THE USE OF THERMOCOUPLES TO EVALUATE LOW TEMPERATURE STRESS TOLERANCE OF FLOWER BUDS OF REDCREST PEACH (PRUNUS PERSICA)

by

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INTRODUCTION

Each winter trees of peach, _Prunus Persica_, are seriously affected by winter injury in Kansas and other parts of the United States. Peaches are susceptible to many types of low temperature injury. The damage to peach flower buds during the winter season has been an important problem in peach crop production. This problem has been given attention in all peach growing regions of Kansas as well as other states.

The peach crop of eastern United States suffered great losses during 1949, 1950, and 1955, due to freezes at blossoming time and after the crop had been set. The areas between Georgia and north Virginia were reported to be seriously affected (32).

During freezing, ice crystals are formed in plant tissues. When water turns to ice, 80 calories of heat per gram of water must be eliminated (4). This energy release causes an influx of heat and a corresponding rise in temperature. This can be measured with thermocouples and recorded with a potentiometer. When the temperature of plant tissues cools, the freezing of water can be plotted. Characteristic freezing curves are observed that indicate the point of ice crystallization. These freezing points are called "isotherms."

The use of thermocouples makes it possible to measure the influx of heat due to ice crystallization in plant tissue at the point where the thermocouple is inserted. If ice crystallization is associated with low temperature injury to plants, then theoretically the temperature at which killing occurs can be measured.

Some methods like the electrical conductivity test, triphenyl tetrazolium chloride test, and visual observation were commonly used by other research workers to determine cold temperature stress tolerance in fruit crops (2, 7, 9, 10, 15, 17, 26, 27, 28, 37, 38). Recently, McLeester, Weiser, and Hall (28) have used thermocouples to determine stress hardiness of woody plant stems.

In this investigation the use of thermocouples to measure low temperature stress tolerance in Redcrest peach flower buds was investigated. The purpose of this study was to find out if chart isotherm can be associated with the killing point of Redcrest peach flower buds. No previous attempt has been made to apply this technique to fruit crops.

REVIEW OF LITERATURE

Nature of Cold Resistance in Plants

General Considerations. Levitt (24) reported that low temperatures injure plant cells by dehydration, impaired metabolism, mechanical injury, and destruction of protoplasm due to ice formation within cells. Injury also occurs when large crystals of ice form in intercellular spaces crushing tissues. Cracks in frozen plant tissues were cited as evidence of this.

Resistance to low temperature injury can be acquired by plants and is a variable factor both among species and plant populations and within the same plant (8, 24, 37). Hardened plants can withstand long exposures to freezing temperatures without injury (8). Resistance to low temperatures is acquired in plants, and the degree of tolerance varies from day to day and throughout the season. Temperature is an important factor influencing plant hardiness (37). It controls the equilibrium of chemical reactions that change the resistance of protoplasm to freezing. Abrupt changes in temperature do not allow plants to harden quickly enough to survive the changing conditions (24).

Moisture is another factor that influences plant hardiness (8, 28). Slightly desicated tissues survived lower temperatures than fully turgid tissues (8). McLeester, Weiser, and Hall (28) determined that the control of water loss by the cell was an important factor of plant hardiness.

Photoperiod is an additional factor related to changes in plant hardiness (44).

Intercellular Ice Formation. Levitt (24) found that ice first forms in the intercellular spaces of plants rather than within the cells. Large masses of ice between cells were more harmful than small ice crystals.

During slow freezing, water moves out of the living portion of cells to the ice crystals in the intercellular spaces (8). Death resulted from a disorganization of the protoplasm since the water for maintenance of structure was depleted. Pure water moved out of the cells to the ice particles and the sap was concentrated. At the killing temperatures, salts may have become toxic to the protoplasm.

Hudson and Idle (21) and Bloch et al. (3) agreed that initial freezing was due to ice crystallization of the free intercellular fluids.

