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EVALUATION OF SPRING FROST CONTROL METHODS AND AN ASSESSMENT OF COLD HARDINESS IN CRANBERRY (Vaccinium macrocarporen Ait.)

A Dissertation Presented

by

FAITH NDLOVU

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

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SEPTEMBER 2015

Plant Biology
EVALUATION OF SPRING FROST CONTROL METHODS AND AN ASSESSMENT OF COLD HARDINESS IN CRANBERRY (*Vaccinium macrocarpurn* Ait.)

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DEDICATION

To my loving family and wonderful fiancé.
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I’m so grateful to my advisor, Dr. Peter Jeranyama for believing in me and giving me the opportunity to do my graduate studies at the University of Massachusetts Amherst. He introduced me to the world of cranberry and it has been an amazing experience! His support, guidance and patience were key to my success and I’ll forever be grateful for that. Secondly, I would like to thank my committee member and Director of the UMass Cranberry Station, Dr. Carolyn DeMoranville for the role she played to ensure that my studies went smoothly. Her passion and expertise in cranberry frost management went a long way into shaping my studies. Despite her busy schedule, she gently encouraged and guided me and invested a lot of time into all my studies and drafts that needed to be revised over and over. Special thanks also to Dr. Michelle Dacosta for giving me access to her lab and for the ideas and suggestions for my studies. Thanks also to Dr. Samuel Hazen for all his support and guidance.

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ABSTRACT

EVALUATION OF SPRING FROST CONTROL METHODS AND AN ASSESSMENT OF COLD HARDINESS IN CRANBERRY (*Vaccinium macrocarpon* Ait.)

SEPTEMBER 2015

FAITH NDLOVU, B.Sc., UNIVERSITY OF ZIMBABWE

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Directed by: Professor Peter Jeranyama

The American cranberry (*Vaccinium macrocarpon* Ait.) is an important temperate woody shrub crop whose fruit has human health benefits. Cranberry acquires cold hardiness in the autumn and loses it in spring, following deacclimation. Frost protection is necessary in cranberry production as a means to reduce bud damage due to low spring temperatures. The objectives of the field studies were to evaluate two methods of sprinkler irrigation for frost protection, the conventional approach consisting of continuous irrigation throughout the night (CON) and intermittent cycling of sprinklers (INT) incorporating cycling on and off throughout the night, by (i) assessing bud damage and yield for cranberry cultivars 'Early Black', 'Howes', and 'Stevens' managed under both methods and (ii) to determine the volume of water used in each method. The objectives of the laboratory studies were to (i) evaluate and quantify carbohydrates and lipids synthesized by cranberry during the cold acclimation period under a controlled environment and to (ii) determine the cold hardiness (*L*T_{50}) and lowest survival temperature (*L*S*T) of buds of economically important cranberry cultivars during acclimation.
For INT to be an effective frost control method, temperature set points should be cultivar specific. Despite differences in bud damage, cranberry yield data did not show any significant differences between the two frost management methods. Since cranberry plants produce more flowers than the number of set fruit that can be supported, the remaining flowers in damaged buds may have been sufficient to produce similar yields in both methods. Substantial water savings were obtained under INT, especially on mild frost nights.

Greater concentrations of total non-structural carbohydrates (TNSC) and membrane stabilizing lipids and a higher fatty acid unsaturation index were associated with low acclimation temperatures. This result suggests the importance of these compounds in increasing cold hardiness in cranberry during acclimation. In addition, a progression of freezing tolerance, determined as the LT$_{50}$ and LST, was noted in the fall for all the cranberry cultivars. Knowledge of bud hardiness in the fall is important in considering the need to protect buds. Differences in hardiness should be considered when implementing frost protection in the fall.
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CHAPTER 1

INTRODUCTION

The American Cranberry

Cranberry \((Vaccinium macrocarpon)\) Ait.) is a temperate, perennial, woody shrub indigenous to North America. It forms terminal mixed buds on short, two to eight inch vertical branches known as uprights, originating from axillary buds on the stolons (Eck, 1990; Sandler, 2008). The indigenous Native American population used cranberry as food, fabric dye and as a healing agent and introduced the plant to the first European settlers. The history of commercial cranberry production dates back to the 1800s. The cranberry industry is economically and aesthetically important in the US, with the sales rising sharply at the Thanksgiving and Christmas holidays.

The importance of cranberry

The berries can be consumed fresh, dried or processed into juices and sauces. Pure cranberry juice is generally known to be low in calories, yet supplying high levels of vitamin C and other important nutrients. Cranberry is associated with health benefits as it contains flavonoids, antioxidants and phytonutrients, useful in the urinary and gastrointestinal tract and the oral cavity (Cranberry Institute, 2015)
At subfreezing temperatures, damage to plants can either be intracellular or extracellular (Guy, 1990). In general, low temperatures are known to disrupt the normal physiological and biosynthetic processes within plants (Linden et al., 2002). Cranberries, like many other temperate shrubs are subject to frost damage at below freezing temperatures. Below freezing temperatures are important limiting factors in cranberry production especially in the spring and fall (Workmaster and Palta, 2006). Potential for bud damage is exacerbated by the location of bogs in low land areas since temperatures tend to be lower in those areas than in the immediate surrounding uplands (DeMoranville, 2008). The risk of damage is more prevalent on clear calm nights when leaves radiate infrared radiation (Sage and Sage, 2002), resulting in significantly lower bog temperatures compared to those in the surrounding uplands.

The freezing process and its importance to cranberry

The formation of intracellular ice through internal nucleation can cause a mechanical disruption of the protoplasmic structure, leading to the desiccation and eventually the death of cells (Levitt, 1980; Wisniewski et al., 2008). The extent of damage due to intracellular freezing depends mainly on how fast the temperature drops and to what level the plant tissue supercools before freezing. In extracellular freezing, ice crystals grow on the external surface and lead to cellular dehydration as water moves out of the cell to form crystals (Guy, 1990). Although frost damage can affect only a small part of plant tissue, sometimes the entire plant can be damaged, resulting in severe yield loss (Rodrigo, 2000).
Damage due to freezing injury is responsible for limited productivity and geographical distribution of both wild and cultivated species of cranberry (Pearce, 2001). In addition, unpredictable weather occurrences make it difficult for plants to survive extreme temperatures (Arora and Rowland, 2011). Over $2 billion crop losses were reported across 21 states for small fruit plants due to unusually low temperatures for an extended period of freezing in April 2007 (Warmund et al., 2008). Although weather patterns vary from year to year, it is common for cold spells to occur within each year. It is therefore important to understand how plants adapt to these low temperatures so that they can better resist freezing damage (Takahashi et al., 2013).

In order to survive freezing temperatures, plants have adopted freezing tolerance and avoidance mechanisms (Levitt, 1980). Tissues relying on freezing avoidance display a deep supercooling whereby some of the cellular water remains liquid far below the freezing point. Supercooling is usually enabled by an effective isolation of a protoplast from the nucleating effect of extracellular ice (Linden et al., 2002; Gusta et al., 1996). Resistance to frost of individual plants shows high variation during the year and among different tissues or parts of the plant (Weiser, 1970). An understanding of frost resistance is fundamental in the production of cranberries.

**Cold hardiness and acclimation in woody perennials**

Hardiness refers to a plant’s ability to survive adverse growing conditions while ‘cold hardiness’ is specifically defined as the ability of plants to withstand sub-freezing temperatures without sustaining significant damage (Linden et al., 2002). It is largely dependent on the ability
of a plant to keep water from leaving its cells and freezing as this can result in severe dehydration to the cells. Cold hardiness depends on the environmental conditions and the time of the year. It is usually thought to occur in three phases namely, acclimation, mid-winter hardness and de-acclimation. Freezing tolerance and avoidance, cold hardiness, and cold acclimation are important concepts with implications in the physiological response of cranberry to frost. Good understanding of these frost resistance mechanisms and the response of the cranberry plant to freezing stress can be the basis for better frost management decisions.

In the *Vaccinium* genus, damage to buds can occur after exposure to freezing temperatures (Lee et al., 2013). Damage to either the vegetative meristem or the floral initials within a cranberry bud can be detrimental to the reproductive phase of cranberries, as it will determine the ultimate berry yield. Previous research in deciduous fruit trees has shown that frost damage is generally most significant in buds, flowers, and developing fruits (Smeeton, 1964). That is also common for other woody perennials like cranberry, where flower buds are formed in late summer and have to tolerate low winter temperatures until the following spring. The ability of plants to survive freezing temperatures differs (Wisniewski et al., 2008) and depends primarily on the development of freezing tolerance. Most plants native to temperate and boreal regions go through a process known as ‘cold acclimation’ (Pagter and Arora, 2013). In this process, an increase in freezing tolerance develops in the plants, mainly through physiological changes that occur in response to low temperatures and short photoperiod in the fall (Xin and Browse 2000).
In cranberry, once maximum hardiness has been attained, the buds lie dormant throughout winter and only become highly susceptible to frost injury in spring as dormancy ends (Workmaster et al., 1999). In late winter and early spring, once chilling hours have been fulfilled, warm temperatures induce the resumption of growth and development. Cranberry buds gradually lose their cold hardiness as plant development and growth resumes (Abdallah and Palta, 1989) and thus the buds become susceptible to spring frost damage. In cranberry, spring freeze damage can be disastrous and can potentially lead to total crop losses in one night (DeMoranville, 2008). Therefore, the development of cold hardiness in the fall and its loss in the spring, along with management techniques for avoiding cold injury, is critical in cranberry production.

**Research undertaken**

Previous studies have extensively investigated the loss of cranberry bud hardiness (deacclimation) in the spring (DeMoranville, 2008; Workmaster and Palta, 2006) but the acquisition of cold hardiness (acclimation) in the fall remains less understood. Abdallah and Palta (1989) and Workmaster and Palta (2009) established a time course for cold acclimation in Wisconsin but there has been no similar study in Massachusetts. To our knowledge there has not been any work characterizing the physiological and biochemical changes that accompany the process of acclimation in cranberry. In this dissertation research we characterized these changes and the time course for cold acclimation in Massachusetts cranberry.

In cranberry production, avoidance of bud damage when temperatures are below the tolerance (hardiness) is accomplished with the application of water through a solid-set sprinkler
irrigation system. This method uses significant resources of water and fuel. In order to increase operational efficiency, a protection method that conserves these resources without loss of effective frost protection is desirable. In this work, we examined the potential for cycling sprinkler irrigation as a conservation practice in cranberry frost management. Field assessments on bud damage, yield, and water and fuel use under both cycling and conventional regimes were conducted in southeastern Massachusetts (cranberry growing region).

This dissertation research focused on an assessment of different methods used by growers to protect their buds from spring frost damage and on increasing our understanding of the physiological changes that occur during cold acclimation in cranberry in the fall. Results from this dissertation have generated new knowledge in cranberry cold acclimation and on ways to manage spring frost while conserving water and energy resources.
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CHAPTER 2

SPRING FROST CONTROL METHOD EFFECTS ON CRANBERRY BUD DAMAGE AND FRUIT YIELD

Abstract

The American cranberry acquires cold hardiness in autumn (fall) and loses it in spring, following deacclimation. As temperatures drop below the hardiness level, cranberry growers use sprinkler irrigation to protect the plant from damage. Two sprinkler frost protection irrigation methods were evaluated; (1) the conventional approach consisting of continuous irrigation throughout the night once the bud temperature tolerance level is reached (CON) and (2) intermittent cycling of sprinklers (INT) started at a temperature tolerance level similar to CON but incorporating cycling on and off throughout the night between two additional set temperatures. Bud damage was assessed for cranberry cultivars 'Early Black', 'Howes', and 'Stevens' managed as either INT or CON prior to deacclimation. Frost monitoring was conducted after one or two frost protection events in spring and late in the spring when danger of frost had passed. In both years and for all cultivar treatment combinations, there was some bud damage prior to the initiation of frost protection and additional damage was observed following frost events. There were no frost protection method differences in bud damage or fruit yield on 'Howes' in both years and in the other cultivars in 2012. 'Stevens' suffered significant bud damage and yield reduction in INT compared to CON after frost events in 2011, which had a colder spring relative to 2012. Intermittent cycling (INT) was effective on all cultivars in 2012, when early spring temperatures were not as cold as in 2011.
Keywords: *Vaccinium macrocarpon*, sprinkler irrigation; intermittent cycling; hardiness; cultivar

**Introduction**

Cranberry (*Vaccinium macrocarpon* Ait.) is an evergreen, woody perennial vine that is indigenous to North America. In midsummer, cranberry plants develop terminal mixed buds, with both vegetative and floral meristems, on vertical branches known as uprights (Eaton and MacPherson, 1978; Eck, 1990). The buds complete development, acclimate, and enter dormancy during the fall. In late winter and early spring, once chilling hours have been met (Eady and Eaton, 1972), warm temperatures induce the resumption of growth and development. Cranberry buds gradually deacclimate as plant development and growth resumes and the buds become susceptible to spring frost damage (Workmaster et al., 1999). Abdallah and Palta (1989) reported that the lowest survival temperature in cranberry buds rising from -18°C in mid-April to -2°C in mid-May, coinciding with the deacclimation period. Workmaster and Palta (2006) showed that the rate of deacclimation within a certain stage of bud development in cranberry was variable among morphologically similar buds, implying that there were internal physiological and anatomical changes occurring that were not necessarily reflected in the visual appearance.

Cranberries are grown in natural or constructed wetlands located on low-lying ground, where the settling of cold air on calm nights increases the danger of damaging low temperatures (DeMoranville, 2008). On clear nights, leaves, including those of cranberry, radiate infrared radiation (Curtis, 1936), further lowering bog temperatures compared to those in surrounding
uplands. The combination of radiational cooling and cold air moving into the low-lying cranberry bogs can result in as much as a 10°C differential between the low-lying bog and the surrounding uplands.

Exposure to spring temperatures lower than the bud hardiness can damage the floral meristem initials and vegetative meristem in a cranberry bud, leading to the potential for severe yield losses. Extent of the damage will determine the potential for recovery. If some of the floral initials have been killed or severely injured, flower number and potential to set fruit will be reduced proportionally. If the vegetative meristem alone is damaged, flowers and berries may form but berries may be aborted due to lack of the photosynthates that are normally produced in the leaf growth that would have developed from the vegetative meristem (DeMoranville, 1998). Therefore, frost protection is critical to cranberry yield by ensuring that both the reproductive and vegetative meristems within the buds are protected from damage. The solid-set sprinkler irrigation system is the most commonly used method of frost protection in cranberry production (Workmaster and Palta, 2009; DeMoranville, 2008). Sprinkler irrigation is also used in orchard management for the prevention of injury during subzero temperatures (Issa, 2012; Ghaemi and Mohammed, 2009).

