436	1.85	<0.0001***
Rep	2	108.24	436	14.18	<0.0001***
Residual	436	7.63	-	-	-

***, $p < 0.001$

The -12^{0}C treatment indicated that the temperature was harsher and not much survival was observed initially, including Melrose (Figure 3.5 a – e). Seventy-seven of the lines had a mean score of 9.0 at 14 days after freezing while at -8^{0}C only twenty-one lines had a mean score of 9.0. ANOVA for -12^{0}C 21 days after freezing showed statistical significance between genotypes which was expected (Table 3.7). Genotypes that are similar to Medora, the spring parent, did not survive the freezing temperatures. No statistical significance was observed between replicates which indicates consistent responses across replicates. The overall mean for the experiement was 6.9 and the CV was 32.5% for data collected 21days after freezing.

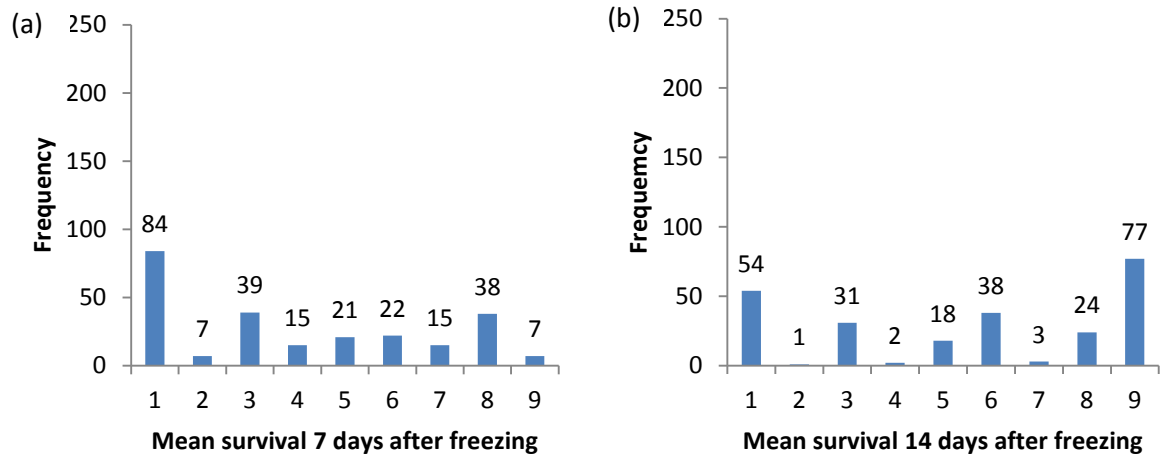

Figure 3.5. Mean survival scores for RILs frozen at -12^{0}C and scored 7 days after freezing (a), 14 days after freezing (b), 21 days after freezing (c), 28 days after freezing (d), and 35 days after freezing (e).

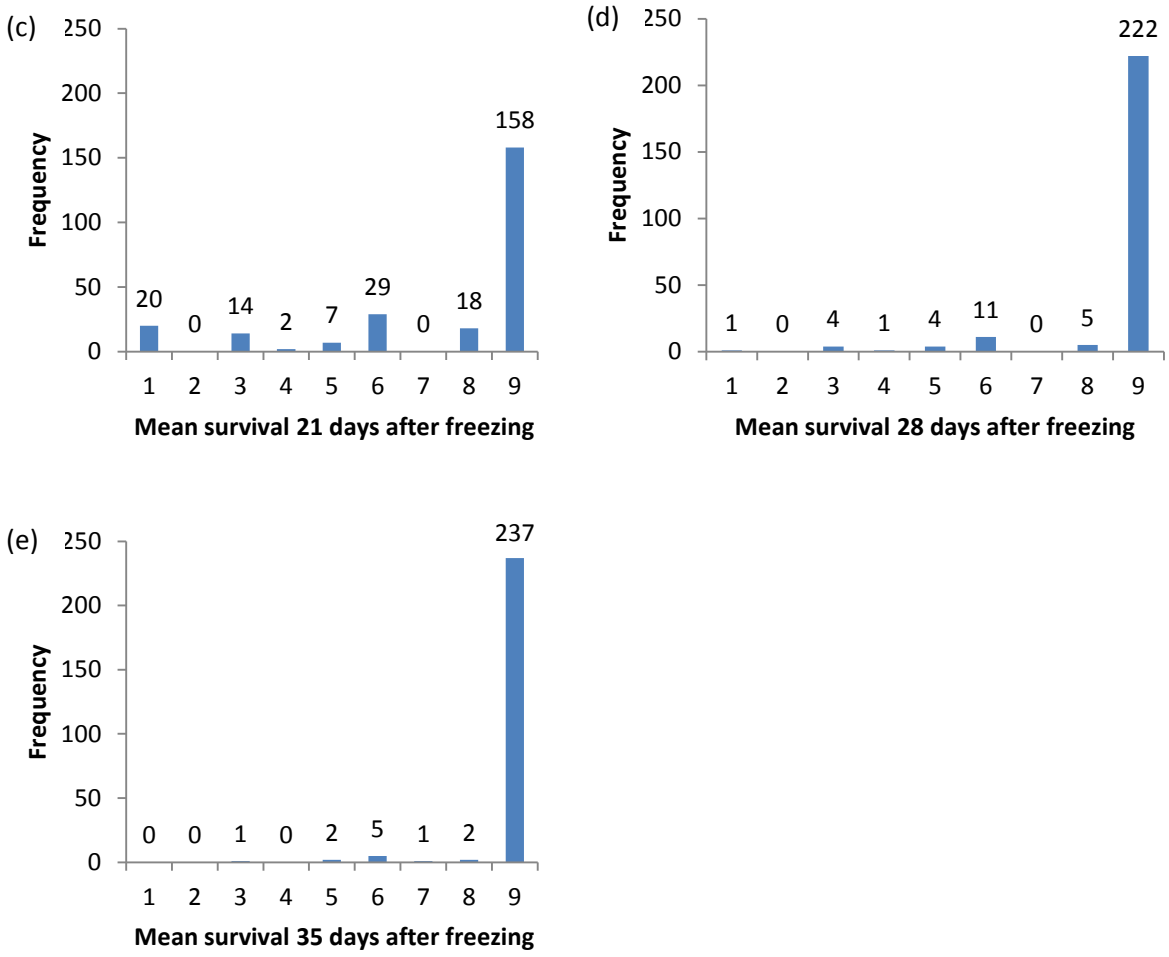

Figure 3.5. Mean survival scores for RILs frozen at -12^{0}C and scored 7 days after freezing (a), 14 days after freezing (b), 21 days after freezing (c), 28 days after freezing (d), and 35 days after freezing (e) (continued).

Table 3.7. ANOVA for 247 RILs tested at -12^{0}C treatment and scored 21 days after freezing.

Source	DF	Mean Square	Error DF	F Value	Pr > F
Genotype	246	16.08	439	2.72	<0.0001***
Rep	2	6.00	439	1.01	0.3634^{ns}
Residual	439	5.92	-	-	-

ns, not significant; ***, $p < 0.001$

A second and third set of experiments with a reduced set of 110 RILs showed similar trends to the first set of experiments and demonstrated that the -12^{0}C treatment was too harsh and little survival was observed. The RILs did not survive the freezing temperatures and died more rapidly than freezing at -8^{0}C. Histograms for the second and third runs are presented in Figures A2- A21.

Mean scores at 7 and 21 days after freezing for the -8^{0}C treatment were compared across all three runs are summarized in Table 3.8. For example, PRIL-2-002 had a mean of 1 at 21 days after freezing during the first run, but increased to 8.7 and 9.0, respectively, during the second and third runs. However, at 7 days after freezing the scores did not increase as drastically across the runs. Many lines showed increased scores or a decreased survival between runs, especially at 7 days after freezing but by 21 days after freezing the differences were lower.

The reduced set of RILs tested in the second and third runs did not represent all suspected winter hardy RILs. PRIL-2-107 was present in all three and performed similarly 7 days after freezing, but had an increased mean in the third run at 21 days after freezing.

Table 3.8. Means across all runs of RILs frozen at -8^{0}C in the greenhouse and scored 7 and 21 days after freezing.

	7 days after freezing				21 days after freezing			
Name	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
MEDORA	6.5	8.4	5.7	6.8	8.3	9.0	9.0	8.8
MELROSE	2.4	1.0	1.0	1.5	3.6	4.1	5.6	4.4
PRIL-2-001	-	9.0	8.0	8.5	-	9.0	9.0	9.0
PRIL-2-002	1.0	3.3	6.0	3.4	1.0	8.7	9.0	6.2
PRIL-2-003	-	1.0	1.0	1.0	-	9.0	9.0	9.0
PRIL-2-004	5.7	5.0	4.5	5.1	6.0	5.0	9.0	6.7
PRIL-2-005	4.0	8.5	8.0	6.8	9.0	9.0	9.0	9.0
PRIL-2-007	2.0	-	-	2.0	6.3	-	-	6.3
PRIL-2-008	-	4.5	8.0	6.3	-	9.0	9.0	9.0
PRIL-2-009	3.0	8.0	8.0	6.3	5.0	9.0	9.0	7.7

- Line not present in experiment

Table 3.8. Means across all runs of RILs frozen at -8^{0}C in the greenhouse and scored 7 and 21 days after freezing (continued).