Intracellular Ice Formation. According to Chandler (8) ice forms in the protoplasm and in the vacuole of cells composing plant tissues that lack hardiness. When more than a very small amount of ice forms in the protoplasm, the cell dies. Levitt (24) stated that intracellular ice formation is almost always lethal to the cell.

Winter Injury Problem in Peaches

General Considerations. Winter killing of peach fruit buds is a limiting factor to a profitable peach crop (9, 36, 41, 43). In Kansas low temperatures during winter cause fruit bud killing (36, 43). Winter injury to peach buds is considered a single limiting factor to peach production in Illinois (9). Savage and

Cowart (41) described winter injury in its various forms as one principal factor affecting peach tree longivity in Georgia.

Edgerton (14) reported that temperature characteristics influence the degree of peach bud hardiness. A rapid rate of temperature drop, a lower minimum temperature, an extended period of low temperature, and a rapid rate of temperature increase have been associated with greater flower bud mortality. Proebsting (36) observed that more peach flower bud damage occurs at low humidities than at high humidities.

The flower bud hardiness of a peach cultivar was the result of the interaction between hereditary factors of that cultivar and environmental conditions (36). Mowry (31) found that bud hardiness in peaches was a heritable character. The average bud hardiness of a cultivar during winter was a useful criterion for selecting parents that transmit bud hardiness.

During spring, as peach buds advance in development they become increasingly susceptible to low temperature. Late blooming cultivars were recommended by Blake and Steelman (2) for sites where late spring frosts occur. Oberle (32) developed peach and nectarine cultivars having more hardiness to frost during the blossom season.

Cullinan and Weinberger (10) found no relationship between rate of fertilizer application and degree of hardiness of peach flower buds. According to Edgerton (13), buds from trees that received heavy nitrogen and late cultivation were less winter hardy than buds from trees given moderate nitrogen. Savage and Cowart (41) found that fertilizer should be enough to give normal growth to trees.

Among Different Cultivars. Campbell (5), Cullinan and Weinberger (10), and Knowlton and Dorsey (22) found that the degree of hardiness of peach flower buds, leaf buds, and wood varied from one cultivar to another. The seasonal variations in cold hardiness of peach flower buds also differed among cultivars (9, 37, 39). Chaplin (9) reported that the difference in hardiness among peach cultivars was not consistent. It varied throughout the year and from year to year as environmental factors varied.

Within the Same Cultivars. Seasonal variations in cold hardiness of peach flower buds occur not only among cultivars but also within the same cultivar (9, 13, 39). Mowry (36) found that different frequencies of temperature fluctuations caused difference in dormant peach bud hardiness of the same cultivar. One peach cultivar may be classified as hardy or tender to low temperatures under different situations.

Proebsting (36) and Edgerton (13) studied the variations in bud hardiness within one peach cultivar. The temperature at which injury occurred was highly variable. Prolonged low temperatures, 28 to 30^{o}F or lower, increased hardiness. Duration of the low temperature was more important than intensity.

At Different Stages of Bud Development. Several research workers (10, 22, 30, 32, 38, 45) found that the hardiness of developing peach flower buds varies as the bud grows during the spring. Proebsting and Mills (39) reported that Elberta flower buds lost hardiness rapidly at the pink stage of bud development.

Mowry (30) reported that open flowers were less hardy than unopened buds. Weaver (45) agreed that retarded bud stages were more hardy during bloom. Further, the time of bloom was not associated with blossom hardiness. Oberle (32) found no correlation between the season of ripening of the fruit and hardiness of the flowers. Blake (1) observed that the flower buds on an individual shoot exhibited a wide range of developmental stages. This variability could be sufficient for some buds to escape damage from a particular low temperature period near bloom.

Proebsting (37) found that as bud development progressed, the temperature lethal to buds increased.

At Various Seasonal Conditions. Mowry (36) reported that peach varieties vary in hardiness during mild winters and severe winters.