The conventional approach to sprinkler frost protection in cranberry has been to initiate irrigation when the temperature in the bed, at vine tip level, approaches the tolerance (hardiness) of the buds and then run the system continuously until after dawn when temperatures rise above the tolerance temperature (DeMoranville, 2008). This method often involves numerous hours of
irrigation with associated large water and fuel usage. In orchard crops, intermittent (cycled) sprinkler irrigation, initiated at a trigger (start) temperature above the bud hardiness, has been used for frost protection with cycles of set duration (Heisey et al., 1994) or based on bud temperature changes (Koc et al., 2000) with varied success. With the goal of reducing water and fuel use, the objective of this research was to compare the effectiveness of continuous (conventional practice) and intermittent (cycling practice) sprinkler protocols as spring frost protection methods for preventing cranberry bud frost damage and cranberry fruit yield losses.

**Materials and Methods**

**Study Layout**

This study was conducted in Bristol, Barnstable and Plymouth counties of southeastern Massachusetts using six commercial cranberry beds that were flooded throughout winter and drained in spring (standard growing practice). Selected bogs varied in age, most of them still had the original vines that were planted more than 50 years ago while others were more recently planted or renovated. All bogs were sanded periodically. The sanding process involves the addition of sand layers to the surface of a bog in order to stimulate organic matter decomposition and suppress fruit rot fungus, among other benefits (DeMoranville and Sandler, 2008). Three sites were utilizing the CON method while the other three were protected using the INT method (see description in the next section). The study was arranged as a split-plot repeated measures design, with sites representing main plots and cultivar sections (strips) within the sites representing sub-plots, subsequently referred to as beds, as illustrated in Fig. 1. Three cranberry cultivars, 'Stevens', 'Howes' and 'Early Black', located on each of the six sites were selected. The
selected cultivars make up more than 80% of commercial production in MA (Caruso, 2008).

Repeated measures refer to the measurements that were taken over time within each bed after selected frost events. Regional temperature minima, collected at the University of Massachusetts Cranberry Experiment Station and archived online at the National Climatic Data Center (NOAA site identifier COOP:192451), were compared to on-bog minima reported to the Cape Cod Cranberry Growers Association (CCCGA) during the spring frost seasons in 2011 and 2012. These data were also used to determine dates when recorded on-bog temperatures in the spring of 2011 and 2012 were lower than the tolerance trigger for initiating frost protection.

**Frost Control Management**

Two frost protection treatments were compared: (i) continuous (conventional) sprinkler irrigation (CON) and (ii) intermittent (cycled) sprinkler irrigation (INT). Frost tolerance was periodically estimated throughout the spring frost season, based on phenological stage of bud development similar to DeMoranville (1998). In both treatments, irrigation was initiated when the temperature at the vine tips reached 1-2°C above the frost tolerance of buds for that cultivar. In the CON approach, once initiated, irrigation water was applied throughout the night and was stopped in the morning when the ambient temperature had risen 1-2°C above the tolerance temperature. In the INT approach, once initiated, the pumps turned off when the temperature at vine tips reached a set point 3-5°C above the initiation trigger temperature, then cycled on and off through the night, restarting at either the initiation temperature or at a second set-point temperature 1-2°C above the initiation trigger. Several such cycles would be possible during the night. In the CON study locations, pumps were stopped and started either manually or via
automation but in both instances, ran throughout the night once initiated. At the INT locations, all pumps were automated and linked to on-bog sensors that triggered the on-off cycles.

**Measurements**

**Buds and yield component sampling protocols**

Buds were collected from April through June along a transect on each bed approximately 24-hrs after both mild and severe frost events, and with approximately 50 buds selected. For both CON and INT beds, transects included areas near a sprinkler head and farther away from it, with buds from both areas combined to make a single sample. Prior to loss of bud dormancy in the spring, and thus prior to any potential for frost injury or need for frost protection, a bud sample was collected to provide a control for any damage that may have existed prior to our monitoring of frost protection events. Thereafter, buds were collected on several days across the season in both years (Table 1). Collected uprights were held at room temperature in the lab and assessed after a 24-hr period. The 24-hr period ensures that frost damage, if any, has been fully expressed within the bud. Since damage is almost always localized in the meristems, bud assessment consisted of looking for internal damage by dissecting the buds under a microscope and performing a visual damage assessment (Arora et al., 2000; Ehlenfeldt et al., 2006). In cranberry buds, freeze injury is indicated by browning of the vegetative meristems, the floral initials, or both. A damage scale, based on degree of browning, was developed and is illustrated in Fig. 2. Buds were collected three times from each bed and evaluated for damage as follows (1) baseline assessment early in the spring, prior to loss of dormant hardiness, to assess the extent of damage that may have occurred either prior to or during the winter or immediately after the removal of
the winter floods, (2) after the first few frost protection events, and (3) after several frost protection events at the end of the spring.

**Yield Evaluation**

At the end of the season, fruit yield was estimated by randomly placing a 25 x 25 cm quadrat at four randomly selected plots on a bed and replicated four times within each bed, in September-October each year, according to Eaton and MacPherson (1978). Berries in each square were collected, separated into usable and damaged/diseased and each group was counted. The usable berries were weighed to determine fresh fruit yield.

**Statistical Analysis**

Because the ordinal data generated in bud assessments did not satisfy the assumptions of normality and homogenous distribution of variances required for ANOVA, bud damage data were analyzed using non-parametric techniques. Data were analyzed using Chi Square (X²) tests in SAS 9.3 (SAS Institute, Cary NC). PROC FREQ was used to generate three-way contingency tables testing significance of association between the frost protection methods, cultivars, and bud damage. Damage levels 2, 3, 4 and 5 (Fig. 2) occurred at low frequency and in order to increase the precision of the analysis, all damage categories were combined and the frequency of damaged buds was compared to undamaged frequency. Yield data collected at the end of the season was analyzed in PROC GLM. Due to significant cultivar and method interaction, we analyzed a reduced model of cultivars within a method. Mean separation by single degree of freedom contrasts was used to further elucidate the response of each cultivar between the two
methods while Fisher’s LSD was used as mean separation test for cultivar within frost protection method.

**Results and discussion**

**Weather conditions**

An analysis of sheltered off bog temperatures in East Wareham for April through June of 2011 and 2012 showed that the years had similar seasonal patterns (Fig. 3). April was the only month with upland temperatures < 0°C in both years. Contrary to the predominantly above-freezing temperatures observed off-bog, reported bog temperatures were substantially lower, especially on calm, clear nights. According to CCCGA data, collected on cranberry bogs (unsheltered sensors), there were 13 frost nights in 2011 and 16 frost nights in 2012. On-bog temperatures were below the hardiness levels of the plants, with tolerances at -7.8°C for 'Early Black' and 'Howes' and -6.7°C for 'Stevens' at the beginning of the 2011 frost season. In 2012, tolerance was at -5.6°C for 'Early Black' and 'Howes' and -3.9°C for 'Stevens' at the beginning of the frost season. In both years, below-zero on-bog temperatures occurred throughout April and the first half of May (Fig.3).

**Bud damage**

An evaluation of buds collected prior to the initiation of frost protection in the spring showed that, in both years and for all cultivar treatment combinations, there was observable damage in buds. In 2011, for all cultivars, there was significant pre-season bud damage in the INT treatment beds compared to CON treatment beds. 'Early Black' had minimal damage (4% in
INT beds versus 2% in CON beds) while there was more damage in 'Howes' (6% in INT versus 3% in CON). 'Stevens' had the highest damage (10%) in the INT treatment, but only 0.5% in the CON beds (Fig. 4A). In 2012, however, there was no difference in pre-season bud damage between the treatments on 'Howes' or 'Stevens', while 'Early Black' had greater damage (5.5% in INT versus 2.3% in the CON beds) (Fig. 5A).

Bud damage observed before the spring frost season had to have occurred either during the previous autumn (fall), winter or in early spring. It is possible that the INT sites coincidentally had more damage or the management of those sites differed from that of the CON sites, resulting in the greater damage. The most likely explanation is the differences in grower practices among the sites since they were not all under one management. If the damage was incurred during the previous fall, it may have been attributable to cycling practices used during that period (the CON sites had not used cycling prior to this study).

By mid-April in both years, deacclimation was determined to have begun and growers were protecting their bogs when temperatures approached the hardiness level as determined by observation of bud phenological stage of development (DeMoranville, 1998). After several frost events, bud damaged was generally greater than that observed in the pre-season evaluations in both methods and for all cultivars (Fig. 4B and 5B). Both methods appeared to offer similar protection for 'Early Black' in both years while in 'Howes' the INT treatment had less damage compared to CON in 2011. Bud damage in 'Stevens' under INT in 2011 was as high as 22% (Fig. 5B) and this was 12% greater than the pre-season baseline. In comparison, damage to 'Stevens'
under CON also increased, but to a lesser degree (from 0.5% to 6%). In 2012, however, there was no frost protection method difference in damage to 'Stevens'. It is not clear why only 'Stevens' incurred substantially more damage in the INT treatment compared to CON in one of the two years. One possible explanation is that the larger buds on 'Stevens' did not always receive adequate water during the brief intermittent irrigation periods under INT to provide sufficient protection. However, this would not explain why in 2012 there was no treatment difference for 'Stevens'. Another explanation could be that the initiating temperature set point was incorrectly selected for the INT 'Stevens' bed in 2011; 'Stevens' tends to be more susceptible to frost damage relative to the other two cultivars for any given phenological stage of bud development (DeMoranville, 1998). It is also notable that the on-bog temperature for the first frost event in 2011 was substantially lower than that of the first event in 2012 (Fig.3). The INT treatment may have been less effective for 'Stevens' when temperatures were below -4°C. At the end of the frost season, percent bud damage in 2011 was also greater for ‘Stevens’ under the INT protocol (Fig. 4C), with most damage being on one or two floral initials. Bud floral initial damage in one or two initials maybe of little economic importance as cranberry buds have four to six floral initials and therefore the undamaged two to four floral initials are expected to flower. In 2012, differences in the methods were only observed in 'Early Black' while 'Howes' and 'Stevens' damage remained low and similar (Fig. 5). However, the damage was low, at only ≤ 4% after several frost events and towards the end of the season (Fig. 5B and 5C).
Yield

Based on an analysis of marketable fruit yield in both years, there was a significant three-way interaction of year × treatment × cultivar ($p \leq 0.0001$), indicating that the yield response for frost control treatment was influenced by both year and cultivar effects. Fruit yield in ‘Howes’ was similar between CON and INT, however, there were significant differences between CON and INT in ‘Early Black’ and ‘Stevens’ in 2011. Both cultivars had higher yields under the CON than INT. There were no cultivar yield differences between frost protection methods in 2012 (Table 2). Many cranberry cultivars exhibit biennial bearing tendencies with individual uprights producing mixed buds (with floral meristems) and purely vegetative buds in alternate years (Eaton and MacPherson, 1978; Strik et al., 1991). In this study, that trend was apparent, particularly for the native cultivar 'Early Black' as shown by CON treatments in 2011 vs. 2012 (Table 2).

Based on the final bud damage assessments in the two years, little or no cultivar yield differences were expected among frost protection methods with the exception of 'Stevens' in 2011. The level of bud damage in ‘Stevens’ in 2011 in INT resulted in significant yield reduction in INT compared to CON (Table 2). However, while post-frost bud assessments of 'Early Black' in 2011 (Fig. 4C) did not predict a treatment effect on yield, the fruit collection data showed a significant treatment effect, with lower yield in the INT treatment. This is not readily explained but there was higher bud damage under INT for that cultivar in 2011 prior to the frost season (Fig. 4A) that may have contributed to the yield differential between the treatments.
Bud damage in the spring has been observed in many woody species (Neuner, 2014; Rowland et al., 2013) but we have shown in this study that the use of irrigation, whether continuously or intermittently, adequately protects cranberry buds from severe losses. Although bud damage was observed in both years, it was minimal with the exception of 2011. 'Stevens' is the only cultivar where observed bud damage in the spring and subsequent yield were consistent, with INT having more damage and lower yield in 2011 (Table 2). Bud damage on one or two floral meristems was the most commonly observed (Fig. 4). Cranberry buds have four to six floral initials and thus the remaining intact initials in the damaged buds would have the ability to produce two to four flowers. Previous research on cranberry fruit development concluded that an individual upright produces adequate carbon resources to support only two berries (Hagidimitriou and Roper, 1994). Therefore the damage most often observed upon bud examination would not necessarily translate into that same level of crop reduction.

There has been interest among Massachusetts cranberry growers in the adoption of frost irrigation cycling (the INT method) as a way to conserve water and save on fuel costs. Previous studies have reported significant water savings accrued from using the INT approach (Heisey et al., 1994; Koc et al., 2000). If this practice is to be fully adopted by cranberry growers, there must be a demonstrable economic advantage in terms of fuel and water usage costs and evidence that bud damage is minimal under this frost protection method. Our data seem to suggest that the INT practice is as effective as CON in protecting 'Howes' from bud damage and subsequent yield reduction. However, the significantly greater bud damage and subsequent lower yield on 'Stevens' and the reduced yield in 'Early Black' protected by INT in 2011 raises questions about
the use of this practice for other cultivars. Since INT was effective on all cultivars in 2012, when early spring temperatures were not as cold as those in 2011, it appears that further research may be needed to define appropriate conditions for the use of the INT practice on 'Early Black' and 'Stevens'. Future studies should also attempt to quantify water and energy savings obtained when switching from CON to INT.
Literature Cited


Table 1. Dates when cranberry buds were collected for frost damage evaluation in the 2011-2 frost seasons in southeast Massachusetts.

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preseason</td>
<td>1-15 April</td>
</tr>
<tr>
<td>Early season</td>
<td>16 April - 23 May</td>
</tr>
<tr>
<td>Late season</td>
<td>24 May - 15 June</td>
</tr>
</tbody>
</table>
Table 2. Effects of frost protection methods, intermittent cycled irrigation (INT) or continuous irrigation (CON), on the yield of three commercial cranberry cultivars in 2011 and 2012.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>2011 INT (Mg ha(^{-1}))</th>
<th>2011 CON (Mg ha(^{-1}))</th>
<th>contrasts(^{\dagger})</th>
<th>2012 INT (Mg ha(^{-1}))</th>
<th>2012 CON (Mg ha(^{-1}))</th>
<th>contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Black</td>
<td>41a(^{\dagger})</td>
<td>61a</td>
<td>*</td>
<td>34b</td>
<td>33b</td>
<td>NS</td>
</tr>
<tr>
<td>Howes</td>
<td>42a</td>
<td>32b</td>
<td>NS</td>
<td>30b</td>
<td>31b</td>
<td>NS</td>
</tr>
<tr>
<td>Stevens</td>
<td>36a</td>
<td>58a</td>
<td>*</td>
<td>52a</td>
<td>49a</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^{\dagger}\)Treatment contrasts (INT vs. CON) within cultivar-year; \(^{\star}\) significant at 0.05;

NS - not significant.

