

CHANGES IN THE FREEZING STRESS RESISTANCE OF THE CRANBERRY LEAF,  
FLOWER BUD, AND FRUIT DURING GROWTH AND DEVELOPMENT

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Abstract

Cranberry (*Vaccinium macrocarpon* Ait.) plant is subject to freezing stress during its life cycle. However, the critical frost killing temperatures for the various plant parts are not well established. We investigated the frost resistance of Searles' cranberry leaves, flower buds, and fruits by controlled freezing. Freezing protocol used was: 1°C/h to reach -4°C, 2°C/h to reach -6°C, and then 4°C/h to reach -24°C. Ice nucleation was initiated at -1°C. Injury was assessed by visual observation and ion leakage.

A sharp increase (from -18°C to -2°C) was observed in the lowest survival temperature (LST) for the flower buds as they lost dormancy and started to open, between late April and early May. A sharp decrease in the LST for newly formed flower buds in the last week of October was found and it reached the lowest LST measured of -22°C in early November. Flowers and small fruits were very susceptible to freezing (no hardier than 0°C). The LST of the fruits changed from 0°C to -8°C and from 0°C to -3°C in 1986 and 1987, respectively, as the fruits changed from green to red stage of development.

Overwintering leaves lost their cold hardiness rapidly from late April to early May (from <-24°C to -7°C). Newly formed leaves changed their hardiness level from 0°C (very early in the growing season) to about -7°C in mid-July, to -24°C in mid-October, and then to <-24°C in mid-December.

Abbreviations: FKT, frost killing temperature; LST, lowest survival temperature.

1. Introduction

Cranberry is a temperate zone, perennial, evergreen, trailing plant. It is grown in bogs (low sites) where a great potential of frost hazard is present. "In Wisconsin there is no truly frost-free period...There is no date, even in July, when there has never been a frost at least in one cranberry marsh" (Dana and Klingbeil, 1966). In spite of the obvious frost hazard problem and that is due to the nature of the plant and the growing sites, the documented information about the freezing stress resistance of all the plant parts is lacking.

Our objectives in this report are: (a) To document the changes in

the frost resistance of the different cranberry plant parts throughout the year. (b) To describe a method for the accurate measurement of the freezing stress resistance in excised cranberry plant parts.

## 2. Materials and methods

### 2.1. Plant materials

Upright shoots (containing overwintering leaves, the present year leaves, flower buds, flowers, and/or fruits) of Searles' cranberry (*Vaccinium macrocarpon* Ait.) were collected from the field every two weeks all the year round (except when the plants were covered with ice in Winter). Uprights were cut to 10 cm long segments (containing all the plant parts that were present at the time of sampling) to be used for freezing.

### 2.2. Freezing the plant parts

Unlike the very quick freezing (1°C/min) that was used in the work of Eaton and Mahrt (1977) that does not resemble the natural freezing, we froze the plant parts slowly and ensured ice initiation at -1°C.

Three segments of the plant uprights were placed in a 70 ml test tube and were frozen in (Forma Scientific Model 2325) ethylene glycol freezing bath. The bath temperature was lowered slowly as the following: 1°C/hour from 0°C to -4°C, 2 °C/h from -4°C to -6°C, and then 4°C/h from -6°C to -24°C. Ice nucleation was initiated at -1°C. The controls were kept on ice at 0°C. Three tubes were removed at each designated temperature and placed on ice for slow thawing overnight. The samples then were placed in a refrigerator at 5°C to allow time for developing the freezing injury symptoms (water soaking and browning).

### 2.3. Assays for freezing injury

#### 2.3.1. Leaves

Due to the apparently heavily waxed cuticle, very thick cell walls (Farag and Palta in this issue), and heavily pigmented leaves in the Fall, it was essential to use two complementary methods to assess the freezing damage (Palta et al., 1978). The two methods used were: (a) Visual observations for water soaking and browning of the tissue. (b) Ion leakage (Dexter et al., 1932).

Intact uprights were visually observed for leaf water soaking and browning. The damage was estimated as a percent water soaking. For electrical conductivity (ion leakage), leaves were detached and cut transversely into three pieces, and then vacuum infiltrated in 20 ml of deionized-distilled H<sub>2</sub>O in 70 ml test tubes. The tubes were then placed for 12 hours in a refrigerator at 5°C and then put at room temperature for one hour. Electrical conductivity was measured using (YSI 3) conductance meter as described in Palta and Li (1978).

The frost killing temperature was determined by plotting the freezing temperatures against the % ion leakage taking into account the % water soaking and browning. The steepest increase in ion leakage that corresponded to 50 to 60% water soaking was considered to be the FKT.

#### 2.3.2. Flower buds, flowers, and fruits

The lowest survival temperature (LST), the lowest temperature at which no damage has occurred, was determined for the flower buds, flowers, and fruits. The LST parameter was chosen to avoid any potential crop loss when recommended for a frost protection program.

The damage to the flower buds and flowers was assessed by observing the dissected parts (longitudinal sections) under a dissecting microscope for browning. The first browning symptoms were observed at the basal portion (the connection between the bud and the stem) and the flower primordia in the flower buds. For flowers, the initial damage was mainly observed in the ovary, the ovules, and the filament. Any browning observed was considered to be lethal.

Fruit damage was determined by water soaking and loss of firmness of the fruit. The initial freezing damage was observed in the distal end of the fruit.

### 3. Results

#### 3.1. Flower buds

Consistent in two years, a sharp change was observed in the LST (from  $-18^{\circ}\text{C}$  in mid-April to  $-2^{\circ}\text{C}$  in early to mid-May) as the buds lost their dormancy and changed the color from red to white. Open buds had LST of  $0^{\circ}\text{C}$ .

Newly formed flower buds had a great variability in their LST early in the Fall and that could be due to the differences in their developmental stages (age). That period was followed by a sharp decrease (became hardier) in the LST of the buds in the last week of October of 1986. The LST was  $-22^{\circ}\text{C}$  in early November.

#### 3.2. Flowers and fruits

Opened and closed flowers had a LST of  $0^{\circ}\text{C}$  and that is in agreement with observation of Reader (1979). The LST of the fruits changed from  $0^{\circ}\text{C}$  to  $-8^{\circ}\text{C}$  and from  $0^{\circ}\text{C}$  to  $-3^{\circ}\text{C}$  in 1986 and 1987, respectively, as the fruits changed from small green to final size red stages of development.

#### 3.3. Leaves

Leaves were the hardiest among all the studied plant parts at any given time. Over wintering leaves lost their freezing stress resistance rapidly from late April ( $-24^{\circ}\text{C}$ ) to early May ( $-7^{\circ}\text{C}$ ). Newly formed leaves changed their hardiness level from  $0^{\circ}\text{C}$  (very early in the growing season) to  $-7^{\circ}\text{C}$  in mid-July, to  $-24^{\circ}\text{C}$  in

mid-October, and then to less than  $-24^{\circ}\text{C}$  in Mid- December.

#### 4. Conclusions

There are dramatic seasonal changes in the leaves and flower buds freezing stress resistance. Open buds, flowers, small fruits, and young leaves have no frost tolerance. Fruit frost hardiness can be as low as  $-8^{\circ}\text{C}$ . In general, it appears that the increase in the frost hardiness of the fruits is related to the increase in anthocyanin and the decrease in chlorophyll contents. In a parallel study (unpublished data) we have found that the increase in the freezing stress resistance of the leaves is correlated to the increase in the unsaturation level of the membrane polar lipid fatty acids.

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