	7 days after freezing				21 days after freezing			
Name	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
PRIL-2-010	3.0	9.0	4.5	5.5	3.7	9.0	9.0	7.2
PRIL-2-011	3.5	1.0	5.7	3.4	8.5	5.0	6.3	6.6
PRIL-2-012	4.0	4.5	1.0	3.2	1.0	9.0	8.5	6.2
PRIL-2-013	5.0	9.0	8.0	7.3	9.0	9.0	9.0	9.0
PRIL-2-014	3.5	4.5	6.0	4.7	5.0	9.0	6.3	6.8
PRIL-2-015	4.0	8.5	4.5	5.7	8.0	9.0	9.0	8.7
PRIL-2-016	5.0	9.0	8.0	7.3	5.0	9.0	9.0	7.7
PRIL-2-017	3.0	8.7	8.0	6.6	3.7	9.0	9.0	7.2
PRIL-2-018	4.0	1.0	1.0	2.0	6.3	9.0	9.0	8.1
PRIL-2-019	1.0	8.7	3.3	4.3	3.3	9.0	9.0	7.1
PRIL-2-020	6.0	1.0	6.0	4.3	9.0	9.0	9.0	9.0
PRIL-2-021	5.7	1.0	8.0	4.9	5.3	9.0	9.0	7.8
PRIL-2-022	-	1.0	1.0	1.0	-	9.0	9.0	9.0
PRIL-2-023	5.3	9.0	8.0	7.4	8.7	9.0	9.0	8.9
PRIL-2-024	3.7	5.0	1.0	3.2	3.7	9.0	9.0	7.2
PRIL-2-025	8.3	8.3	8.3	8.3	9.0	9.0	9.0	9.0
PRIL-2-026	8.5	3.3	6.0	5.9	9.0	9.0	9.0	9.0
PRIL-2-027	4.7	5.0	5.0	4.9	6.3	9.0	9.0	8.1
PRIL-2-028	7.7	4.5	6.0	6.1	9.0	9.0	9.0	9.0
PRIL-2-029	7.7	8.7	6.3	7.6	9.0	9.0	8.3	8.8
PRIL-2-030	6.7	3.3	3.3	4.4	8.7	8.7	9.0	8.8
PRIL-2-031	5.0	5.0	3.3	4.4	6.3	9.0	9.0	8.1
PRIL-2-032	4.7	3.3	8.0	5.3	8.3	9.0	9.0	8.8
PRIL-2-033	6.7	9.0	8.0	7.9	6.3	9.0	9.0	8.1
PRIL-2-034	7.0	8.5	5.7	7.1	9.0	9.0	9.0	9.0
PRIL-2-037	5.0	8.0	5.7	6.2	7.0	9.0	9.0	8.3
PRIL-2-038	6.7	8.3	8.5	7.8	8.7	9.0	9.0	8.9
PRIL-2-039	8.0	8.5	8.0	8.2	9.0	9.0	9.0	9.0
PRIL-2-040	5.0	8.3	8.0	7.1	9.0	9.0	9.0	9.0
PRIL-2-041	8.7	-	-	8.7	6.3	-	-	6.3
PRIL-2-042	4.0	9.0	8.0	7.0	9.0	9.0	9.0	9.0
PRIL-2-043	5.3	8.3	1.0	4.9	8.3	9.0	9.0	8.8
PRIL-2-044	8.3	-	8.0	8.2	9.0	-	9.0	9.0
PRIL-2-045	6.3	8.0	8.0	7.4	9.0	9.0	9.0	9.0
PRIL-2-046	6.0	8.0	8.0	7.3	9.0	9.0	9.0	9.0

- Line not present in experiment

Table 3.8. Means across all runs of RILs frozen at -8^{0}C in the greenhouse and scored 7 and 21 days after freezing (continued).

	7 days after freezing				21 days after freezing			
Name	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
PRIL-2-047	8.3	3.7	8.3	6.8	9.0	6.3	9.0	8.1
PRIL-2-048	7.7	1.0	4.5	4.4	9.0	4.3	9.0	7.4
PRIL-2-049	8.3	5.3	8.0	7.2	8.7	9.0	9.0	8.9
PRIL-2-050	6.0	8.7	5.0	6.6	6.3	9.0	9.0	8.1
PRIL-2-051	9.0	-	-	9.0	9.0	-	-	9.0
PRIL-2-052	8.7	8.7	5.7	7.7	9.0	9.0	9.0	9.0
PRIL-2-053	8.3	3.3	3.3	5.0	9.0	9.0	9.0	9.0
PRIL-2-054	8.0	8.3	5.7	7.3	6.3	9.0	8.7	8.0
PRIL-2-055	7.0	3.3	5.7	5.3	9.0	9.0	9.0	9.0
PRIL-2-056	9.0	8.0	3.3	6.8	9.0	9.0	9.0	9.0
PRIL-2-057	9.0	6.3	3.7	6.3	9.0	9.0	9.0	9.0
PRIL-2-058	5.7	-	-	5.7	8.7	-	-	8.7
PRIL-2-059	8.3	6.0	3.3	5.9	9.0	9.0	9.0	9.0
PRIL-2-061	8.0	5.7	5.7	6.4	9.0	9.0	9.0	9.0
PRIL-2-062	7.7	8.5	8.3	8.2	9.0	9.0	9.0	9.0
PRIL-2-063	6.3	4.5	4.5	5.1	6.3	9.0	9.0	8.1
PRIL-2-064	7.7	9.0	8.5	8.4	9.0	9.0	9.0	9.0
PRIL-2-065	9.0	9.0	8.5	8.8	9.0	9.0	9.0	9.0
PRIL-2-066	8.0	1.0	1.0	3.3	9.0	9.0	9.0	9.0
PRIL-2-067	8.3	-	-	8.3	9.0	-	-	9.0
PRIL-2-068	6.0	3.7	1.0	3.6	6.3	9.0	8.5	7.9
PRIL-2-069	7.3	4.5	4.0	5.3	6.3	9.0	9.0	8.1
PRIL-2-070	5.0	6.0	8.0	6.3	9.0	9.0	9.0	9.0
PRIL-2-071	6.5	8.0	5.7	6.7	9.0	9.0	9.0	9.0
PRIL-2-072	3.0	6.3	3.3	4.2	6.0	9.0	9.0	8.0
PRIL-2-073	3.3	8.0	8.5	6.6	6.3	9.0	9.0	8.1
PRIL-2-074	4.0	9.0	8.0	7.0	9.0	9.0	9.0	9.0
PRIL-2-075	-	8.7	9.0	8.8	-	9.0	9.0	9.0
PRIL-2-076	4.0	1.0	3.3	2.8	5.0	6.3	9.0	6.8
PRIL-2-077	7.0	-	-	7.0	8.5	-	-	8.5
PRIL-2-078	3.0	9.0	8.0	6.7	9.0	9.0	9.0	9.0
PRIL-2-079	4.0	5.0	1.0	3.3	8.5	9.0	9.0	8.8
PRIL-2-080	7.0	3.3	3.3	4.6	8.3	9.0	9.0	8.8
PRIL-2-081	3.5	8.0	1.0	4.2	9.0	9.0	9.0	9.0
PRIL-2-082	2.7	3.3	6.0	4.0	6.0	9.0	9.0	8.0

- Line not present in experiment

Table 3.8. Means across all runs of RILs frozen at -8^{0}C in the greenhouse and scored 7 and 21 days after freezing (continued).

	7 days after freezing				21 days after freezing			
Name	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
PRIL-2-083	2.7	1.0	1.0	1.6	6.0	9.0	9.0	8.0
PRIL-2-084	2.7	-	-	2.7	8.7	-	-	8.7
PRIL-2-085	4.3	3.3	3.3	3.7	5.7	9.0	9.0	7.9
PRIL-2-086	7.0	-	-	7.0	9.0	-	-	9.0
PRIL-2-087	1.0	6.0	1.0	2.7	9.0	6.3	9.0	8.1
PRIL-2-088	1.0	-	-	1.0	9.0	-	-	9.0
PRIL-2-089	3.7	5.0	3.3	4.0	3.3	9.0	9.0	7.1
PRIL-2-090	3.3	6.3	5.7	5.1	6.0	6.3	9.0	7.1
PRIL-2-091	1.0	-	-	1.0	1.0	-	-	1.0
PRIL-2-092	3.3	7.0	1.0	3.8	8.7	6.3	9.0	8.0
PRIL-2-093	5.0	9.0	8.0	7.3	9.0	9.0	9.0	9.0
PRIL-2-094	1.0	-	-	1.0	8.5	-	-	8.5
PRIL-2-095	3.7	9.0	4.5	5.7	6.0	9.0	9.0	8.0
PRIL-2-096	4.7	8.0	8.0	6.9	8.0	9.0	9.0	8.7
PRIL-2-097	2.3	8.0	3.3	4.6	9.0	9.0	9.0	9.0
PRIL-2-098	5.0	5.0	3.3	4.4	9.0	5.0	9.0	7.7
PRIL-2-099	5.0	5.0	8.5	6.2	8.0	9.0	9.0	8.7
PRIL-2-100	5.3	-	-	5.3	8.7	-	-	8.7
PRIL-2-101	2.5	8.5	8.0	6.3	5.0	9.0	9.0	7.7
PRIL-2-102	3.0	8.3	1.0	4.1	8.7	9.0	9.0	8.9
PRIL-2-103	3.5	4.5	8.0	5.3	8.5	9.0	9.0	8.8
PRIL-2-104	6.7	8.0	8.0	7.6	9.0	9.0	9.0	9.0
PRIL-2-105	3.3	8.0	8.0	6.4	9.0	9.0	9.0	9.0
PRIL-2-106	2.5	-	-	2.5	9.0	-	-	9.0
PRIL-2-107	3.3	1.0	1.0	1.8	4.3	3.7	6.3	4.8
PRIL-2-108	5.0	8.3	5.7	6.3	8.3	9.0	9.0	8.8
PRIL-2-109	3.7	1.0	6.0	3.6	8.7	9.0	9.0	8.9
PRIL-2-110	1.0	3.7	1.0	1.9	9.0	6.3	8.7	8.0
PRIL-2-111	4.0	4.5	1.0	3.2	9.0	9.0	9.0	9.0
PRIL-2-112	3.3	5.7	1.0	3.3	7.0	9.0	9.0	8.3
PRIL-2-113	6.0	5.0	4.5	5.2	8.3	9.0	9.0	8.8
PRIL-2-114	4.7	5.7	3.3	4.6	9.0	9.0	9.0	9.0
PRIL-2-115	6.0	5.7	1.0	4.2	9.0	9.0	9.0	9.0
PRIL-2-116	6.3	8.0	3.3	5.9	9.0	9.0	9.0	9.0
PRIL-2-117	7.7	6.3	4.5	6.2	9.0	9.0	5.0	7.7

- Line not present in experiment

Table 3.8. Means across all runs of RILs frozen at -8^{0}C in the greenhouse and scored 7 and 21 days after freezing (continued).