\(^{\dagger}\)Means followed by the same letter(s) within a column are not significantly different, based on Fisher’s protected LSD (\(\alpha=0.05\))
Fig. 1. Illustration of the split-plot repeated measures design, where cranberry bog sites are main plots and cultivar strips (beds) are split plots within the site. Measurements are repeated three times on each bed, within the frost season.
Fig. 2. Cross-sectional images of cranberry buds evaluated under a microscope, illustrating different levels of frost damage: (1) no damage in either floral or vegetative meristems, (2) no damage in the vegetative meristem but some damage in one or two floral meristems, (3) damage in all floral meristems, (4) damage in the vegetative meristem and (5) all internal tissues damaged (dead).
Fig. 3. Minimum daily temperatures recorded at the University of Massachusetts Cranberry Station in East Wareham, Massachusetts (NOAA site identifier COOP:192451) and on-bog minimum temperatures reported to the Cape Cod Cranberry Growers Association in April, May, and June of 2011 and 2012. Asterisks indicate nights with dangerous frost warnings issued.
Fig. 4. Bud damage on beds of the cranberry cultivars ‘Early Black’, ‘Howes’ and ‘Stevens’ under two frost protection treatments in 2011: intermittent cycled irrigation (INT) or continuous irrigation (CON). Damage evaluations were (A) prior to the beginning of the season, (B) early in the frost season after at least one frost occurrence, (C) late in the spring after several frost protections events. Solid bars represent the percent damage in 50-bud samples, error bars are +/- standard error of the mean.
Fig. 5. Bud damage on beds of the cranberry cultivars ‘Early Black’, ‘Howes’ and ‘Stevens’ under two frost protection treatments in 2012: intermittent cycled irrigation (INT) or continuous irrigation (CON). Damage evaluations were (A) prior to the beginning of the season, (B) early in the frost season after at least one frost occurrence, (C) late in the spring after several frost protections events. Solid bars represent the percent damage in 50-bud samples, error bars are +/- standard error of the mean.
CHAPTER 3

EVALUATION OF AUTOMATED IRRIGATION CYCLING IN CRANBERRY FROST PROTECTION

Abstract

Frost protection is a necessary practice during cranberry production in order to reduce losses due to low temperatures. In the spring of 2013 and 2014, an experiment was conducted in southeastern Massachusetts to evaluate two common frost protection practices, continuous sprinkling (CON), the conventional method, and automated intermittently cycled irrigation (INT). Objectives included: 1) assess the efficacy of the INT practice for cranberry frost protection by evaluating cranberry bud damage and crop yield following frost events and 2) quantify the amount of water applied using INT and CON protocols for both mild and severe frost events. Cranberry bud damage was assessed from cultivars ‘Stevens’, ‘Howes’ and ‘Early Black’ by cutting and examining buds collected from beds managed with INT or CON methods. Water use was monitored using flow meters installed on the pump output and logging the volume of water used. Yield was determined for each combination of cultivar and frost protection method. Minimal damage was observed across cultivars before the beginning of the 2013 spring frost season. After more than 14 dangerous frost warnings, bud damage levels had increased to a high of 13% for ‘Early Black’ under INT and 8% for ‘Stevens’ also under INT. Cumulative water savings of 35% and 77%, respectively, were calculated for 2013 and 2014 at the end of each season. Substantial water savings under INT were noted on mild nights. On nights predicted to have a dangerous frost, both methods ran sprinklers from evening until sunrise the next morning, hence there were no differences in water use. Despite differences in
observed bud damage, yields were similar between the two frost protection methods within each cultivar for both years. We conclude that use of INT versus CON did not affect cranberry yield; however, considerable water savings were realized using INT.

**Keywords:** *Vaccinium macrocarpon*; intermittent sprinkling; water savings; bud damage; crop yield

**Introduction**

Automated irrigation has been introduced in cranberry management and consists of computerized equipment connected to a sprinkler irrigation system and sensors. When using this equipment for frost protection, start and stop of the irrigation system can be programmed for set temperatures, and then triggered based on readings from sensors placed on the coldest spots of the beds. Although sprinkler automation has been used for vegetables, strawberries, orchards and other plant systems for many years (Koc et al., 2000), it was only introduced in 2005 in cranberry (*Vaccinium macrocarpon* Ait.) production in Massachusetts.

Attempts have been made to evaluate the use of automated irrigation for frost protection in other plant systems (Heinemann et al., 1992; Heisey et al., 1994) but in cranberry there is only one report documenting findings from commercial beds (CCCGA, 2007). That report described savings of water, fuel, miles driven, and labor, along with increased safety and pump longevity when growers installed automation and used it to initiate starting and stopping of sprinkler irrigation systems used in frost protection. As a result of that study, more growers installed automation and some began to experiment with cycling the sprinklers on and off during frost
protection events. This was a change from the standard practice of starting the system when the
temperature dropped to near the critical tolerance temperature of the buds and then running the
system throughout the night until ambient temperatures rose above the tolerance in the morning
(Gates-Allen, 2009). While those growers were satisfied with the outcome of their cycling
experiments, the question of effectiveness of irrigation cycling in preventing freeze damage
remains pertinent and this led to the reluctance by some growers to adopt this approach.

A major concern in cycling is the possibility of bud damage during periods when
sprinklers are not running. If cycling is implemented correctly, significant fuel and water savings
are possible. Therefore, there is an urgency to study cycling in detail, particularly automated
cycling, so as to further understand its effectiveness in frost prevention while also assessing
water savings. This study focused on evaluating the efficacy of intermittent irrigation cycling
under an automated system (INT), compared to the conventional approach of running
continuously through the night (CON), with specific emphasis on bud damage and water usage.

Our objective was to evaluate the effects of automated cycling on the degree of
observable bud damage under mild and severe frosts. Mild frosts are characterized by
temperatures gradually decreasing through the night and briefly dropping below the plant's
hardiness temperature, while in a severe frost, temperatures are likely to drop rapidly to
significantly low temperatures where they remained for longer periods, usually lasting
throughout the night. A further objective was to compare water use between sites using the
conventional approach (no cycling) and those using cycling for frost protection during both types
of frost. Our hypothesis was that cycling saves water and fuel and is highly effective in protecting buds against frost damage.

**Materials and Methods**

**Study Layout**

This study was conducted in Bristol, Barnstable and Plymouth counties of southeastern Massachusetts using six commercial cranberry beds, three of which used the CON method of frost protection, while the other three used INT. One of the sites with both frost protection methods was used for the evaluation of water use. At all six sites, buds were collected between April and June (Table 3) and assessed for damage, and berry yields were sampled as described in Chapter 2. Ambient temperature minima were collected from the UMass Cranberry Station and reported on-bog temperatures were gathered by the Cape Cod Cranberry Growers Association (CCCGA) as described in Chapter 2.

**Measuring water use**

Selection of the site for water use comparisons was based on the ability to compare the two frost protection methods side-by-side at the same farm location. The site chosen was a cranberry bog built in the 1920s and is a naturally wet peat bottom bog with the ditches of drier sections kept high to reduce the necessity of irrigation. The sprinkler systems at this site consist of Rain Bird (35A-TNT; Rain Bird Co., Azusa, California) impact heads with uniform 50’ X 60’ spacing and a coefficient of uniformity of approximately 68%. Flow meters (McPropeller; McCrometer, Inc., Hemet, California) were connected to the discharge pipe from the pumps.
assigned to the CON and INT practices (Fig. 6) and recorded the volume of water used when the pump was run. Flow volumes were recorded before and after a frost event to determine the volume of water used in each event throughout the season and pump run times were manually recorded before and after the readings. Water use under INT was documented and compared to CON irrigation.

Data analysis

Bud damage data were analyzed using Chi Square ($X^2$) tests in SAS 9.3 (SAS Institute, Cary NC). PROC FREQ was used to generate three-way contingency tables that showed the measures of association between the method of frost protection, cultivars and the damage score. The yield was analyzed as described in Chapter 2, for both grouped sites and separately for the site where water use was evaluated. The student’s paired t-test was used to compare the means of water use per frost event in sites using CON and INT approaches over the season.

Results and Discussion

Temperature varied between years and months, with lowest temperatures experienced in the month of April, both at the East Wareham sheltered, upland location and on the bogs for the two years of the study (Fig. 7). The monthly average temperature in April, May and June did not differ significantly between 2013 and 2014. Bog temperatures were significantly lower than those recorded at East Wareham (non-bog) likely due to radiational cooling (DeMoranville, 2008). Consequently, in both years, April and May bog temperatures were often below zero (Fig.
The 2013 frost season had a total of 24 frost warnings, with 58% of them occurring early in the season while the 2014 season had a total of 19 frost warnings.

**Bud damage**

In 2013, minimal damage was observed across cultivars before the beginning of the season. Prior to the occurrence of frost, ‘Stevens’ under CON conditions had the highest bud damage (6%) compared to ‘Early Black’ (2%), the lowest under either method (Fig. 8A). By 2013 mid-season, after 14 frost warnings had been issued, bud damage levels had increased to 13% for ‘Early Black’ and 8% for ‘Stevens’ under INT (Fig. 8B). The end of the season was characterized by less frequent frost nights, with mild and moderate temperatures. In the end of frost season evaluation, damage was relatively low in our evaluations, with bud damage at 5% and 4% respectively, for ‘Stevens’ and ‘Early Black’ under INT (Fig. 8C). There were no bud damage differences between methods for ‘Howes’ at the end of the season. Overall, in 2013, ‘Stevens’ and ‘Early Black’ had more damage under INT while ‘Howes’ had similar damage under either method.

In 2014, preseason damage was slightly higher than in 2013 and ‘Howes’ had 14% damage in INT vs. 7% for CON (Fig. 9A). After several frost nights including a possible frost predicted to be -2.2°C on 18 May, collected buds showed no method difference in ‘Howes’ or ‘Early Black’ and greater damage in CON vs. INT for ‘Stevens’ (Fig. 9B). In 2014, only ‘Stevens’ showed differences between the methods with CON having higher damage, likely incurred in the pre-season period. Differences between cultivars may be due to their different
tolerances and bud size. Also, the set points chosen by the growers for INT may only be ideal for ‘Howes’ which was shown to have low and similar damage under both methods.

Yield

There were no significant differences in cranberry yield between the two frost protection methods within each cultivar for either year when compared across at all the sites in this study, despite the high bud damage of ‘Stevens’ and ‘Early Black’ under INT (Table 4). This is likely because damage was most often observed on only one or two floral initials within a bud, giving the remaining ones an opportunity to develop. Previous research (Hagidimitriou and Roper, 1994) showed that each cranberry upright produces only adequate carbohydrate to support an average of two berries. The only significant differences (P ≤ 0.001) were observed among cultivars. As would be expected, yield for the hybrid ‘Stevens’ was consistently higher than that of either ‘Howes’ or ‘Early Black’, native selections (Table 4). The robust ‘Stevens’ had a numerically lower yield value under CON but it was not statistically different from INT, despite higher bud damage under INT in 2013. These yield similarities within cultivar for the frost protection methods corroborate our hypothesis that the use of INT does not affect cranberry yield.

A closer look at the one site where water use was evaluated showed higher yields under INT than CON for ‘Early Black’ in 2013 and no difference between methods for the other two cultivars (Table 5). This was contrary to the bud damage observed in INT after several frost events and even at the end of the season. At 17.1 Mg ha⁻¹, this was the lowest yield observed
throughout the studies implying that other factors could have been responsible for the low yield. The CON method generally introduces more water on a bed due to continuous application and this can lead to the saturation of the root zone. As a wetland plant, cranberries have some tolerance to the lack of oxygen, however, persistent saturation of the root zone has a negative effect on respiration due to oxygen deficiency around the roots. This interferes with metabolism by causing the accumulation of toxic end products of anaerobic metabolism and the lack of substrates for respiration (Drew, 1997). It then results in poor root development, poor nutrient uptake and poor nitrogen utilization (DeMoranville, 2007). These factors will affect general plant health and development which can then influence the yield potential. Moreover, on the ‘Early Black’ CON bed we also observed patches that looked like die back caused by Phytophthora root rot. Both these factors could have contributed to lower yields observed in ‘Early Black’ under CON in 2013. Poor drainage can also promote fruit rot, further reducing yields. We generally observed higher fruit rot in CON beds (data not shown).

**Water use**

There were significant differences ($p = 0.01$) between the CON and INT methods for water use in both years. In 2013, the greatest differences in water use were between CON and INT were recorded on 18, 21, 25 April and 2, 17, 19 and 27 May (Fig. 10).

Cultivar tolerance at the beginning of the season were -6.7°C, -7.8°C and -6.7°C for ‘Early Black’, ‘Howes’ and ‘Stevens’, respectively. By the end of the season, tolerance had risen to -1.4°C for all cultivars. The early season was characterized by ‘intense’ or ‘dangerous frost
nights’ where temperatures are well below the trigger temperatures. On those nights, growers are generally reluctant to cycle as there is a chance that the sprinkler heads will freeze and result in inadequate protection and high levels of bud damage. In the early morning hours on 21 April 2013, temperatures dropped below the plant tolerance for about one hour. During that event both methods had similar water use. During a frost event the following day however, the CON method site used about 44,900 gal/acre of water, while the INT site used only about 5,400 gal/acre (Fig. 10). This large differential was unexpected early in the season when frost events are often severe, growers often choose not to cycle, and even with cycling, the off periods are generally brief. One explanation is that the grower used a lower start temperature in the INT beds so that some savings were due to an initial start later into the night compared to that in the CON bog. A similar pattern was noted on 21 April, 2014, also resulting in extremely high water use in CON compared to INT.

From 26 April 2013 - 6 May 2013, the CCCGA frost alert system predicted dangerous frost conditions on each of those days. This likely was the period when the differential bud damage observed on ‘Early Black’ and ‘Stevens’ occurred. ‘Howes’ did not have much damage during that period. Cultivar tolerances had increased from -5.6°C in ‘Howes’ and -3.9°C in both ‘Early Black’ and ‘Stevens’ to -1.4°C in all cultivars on 6 May. On 4, 14, 15 and 16 May, water use was similar between the methods although the amounts varied between the days (Fig. 10). The greatest water use was on 4 May while the lowest was on 16 May. It was observed that on those four nights, temperatures were particularly low with predictions for a dangerous frost lasting throughout the night. On 4 May, pumps were turned on from early evening (8 p.m.) to
early morning (6 a.m.) under both methods giving no advantages in water savings in INT (Fig. 10). Possibly, the INT protocol was not cycled on any of those four nights as most growers are reluctant to cycle when temperatures are extremely low and well below bud tolerance. Results from extremely cold nights seem to suggest that it may not be feasible to cycle when a dangerous frost occurs.