Cullinan and Weinberger (10) found that as a peach tree enters the dormant season its buds become progressively more resistant to low temperatures. Chaplin (9) and Meader (26) found that the killing point of peach fruit buds fluctuates directly with the temperature changes during the winter months. Proebsting (37) found that the large differences in hardiness of Elberta peach fruit buds occur within one dormant season. Further, the seasonal hardiness of those buds differed from year to year.

The air temperatures during the dormant season affected hardiness of Elberta peach fruit buds at Yakima Valley, Washington (38), but this relationship was not close enough to be of any value.

At Different Locations. Mowry (36) reported that flower bud hardiness was influenced by temperature variability within and between climatic regions at different latitudes. Mowry (29) agreed that dormant flower buds of one peach cultivar differ in hardiness when grown in a northern latitude as compared to those grown in a southern latitude.

Methods of Evaluating Stress Tolerance of Plants

Electrical Conductivity Test. The electrical conductivity test has been used by several workers to evaluate low temperature stress tolerance of plants (7, 11, 12, 15, 17, 18, 23, 46, 47, 48, 49). Filinger and Cardwell (17) studied the winter hardiness of red raspberry canes by reading the electrolytic resistance of plants or plant part. They found this technique to be a rapid method. The apparatus was portable and could be taken to the field to study plants after adverse weather. The plant was not destroyed when determinations were made. Campbell and Ghosheh (7) also used the electrical conductivity method to study hardiness of grapes.

Donoho and Walker (12) found that in Elberta peach the electrical conductance of twigs from cold-injured trees was greater than those from uninjured trees. Lapins (23) studied the cold hardiness of apple seedlings by using the same method. Significant differences were found in hardiness among various seedling progenies.

Emmert and Howlett (15) studied the electrolytic determinations of the resistance of 55 apple varieties to low temperature. They found Garnet crab apple hardier than McIntosh apple in the fall.

TTC (Triphenyl Tetrazolium Chloride) Test. The TTC (triphenyl tetrazolium chloride) test can determine freeze damage to plant tissues (33, 34, 35, 40, 42).

Purcell and Young (40) used the TTC test to determine whether living and dead bark of citrus tree could be distinguished. About four square inches of bark was cut into three pieces. One piece was tested immediately, another was submerged in boiling water for 1 minute before exposure to -20°F for 30 minutes and thawing. Results showed that inside surfaces of untreated (alive) plug developed a uniform red color during incubation. Frozen and boiled (dead) samples developed no color. If the red formazan derivative was produced by the TTC reduction, the tissue was considered to be viable.

Visual Observations. Several workers (2, 5, 6, 9, 10, 26, 37) found visual observations to be a simple and accurate method to measure low temperature stress injury.

Campbell and Lingle (6) visually examined the signs of winter injury in strawberry plants. Dead tissue oxidized quickly and became brown, distinguishing it from living tissue.

Campbell (5) studied the winter hardiness of more than 30 different peach cultivars by visual observations. The injury was rated as light, medium, and severe, depending on the degree of severity of winter injury to twigs, leaf buds, and sapwood. The discoloration of investigated sapwood of all trees ranged from dark walnut to various shades of brown color.

In artificial freezing tests of peach fruit buds Chaplin (9) determined the fruit bud injury by cutting the thawed buds transversely at midpoint with a razor blade. Cullinan and Weinberger (10) reported that the amount of injury and death to tissues could be detected easily by sectioning the bud through the floral axis noting the browning of the injured parts. Microscopic observations were frequently used to determine the degree of injury.

Use of Thermocouples to Measure Stress Tolerances

Lorenzen (25) considered thermocouples relatively accurate for temperature measurements in agricultural research. Esau (16) fully explained the construction and application of thermocouples for temperature recording of tree buds and fruits. He suggested that the completed thermocouples should be placed in a glass tube for protection against accidental damage.

McLeester, Weiser, and Hall (27) studied seasonal variations in freezing curves of stem sections of redosier dogwood, Cornus stolonifera, by the use of thermocouples. Different seasonal curves had different numbers of freezing points. In a similar experiment (28) the presence or absence of two freezing points in the second freezing cycle was used as a criterion for establishing viability.