	7 days after freezing				21 days after freezing			
Name	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
PRIL-2-118	6.3	5.7	3.3	5.1	6.3	9.0	9.0	8.1
PRIL-2-119	7.0	8.3	5.7	7.0	9.0	9.0	9.0	9.0
PRIL-2-120	6.3	8.3	4.5	6.4	8.7	9.0	9.0	8.9
PRIL-2-121	4.5	3.7	8.0	5.4	9.0	6.3	9.0	8.1
PRIL-2-122	6.7	1.0	1.0	2.9	9.0	9.0	9.0	9.0
PRIL-2-123	1.0	-	-	1.0	9.0	-	-	9.0
PRIL-2-124	6.7	4.5	5.0	5.4	9.0	8.5	9.0	8.8
PRIL-2-125	4.0	1.0	1.0	2.0	9.0	9.0	9.0	9.0
PRIL-2-126	6.5	8.0	8.0	7.5	9.0	9.0	9.0	9.0
PRIL-2-127	8.0	5.7	3.3	5.7	9.0	9.0	9.0	9.0
PRIL-2-128	6.0	8.0	5.7	6.6	8.7	9.0	9.0	8.9
PRIL-2-129	5.0	-	-	5.0	9.0	-	-	9.0
PRIL-2-130	6.0	-	-	6.0	9.0	-	-	9.0
PRIL-2-131	5.3	-	-	5.3	9.0	-	-	9.0
PRIL-2-132	4.0	-	-	4.0	6.3	-	-	6.3
PRIL-2-133	6.0	-	-	6.0	9.0	-	-	9.0
PRIL-2-134	4.7	-	-	4.7	8.7	-	-	8.7
PRIL-2-135	6.0	-	-	6.0	9.0	-	-	9.0
PRIL-2-136	3.3	-	-	3.3	3.3	-	-	3.3
PRIL-2-137	2.0	-	-	2.0	3.7	-	-	3.7
PRIL-2-138	3.0	-	-	3.0	5.0	-	-	5.0
PRIL-2-139	5.0	-	-	5.0	6.0	-	-	6.0
PRIL-2-140	2.0	-	-	2.0	3.3	-	-	3.3
PRIL-2-141	3.0	-	-	3.0	9.0	-	-	9.0
PRIL-2-142	2.7	-	-	2.7	6.0	-	-	6.0
PRIL-2-144	2.3	-	-	2.3	3.3	-	-	3.3
PRIL-2-145	1.0	-	-	1.0	1.0	-	-	1.0
PRIL-2-146	1.7	-	-	1.7	1.0	-	-	1.0
PRIL-2-147	5.0	-	-	5.0	8.7	-	-	8.7
PRIL-2-148	7.3	-	-	7.3	9.0	-	-	9.0
PRIL-2-149	3.3	-	-	3.3	8.7	-	-	8.7
PRIL-2-150	4.0	-	-	4.0	6.3	-	-	6.3
PRIL-2-151	3.3	-	-	3.3	9.0	-	-	9.0
PRIL-2-152	4.0	-	-	4.0	8.0	-	-	8.0
PRIL-2-153	1.0	-	-	1.0	1.0	-	-	1.0

- Line not present in experiment

Table 3.8. Means across all runs of RILs frozen at -8^{0}C in the greenhouse and scored 7 and 21 days after freezing (continued).

	7 days after freezing				21 days after freezing			
Name	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
PRIL-2-154	1.0	-	-	1.0	6.0	-	-	6.0
PRIL-2-155	2.3	-	-	2.3	6.3	-	-	6.3
PRIL-2-156	5.3	-	-	5.3	8.7	-	-	8.7
PRIL-2-157	2.7	-	-	2.7	5.0	-	-	5.0
PRIL-2-158	5.7	-	-	5.7	9.0	-	-	9.0
PRIL-2-159	5.3	-	-	5.3	8.3	-	-	8.3
PRIL-2-160	2.7	-	-	2.7	8.0	-	-	8.0
PRIL-2-161	6.5	-	-	6.5	9.0	-	-	9.0
PRIL-2-162	5.3	-	-	5.3	8.7	-	-	8.7
PRIL-2-163	5.5	-	-	5.5	8.5	-	-	8.5
PRIL-2-164	2.3	-	-	2.3	6.0	-	-	6.0
PRIL-2-165	5.3	-	-	5.3	8.3	-	-	8.3
PRIL-2-166	5.0	-	-	5.0	8.7	-	-	8.7
PRIL-2-167	5.0	-	-	5.0	8.7	-	-	8.7
PRIL-2-168	4.3	-	-	4.3	8.3	-	-	8.3
PRIL-2-169	1.0	-	-	1.0	8.5	-	-	8.5
PRIL-2-170	3.5	-	-	3.5	9.0	-	-	9.0
PRIL-2-171	4.7	-	-	4.7	9.0	-	-	9.0
PRIL-2-172	3.0	-	-	3.0	3.7	-	-	3.7
PRIL-2-173	4.3	-	-	4.3	6.0	-	-	6.0
PRIL-2-174	5.5	-	-	5.5	9.0	-	-	9.0
PRIL-2-175	4.0	-	-	4.0	5.7	-	-	5.7
PRIL-2-176	5.5	-	-	5.5	8.5	-	-	8.5
PRIL-2-177	4.0	-	-	4.0	8.3	-	-	8.3
PRIL-2-178	3.7	-	-	3.7	3.3	-	-	3.3
PRIL-2-179	3.0	-	-	3.0	4.5	-	-	4.5
PRIL-2-180	1.0	-	-	1.0	1.0	-	-	1.0
PRIL-2-181	5.5	-	-	5.5	5.0	-	-	5.0
PRIL-2-182	4.3	-	-	4.3	3.3	-	-	3.3
PRIL-2-183	5.0	-	-	5.0	8.0	-	-	8.0
PRIL-2-184	2.0	-	-	2.0	3.3	-	-	3.3
PRIL-2-185	3.7	-	-	3.7	6.0	-	-	6.0
PRIL-2-186	2.3	-	-	2.3	6.0	-	-	6.0
PRIL-2-187	4.3	-	-	4.3	9.0	-	-	9.0
PRIL-2-188	3.7	-	-	3.7	8.3	-	-	8.3

- Line not present in experiment

Table 3.8. Means across all runs of RILs frozen at -8^{0}C in the greenhouse and scored 7 and 21 days after freezing (continued).

	7 days after freezing				21 days after freezing			
Name	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
PRIL-2-189	5.5	-	-	5.5	9.0	-	-	9.0
PRIL-2-190	5.0	-	-	5.0	9.0	-	-	9.0
PRIL-2-191	2.0	-	-	2.0	3.7	-	-	3.7
PRIL-2-192	4.0	-	-	4.0	9.0	-	-	9.0
PRIL-2-193	1.0	-	-	1.0	1.0	-	-	1.0
PRIL-2-194	1.0	-	-	1.0	3.7	-	-	3.7
PRIL-2-195	9.0	-	-	9.0	9.0	-	-	9.0
PRIL-2-196	3.3	-	-	3.3	8.7	-	-	8.7
PRIL-2-197	2.7	-	-	2.7	6.0	-	-	6.0
PRIL-2-198	1.0	-	-	1.0	8.7	-	-	8.7
PRIL-2-199	3.7	-	-	3.7	8.3	-	-	8.3
PRIL-2-200	3.5	-	-	3.5	8.0	-	-	8.0
PRIL-2-201	2.3	-	-	2.3	8.3	-	-	8.3
PRIL-2-202	4.0	-	-	4.0	6.3	-	-	6.3
PRIL-2-203	3.5	-	-	3.5	5.0	-	-	5.0
PRIL-2-204	5.5	-	-	5.5	9.0	-	-	9.0
PRIL-2-205	4.5	-	-	4.5	9.0	-	-	9.0
PRIL-2-206	6.0	-	-	6.0	9.0	-	-	9.0
PRIL-2-207	4.3	-	-	4.3	8.7	-	-	8.7
PRIL-2-208	6.0	-	-	6.0	9.0	-	-	9.0
PRIL-2-209	3.5	-	-	3.5	5.0	-	-	5.0
PRIL-2-210	5.0	-	-	5.0	8.7	-	-	8.7
PRIL-2-212	2.3	-	-	2.3	3.7	-	-	3.7
PRIL-2-213	5.3	-	-	5.3	9.0	-	-	9.0
PRIL-2-214	4.3	-	-	4.3	7.7	-	-	7.7
PRIL-2-215	6.5	-	-	6.5	9.0	-	-	9.0
PRIL-2-216	3.0	-	-	3.0	9.0	-	-	9.0
PRIL-2-217	4.7	-	-	4.7	6.3	-	-	6.3
PRIL-2-218	4.7	-	-	4.7	6.3	-	-	6.3
PRIL-2-219	6.0	-	-	6.0	6.3	-	-	6.3
PRIL-2-220	6.0	-	-	6.0	9.0	-	-	9.0
PRIL-2-221	5.5	-	-	5.5	9.0	-	-	9.0
PRIL-2-224	5.3	-	-	5.3	9.0	-	-	9.0
PRIL-2-225	2.0	-	-	2.0	3.3	-	-	3.3
PRIL-2-226	5.0	-	-	5.0	9.0	-	-	9.0

- Line not present in experiment

Table 3.8. Means across all runs of RILs frozen at -8^{0}C in the greenhouse and scored 7 and 21 days after freezing (continued).