Maximum water savings for INT are incurred on nights when the temperature dipped just below the plant tolerance temperature and short run times were sufficient to raise the temperature of the plants. Despite the substantial savings, there were instances in our study when water use under INT was more than CON, probably due to differences in bed temperature. This was observed on 27 April 2013 and 16 May 2013 and both those days were predicted to be frost nights, with bog temperature at -5°C and -1°C, respectively (Fig. 10). Protection on those nights may have been inadequate under conventional as they ran irrigation pumps for shorter periods, indicating that pumps were turned on closer to morning.

In 2014, the season began on 16 April and the first two days were predicted to have dangerous frost with temperatures dropping to -9.4 and -8.9°C, respectively. While high water use was observed under the CON method on those days, the INT did not have equally high water use; as those pumps probably cycled. There may have been differences in bed temperature, leading to these drastic differences in the amount of water used. This pattern was also observed on 6 May and 30 May, days which had high water use for CON, relative to INT (Fig. 11).
On average, about 35% more water was used in CON compared to the INT method in the 2013 spring frost season (Fig. 12). This calculation includes the additional savings from two days when the INT beds did not implement frost protection at all. More water was used in 2013 as there were more frost nights. Savings in 2014 (Fig. 12) were even more substantial, with as much as 77% less water used under INT than CON for the season.

Several commercial automatic controllers aimed at regulating soil water content have since been manufactured and examined (Bhosale and Dixit, 2012). Goumopoulos et al. (2014) designed an adaptable decision support system combined with a wireless sensor/actuator network to implement autonomous closed-loop zone-specific irrigation while Miller et al., 2014 demonstrated the utility of multi-sensor capacitance probes. However, there are limited studies that have demonstrated the use of automation and cycling to save water during frost protection.

A previous study in an apple orchard tested both the software and hardware for an early automated irrigation system originally designed for the frost protection of strawberries (Heinemann et al., 1992). Contrary to the cranberry automated system where the cycling was controlled by fluctuating bog temperatures, their regimen was fixed. They determined a fixed and conservative intermittent cycle which was designed to generate one revolution of the sprinkler head. The start point was the temperature where plant or air temperature was below the adjusted critical temperature of 1-1.5°C (Heisey et al., 1994). This kind of cycling could be detrimental to cranberry frost protection since bog temperatures can drop quickly and remain low for long periods before they rise. Longer cycle intervals, based on bed temperature are necessary
to ensure adequate protection in cranberry frost protection. While their findings showed 75% water savings compared to the conventional method on one particular night, Heisey et al. (1994) reported near freezing bud temperatures caused by lingering ice in the morning and slight damage in the leaves. This implied ineffectiveness of frost protection, which they attributed to inadequate water distribution mainly caused by short cycles and orchard trellis structures (Heisey et al., 1994).

This study has practical implications for cranberry growers in Massachusetts. Findings have demonstrated that there are water savings associated with INT and with reduced irrigation pumping time fuel consumption and its associated emissions should also be reduced. There is also evidence that bud damage is minimal under this frost protection method. Having successfully equipped growers with the knowledge that the intermittent automated method of irrigation, with cycling (INT), equally protects cranberry buds against frost damage compared to running sprinklers through the night, growers now have an opportunity to re-evaluate their frost protection and management practices.
Literature Cited


Table. 3. Dates when cranberry buds were collected for frost damage evaluation in the 2013-2014 frost seasons in southeastern Massachusetts.

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preseason</td>
<td>1-15 April</td>
</tr>
<tr>
<td>Early season</td>
<td>16 April - 23 May</td>
</tr>
<tr>
<td>Late season</td>
<td>24 May - 15 June</td>
</tr>
</tbody>
</table>
Table 4. Effects of frost protection methods, intermittent cycled irrigation (INT) or continuous irrigation (CON), on the yield of three commercial cranberry cultivars from grouped sites in 2013 and 2014.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>INT</th>
<th>CON</th>
<th>contrasts†</th>
<th>INT</th>
<th>CON</th>
<th>contrasts†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Black</td>
<td>22.2b</td>
<td>23.2a</td>
<td>NS</td>
<td>25.3b</td>
<td>29.3b</td>
<td>NS</td>
</tr>
<tr>
<td>Howes</td>
<td>26.8b</td>
<td>26.3a</td>
<td>NS</td>
<td>37.9a</td>
<td>36.5ab</td>
<td>NS</td>
</tr>
<tr>
<td>Stevens</td>
<td>38.3a</td>
<td>30.0a</td>
<td>NS</td>
<td>42.9a</td>
<td>39.8a</td>
<td>NS</td>
</tr>
</tbody>
</table>

Method contrasts show the frost protection method effect on cultivar based on single degree of freedom contrasts.

†Treatment contrasts (INT vs. CON) within cultivar-year; * - significant at 0.05;

NS - Nonsignificant at p = 0.05.

†Means followed by the same letter(s) within a column are not significantly different, based on Fisher’s protected LSD (α = 0.05)
Table 5. Effects of frost protection methods, intermittent cycled irrigation (INT) or continuous irrigation (CON), on the yield of three commercial cranberry cultivars for the individual site used to assess water use in southeastern MA, 2013 and 2014.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>INT</th>
<th>CON</th>
<th>contrasts†</th>
<th>INT</th>
<th>CON</th>
<th>contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Black</td>
<td>27.3a</td>
<td>17.1b</td>
<td>*</td>
<td>21.0a</td>
<td>18.7b</td>
<td>NS</td>
</tr>
<tr>
<td>Howes</td>
<td>31.7a</td>
<td>27.6ab</td>
<td>NS</td>
<td>41.1a</td>
<td>33.9a</td>
<td>NS</td>
</tr>
<tr>
<td>Stevens</td>
<td>34.9a</td>
<td>30.2a</td>
<td>NS</td>
<td>53.1a</td>
<td>42.8a</td>
<td>NS</td>
</tr>
</tbody>
</table>

Method contrasts show the frost protection method effect on cultivar based on single degree of freedom contrasts.

†Treatment contrasts (INT vs. CON) within cultivar-year; * - significant at 0.05;

NS - Nonsignificant at p = 0.05.

†Means followed by the same letter(s) within a column are not significantly different, based on Fisher’s protected LSD (α = 0.05)
Fig. 6. Image of the (A) McCrometer propeller flow meter connected to a discharge pipe on an engine and (B) closer look at a reading.
Fig. 7. Minimum daily temperatures recorded at the University of Massachusetts Cranberry Station in East Wareham, Massachusetts (NOAA site identifier COOP:192451) and on-bog minimum temperatures reported to the Cape Cod Cranberry Growers Association in April, May, and June of 2013 and 2014. Asterisks indicate nights with dangerous frost warnings issued.
Fig. 8. Bud damage on beds of the cranberry cultivars ‘Early Black’, ‘Howes’ and ‘Stevens’ under two frost protection treatments in 2013: intermittent cycled irrigation (INT) or continuous irrigation (CON). Damage evaluations were (A) prior to the beginning of the season, (B) early in the frost season after at least one frost occurrence, (C) late in the spring after several frost protections events. Solid bars represent the percent damage in 50-bud samples, error bars are +/- standard error of the mean.
Fig. 9. Bud damage on beds of the cranberry cultivars ‘Early Black’, ‘Howes’ and ‘Stevens’ under two frost protection treatments in 2014: intermittent cycled irrigation (INT) or continuous irrigation (CON). Damage evaluations were (A) prior to the beginning of the season, (B) late in the spring after several frost protection events. Solid bars represent the percent damage in 50-bud samples, error bars are +/- standard error of the mean.
Fig. 10. Average volume of water used per frost event in beds under continuous (CON) and cycling (INT) frost protection methods shown by black and grey lines respectively. Measurements were taken using McCrometer propeller flow meters over the 2013 frost season.

Fig. 11. Average volume of water used per frost event in beds under continuous (CON) and cycling (INT) frost protection methods shown by black and grey lines respectively. Measurements were taken using McCrometer propeller flow meters over the 2014 frost season.
Fig. 12. Total average volume of water used during frost protection in the 2013 and 2014 seasons under continuous (CON) and cycling (INT) frost protection methods shown by dark grey and light grey bars respectively, Massachusetts. Significant differences in water use, shown by standard error bars were observed between the two methods in both years.
CHAPTER 4

EFFECT OF ACCLIMATION TEMPERATURE ON CARBOHYDRATE AND LIPID CONCENTRATION IN FOUR CRANBERRY CULTIVARS

Abstract

The American cranberry (Vaccinium macrocarpon Ait.) is a temperate woody sub shrub whose buds are susceptible to freeze damage. The plant goes through a period of acclimation in the fall as temperatures decline and days shorten. That results in an increase in cold hardiness and the ability to withstand winter temperatures without incurring freeze damage in its tissues. In many temperate plants, acclimation includes the synthesis of carbohydrates and lipids that are thought to reduce cellular dehydration and maintain membranes in a liquid-gel crystalline state. Carbohydrate and lipid changes in cranberry, in response to acclimating temperatures were determined in potted plants of cranberry cultivars ‘Demoranville’, ‘Howes’, ‘Mullica Queen’ and ‘Stevens’. Cranberry cultivars were subjected to four sequentially applied chilling temperatures (15, 6, 2 and -2°C for 14 d each) in a growth chamber. After each 14 d acclimation period, uprights from each cultivar were removed from the pots and leaf and stem tissues were analyzed for total non-structural carbohydrates (TNSC), monosaccharide carbohydrates (fructose and glucose), sucrose, and starch. Sample of leaves were analyzed for galactolipids, phospholipids and lyso-group lipids. Total nonstructural carbohydrates, sucrose, fructose, and glucose concentrations in leaves increased while starch concentrations decreased as the acclimation temperature decreased. In stems, TNSC and sucrose concentrations generally increased as acclimation temperature decreased. Maximum increase in synthesized TNSC in leaves occurred between 6 to 2°C resulting in an 80% increase across all cultivars. The lipid analyses identified
eleven polar lipid groups of three types: galactolipids (77.6 % total polar lipids); phospholipids (22 % total polar lipids); and lyso-groups (0.4 % total polar lipids). Digalactosyldiacylglycerol (DGDG) increased in leaves of all cultivars as acclimation temperatures decreased. At -2°C, leaves of most cultivars showed decreases in phosphatidylserine (PS) concentrations compared to those in plants treated at 15°C. The cultivar ‘Stevens’ had substantial increases in lyso-group lysophosphatidylcholine from 15°C to -2°C (0.1 to 0.4 mol %, respectively) but in general there was no consistent trend within the lyso-group. We concluded that compounds that increased in response to acclimating temperatures are important in increasing cold hardiness in cranberry.

**Keywords:** *Vaccinium macrocarpon*, cold acclimation, total non-structural carbohydrates (TNSC); double bonds; membrane stability

**Introduction**

Cranberry (*Vaccinium macrocarpon* Ait.) is a cool-season temperate woody sub shrub native to North America. It is susceptible to low temperatures that may cause freezing injury of buds, leaves and berries. Freezing injury, damage inflicted by sub-zero temperatures, causes severe cellular dehydration and is a major abiotic stress (Xin and Browse, 2000). As temperatures decrease, the plasma membrane may become dehydrated and rigidify, resulting in the loss of membrane integrity and cell function (Palta, 1990; Xin and Browse, 2000). At the whole plant level, freezing injury causes permanent damage to leaves and buds, impairs growth and may result in the death of plants. In order to withstand sub-freezing winter temperatures without sustaining significant damage, many woody perennials, including cranberry, go through
the process of cold acclimation; in the spring these plants must resist premature deacclimation, which equates to loss of hardiness (Arora and Rowland, 2011; Rodrigo, 2000).

Cold acclimation is an adaptive mechanism that allows plants to increase freezing tolerance after exposure to low, non-freezing temperatures and short photoperiods (Levitt, 1980; Rowland et al., 2005; Thomashow, 1999). These environmental cues occur in the fall and early winter, leading to structural, biochemical, physiological, molecular and metabolic changes in plants. These changes increase the ability of plants to tolerate low temperatures and, thus, increase levels of cold hardiness (Hao et al., 2009; Panta et al., 2004). Some commonly observed physiological changes include: 1) modified lipid membrane composition; and 2) increased concentrations of compatible solutes including proline, betaine, polyols, soluble sugars (Kishitani et al., 1994), amino acids, organic acids, hormones, antioxidants and protective proteins (Xin and Browse, 2000). It is common for cultivars of the same species to exhibit some differences in the maximum attainable hardiness (McNamara and Pellett, 1998).

In highbush blueberry (Vaccinium corymbosum L.), researchers identified three dehydrins, hydrophilic and thermostable proteins, of 65, 60, and 14 kDa size that increased with cold acclimation and decreased with deacclimation and resumption of growth (Arora et al., 1997) suggesting that they are synthesized during cold acclimation, in response to the changing environmental conditions. Carbohydrates and lipids are also among the critical physiological compounds involved in the development of frost resistance and survival in freezing temperatures (Bohn et al., 2007).
Changes in carbohydrates have been noted during cold acclimation in grapes (*Vitis vinifera* L.) (Hamman et al., 1996), guava (*Psidium guajava* L.) (Hao et al., 2009), wheat (*Triticum aestivum* L.) (Skinner and Garland-Campbell, 2008), grasses (Dionne et al., 2001; Espevig et al., 2011; Hoffman et al., 2010), and *Vaccinium* (Hagidimitriou and Roper, 1994; Lee et al., 2013; Spann, 2004; Taulavuori et al., 2001). Glucose and fructose measured in 21 blueberry cultivars was found to be positively correlated with levels of cold hardiness (Lee et al., 2013). A study of changes in cranberry nonstructural carbohydrates throughout the year, of both above and below ground tissue, showed variability in carbohydrate amounts based on the time of year (Hagidimitriou and Roper, 1994).

Membrane composition, structure, and function are important in cold acclimation as they directly influence plant adaptation to low temperatures (Szalai et al., 2001). As cold tolerant plants gradually acclimate to low temperatures, structural and biochemical changes in membrane lipid help to increase the stability of cell structure and metabolism at low temperatures. Lipids, an important constituent of cellular membranes, tend to transition from liquid-crystalline to gel phase as a primary response to chilling injury, occurring at low and non-freezing temperatures (Lyons, 1973). As temperatures drop below zero, there is a further reduction in membrane fluidity and subsequent changes in lipid head groups, acyl chain length, backbone, and lipid fluidity (Palta, 1990). Modifications in plasma membrane lipids are necessary for the development of frost resistance and survival at subzero temperatures (Steponkus, 1984).

Increased freezing tolerance is associated with unsaturated fatty acids, which are known
to increase in concentrations in response to internal and external cues during acclimation.