Graham and Mullin (20) found three freezing points in the freeze curve of deciduous Azalea stems. The third freezing point, occurring at -37^{o} to 42^{o}C in midwinter, was the killing point. They completely agreed upon the killing point shown by the freezing curve (19). They further stated that the thermocouple technique saved time and material. They described it as a simple and accurate method. Esau (16) stated that the main advantage of using the thermocouple is its ability to measure actual temperature inside the tissue of a plant.

MATERIALS AND METHODS

The plant materials collected for this study during the winter of 1969-70 were Redcrest peaches grown at the Horticulture Farm of Kansas State University, Manhattan, Kansas. The trees were four years old and in good vigor. Healthy branches were selected at random throughout the dormant season and until the swollen stage of bud development. The branches were cut and tied in a bundle, placed in a moist plastic bag to keep them fresh, and kept in the laboratory in a refrigerator until used. Before freezing the branches were cut into pieces 4 to 5 inches in length. Each twig had several flower buds on it.

The thermocouples were made in the laboratory, using a fine gauge copper and constantan wires. They were constructed with a long pointed tip as illustrated in Fig. 1.

For temperature recording a Honeywell Model Electronik 16 multipoint strip chart recording potentiometer was used (Fig. 2). The chart range of temperature was $-50^{\circ}F$ to $+100^{\circ}F$. A chart speed of 8 inches/hour was used.

To insert thermocouples a cut was made with a razor blade to remove the tip of the fruit bud, and the pointed end of the thermocouple was inserted in the bud (Fig. 3). The twig and thermocouple wire were secured tightly with electrician's tape. The electrician's tape held the thermocouple point in the bud tip and could be easily removed after freezing.

For most of the freezing tests six thermocouples were used,

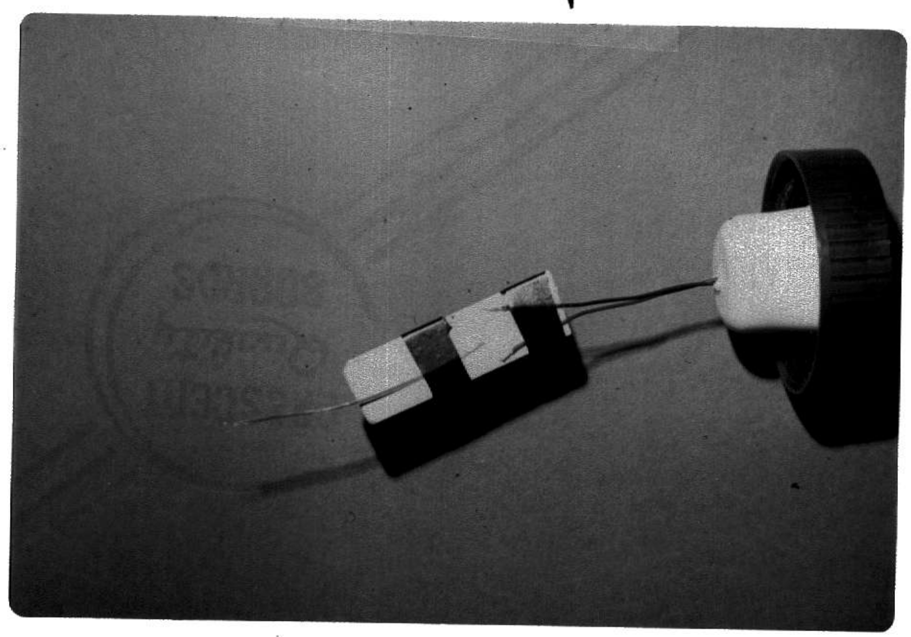

Fig. 1. A thermocouple showing pointed end ready to be inserted in the bud.