	7 days after freezing				21 days after freezing			
Name	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
PRIL-2-228	3.7	-	-	3.7	6.7	-	-	6.7
PRIL-2-229	1.0	-	-	1.0	1.0	-	-	1.0
PRIL-2-230	2.0	-	-	2.0	3.3	-	-	3.3
PRIL-2-231	4.5	-	-	4.5	8.5	-	-	8.5
PRIL-2-233	5.7	-	-	5.7	9.0	-	-	9.0
PRIL-2-234	4.5	-	-	4.5	5.0	-	-	5.0
PRIL-2-235	4.0	-	-	4.0	8.5	-	-	8.5
PRIL-2-238	5.0	-	-	5.0	9.0	-	-	9.0
PRIL-2-239	3.5	-	-	3.5	5.0	-	-	5.0
PRIL-2-240	5.3	-	-	5.3	8.7	-	-	8.7
PRIL-2-241	2.3	-	-	2.3	8.3	-	-	8.3
PRIL-2-242	2.5	-	-	2.5	4.5	-	-	4.5
PRIL-2-243	2.3	-	-	2.3	3.7	-	-	3.7
PRIL-2-244	2.0	-	-	2.0	1.0	-	-	1.0
PRIL-2-245	4.7	-	-	4.7	9.0	-	-	9.0
PRIL-2-246	4.0	-	-	4.0	5.7	-	-	5.7
PRIL-2-247	3.3	-	-	3.3	3.3	-	-	3.3
PRIL-2-248	4.3	-	-	4.3	8.0	-	-	8.0
PRIL-2-249	2.0	-	-	2.0	8.0	-	-	8.0
PRIL-2-250	1.0	-	-	1.0	6.0	-	-	6.0
PRIL-2-251	4.7	-	-	4.7	6.3	-	-	6.3
PRIL-2-252	3.0	-	-	3.0	9.0	-	-	9.0
PRIL-2-253	2.7	-	-	2.7	9.0	-	-	9.0
PRIL-2-254	5.0	-	-	5.0	9.0	-	-	9.0
PRIL-2-255	2.0	-	-	2.0	8.5	-	-	8.5
PRIL-2-256	5.0	-	-	5.0	9.0	-	-	9.0
PRIL-2-257	1.7	-	-	1.7	5.7	-	-	5.7
PRIL-2-258	2.7	-	-	2.7	5.7	-	-	5.7
PRIL-2-259	2.7	-	-	2.7	3.7	-	-	3.7
PRIL-2-260	2.3	-	-	2.3	9.0	-	-	9.0
PRIL-2-261	2.0	-	-	2.0	3.3	-	-	3.3
PRIL-2-262	3.0	-	-	3.0	3.3	-	-	3.3
PRIL-2-263	5.7	-	-	5.7	8.7	-	-	8.7
PRIL-2-265	2.3	-	-	2.3	3.3	-	-	3.3
PRIL-2-266	1.0	-	-	1.0	3.3	-	-	3.3

- Line not present in experiment

Table 3.8. Means across all runs of RILs frozen at -8^{0}C in the greenhouse and scored 7 and 21 days after freezing (continued).

	7 days after freezing				21 days after freezing			
Name	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
PRIL-2-267	4.3	-	-	4.3	6.0	-	-	6.0

- Line not present in experiment

Discussion

Winter pea would be beneficial for growers' rotations because of the benefits of a legume and a fall-sown crop as other winter crops have been shown to be beneficial for rotations. Winter pea has potential for higher yields than spring-sown pea if hardiness is sufficient for survival. Identifying winter hardy pea lines is the first step in developing winter pea as a viable crop option. Winter hardiness can be evaluated in the field under natural conditions and in the greenhouse under artificial conditions using an established protocol that best predicts hardiness, but all lines must be tested in the field for true winter hardiness and the ability to withstand other stresses, such as diseases. Data from this experiment could be used in future studies. RILs identified in the greenhouse should be field tested to eliminate false positives. Some of the RILs identified in the greenhouse did perform well in 2011; however, the experiment should be replicated to determine if winter hardiness is present.

Field testing is an important aspect of determining winter hardiness because field conditions cannot fully be followed in the greenhouse. Field studies are exposed to many stresses including, water, disease, pest, and weed pressure that greenhouse grown plants are not exposed to. Greenhouse plants have adequate water and temperature conditions that could make it easier for plants to survive. Controlled environmental conditions tend to be milder and have a shorter

duration of freezing. Greenhouse plants are also not exposed to the same freeze- thaw cycles that would be experienced in the field.

The protocol used for the greenhouse study was based on previous work on winter hardiness in pea and each experiment lasted eleven weeks. Four weeks of acclimation was chosen because it showed the greatest potential for survival. However, a study completed to help optimize the protocol was conducted and three weeks of acclimation was determined to be a better indicator of survival. Four weeks of acclimation showed decreased survival when compared with three weeks of acclimation. Based on results from the acclimation study to optimize the protocol, it was also determined that 21 days after freezing was sufficient for making decisions and 35 days after freezing is not necessary. The optimized protocol brings the total time required down to eight weeks, which saves three weeks for every experiment.

Conclusion

Predicting potential winter hardy lines was conducted in controlled conditions and the field. PRIL-2-107, PRIL-2-146, PRIL-2-180, PRIL-2-184, PRIL-2-194, PRIL-2-225, and PRIL-2-230 had good field and greenhouse performance which indicates the potential for successful predictions of winter hardy lines. Further field testing is needed on all lines to test winter hardiness and to verify resistance to other factors that are not able to be tested in controlled conditions. Factors in the field include disease, insect, and weed pressure, and water stresses. These stresses can be found individually or in any combination. Greenhouse plants are not exposed to many of these stresses

The controlled environment experiments turned out as expected with many lines not surviving. Also, testing three temperatures helped to determine one optimal temperature for testing in artificial conditions. The highest temperature (-4^0C) and the lowest temperature (-

12^{0}C) were too mild or too harsh, respectively. Other temperatures around -8^{0}C could also be tested to determine if a better test temperature can be found.

The results from this study did identify some lines that have potential winter hardiness. These lines should be further tested and evaluated for disease resistance and yield. Selection for superior quality traits must also be maintained as winter pea cultivars are being developed. The nutritional characteristics of winter pea must be evaluated to maintain adequate quality.

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APPENDIX

Table A1. Mean spring survival scores for all RILs in 2011-2012.

Name	Mean spring survival	Name	Mean spring survival
PRIL-2-230	3.0	PRIL-2-012	9.0
PRIL-2-107	4.0	PRIL-2-013	9.0
PRIL-2-194	4.0	PRIL-2-016	9.0
MELROSE	5.0	PRIL-2-018	9.0
PRIL-2-146	5.0	PRIL-2-019	9.0
PRIL-2-180	5.0	PRIL-2-020	9.0
PRIL-2-184	5.0	PRIL-2-021	9.0
PRIL-2-225	5.0	PRIL-2-022	9.0
PRIL-2-068	5.5	PRIL-2-025	9.0
PRIL-2-091	5.5	PRIL-2-026	9.0
PRIL-2-212	5.5	PRIL-2-028	9.0
PRIL-2-006	6.0	PRIL-2-029	9.0
PRIL-2-073	6.5	PRIL-2-032	9.0
PRIL-2-109	6.5	PRIL-2-033	9.0
PRIL-2-159	6.5	PRIL-2-039	9.0
PRIL-2-201	6.5	PRIL-2-040	9.0
PRIL-2-202	6.5	PRIL-2-042	9.0
PRIL-2-244	6.5	PRIL-2-044	9.0
PRIL-2-007	7.0	PRIL-2-045	9.0
PRIL-2-050	7.0	PRIL-2-046	9.0
PRIL-2-055	7.0	PRIL-2-047	9.0
PRIL-2-095	7.0	PRIL-2-049	9.0
PRIL-2-160	7.0	PRIL-2-051	9.0
PRIL-2-165	7.0	PRIL-2-052	9.0
PRIL-2-203	7.0	PRIL-2-056	9.0
PRIL-2-243	7.0	PRIL-2-057	9.0
PRIL-2-246	7.0	PRIL-2-058	9.0
PRIL-2-259	7.0	PRIL-2-061	9.0
PRIL-2-015	7.5	PRIL-2-062	9.0
PRIL-2-023	7.5	PRIL-2-063	9.0
PRIL-2-092	7.5	PRIL-2-064	9.0
PRIL-2-110	7.5	PRIL-2-067	9.0
PRIL-2-130	7.5	PRIL-2-069	9.0
PRIL-2-135	7.5	PRIL-2-070	9.0
PRIL-2-150	7.5	PRIL-2-072	9.0
PRIL-2-154	7.5	PRIL-2-074	9.0

Table A1. Mean spring survival scores for all RILs in 2011-2012 (continued).

Name	Mean spring survival	Name	Mean spring survival
PRIL-2-179	7.5	PRIL-2-075	9.0
PRIL-2-186	7.5	PRIL-2-077	9.0
PRIL-2-191	7.5	PRIL-2-080	9.0
PRIL-2-220	7.5	PRIL-2-081	9.0
PRIL-2-229	7.5	PRIL-2-083	9.0
PRIL-2-011	8.0	PRIL-2-086	9.0
PRIL-2-017	8.0	PRIL-2-087	9.0
PRIL-2-027	8.0	PRIL-2-088	9.0
PRIL-2-034	8.0	PRIL-2-090	9.0
PRIL-2-038	8.0	PRIL-2-093	9.0
PRIL-2-053	8.0	PRIL-2-099	9.0
PRIL-2-054	8.0	PRIL-2-100	9.0
PRIL-2-084	8.0	PRIL-2-103	9.0
PRIL-2-089	8.0	PRIL-2-104	9.0
PRIL-2-096	8.0	PRIL-2-105	9.0
PRIL-2-098	8.0	PRIL-2-111	9.0
PRIL-2-102	8.0	PRIL-2-112	9.0
PRIL-2-108	8.0	PRIL-2-113	9.0
PRIL-2-123	8.0	PRIL-2-114	9.0
PRIL-2-125	8.0	PRIL-2-115	9.0
PRIL-2-136	8.0	PRIL-2-116	9.0
PRIL-2-137	8.0	PRIL-2-117	9.0
PRIL-2-172	8.0	PRIL-2-119	9.0
PRIL-2-182	8.0	PRIL-2-121	9.0
PRIL-2-209	8.0	PRIL-2-122	9.0
PRIL-2-214	8.0	PRIL-2-127	9.0
PRIL-2-223	8.0	PRIL-2-128	9.0
PRIL-2-239	8.0	PRIL-2-129	9.0
PRIL-2-241	8.0	PRIL-2-131	9.0
PRIL-2-261	8.0	PRIL-2-132	9.0
PRIL-2-249	8.3	PRIL-2-133	9.0
PRIL-2-009	8.5	PRIL-2-134	9.0
PRIL-2-010	8.5	PRIL-2-138	9.0
PRIL-2-014	8.5	PRIL-2-139	9.0
PRIL-2-024	8.5	PRIL-2-143	9.0
PRIL-2-031	8.5	PRIL-2-145	9.0

Table A1. Mean spring survival scores for all RILs in 2011-2012 (continued).