Previous studies have shown an increase in the proportion of unsaturated fatty acids at lower temperatures (Szalai et al., 2001). The unsaturated fatty acids create kinks that prevent the tight packing of phospholipids and therefore increase membrane fluidity and stability during desiccation (Campos et al., 2003) leading to increased freezing tolerance (Martz et al., 2006). In an evaluation of the efficiency of low-temperature hardening in tobacco (Nicotiana tabacum L.) leaves, Popov et al. (2012) found an accumulation of 18:2n-6 and 18:3n-3 while 16:0 and other saturated fatty acids decreased. In crowns of a hardened, frost tolerant wheat cultivar, the percentage of fatty acid unsaturation in phosphatidylethanolamine (PE) was greater than that of a less frost tolerant cultivar (Szalai et al., 2001). Similar findings were seen in crowns of perennial ryegrass (Lolium perenne L.), where a freezing-tolerant accession had a higher ratio of membrane stabilizing lipids and unsaturated fatty acid content than the freezing-susceptible accessions (Hoffman et al., 2010).

We hypothesized that: 1) acclimation temperature affects development of hardiness in cranberry by affecting the levels of total non-structural carbohydrates (TNSC) and lipids synthesized during the cold acclimation period; and 2) the response differs among cranberry cultivars. Therefore, our objectives were to identify and quantify the carbohydrates and lipids that are synthesized during cold acclimation in four cranberry cultivars.
Materials and Methods

Plant Material

Stem cuttings from four cranberry cultivars planted at the UMass Cranberry Station in East Wareham, Massachusetts (lat. 41°45’N, long. 70°40’W) were made on 1 April 2010 for use in propagation. We selected two recently released hybrid cultivars, ‘Demoranville’ and ‘Mullica Queen’, a native cultivar ‘Howes’, and an older hybrid, ‘Stevens’. For each of the cultivars, four cuttings per pot were planted in 10 x 10 cm containers (diameter x depth) filled with 3:1 (v:v) sand peat moss mix. Potted cuttings were placed under an overhead mist system in a greenhouse. Plants were fertilized four weeks after planting with one scoop (approximately 1.25 g) of Osmocote slow release fertilizer (14N–6P–11K) (Scotts, Marysville, OH), applied on the surface of the soil. Plants were transferred to a hoop house on 1 Nov. 2010 to satisfy the chilling requirement and returned to the greenhouse on 1 April 2011. Cranberry, like other temperate perennials, requires a period of cool temperatures to enter dormancy, resist premature deacclimation during brief warm spells, prevent delays in bud break and ensure normal leaf and fruit development (Fu et al., 2012). On 1 Oct. 2011, the potted cuttings were used for the acclimation study.

Acclimation temperature treatments

The temperature regimes (15, 6, 2 and -2°C) for the controlled-temperature acclimation study were selected based on calculations of the average ambient temperatures for September, October and November in East Wareham, Massachusetts during the years 2008 to 2011, plus a -2°C treatment. Although the average temperatures do not get as low as -2°C during those
months, temperature minima during the period are often that low, and previous studies have shown that sub-zero temperatures may be need to achieve full cold acclimation potential in woody perennials (Arora and Rowland, 2011). Sixteen pots from each cultivar were used in this experiment so that a total of 64 pots were placed in the growth chamber. The pots were exposed to a series of cold acclimation treatments, consisting of sequentially exposing plants to 15, 6, 2 and -2°C for 14 days at each temperature in a controlled Percival Growth Chamber (PGC-10; Percival Scientific, Perry, IA). In the growth chamber, plants were watered periodically to maintain sufficient soil moisture and prevent water stress. Other specific conditions in the growth chamber included change in day length (combination of cool-white fluorescent lamps and incandescent light) and light intensity as follows: 15°C day/night, 12-h photoperiod and photosynthetic photon flux density (PPFD) of 500 µmol m⁻² s⁻¹ for 14 days, 6°C day/night, 10-h photoperiod and PPFD of 250 µmol m⁻² s⁻¹ for 14 days, 2°C day/night, 10-h photoperiod and PPFD of 250 µmol m⁻² s⁻¹ for 14 days and -2°C day/night in total darkness for 14 d. At the end of each 14 d single temperature acclimation period, four replicate pots of each cultivar were removed from the chamber. Approximately 20 cranberry leaves were collected from each pot and subjected to lipid analysis while the remaining plants in that same pot were separated into leaves and stems for carbohydrate analysis.

**Carbohydrate Extraction and Analysis**

Above-ground plant material in each pot was separated into leaves and stems and weighed for fresh mass. The biomass was then dried at 60°C for a minimum of three days to obtain dry weight and subsequently ground to 40 mesh size using a Wiley mill (3383-L10;
Thomas Scientific, Swedesboro, NJ). Ground leaf and stem samples were stored at room temperature prior to carbohydrate analysis. Carbohydrates and starch in leaf and stem samples were analyzed by high pressure liquid chromatography (HPLC) using elution methods developed by Botelho and Vanden Heuvel (2005), with the exception of a change to Empower software (Waters Corp., Milford, MA) for integrating peaks. Standards were prepared separately and were analyzed simultaneously with the samples. Carbohydrates in the samples were quantified based on comparison of their HPLC peaks and those of the standards.

**Lipid Extraction and Analysis**

Lipids were extracted from the leaf samples according to a modified procedure of Welti et al., (2002). Scintillation vials were filled with 3 ml of isopropanol and 0.01% butylated hydroxytoluene (BHT) and placed in a 75°C water bath. Cranberry leaves were placed into these test tubes and incubated for 15 min. After cooling, 1.5 ml chloroform was added to each test tube, shaken at room temperature for at least five hours to allow layers to separate and the lower layer (chloroform plus lipids) transferred into new test tubes using Pasteur pipettes. A 4 ml aliquot of 2 parts chloroform: 1 part methanol with 0.01% BHT was added to the original tubes containing the plant tissue and the extraction process repeated five times. One ml of 1M KCL was added to the lipid extract and the tubes centrifuged for 10 min at 5,000 RPM. Next, 2 ml of distilled water was added and the mixture was vortexed and then centrifuged for 10 min at 5,000 RPM. The solvent was evaporated under nitrogen to a small volume (less than 4.0 ml) for 4 h then the samples were transferred to 2 ml vials with Teflon-lined screw cap and evaporated completely. Vials were stored at -80°C until ready for shipping to Kansas Lipidomics Research
Center (Kansas State University, Manhattan, KS) for mass spectrometry. There, a polar lipid profile was generated using electrospray ionization coupled with tandem mass spectrometry (ESI-MS/MS).

**Statistical analysis**

The experiment was conducted as a completely randomized design (CRD) replicated four times; the 64 pots (16 per cultivar) were arranged randomly in the growth chamber. Data were subjected to analysis of variance using PROC GLM (SAS version 9.3; SAS Institute, Cary, NC) where appropriate with mean separation by Fisher’s protected least significant difference test. For each cultivar at each acclimation temperature, fatty acid unsaturation index (FAUI) was calculated for each lipid group. An equation was used (Zhang et al, 2009):

$FAUI = \text{sum of } \left(\frac{\text{mol} \% \text{ of each lipid species in a class } \times N}{\text{mol} \% \text{ of the lipid class}}\right)$

where N is the number of double bonds of the species and class is the lipid species within a head group.

**Results and Discussion**

**Carbohydrate response to acclimation temperature**

The HPLC analysis separated three water soluble carbohydrates (sucrose, glucose and fructose) and starch; together these constituted TNSC. TNSC increased with a decrease in acclimation temperature. There was a significant interaction ($P \leq 0.001$) between organ and acclimation temperature, indicating that acclimation temperature influenced leaves and stems differently. The TNSC content in leaves was higher than that in stems (Figs. 13 and 14).
The most abundant carbohydrates in cranberry leaves and stems were sucrose and fructose, which, as acclimation temperatures were lowered, increased while starch concentrations declined. These sugars also were predominant in blueberry (Lee et al., 2013), a woody perennial in the same genus as cranberry. We observed a sharp decline of starch which was concomitant with a sharp increase of sucrose levels as the acclimation temperature was changed from 15 to 6°C. As the acclimation temperature was reduced, ‘Mullica Queen’ had the greatest decline in starch levels (80%) while ‘Demoranville’ had the lowest (50%) (Fig. 13). Throughout the study, increases in water soluble sugars were accompanied by decreases in starch levels in both leaves and stems as temperature gradually decreased. Our findings agreed with those of Nagao et al. (2005), but were somewhat contrary to observations in tomato (*Solanum lycopersicum* L.) which increased both sugar and starch as temperatures declined (Klopotek and Klaring 2014; Seginar and Gent, 2012). Generally, there are species-specific differences in carbohydrate metabolism, especially during cold acclimation (Lee et al., 2013).

In this study, the temperature treatments also included a gradual reduction of day length, ending with total darkness in the -2°C treatment. Leaf starch content is influenced by the expression of starch-mobilizing genes which are circadian regulated (Lu et al., 2005). However, this may not have any influence on plants that were kept in the dark. Starch degradation in circadian patterns is linked to sucrose synthesis and we found that sucrose increased markedly during cold acclimation of cranberry. After cranberry plants were exposed to 15°C for 14 days, the levels of sucrose, fructose, and glucose were similar in leaves (Fig. 13) while in stems, sucrose was substantially higher compared to the other sugars (Fig. 14). However, starch in both
tissues was found at higher concentrations than sucrose. The TNSC content at 15°C corresponded to values reported for cranberry exposed to similar conditions (Vanden Heuvel and Davenport, 2005).

After an additional 14 days at 6°C, glucose and fructose concentrations were little changed but there was an average increase of about 80% in sucrose levels and a similar drop in starch levels in leaves of all cultivars. After a further 14 d at 2°C, sucrose, fructose, and to a lesser extent, glucose, continued to increase as starch decreased in the leaves. The increase in sucrose, fructose and glucose levels in response to decreasing temperature is consistent with previous studies (Dionne et al., 2001; Espevig et al., 2011), although some researchers have suggested that the high levels of these sugars do not necessarily coincide with increases in cold hardiness (Hamman et al., 1996).

There was little change in TNSC between the 2 and -2°C treatments. In other plants, accumulation of carbohydrates and other compatible solutes occurs at non-freezing temperatures (Livingstone et al., 2009) while subfreezing temperatures are followed by additional cellular modifications that increase membrane stability (Herman et al., 2006). In stems, the increase in sucrose levels from 15 to -2°C was about 50% for all cultivars (Fig. 14). At acclimating temperatures (<15°C in leaves, <6°C in stems), it appears that starch is converted to sucrose, indicating a possible role for that sugar in the development of hardiness.
Our findings are supported by studies that have found sucrose to regulate cold-acclimation-associated gene expression in *Arabidopsis* (Rekarte-Cowie et al., 2008). During cold acclimation of red raspberry (*Rubus idaeus* L.), sucrose also was found in high concentrations (Palonen et al., 2000). Sucrose has been proposed to play a role in maintaining membrane integrity and preventing any structural changes in soluble proteins by maintaining membrane phospholipids in the liquid crystalline phase during dessication and cold stress (Farrant et al., 1993, Gusta et al., 1996). Sucrose may also prevent the adhesion of ice to critical cellular tissue during freezing (Olien, 1984).

In leaves, the total amount of TNSC, particularly fructose and sucrose, increased steadily for all cultivars as the acclimation temperature was decreased and as plants were exposed to sub-zero temperatures we observed TNSC concentrations as high as 17.6% of dry matter (Fig. 13, 'Stevens' at -2°C). This suggests that sucrose and fructose synthesis in cranberry is associated with freezing tolerance and plant hardiness. By comparison, highest TNSC concentration in stems was about 10% (Fig. 14, 'Demoranville' at -2°C). While total TNSC concentration was not significantly different between leaves and stems in the 15 and 6°C treatments, the TNSC concentration in leaves were twice that of stems at 2 and -2°C. This implies that leaves may be more sensitive to temperature changes than woody stems, which are more hardy and resistant to abiotic stresses.
Lipid response to different acclimation temperatures

Lipids are a main constituent in cell membranes and play a critical role in the development of plant tolerance to low temperatures (Welti et al., 2002). To our knowledge, this is the first time lipid changes in cranberry during cold acclimation have been evaluated.

Using the ESI-MS/MS, we assessed changes in lipid content during cold acclimation in cranberry. Eleven lipid groups were identified in cranberry leaves: digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylinerine (PS), phosphatidylethanolamine (PE), lysophosphatidylglycerol (Lyso-PG), lysophosphatidylcholine (Lyso-PC) and lyso-phosphatidylethanolamine (Lyso-PE). The most abundant polar lipids, MGDG, DGDG and PC, comprised 88% of the total lipids, while PE, PG and PI accounted for about 11%, and PA, PS and the Lyso groups were present in trace amounts.

Galactolipids, MGDG and DGDG accounted for more than 70% of the total polar lipids in cranberry leaves. This was similar to the findings in a study of several coffee (Coffea) cultivars exposed to cold conditions; galactolipid levels between 75 and 85% were reported, with MGDG in higher levels than DGDG (Partelli et al., 2011). However, in several grass species these lipids were found in much lower concentrations: trace amounts in wheat (Triticum aestivum L.) (Szalai et al., 2001), 15-20% of total lipids in perennial ryegrass crowns (Hoffman et al., 2010), and 20-40% in zoysiagrass rhizomes (Zhang et al., 2009). Galactolipids are major chloroplast lipids (Douce and Joyard, 1980), representing up to 80% of thylakoid membrane
glycerolipids (Siebertz et al., 1980) and containing high levels of polyunsaturated fatty acids.

We observed significant differences in galactolipid accumulation between cold acclimation temperatures and among cultivars (Table 6). ‘Demoranville’ or ‘Mullica Queen’ had similar DGDG levels at 15 or 6°C, but significant increases were observed when the plants were exposed to 2 and -2°C. For ‘Howes’ and ‘Stevens’ no significant differences were observed among the 15, 6 and 2°C treatments, however, an increase in DGDG at -2°C was observed. DGDG levels in the -2°C treatment increased from those in the non-acclimating 15°C treatment for all cultivars. This increase suggests that DGDG, a bilayer-forming lipid normally associated with thylakoid membranes, may be necessary for cold acclimation in cranberry as it is in other crops (Campos et al., 2003). In addition, DGDG has been reported to enhance control of ionic permeability in the chloroplasts, minimizing changes in the lipid environment and hence preserving activity of membrane proteins (Navari-Izzo et al., 1995). The DGDG galactolipid may be primarily responsible for stabilizing the membrane at lower temperatures by stabilizing photosynthetic complexes in the thylakoids (Klaus et al., 2002).

Monogalactosyldiacylglycerol was the most abundant lipid and accounted for 43% of the total polar lipids. The ratio of DGDG/MDGD generally was higher at -2°C than at 2, 6 or 15°C (Table 6). MGDG showed a decreasing trend with decreased temperatures for some cultivars but differences were not significant (Table 6). This was expected since MGDG has a greater tendency to form HII phase structures, which are known to result in the instability of membranes (Campos et al., 2003).
Phospholipid response to changes in acclimation temperature was varied (Table 6). PA, usually associated with membrane injury (Welti et al., 2002), increased with decreasing temperature but the amounts were very small. PA levels increased as the acclimation temperature was decreased from 15°C to -2°C for all cultivars. For ‘Demoranville’ and ‘Stevens’, PA levels were statistically different at -2°C compared to those at 15°C. The increase in PA values at -2°C and 15°C only were significant for the cultivar, ‘Demoranville’. A temperature relationship similar to that for PA was seen for PG in ‘Howes’. PG plays a critical role in photosynthesis by helping to maintain structural integrity of the QB-binding site of the PSII reaction center (Varkonyi et al., 2002; Hagio et al., 2000).