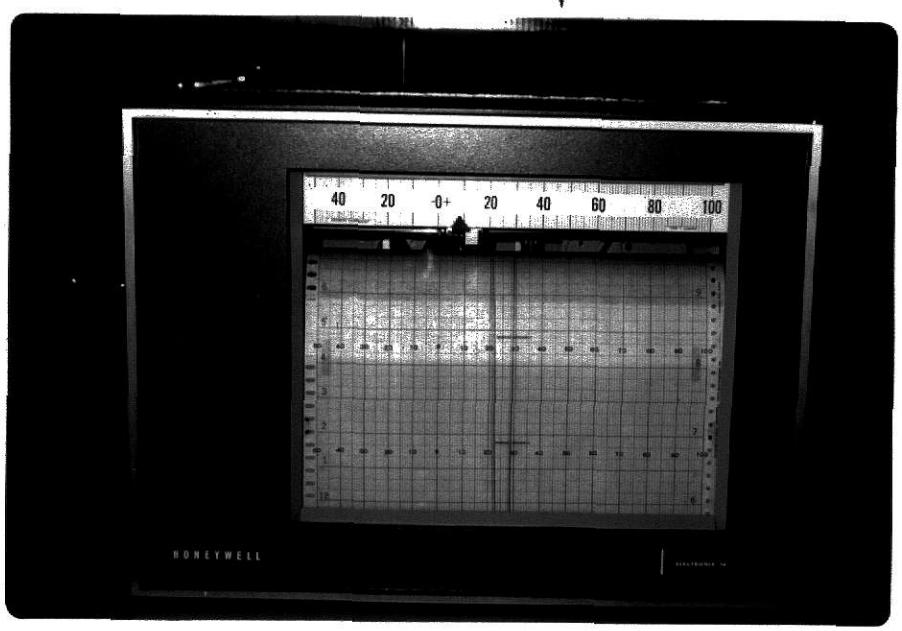

Fig. 2. Multipoint strip chart recording potentiometer which records the freezing temperatures of buds by means of thermocouples.

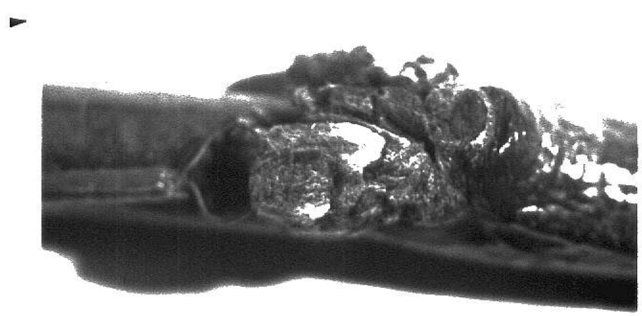

Fig. 3. Thermocouple after inserting in the flower bud tip.

unless any thermocouple failed to perform by getting broken or by being misplaced.

In each thermos bottle there was one thermocouple. In initial experiments each thermos bottle contained four twigs bearing buds, including that twig in which the thermocouple was inserted in a fruit bud. In later experiments thermos bottles contained one twig since only one bud was studied to obtain more accurate results on freeze injury relative to recorded temperatures.

The place of junction of the thermocouple and bud tip was kept uncovered to determine whether the thermocouple remained in the bud throughout the freezing procedure (Fig. 3). A small hole was made in the lid of each thermos bottle stopper to allow the thermocouple wires to pass through as shown in Figs. 1 and 4.

The temperature in the thermos bottle was lowered at a rate between 9 and 27°F/hour, until experimented temperature had been reached. Following removal from the freezer, these thermos bottles were transferred to a refrigerator at 33°F for 12 hours for slow thawing of tissues. The tissues were removed from the thermos bottles and kept for 24 hours in a sealed chamber containing water for complete thawing under humid conditions at room temperature (28, 39).