Name	Mean spring survival	Name	Mean spring survival
PRIL-2-037	8.5	PRIL-2-148	9.0
PRIL-2-043	8.5	PRIL-2-151	9.0
PRIL-2-065	8.5	PRIL-2-152	9.0
PRIL-2-066	8.5	PRIL-2-153	9.0
PRIL-2-071	8.5	PRIL-2-155	9.0
PRIL-2-076	8.5	PRIL-2-157	9.0
PRIL-2-078	8.5	PRIL-2-158	9.0
PRIL-2-079	8.5	PRIL-2-162	9.0
PRIL-2-082	8.5	PRIL-2-164	9.0
PRIL-2-085	8.5	PRIL-2-166	9.0
PRIL-2-094	8.5	PRIL-2-167	9.0
PRIL-2-097	8.5	PRIL-2-170	9.0
PRIL-2-101	8.5	PRIL-2-171	9.0
PRIL-2-106	8.5	PRIL-2-173	9.0
PRIL-2-118	8.5	PRIL-2-174	9.0
PRIL-2-120	8.5	PRIL-2-175	9.0
PRIL-2-124	8.5	PRIL-2-176	9.0
PRIL-2-140	8.5	PRIL-2-178	9.0
PRIL-2-141	8.5	PRIL-2-183	9.0
PRIL-2-142	8.5	PRIL-2-185	9.0
PRIL-2-144	8.5	PRIL-2-187	9.0
PRIL-2-149	8.5	PRIL-2-189	9.0
PRIL-2-156	8.5	PRIL-2-190	9.0
PRIL-2-163	8.5	PRIL-2-192	9.0
PRIL-2-168	8.5	PRIL-2-193	9.0
PRIL-2-169	8.5	PRIL-2-198	9.0
PRIL-2-177	8.5	PRIL-2-199	9.0
PRIL-2-181	8.5	PRIL-2-204	9.0
PRIL-2-188	8.5	PRIL-2-205	9.0
PRIL-2-195	8.5	PRIL-2-207	9.0
PRIL-2-196	8.5	PRIL-2-213	9.0
PRIL-2-197	8.5	PRIL-2-215	9.0
PRIL-2-200	8.5	PRIL-2-216	9.0
PRIL-2-206	8.5	PRIL-2-218	9.0
PRIL-2-208	8.5	PRIL-2-226	9.0
PRIL-2-210	8.5	PRIL-2-227	9.0

Table A1. Mean spring survival scores for all RILs in 2011-2012 (continued).

Name	Mean spring survival	Name	Mean spring survival
PRIL-2-217	8.5	PRIL-2-231	9.0
PRIL-2-219	8.5	PRIL-2-233	9.0
PRIL-2-221	8.5	PRIL-2-234	9.0
PRIL-2-222	8.5	PRIL-2-235	9.0
PRIL-2-224	8.5	PRIL-2-238	9.0
PRIL-2-228	8.5	PRIL-2-240	9.0
PRIL-2-247	8.5	PRIL-2-242	9.0
PRIL-2-250	8.5	PRIL-2-245	9.0
PRIL-2-251	8.5	PRIL-2-248	9.0
PRIL-2-252	8.5	PRIL-2-253	9.0
PRIL-2-255	8.5	PRIL-2-254	9.0
PRIL-2-258	8.5	PRIL-2-256	9.0
PRIL-2-265	8.5	PRIL-2-257	9.0
MEDORA	9.0	PRIL-2-260	9.0
PRIL-2-001	9.0	PRIL-2-262	9.0
PRIL-2-002	9.0	PRIL-2-263	9.0
PRIL-2-003	9.0	PRIL-2-266	9.0
PRIL-2-004	9.0	PRIL-2-267	9.0
PRIL-2-005	9.0		

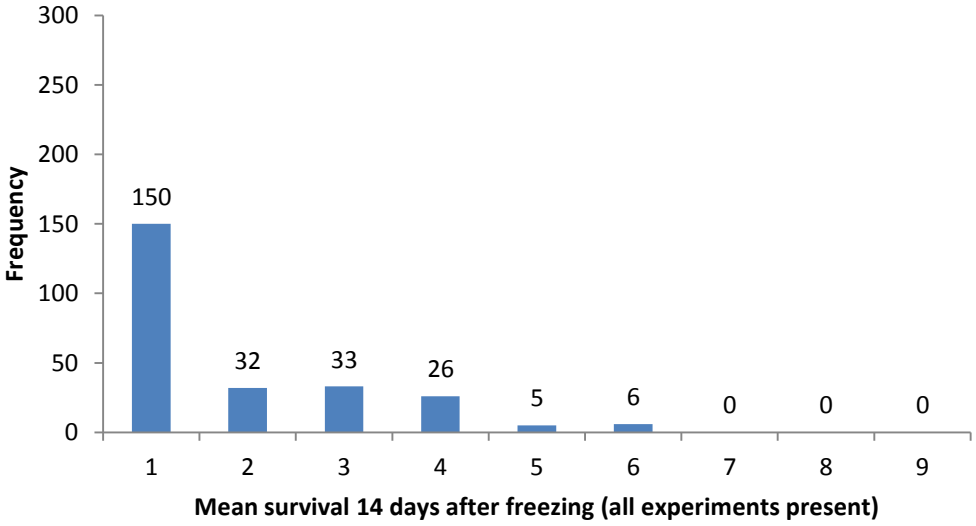

Figure A1. -4^{0}C 14 days after freezing full data set with all experiments present.

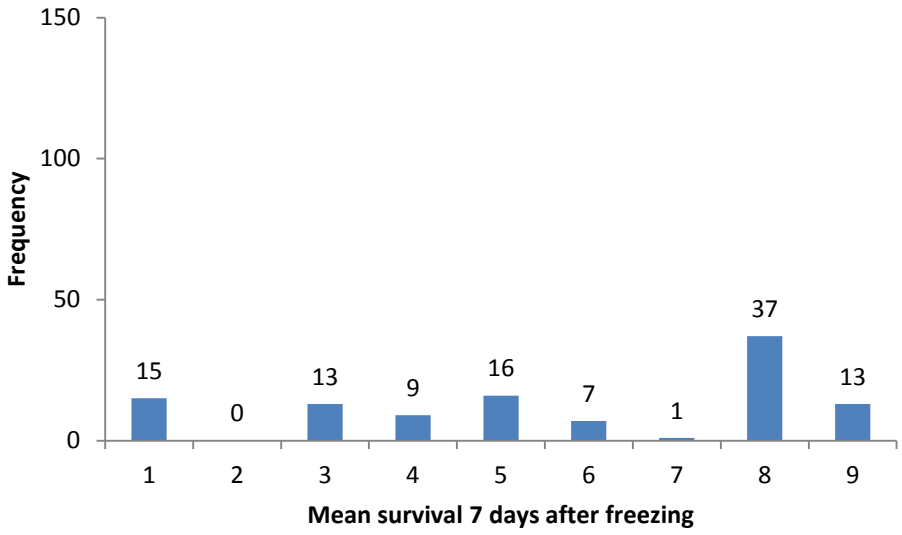

Figure A2. Means of PRIL-2 survival in the greenhouse from the second run at -8^{0}C 7 days after freezing.

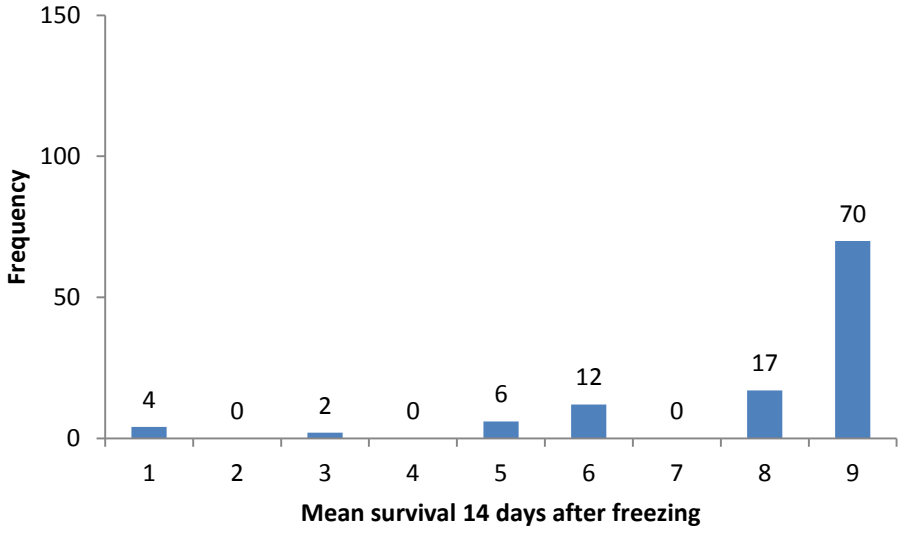

Figure A3. Means of PRIL-2 survival in the greenhouse from the second run at -8^{0}C 14 days after freezing.