For all cultivars, PC levels fluctuated with temperature and the lowest levels were observed at -2°C, which was significantly lower than 15°C for ‘Howes’ and ‘Stevens’. PI was variable and tended to be significantly lower at -2°C compared to 15°C in ‘Demoranville’ and ‘Mullica Queen’. PE response seemed to be similar to that of PC. ‘Howes’ and ‘Stevens’ had significantly less PE at -2°C than at 15°C. However, the PC to (PA+PE) ratio decreased at lower acclimation temperatures as higher values were noted at 15°C compared to -2°C. It previously was speculated that a high PC/PE ratio was beneficial for plant survival at low temperatures (Uemura et al., 1995). The decreases in PC and PE levels at lower acclimation temperature (Table 6) were contrary to what was found in studies of other species, where PC and PE attributed to higher total lipid composition (Campos et al., 2003; Hoffman et al., 2010, Partelli et al., 2011). PC is known as a bilayer-forming lipid that stabilizes the membrane (Welti et al., 2002) and it is not clear why concentrations declined with decreasing acclimation temperature. In
a salt cress (*Thellungiella salsuginea* Pall.) study, changes in lyso-phospholipids were negligible (Zhang et al., 2013), while in *Arabidopsis* there was a 7-10 fold increase during freezing (Li et al., 2008; Welti et al., 2002). It is possible that lyso-phospholipids and the phospholipids (Table 6) do not play any significant role in the cold acclimation of cranberry.

A high fatty acid unsaturation index (FAUI) indicates high levels of unsaturated fatty acids that reduce rigidity in membranes, reducing their tendency to form non-bilayer structures and thus enhancing membrane stability at low temperatures. The greatest FAUI for DGDG was observed at the lowest acclimation temperature in all cultivars with the exception of 'Stevens' (Table 7). The increase in the quantity of unsaturated fatty acids with the decline in temperature suggests that the cranberry plant adapts as temperatures are lowered by synthesizing protective lipids that increase membrane fluidity and hence protect it from freezing damage. There were significant differences in phospholipid FAUI among acclimation temperatures, differences were more distinct between 15 and -2°C (Table 7). For all evaluated lipids, there was significantly higher unsaturation at -2°C than at 15°C, while there were variations at the other temperatures.

All four cultivars exhibited differences in their individual responses to acclimation temperature. In general, sucrose, glucose and fructose, particularly in the leaves, increased with a decline in acclimation temperature (from 15°C to -2°C) corresponding with decreased starch concentration. Maximum carbohydrate and lipid accumulation occurred between 6 and 2°C while the transition from 2 to -2°C was not significant. Although eleven lipids were identified in cranberry leaves following acclimation treatments, galactolipids DGDG and MGDG were found
in the greatest amounts. DGDG increased significantly when temperatures were gradually
decreased, implying that this major lipid constituent is involved in cranberry cold acclimation.
In conclusion, this study indicates the strong likelihood that the development of hardiness during
cranberry acclimation involves the accumulation of sugars, particularly sucrose and fructose, and
the galactolipid DGDG.
Literature Cited


Table 6. Polar lipid content in leaf tissues of four cranberry cultivars in response to four acclimation temperatures (15, 6, 2 and -2°C).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Atemp</th>
<th>Galactolipids</th>
<th></th>
<th>Phospholipids</th>
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<th>Lyso-group</th>
</tr>
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<tr>
<td></td>
<td></td>
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<td>MGDG</td>
<td>DGDG/ MGDG</td>
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<td>PE</td>
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<tr>
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<td>2</td>
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<td></td>
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<td>4.8c</td>
</tr>
</tbody>
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†For each cultivar, means followed by the same letter(s) within a column are not significantly different based on Fisher’s protected LSD (α = 0.05)

DM, Demoranville; HW, Howes; MQ, Mullica Queen and ST, Stevens

‡Atemp, acclimation temperature; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; PG, phosphatidylglycerol; PS, phosphatidylinserine; PI, phosphatidylinositol; Lyso-PG, lyso phosphatidylglycerol; Lyso-PC, lysophosphatidylcholine; Lyso PE, lysophosphatidylethanolamine.
Table 7. Fatty acid unsaturation index (FAUI) for polar lipids in four cranberry cultivars in response to four acclimation treatments (15, 6, 2 and -2°C). FAUI = sum of [(mol % of each lipid species in a class × N)/mol % of the lipid class]

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Atemp</th>
<th>Galactolipids</th>
<th>Phospholipids</th>
<th>Lyso-group</th>
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†For each cultivar, means followed by the same letter(s) within a column are not significantly different based on Fisher’s protected LSD (α = 0.05)

DM, Demoranville; HW, Howes; MQ, Mullica Queen and ST, Stevens

†Atemp, acclimation temperature; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; PG, phosphatidylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol; Lyso-PG, lyso phosphatidylglycerol; Lyso-PC, lysophosphatidylcholine; Lyso PE, lysophosphatidylethanolamine.
Fig. 13. Changes in carbohydrate content for leaves of four cranberry cultivars, ‘Demoranville’, ‘Howes’, ‘Mullica Queen’ and ‘Stevens’, in response to four acclimation temperatures (15, 6, 2 and -2°C). Significant differences were noted between acclimation temperatures.
Fig. 14. Changes in carbohydrate content for stems of four cranberry cultivars, ‘Demoranville’, ‘Howes’, ‘Mullica Queen’ and ‘Stevens’, in response to four acclimation temperatures (15, 6, 2 and -2°C). Significant differences were noted between acclimation temperatures.
CHAPTER 5

EVALUATION OF COLD HARDINESS CHANGES DURING ACCLIMATION IN CRANBERRY CULTIVARS

Abstract

Freezing injury is a major abiotic stress affecting cranberry (*Vaccinium macrocarpon* Ait.). While changes in bud hardiness during spring deacclimation are well known, bud acclimation in the fall has received minimal study. An experiment was designed to assess the timing of acclimation and degree of cold hardiness for terminal buds of six cranberry cultivars collected from a cranberry field in East Wareham, Mass. or grown in pots in a greenhouse then acclimated under controlled conditions in a growth chamber (15, 6, 2 or -2°C and varied light intensity for seven days at each temperature). Field-collected samples were taken from mid-September through mid-December, 2014. Cranberry uprights with terminal buds were exposed to a series of freezing temperatures and then evaluated for visible damage. LT50 (the temperature at which 50% of the buds were damaged) across treatments was determined using the Gompertz function and the lowest survival temperature (LST) was determined directly from visual assessments. In the field-acclimated buds, LT50 and LST temperatures declined with each successive freeze test and reached a steady state by 19 Nov. (LT50) or Dec. 3 (LST). The exception was in the first week of Oct. when the field was flooded for harvest: during that period hardiness ceased to increase, resuming its downward progression a week later. Cultivars differed in both hardiness progression and degree. Based on LT50 values of buds acclimated at -2°C in controlled conditions prior to freeze testing, the cultivars ranked as follows (in order starting
with the most hardy): ‘Early Black’, ‘Crimson Queen’, ‘Demoranville’, ‘Mullica Queen’, ‘Howes’ and ‘Stevens’. In both the field- and growth-chamber-acclimated buds, a quadratic trend was observed for LT$_{50}$ vs. date across all cultivars. By early December, the LST range was between -24 to -18°C across cultivars in both acclimation conditions.

**Keywords:** freezing tolerance, frost damage, *Vaccinium macrocarpon*, lowest survival temperature, LT$_{50}$

**Introduction**

Freezing injury is a major abiotic stress to plants native to temperate regions. To survive exposure to low temperatures, such plants must develop freezing tolerance (Pagter and Arora, 2013), defined as the ability to withstand low temperatures without sustaining damage to tissues. Freeze tolerance of perennial temperate zone plants is not consistent throughout the year and winter survival depends mainly on the level of tolerance acquired via cold acclimation before the onset of freezing temperatures (Ehlenfeldt et al., 2012). Cold acclimation is a gradual process that enables plants to survive increasingly lower temperatures until they reach maximum hardiness. The process is genetically controlled and varies among cultivars in the *Vaccinium* genus (Rowland et al., 2013).

In cranberry (*Vaccinium macrocarpon* Ait), floral buds for the following year are initiated in the summer, continue to develop into the fall, and must become cold hardy in order to survive the winter. Although frost protection of the buds in the spring is routinely required and
practiced in commercial cranberry production (DeMoranville, 2008), limited knowledge regarding the progression of cold acclimation in the fall raises questions regarding the need to implement frost protection of the buds in the fall. When cranberry growers implement frost protection in the fall, they do so to protect the ripening fruit and may be incidentally protecting the buds. Early work on the progression of cranberry cold hardiness in the fall (Abdallah and Palta, 1989) was conducted in Wisconsin on 'Searles', a native cultivar no longer widely grown in Wisconsin and not grown in any of the other cranberry production regions. A more recent Wisconsin study (Workmaster and Palta, 2006), of fall cranberry bud hardiness in 'Stevens' showed a progression in tolerance, from \(-10 ^\circ C\) in mid-Sept. and reaching \(-25 ^\circ C\) by early Nov.

Recently, new cultivars and processing requirements have resulted in earlier harvest after which frost protection ceases. It is therefore critical to understand the timing of acclimation and the acquisition of hardiness of cranberry buds in the fall in regions other than Wisconsin and for additional cranberry cultivars to determine the extent that fall protection of the buds may be necessary.

Field-grown cranberry cultivars ‘Ben Lear’, ‘Early Black’, ‘Howes’ and ‘Stevens’ differed in their deacclimation (the loss of hardiness) in the spring based on a comparison of their cold tolerances (hardiness) at specific developmental stages (DeMoranville, 2008). New cranberry cultivars, ‘Crimson Queen’, ‘Demoranville’ and ‘Mullica Queen’ were released by Rutgers University in 2006-2007 (Johnson-Cicalese and Vorsa, 2006; Clark and Finn, 2010) and have been planted on a limited basis on commercial beds in Massachusetts. However, little is
known about their cold hardiness levels, how they compare with native cultivars such as ‘Howes’ or the commonly grown hybrid, ‘Stevens’, or the timetable for acclimation in the fall for any of these cultivars.

Several techniques have been used to study the response of buds to freezing temperatures. These include (i) differential thermal analysis (freezing exotherms) alone or in combination with a 2, 3, 5-triphenyltetrazolium chloride reduction assay (Takeda et al., 1993), (ii) visual assessments (Ehlenfeldt et al., 2006), and (iii) electrolyte leakage (Lee et al., 2013). Outcomes of freezing tests are most commonly presented as LT₅₀, the temperature at which 50% of the subject tissues are damaged. However, previous cranberry studies (Abdallah and Palta, 1989; Workmaster and Palta, 2006) have reported LST, the lowest survival temperature, that temperature at which there is no observable damage but below which damage was observed.

In this study, we hypothesized that, during acclimation in the field in Massachusetts, cranberry cultivars would develop hardiness differentially in response to cold temperatures. The objectives of this research were: (1) to determine the LT₅₀ and the LST during cold acclimation of cranberry buds grown in the field and under a controlled environment and (2) to compare the cold acclimation responses of six cranberry cultivars commonly grown in southeastern Massachusetts.
Materials and Methods

Field sample collection

The experiment was conducted from September (pre-harvest) to December 2014, ending just before the winter flood was applied. Samples were collected from a seven-year-old bog located at the UMASS Cranberry Station (East Wareham, MA; lat. 41°45’N, long. 70°40’W) on 17 and 24 September, 1, 8, 15, 22 and 29 October, 12 and 19 November, 3 and 10 December, 2014. Cranberry cultivars ‘Crimson Queen’, ‘Demoranville’, ‘Early Black’, ‘Howes’, ‘Mullica Queen’, and ‘Stevens’ (Table 8) were included in the study. For each cultivar, the terminal 6-cm of an upright stem, including the terminal bud, was cut from actively growing plants and immediately brought to the laboratory for freeze tests.

Growth chamber experiment

The controlled temperature experiment was conducted in growth chambers using one-year-old potted plants that had been grown and held in a greenhouse on site, with the same six cultivars as in the field sampling. Each pot contained, on average, 6 budded uprights. Thirty-two pots of each cultivar were transferred from the greenhouse to two controlled growth chambers (PGC-10; Percival Scientific, Perry, IA) and sequentially taken through seven day periods at each of four temperature settings (15, 6, 2 and -2°C) to simulate fall acclimation. Other specific conditions in the growth chamber included change in day length (combination of cool-white fluorescent lamps and incandescent light) and light intensity as follows: 15°C: 12-h photoperiod and photosynthetic photon flux density (PPFD) of 500 µmol m⁻² s⁻¹; 6°C: 10-h photoperiod and PPFD of 250 µmol m⁻² s⁻¹; 2°C: 10-h photoperiod and PPFD of 250 µmol m⁻² s⁻¹; and -2°C in
darkness. The settings for the first three temperatures were chosen to mimic conditions in Massachusetts during the fall. The last setting of -2°C was included because previous studies have shown that sub-zero temperatures may be needed to achieve full cold acclimation potential in woody perennials (Arora and Rowland, 2011). Following each 7-day acclimation period, uprights were cut from the potted plants for freeze testing.

**Controlled freezing tests**

Freezing tests were carried out simultaneously on uprights excised from the field-grown and potted plants. While some researchers have evaluated both the whole plant and excised shoots to determine frost tolerance (Rowland et al., 2013), it is also common to examine hardiness using solely individual plant parts (Hoffman et al., 2010; Lisek, 2012). Since ice nucleation is considered crucial for ensuring accuracy of freezing tests (Workmaster et al., 1999), individual uprights from each cultivar (6 uprights total) were wrapped in a moistened cheese cloth and sealed in aluminum foil to make one replicate packet (Fig. 15). There were four replicate packets each of field-grown and growth chamber uprights for each freeze test temperature. Samples were placed inside a programmable freezer (T10RS-1.5; Tenney Environmental, Williamsport, PA) at -2°C overnight to equilibrate prior to freeze tests, modeled on methods developed for blackberry (Bell et al., 1995; Cortell and Strik, 1997). The following morning they were subjected to a step-wise lowering of temperature at a rate of 2°C per hour from -2°C, stopping for 1 hour at each test temperature (-8°C, -12°C, -15°C, -18°C, -24°C, -30°C and -36°C) until the minimum temperature of -36°C had been reached (Bell et al., 1995). At the end of each one-hour hold, four replicate packets were removed and transferred to a cold room...
(4°C) to allow gradual thawing. The following day, the packets were placed in a plastic bag on the laboratory bench (22.5°C) for about 12 hours until they were evaluated (Bell et al., 1995; Cortell and Strik, 1997).