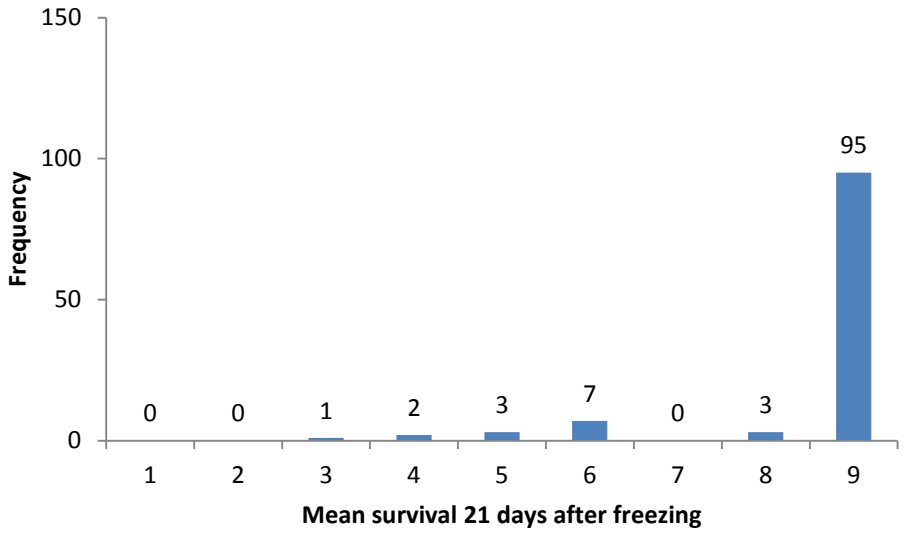

Figure A4. Means of PRIL-2 survival in the greenhouse from the second run at -8^{0}C 21 days after freezing.

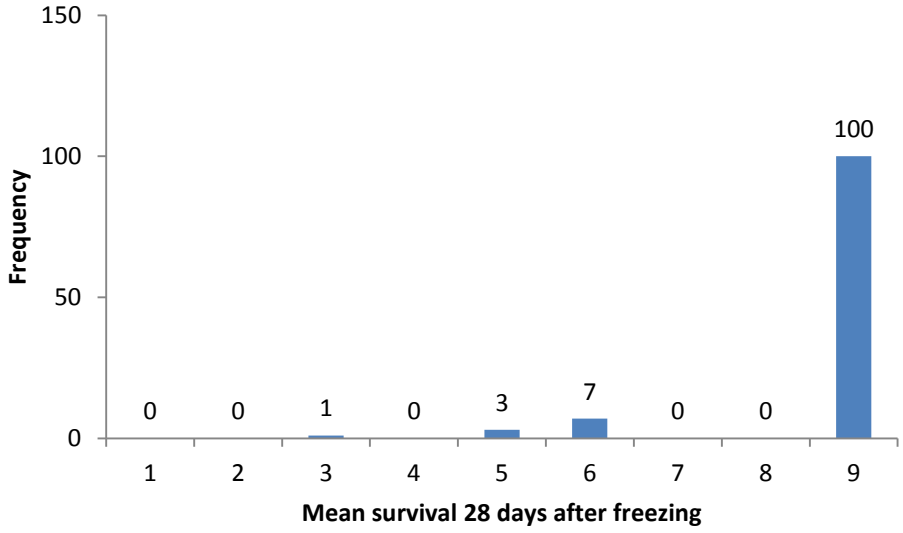

Figure A5. Means of PRIL-2 survival in the greenhouse from the second run at -8^{0}C 28 days after freezing.

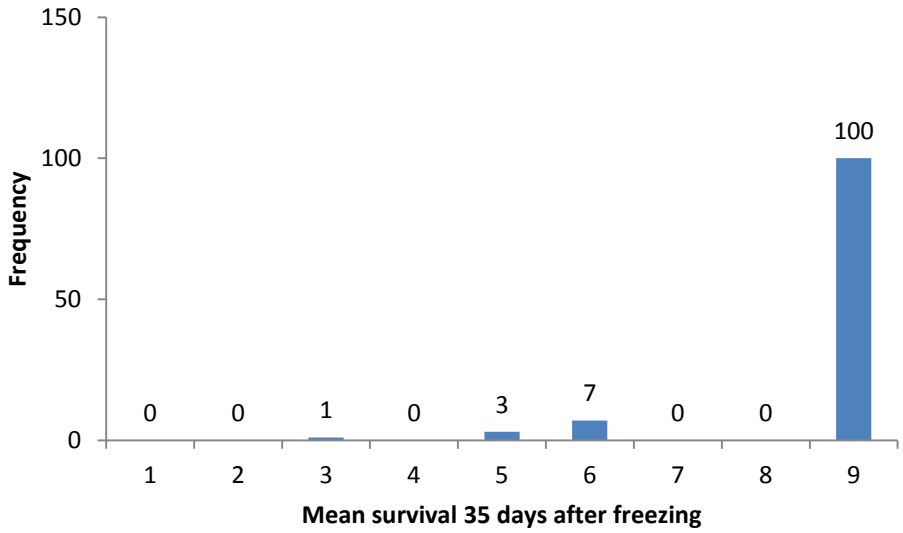

Figure A6. Means of PRIL-2 survival in the greenhouse from the second run at -8^{0}C 35 days after freezing.

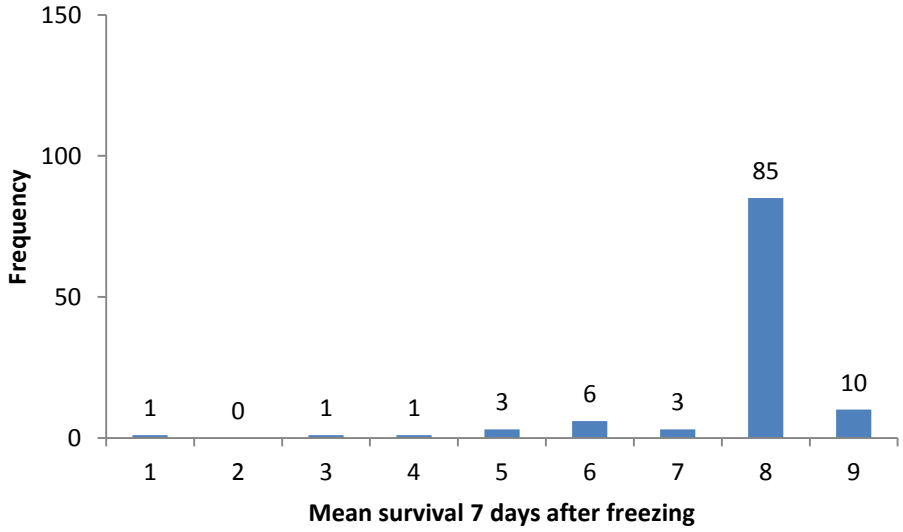

Figure A7. Means of PRIL-2 survival in the greenhouse from the second run at -12^{0}C 7 days after freezing.

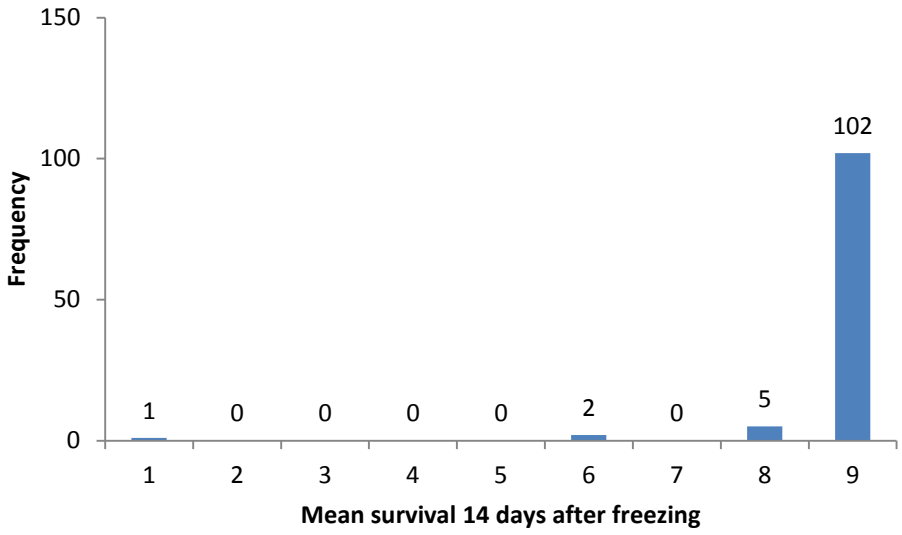

Figure A8. Means of PRIL-2 survival in the greenhouse from the second run at -12^{0}C 14 days after freezing.

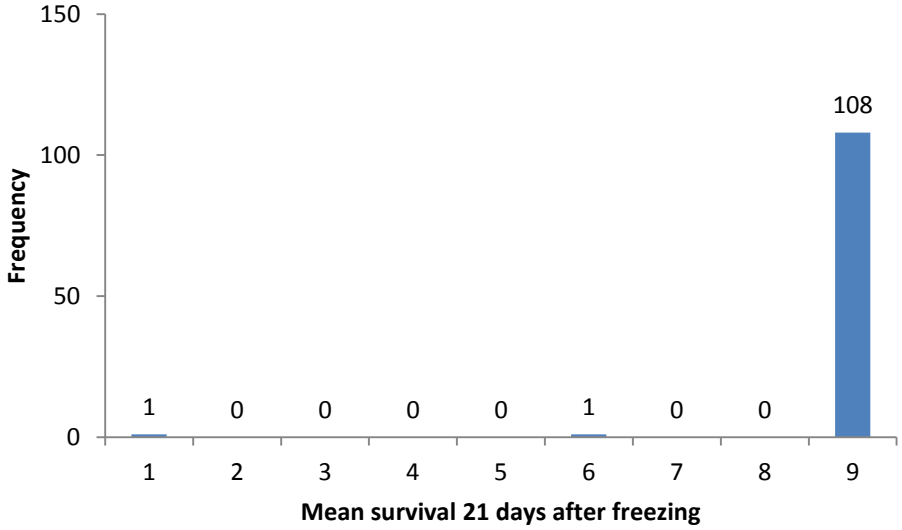

Figure A9. Means of PRIL-2 survival in the greenhouse from the second run at -12^{0}C 21 days after freezing.

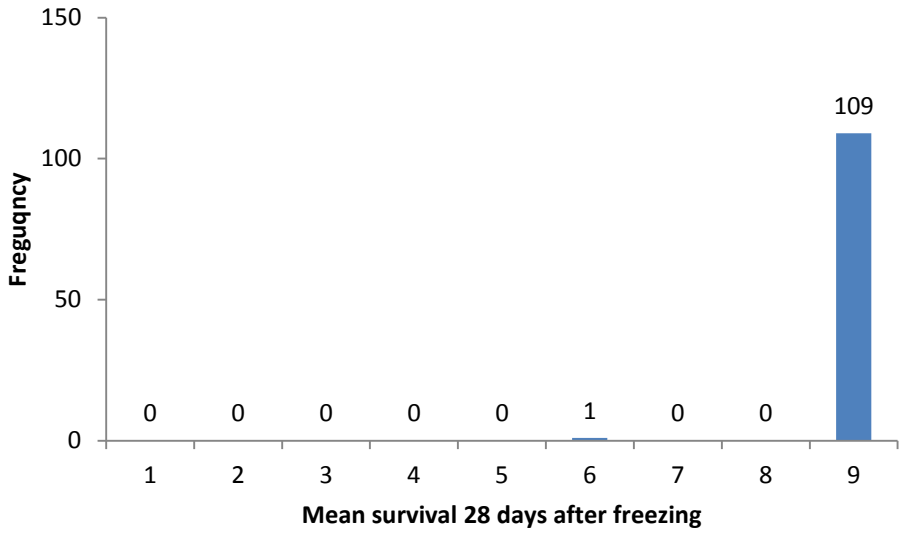

Figure A10. Means of PRIL-2 survival in the greenhouse from the second run at -12^{0}C 28 days after freezing.

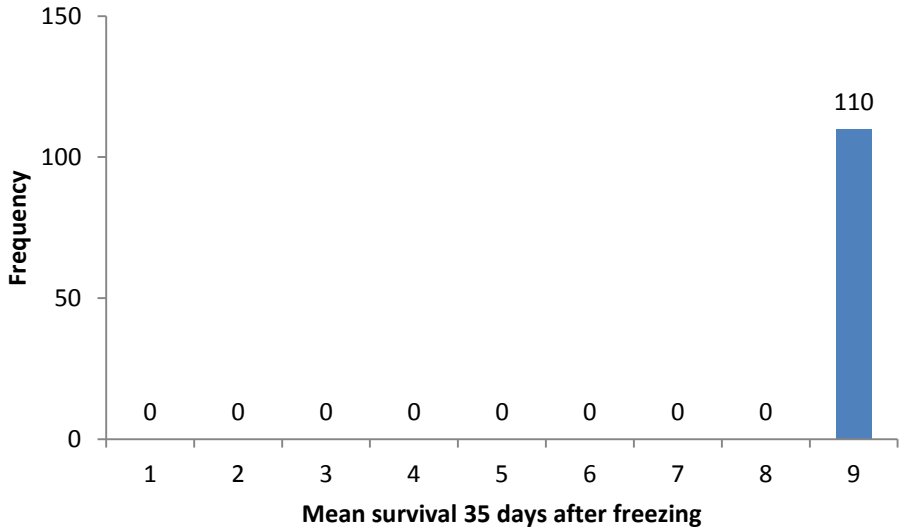

Figure A11. Means of PRIL-2 survival in the greenhouse from the second run at -12^{0}C 35 days after freezing.

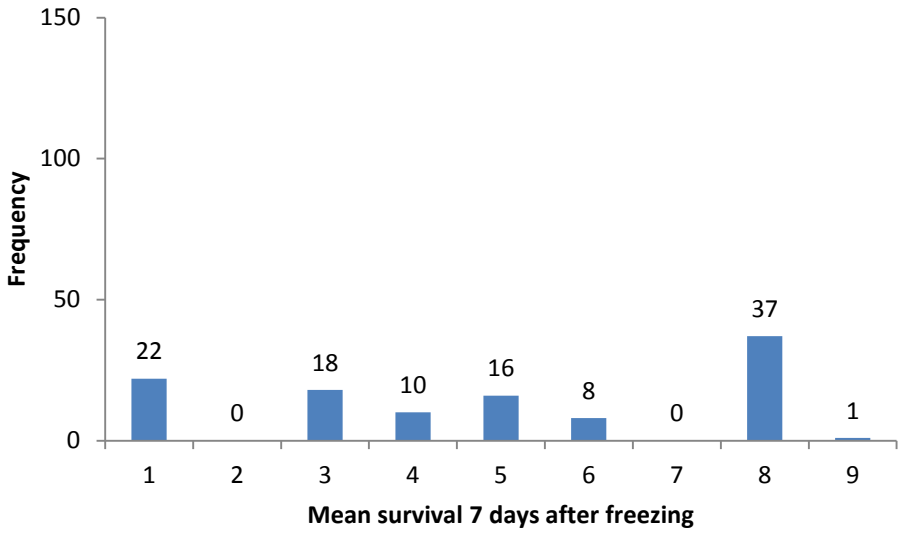

Figure A12. Means of PRIL-2 survival in the greenhouse from the third run at -8^{0}C 7 days after freezing.

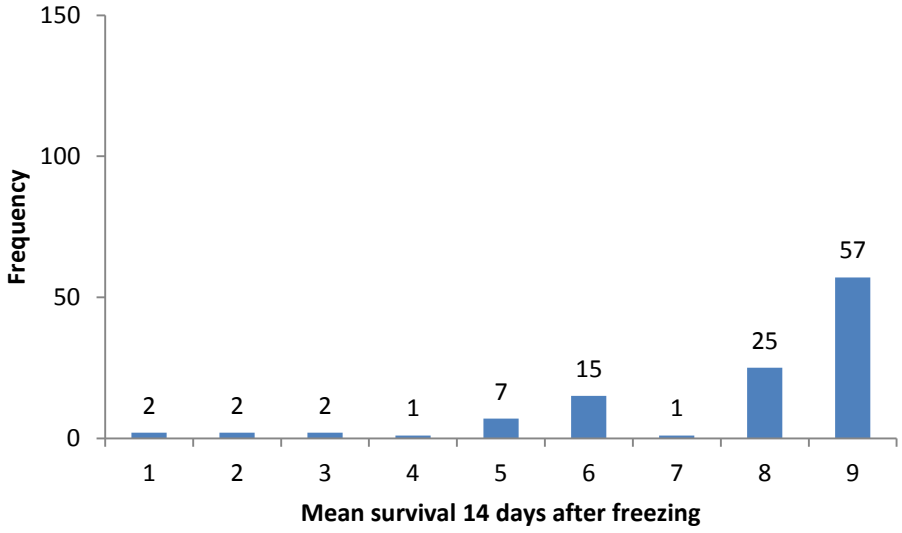

Figure A13. Means of PRIL-2 survival in the greenhouse from the third run at -8^{0}C 14 days after freezing.

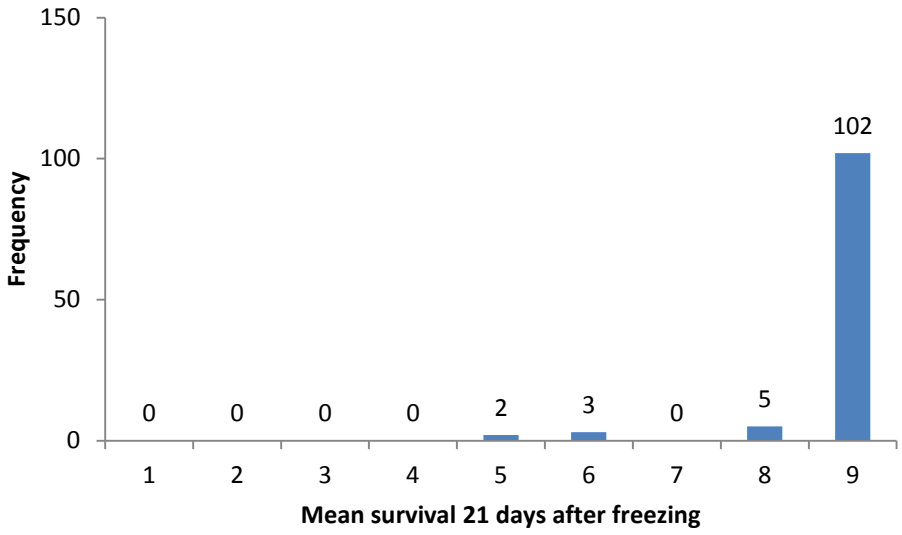

Figure A14. Means of PRIL-2 survival in the greenhouse from the third run at -8^{0}C 21 days after freezing.

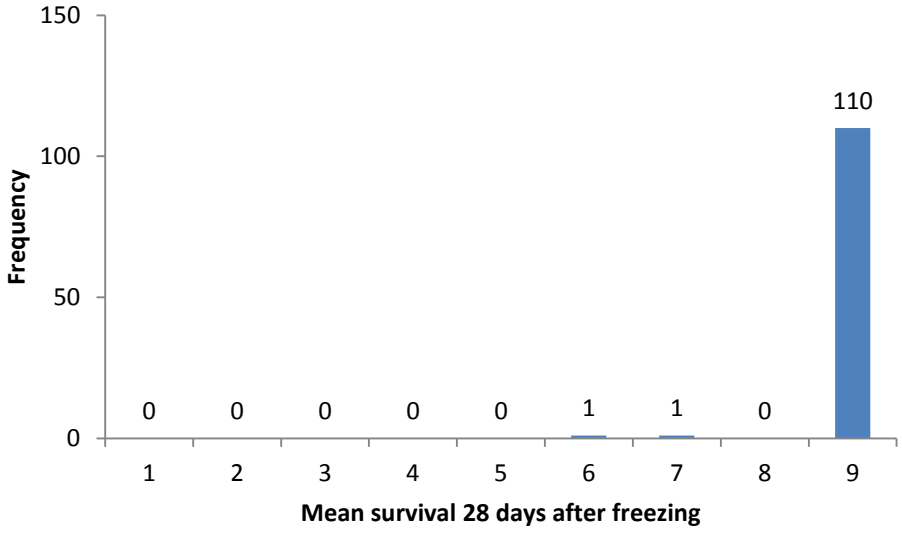

Figure A15. Means of PRIL-2 survival in the greenhouse from the third run at -8^{0}C 28 days after freezing.