**Bud injury evaluation**

Damage, defined as internal browning of the bud, was determined by dissecting and observing the cranberry buds under a microscope at the completion of the freezing test. Buds were rated and scored based on the extent of damage (0% = green and healthy, 25% = watery and brown bud scales, 50% = browning in 50% of the bud, 75% = browning in 75% of the bud, and 100% = black or dead bud). Control samples were kept in the cold room at 4°C (no exposure to the freezing tests) and were assessed along with the treated samples. The selected temperature range was based on pretests conducted prior to the initiation of experimentation to estimate the temperature that results in bud injury. The LT$_{50}$ values for each cultivar were estimated by fitting the first derivative $-\log((\log(100)-\log(50))/b)/k)$ of the Gompertz function derived from Lim et al. (1998), [$Y= a\exp (-b\exp -kT)$ where: $a$ = upper asymptote, $b$ and $k$ = negative growth rates, $\exp$ = exponential and $T$ = temperature] to the percentage of bud injury versus freeze test temperature and then computing the predicted value from the regression equation, according to Wilson et al., (2014). We made an assumption that at -36°C, all buds would be killed (total browning of buds) and therefore there was no need to estimate parameter $a$, as we designated the upper asymptote to be 100. The constants $b$ and $k$ are derived from statistical analysis using PROC NLIN in SAS. Throughout the study, the assumption was correct as we observed total kill of the buds at -36°C.
In addition to calculating the LT_{50}, we also used visual observation of the buds to determine the LST (lowest survival temperature) of the cranberry buds from the freeze tests (Abdallah and Palta, 1989). LST, in contrast to LT_{50}, determines the economic tolerance of the plant to cold; that is, the temperature threshold above which no tissue damage is observed. For determining the LST, any observation of browning following the freeze test was considered to be lethal (Abdallah and Palta, 1989).

**Statistical analysis**

Data was analyzed in SAS 9.3 (SAS Institute Inc., Cary, NC) using Proc NLIN and bootstrapping methods. The LT_{50} was derived by fitting an individual bud damage replicate to the first derivative of the Gompertz equation (\( Y = ae^{b(eT)} \)). Regression analysis was done on the generated means to determine the trend that best fit the data. Mean separation for cultivars within dates was done using Fisher’s protected least significant difference (LSD) test at \( P \leq 0.05 \), while mean separation for dates within each cultivar was done using orthogonal contrasts.

**Results and Discussion**

This study examined bud acclimation time course in six cranberry cultivars, through controlled freezing tests using detached uprights acclimated in the field or in controlled growth-chamber conditions. Freezing tests have previously been used to screen winter survival potential for different small fruit genotypes (Linden et al., 2002; Ehlenfeldt et al., 2012).
The upland air temperatures at the E. Wareham, MA field site were monitored for the duration of the study (Fig. 16). Weather data showed a gradual decrease of both the average and the average minimum temperature from September to December. The average minimum temperature was 12.5, 9.3, 0.8 and 0.3°C for September, October, November and December, respectively. Temperature ranges were 3.3 to 22.2°C in September, 1.7 to 17.8°C in October, -7.2 to 7.8°C in November and -9.4 to 7.8°C in December (Fig. 16).

**Fitting the Gompertz function**

The Gompertz function is an asymmetric sigmoid function, which was determined to have a better fit when compared with the Richards function in estimating freezing injury in *Rhododendron* (Lim et al., 1998). It had been used successfully to estimate freezing stress resistance in cranberry (Workmaster and Palta, 2006). Using this function, the LT$_{50}$ was derived by fitting bud damage (Y) and temperature (X) to the equation (Y = 100e$^{be^{-kT}}$) (Fig. 17 and 18). Values of LT$_{50}$ obtained using this procedure were only slightly different from those obtained by plotting the raw data. A similar result was observed in the estimation of freezing tolerance in blueberry genotypes and could be explained by the fact that quadratic models tend to fit the data to a symmetric smoothed curve (Ehlenfeldt et al., 2012). The response of three cultivars, ‘Crimson Queen’, ‘Howes’ and ‘Stevens’, under field conditions is illustrated (Fig. 17). As test temperatures decreased, bud damage increased for field-collected samples. The greenhouse-grown plants responded similarly to field-grown plants although the plotted line had a different slope (Fig. 18). Differences in slopes are expected due to different acclimation conditions, temperature and daylength, in the field vs. in the growth chamber. Declining acclimation
temperatures and the corresponding decline in daylength, resulted in hardier buds. Various
cultivar responses to declining test temperature indicated the different freezing tolerance of each
cultivar. Plants acclimated at 15°C were less tolerant than plants acclimated at lower
temperatures and subsequent exposure to -18°C resulted in increased bud damage (range = 75 to
81.3%) compared to 2°C acclimated plants (range = 0 to 25%).

Trend analysis assessment

The change in LT$_{50}$ over time was determined by plotting against day number (DOY) and
evaluating linear and quadratic trends from single degree of freedom comparisons. The trend
analysis showed a significant quadratic trend ($p \leq 0.001$) of LT$_{50}$ with DOY for field samples
(Fig. 19). The fitted equations predicting LT$_{50}$ versus day of year were calculated as $Y=
0.00103x^2 - 0.8144x + 131.8486$ ($r^2=0.95$) for field-grown samples. There was a progression of
freezing tolerance, with increasingly lower temperatures required to cause 50% damage within a
bud, until maximum hardiness was reached in early December.

Early fall season cultivar responses

Values of LT$_{50}$ and LST showed a progression of increasing freezing tolerance for all
cultivars. During the first week of field sampling (mid-September), cranberry cultivars had LT$_{50}$
ranging from -12.9°C in ‘Early Black’ to -10.1°C in ‘Howes’ (Table 9). The initial values for
these field-acclimated samples were higher than those obtained when cranberry uprights were
acclimated at a fixed temperature of 15°C; with a range of -16.1°C in ‘Early Black’ to -12.8°C in
‘Demoranville’ (Table 10). The acquisition of cold hardiness in the field was likely slowed down
by the harvest flood, which was applied on 1 Oct. and removed on 7 Oct. 2014. During that period, the uprights were submerged under 15 to 18°C water. We did not observe any progression in cold hardiness for any cultivar during the flood period (Table 9, compare 1 Oct. to 8 Oct.). In Wisconsin, Workmaster and Pálta (2006) showed a temporary loss of hardiness in 'Stevens' during the harvest flood, while in this study the progression was only arrested. Most of the cultivars recovered by the following week of sampling and continued the development of hardiness except for ‘Crimson Queen’ and ‘Demoranville’ that took longer than the rest of the cultivars to resume their progression. By mid-December, the LT50 range for field samples was between -26.0°C in ‘Howes’ and -27.4°C in ‘Early Black’. All cultivars had reached maximum hardiness by 19 Nov. (Table 9).

The role of temperature and daylength in cold acclimation

Decreasing the growth chamber acclimation temperature from 15°C to 6°C resulted in a sharp decline in LT50 across cultivars, to -24.9, -22.2, -20.1, -19.8, -19.3 and -18.7°C for ‘Early Black’, ‘Crimson Queen’, ‘Stevens’, ‘Mullica Queen’, ‘Howes’ and ‘Demoranville’, respectively (Table 10). However, the lower temperature was paired with a 10-h photoperiod, while the 15°C treatment was combined with a 12-h photoperiod. Little additional hardiness was achieved by further lowering the temperature (Table 10). Reduction in temperature and daylength (compare the 15°, 6°, and 2°C treatments) significantly increased the degree of hardiness achieved but this experimental design cannot separate the temperature and daylength effects. The C-repeat binding factor (CBF) pathway has extensively been described as being the cold regulatory, metabolic pathway, directly responsible for plant cold acclimation (Thomashow, 2010; Lee and
Thomashow, 2012). Its importance and role in increasing freezing tolerance, particularly under short days was highlighted in Arabidopsis (Lee and Thomashow, 2012), thus the role of daylength in our results cannot be discounted.

Based on LT$_{50}$ values obtained in early December (Table 9), the cultivars ranked as follows (in order of decreasing cold hardiness): ‘Early Black’, ‘Demoranville’, ‘Crimson Queen’, ‘Mullica Queen’, ‘Howes’ and ‘Stevens’. Variations in cold tolerance among cultivars had been observed in blueberry (Vaccinium corymbosum) shoots (Lee et al., 2013). Buds and open flowers had LT$_{50}$ values ranging from -24.9°C to -13.7°C in rabbiteye (V. ashei) cultivars (Ehlenfeldt et al., 2006; Rowland et al., 2013). Recent studies on the effects of cold hardiness on blueberry bud survival also showed cultivar differences (Ehlenfeldt and Vinyard, 2015).

**Lowest survival temperatures**

The LST has economic implications for bud protection in cranberry, above this temperature no damage is incurred. LST has been used as a measure of cranberry hardiness in Wisconsin (Workmaster and Palta, 2006; Abdallah and Palta, 1989). In this study, control field samples exposed to 4°C did not incur any damage. This was expected, as there have not been previous reports of damage to any cranberry plant tissues when temperatures were above freezing. However, buds collected on 17 Sep. and exposed to the freezing test temperature of -8°C incurred 100% damage in buds for all cultivars (Table 11). On 24 Sep. the LST range had decreased, as all cultivars could tolerate -8°C without damage but incurred some level of damage at -12°C (Table 11). The acclimation process was gradual and for most of October, the LST did
not drop below -12°C (Table 11). In Wisconsin, ‘Stevens’ was reported to have an LST of -26°C at the beginning of November (Workmaster and Palta, 2006), while ‘Searles’ was reported to have an LST of -24°C (Abdallah and Palta, 1989). This is contrary to our findings, where the LST did not reach -24°C for any cultivar, including ‘Stevens’, by early December. Since all cultivars did not increase hardiness (based on no change in LST) after 3 Dec. in this study, it is likely that the LST values reported here were maintained until deacclimation. The difference in LST may be due to different climatic conditions between Massachusetts and Wisconsin. Growth-chamber-acclimated samples had LST values between -12 and -8°C after one week at 15°C and after acclimation at -2°C showed damage at the -24°C test temperature (Table 12).

The native cultivar ‘Early Black’ has smaller leaves and smaller buds than other cultivars. It was consistently the hardiest cultivar throughout the study, especially compared to ‘Howes’ or ‘Stevens’, which were among the least hardy cultivars. However, the small berries of ‘Early Black’ contribute to its fairly low yield potential. The newer ‘Crimson Queen’, known for its high yield potential and relatively large berries was also among the hardiest cultivars, ranking higher than one of its parents, ‘Stevens’, the current dominant hybrid in Massachusetts production. ‘Demoranville’, another new cultivar, was also among the hardiest cultivars, although its rate of hardiness acquisition was slower than most of the other cultivars.

Cranberry cultivar cold tolerances have been investigated in the spring and have been shown to vary with stage of phenological development and by cultivar within stage. For example, at the cabbage head stage, described as a pronounced swelling marked by displacement
of bud scales (Workmaster and Palta, 1998), ‘Early Black’ and ‘Howes’ had a frost tolerance of -3.9°C while ‘Ben Lear’ had a tolerance of -2.8°C (DeMoranville, 2008). To our knowledge, there are no studies on frost tolerance evaluation of the new cultivars ‘Crimson Queen’, ‘Demoranville’ and ‘Mullica Queen’ in either the spring or fall.

Several studies have been conducted to investigate the hardiness of other Vaccinium species including highbush blueberry (V. corymbosum) hydrids (Lee et al., 2012; Lee et al., 2013; Rowland et al., 2013) and rabbiteye blueberry (V. ashei) (Ehlenfeldt et al., 2006), to inform plant breeding programs. Contemporary cranberry cultivars are not necessarily selected for cold tolerance but rather for other desirable traits such as high yield, anthocyanin production, stolon vigor and fruit rot resistance (McCown and Zeldin, 2003; Johnson-Cicalese and Vorsa, 2006). Although these factors are critical when choosing cultivars for commercial production, it may be necessary to also emphasize cold hardiness. The use of hardier cultivars can potentially reduce losses due to frost damage. The level of hardiness achieved during the fall will likely influence the rate of deacclimation and frost tolerance in spring when cranberry buds are most susceptible to low temperatures.

Based on LT$_{50}$ and LST values and calculated trends, there are differences among cranberry cultivars in the timing of the acquisition of freezing tolerance, i.e. acclimation. This has potential practical implications in determining whether or not frost management to protect buds should be implemented in the fall. Full size berries are susceptible to damage at low temperatures in the fall and cranberry growers implement frost protection when temperatures are
below -2.2 °C in early September and below -3.9 °C later in the month (DeMoranville, 2008). We observed an LST for buds ranging from -8°C to 4°C in mid-September. Since this period coincides with frost protection of berries, buds may be inadvertently protected. However, if growers harvest early to meet processing demands or for early ripening cultivars such as 'Crimson Queen' and 'Demoranville', and then cease frost management, post-harvest bud damage could be incurred prior to the development of full hardiness in November. Monitoring of post-harvest bed temperatures and implementing frost management should be considered when harvesting early.
Literature Cited


Table. 8. Genetic source and origin of six *Vaccinium macrocarpon* cultivars used in frost tolerance experiments.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Parental source</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crimson Queen†</td>
<td>Stevens x Ben Lear</td>
<td>New Jersey</td>
</tr>
<tr>
<td>Demoranville†</td>
<td>Franklin x Ben Lear</td>
<td>New Jersey</td>
</tr>
<tr>
<td>Early Black</td>
<td>native</td>
<td>Massachusetts</td>
</tr>
<tr>
<td>Howes</td>
<td>native</td>
<td>Massachusetts</td>
</tr>
<tr>
<td>Mullica Queen†</td>
<td>LeMunyon x #35</td>
<td>New Jersey</td>
</tr>
<tr>
<td>Stevens†</td>
<td>McFarlin x Potter</td>
<td>Beltsville, Maryland USDA facility</td>
</tr>
</tbody>
</table>

†cranberry hybrid
Table 9. Effects of date on the cold hardiness of six cranberry cultivars growing in the field, in the 2014 fall season, E. Wareham, MA.