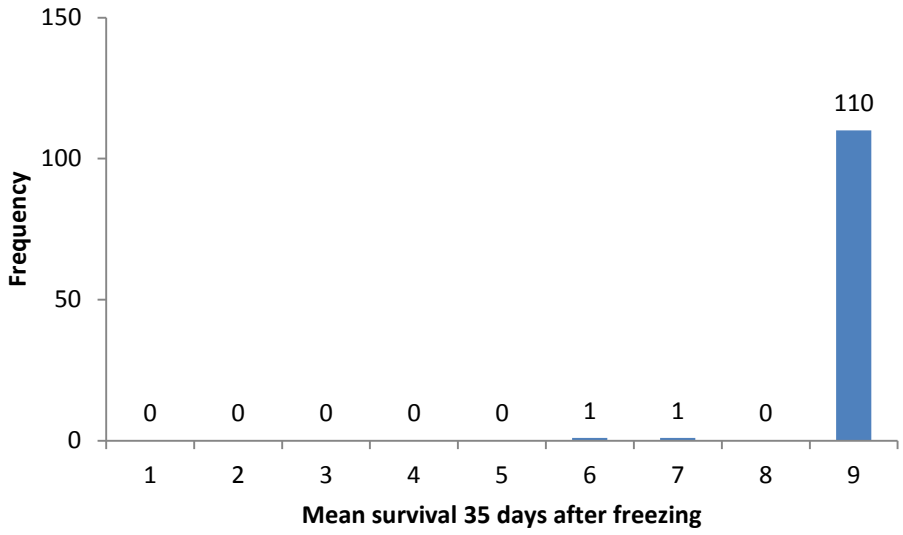

Figure A16. Means of PRIL-2 survival in the greenhouse from the third run at -8^{0}C 35 days after freezing.

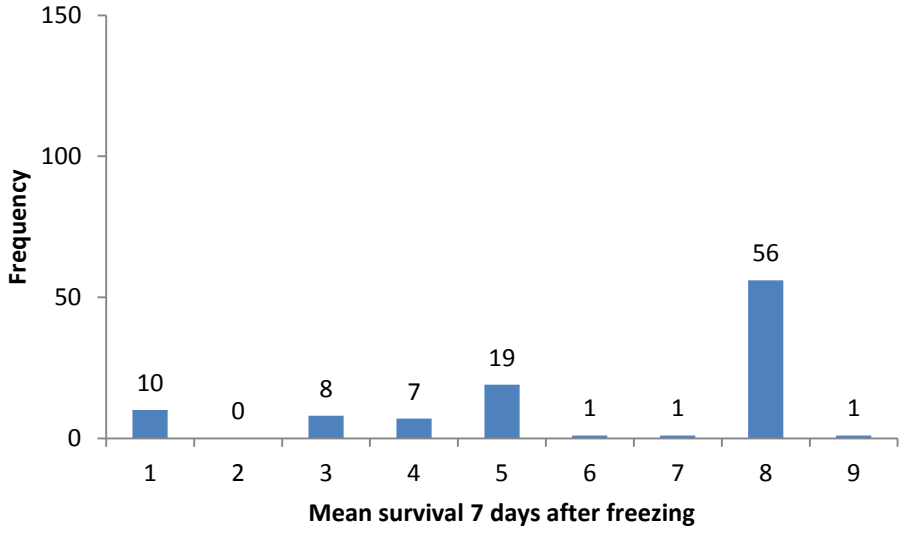

Figure A17. Means of PRIL-2 survival in the greenhouse from the third run at -12^{0}C 7 days after freezing.

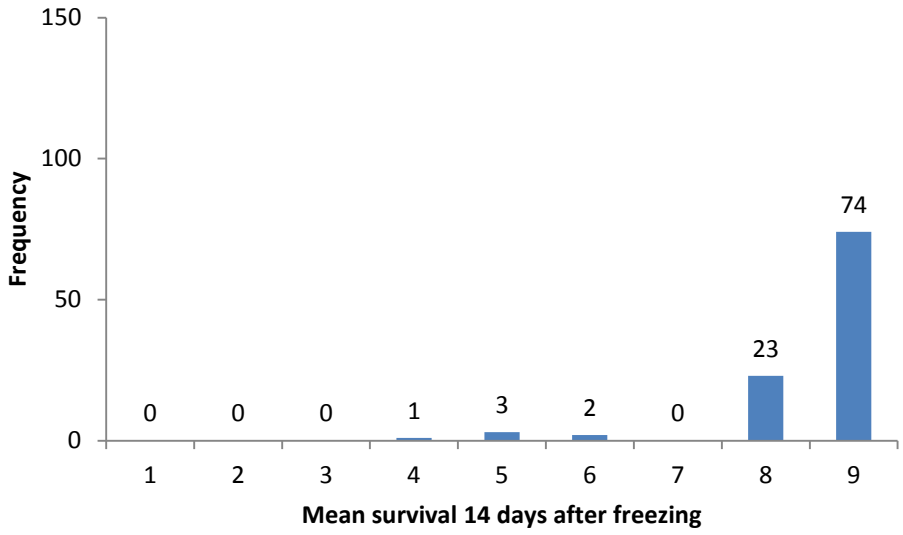

Figure A18. Means of PRIL-2 survival in the greenhouse from the third run at -12^{0}C 14 days after freezing.

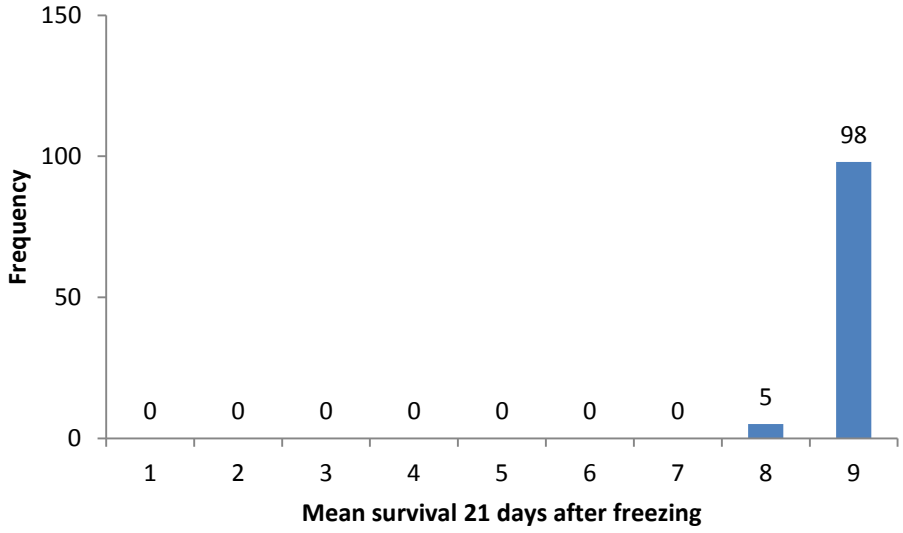

Figure A19. Means of PRIL-2 survival in the greenhouse from the third run at -12^{0}C 21 days after freezing.

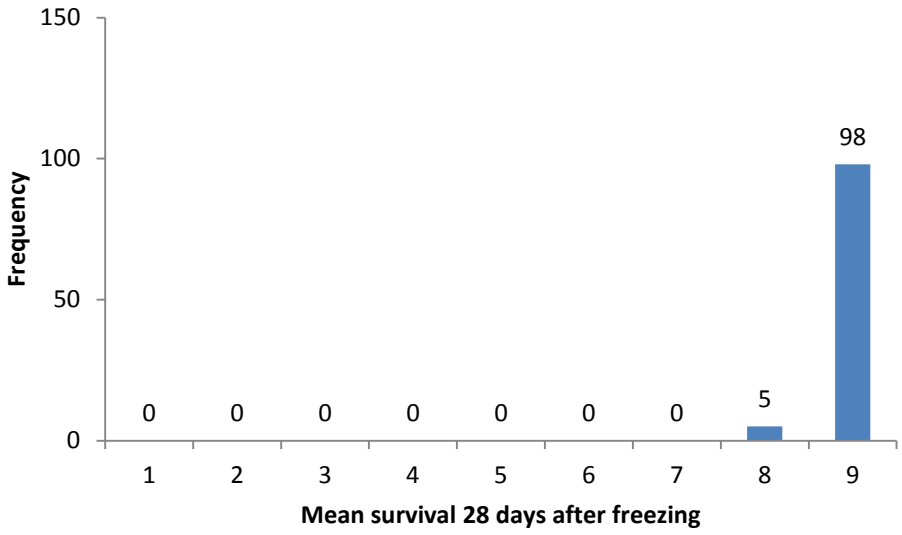

Figure A20. Means of PRIL-2 survival in the greenhouse from the third run at -12^{0}C 28 days after freezing.

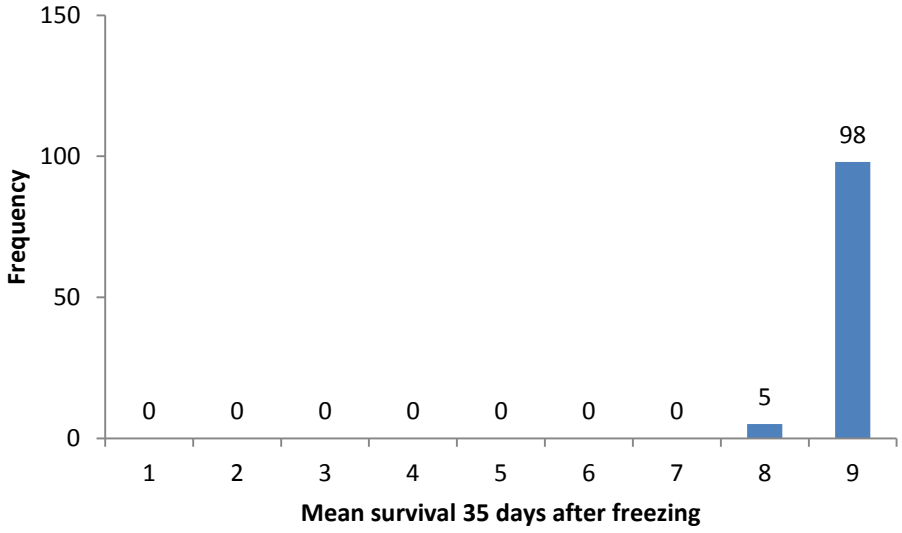

Figure A21. Means of PRIL-2 survival in the greenhouse from the third run at -12^{0}C 35 days after freezing.