<table>
<thead>
<tr>
<th>Date</th>
<th>Cultivars</th>
<th>+CQ</th>
<th>DM</th>
<th>EB</th>
<th>HW</th>
<th>MQ</th>
<th>ST</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+LT$_{50}$ (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 September</td>
<td></td>
<td>-12.1</td>
<td>-10.4</td>
<td>-12.9</td>
<td>-10.1</td>
<td>-10.6</td>
<td>-11.6</td>
<td>1.75</td>
</tr>
<tr>
<td>1 October</td>
<td></td>
<td>-14.5</td>
<td>-14.5</td>
<td>-15.7</td>
<td>-13.1</td>
<td>-13.2</td>
<td>-13.8</td>
<td>1.29</td>
</tr>
<tr>
<td>8 October</td>
<td></td>
<td>-14.7</td>
<td>-14.9</td>
<td>-15.6</td>
<td>-13.6</td>
<td>-14.2</td>
<td>-13.8</td>
<td>0.90</td>
</tr>
<tr>
<td>15 October</td>
<td></td>
<td>-15.6</td>
<td>-16.1</td>
<td>-18.7</td>
<td>-16.7</td>
<td>-15.5</td>
<td>-16.1</td>
<td>1.20</td>
</tr>
<tr>
<td>29 October</td>
<td></td>
<td>-22.3</td>
<td>-20.5</td>
<td>-23.1</td>
<td>-21.7</td>
<td>-19.7</td>
<td>-21.6</td>
<td>2.26</td>
</tr>
<tr>
<td>19 November</td>
<td></td>
<td>-25.5</td>
<td>-25.3</td>
<td>-26.8</td>
<td>-24.5</td>
<td>-24.3</td>
<td>-24.8</td>
<td>1.94</td>
</tr>
<tr>
<td>10 December</td>
<td></td>
<td>-26.1</td>
<td>-26.4</td>
<td>-27.4</td>
<td>-26.0</td>
<td>-25.5</td>
<td>-26.0</td>
<td>1.18</td>
</tr>
</tbody>
</table>

**Selected contrasts**
- 17 Sep vs 1 Oct: * ** * * * *
- 1 Oct vs 8 Oct: NS NS NS NS NS NS NS
- 8 Oct vs 15 Oct: NS NS ** * * *
- 29 Oct vs 19 Nov: * ** ** * ** *
- 19 Nov vs 10 Dec: NS NS NS NS NS NS NS

+‡CQ, Crimson Queen; DM, Demoranville; EB, Early Black; HW, Howes; MQ, Mullica Queen and ST, Stevens

+‡LT$_{50}$: temperature at which 50% damage occurs.

NS, ***, * Nonsignificant, significant at $P \leq 0.01$ and significant at $P \leq 0.05$, respectively.
Table 10. Acclimation temperature effects on the cold hardiness ($LT_{50}$) of six cranberry cultivars acclimated at four temperatures settings (15, 6, 2 and -2°C) in a growth chamber.

<table>
<thead>
<tr>
<th>Acclimation Temperature (°C)</th>
<th>†CQ</th>
<th>DM</th>
<th>EB</th>
<th>HW</th>
<th>MQ</th>
<th>ST</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>-14.8</td>
<td>-12.8</td>
<td>-16.1</td>
<td>-13.6</td>
<td>-13.5</td>
<td>-14.9</td>
<td>1.36</td>
</tr>
<tr>
<td>6</td>
<td>-22.2</td>
<td>-18.7</td>
<td>-24.9</td>
<td>-19.3</td>
<td>-19.8</td>
<td>-20.1</td>
<td>2.02</td>
</tr>
<tr>
<td>2</td>
<td>-22.9</td>
<td>-22.6</td>
<td>-27.2</td>
<td>-21.3</td>
<td>-23.8</td>
<td>-22.0</td>
<td>1.86</td>
</tr>
<tr>
<td>-2</td>
<td>-26.7</td>
<td>-26.2</td>
<td>-28.2</td>
<td>-25.5</td>
<td>-25.8</td>
<td>-25.0</td>
<td>1.18</td>
</tr>
</tbody>
</table>

**Selected contrasts**

15 vs 6 ** ** ** ** ** **
6 vs 2 NS * * * * *
2 vs -2 * * NS * * *

†CQ, Crimson Queen; DM, Demoranville; EB, Early Black; HW, Howes; MQ, Mullica Queen and ST, Stevens

‡$LT_{50}$: temperature at which 50% damage occurs.

NS, **, * Nonsignificant, significant at $P \leq 0.01$ and significant at $P \leq 0.05$, respectively.
Table 11. Visually estimated lowest survival temperature (LST) ranges for cranberry cultivars growing in the field in E. Wareham, Massachusetts. Samples were collected from mid-September through December. Buds were evaluated under a microscope and any observed browning was considered to be damage.

<table>
<thead>
<tr>
<th>Date</th>
<th>LST range</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 September</td>
<td>-8°C ≤ LST ≤ 4°C</td>
</tr>
<tr>
<td>24 September</td>
<td>-12°C ≤ LST ≤ -8°C</td>
</tr>
<tr>
<td>1 October</td>
<td>-12°C ≤ LST ≤ -8°C</td>
</tr>
<tr>
<td>8 October</td>
<td>-12°C ≤ LST ≤ -8°C</td>
</tr>
<tr>
<td>15 October</td>
<td>-12°C ≤ LST ≤ -8°C</td>
</tr>
<tr>
<td>22 October</td>
<td>-12°C ≤ LST ≤ -8°C</td>
</tr>
<tr>
<td>29 October</td>
<td>-15°C ≤ LST ≤ -12°C</td>
</tr>
<tr>
<td>12 November</td>
<td>-18°C ≤ LST ≤ -15°C</td>
</tr>
<tr>
<td>19 November</td>
<td>-18°C ≤ LST ≤ -15°C</td>
</tr>
<tr>
<td>3 December</td>
<td>-24°C ≤ LST ≤ -18°C</td>
</tr>
<tr>
<td>10 December</td>
<td>-24°C ≤ LST ≤ -18°C</td>
</tr>
</tbody>
</table>
Table. 12. Visually estimated lowest survival temperature (LST) ranges for six cranberry cultivars that were exposed to acclimation temperatures; 15, 6, 2 and -2°C. Buds were evaluated under a microscope and any observed browning was considered to be damage.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>15°C</th>
<th>6°C</th>
<th>2°C</th>
<th>-2°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crimson Queen</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-18°C ≤ LST ≤ -15°C</td>
<td>-24°C ≤ LST ≤ -18°C</td>
</tr>
<tr>
<td>Demoranville</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-15°C ≤ LST ≤ -12°C</td>
<td>-24°C ≤ LST ≤ -18°C</td>
</tr>
<tr>
<td>Early Black</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-18°C ≤ LST ≤ -15°C</td>
<td>-24°C ≤ LST ≤ -18°C</td>
<td>-24°C ≤ LST ≤ -18°C</td>
</tr>
<tr>
<td>Howes</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-18°C ≤ LST ≤ -15°C</td>
<td>-24°C ≤ LST ≤ -18°C</td>
</tr>
<tr>
<td>Mullica Queen</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-15°C ≤ LST ≤ -12°C</td>
<td>-24°C ≤ LST ≤ -18°C</td>
<td>-24°C ≤ LST ≤ -18°C</td>
</tr>
<tr>
<td>Stevens</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-12°C ≤ LST ≤ -8°C</td>
<td>-18°C ≤ LST ≤ -15°C</td>
<td>-24°C ≤ LST ≤ -18°C</td>
</tr>
</tbody>
</table>
Fig 15. Six individual cranberry uprights from the cultivars, ‘Crimson Queen’, ‘Demoranville’, ‘Early Black’, ‘Howes’, ‘Mullica Queen’ and ‘Stevens’ were collected in E. Wareham, MA and (A) tagged for identification, (B) wrapped in a moistened cheese cloth and sealed in aluminum foil to make one replicate packet for testing in a controlled temperature freezer.
Fig. 16. Minimum daily temperatures from mid-Sep. to mid-Dec. at the University of Massachusetts Cranberry Station, E. Wareham, Mass. (data archived online at the National Climatic Data Center: NOAA site identifier COOP:192451).
Fig. 17. Percent bud damage ($Y$) in field-collected cranberry buds exposed to freezing temperatures ($T$) fitted to the first derivative of the Gompertz equation; $Y = 100e^{-be^{-kT}}$ for Sep., Oct., Nov. and Dec. The constants $b$ and $k$ are derived from statistical analysis using PROC NLIN in SAS. Samples were collected from a cranberry bed in E. Wareham, Mass. Data for the native cultivar, ‘Howes’, the older hybrid, ‘Stevens’ and the newer hybrid, ‘Crimson Queen’ are shown.
Fig. 18. Percent bud damage (Y) in cranberry buds from 6 cultivars exposed to controlled freezing temperatures (T) following acclimation in a controlled environment (growth chamber) fitted to the first derivative of the Gompertz equation: $Y = 100e^{-be^{-kT}}$. The constants $b$ and $k$ are derived from statistical analysis using PROC NLIN in SAS. Potted cranberry plants were acclimated at 15°C, 6°C, 2°C or -2°C prior to removal of budded uprights that were then used for the freezing tests.
Fig. 19. Freezing tolerance (LT$_{50}$) for six cranberry cultivars. For field-grown samples, data best fit a quadratic trend line ($p \leq 0.001$) with the regression equation: $Y = 0.00103x^2 - 0.8144x + 131.8486$. LT$_{50}$ for growth-chamber-acclimated samples also fit a quadratic trend line ($p \leq 0.001$) with the regression equation: $Y = 0.01849x^2 - 11.5806x + 1787.092$ (data not shown).
SUMMARY

Frost protection remains one of the most critical processes in cranberry production and if implemented correctly, can reduce bud damage and associated costs. Cranberry growers in Massachusetts can confidently adopt the INT (cycling) method of spring frost protection for its ability to protect buds without impacting fruit yield relative to the conventional method and most importantly for its high water and possible energy savings. The ability to have adequate bud protection with less water gives an advantage in benign environmental stewardship. However, it is recommended that set points of the least tolerant cultivar should be used to ensure maximum bud protection across cultivars. Cultivar differences were noted with regards to bud damage susceptibility and yield potential and these are likely inherent. Additional evaluation of set points used in cycling protocols is the logical next step. If initiation set points can be safely set near the hardiness temperature, maximum savings of water and fuel can be achieved. Knowledge of spring deacclimation will be critical to developing such protocols.

Carbohydrate and lipid concentrations synthesized during acclimation varied among cultivars at a series of decreasing acclimation temperatures. Soluble sugars, sucrose, glucose and fructose, and the lipids, digalactosyldiacylglycerol (DGDG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were found at higher concentrations at lower acclimation temperatures and these compounds played a critical role in cold acclimation. However, inclusion of daylength in the acclimation protocol probably confounded the temperature effects in the
controlled growth chamber study. There were no clear cultivar differences; they all seemed to follow the same pattern in their response to acclimation temperature.

A comparison of our assessment of freezing tolerance during acclimation to the data from our studies of carbohydrates and lipids during acclimation showed a direct relationship between the synthesized compounds and levels of tolerance. For example, the ‘Demoranville’ cultivar showed an increase in the membrane stabilizing lipids and ranked among the hardiest cultivars after freeze tests while ‘Stevens’ did not change the concentrations of the major galactolipid, DGDG in response to acclimation temperature and ranked among the least hardy cultivars. Unfortunately, some of the cultivars tested for freezing tolerance were not evaluated for carbohydrate and lipid synthesis so our conclusion that there is a relationship is preliminary. Lipid or carbohydrate concentration cannot be independently used as a measure of freezing tolerance; based on our studies to date, other factors would have to be considered. However, we have established that the new high yielding cultivars are at least as hardy as the commonly planted ‘Stevens’ and have the potential for increasing yield with no increase in risk of frost damage. While we have studied the response of the cultivars during the cold acclimation period, the next step will be to research the deacclimation process (spring) so as to fully understand freezing tolerance and LST of buds, cultivar interactions, and any relationship between acclimation and deacclimation responses among cultivars.

The presence of preseason damage that we observed on the bogs, together with the knowledge that buds are formed in summer for the following season and the observation that full
hardiness is not achieved until at least November, lead us to the conclusion that it may be necessary for the growers to implement frost protection in the fall, with the goal to protect buds. Currently, in Massachusetts growers do protect the fruit (berries) on cold fall nights but once they harvest a bed, they suspend frost management, leaving buds unprotected. In some cases the buds may not have yet acquired the necessary hardiness to withstand low temperatures without damage. Fall hardiness of buds must be considered on beds where fruit is harvested in the early fall.
APPENDIX A

REGRESSION EQUATIONS USED TO FIT THE GOMPERTZ FUNCTION \((Y = ae^{be^{-kT}})\) FOR FREEZE TESTS CONDUCTED ON SIX CRANBERRY CULTIVARS IN THE FALL OF 2014 (FIG. 17). BUD DAMAGE \((Y)\) WAS PLOTTED AGAINST FREEZING TEST TEMPERATURE \((T)\) FOR FIELD-ACCLIMATED BUDS COLLECTED IN SEP., OCT., NOV. AND DEC IN E. WAREHAM, MASS. THE CONSTANTS \(b\) AND \(k\) ARE DERIVED FROM STATISTICAL ANALYSIS USING PROC NLIN IN SAS. WE DESIGNATED THE UPPER ASYMPTOTE \(a\) TO BE 100 AS WE ASSUMED THAT AT -36°C, BUDS WOULD BE KILLED.

<table>
<thead>
<tr>
<th>Date</th>
<th>Cultivar</th>
<th>Equation</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 September 2014</td>
<td>Crimson Queen</td>
<td>(Y = 100e^{13.7e0.2T})</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Demoranville</td>
<td>(Y = 100e^{6e0.2T})</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Early Black</td>
<td>(Y = 100e^{16.9e0.3T})</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Howes</td>
<td>(Y = 100e^{7.3e0.3T})</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Mullica Queen</td>
<td>(Y = 100e^{18.0e0.3T})</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Stevens</td>
<td>(Y = 100e^{10.2e0.2T})</td>
<td>0.99</td>
</tr>
<tr>
<td>15 October 2014</td>
<td>Crimson Queen</td>
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APPENDIX B

REGRESSION EQUATIONS USED TO FIT THE GOMPERTZ FUNCTION \( Y = ae^{bc^kT} \) FOR FREEZE TESTS CONDUCTED ON SIX CRANBERRY CULTIVARS IN THE FALL OF 2014 (FIG. 18). BUD DAMAGE \( Y \) WAS PLOTTED AGAINST FREEZING TEST TEMPERATURE \( T \) FOR PLANTS THAT WERE PREVIOUSLY ACCLIMATED AT 15˚C, 6˚C, 2˚C AND -2˚C. THE CONSTANTS \( b \) AND \( k \) ARE DERIVED FROM STATISTICAL ANALYSIS USING PROC NLIN IN SAS. WE DESIGNATED THE UPPER ASYMPTOTE \( a \) TO BE 100 AS WE ASSUMED THAT AT -36˚C, BUDS WOULD BE KILLED.

<table>
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<th>Acclimation temperature (˚C)</th>
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<th>Equation</th>
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BIBLIOGRAPHY


Smeeton, R. 1964. Late spring frost damage to apple shoots in the nursery. E. Malling Research Station, A47.