Upon removal from the humid chamber, the buds were sectioned and examined for evidence of injury. Visual observations were made with the help of a 2" x 4" magnifying lens. The injury rating was done on the basis of brown color of apex

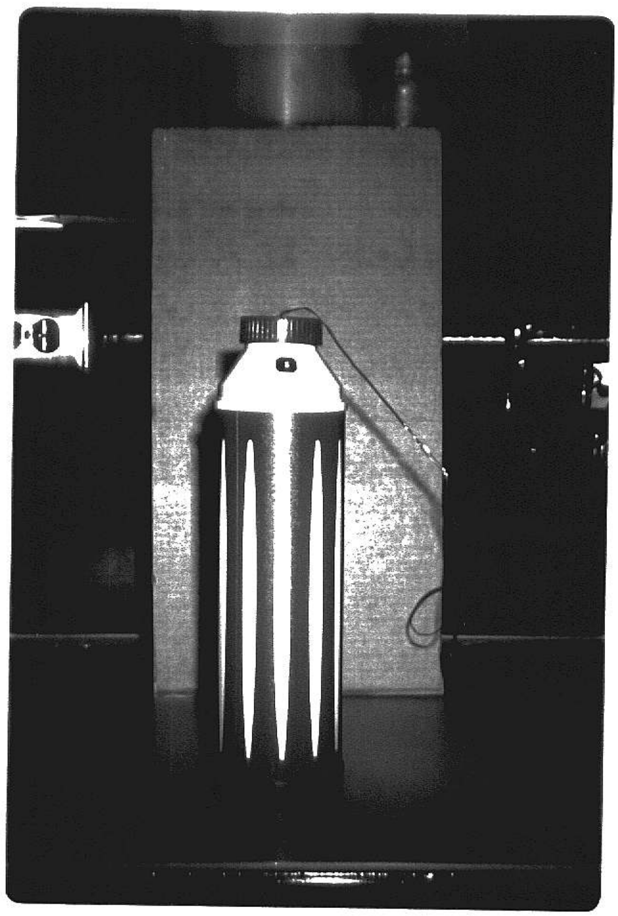

Fig. 4. Thermos bottle ready to put in freezer after having thermocouple and bud in it.

and pistil in dormant and swollen buds, respectively. All buds were classified as alive, 1; damaged, 2; or dead, 3. The data were presented either as percentages or as individual bud observation.

Each potentiometer chart was labeled according to the number and date of freeze test conducted. Also plant materials were kept in bundles and labeled according to thermocouple number and date of freeze test conducted.

The data taken from charts were: temperature at which each isotherm occurred in ^{o}F; height (rise in temperature) of each isotherm in ^{o}F; distance isotherms traveled in inches until reached at their original temperatures; maximum and minimum temperatures in ^{o}F recorded by each thermocouple on the chart; the whole chart distance traveled by each thermocouple in inches.

The returning time to reach at original temperature of each isotherm was calculated by the following formula:

$$\frac{\text{Distance thermocouple traveled in inches x 60}}{\text{Chart speed (8"/hr)}}$$

= Returning time in minutes

The rate of temperature drop for each thermocouple during freeze test was calculated as follows:

$$\frac{\text{(Max. temp. }^{o}\text{F - min. temp. }^{o}\text{F) x chart speed (8"/hr)}}{\text{Distance thermocouple traveled in inches}}$$

= Rate of temperature drop in degree/hr.

RESULTS

Characteristics of Freeze Curves of Peach Flower Buds

In Fig. 5 the typical freeze curve of dormant Redcrest peach flower bud is represented (green). Isotherm I occurred at 20°F. The plateau of isotherm I is shown. Isotherm II occurred at 1°F. No additional characteristics of the curve are important.

Table 1 describes the important characteristics of freeze curves as measured by thermocouples inserted into dormant flower buds during controlled freezing tests. The average rate of temperature drop was 12.1°F per hour. Isotherm I occurred at about the same temperature, 16°F. The average height of isotherm I, which is the rise in temperature after influx of heat, was 3.9°F. The average returning time of isotherm I, which is the time taken to reach the original temperature where isotherm occurred, was 11.9 minutes and varied. Isotherm II occurred at -2.3°F and varied. The average height of isotherm II was 2.8°F. The returning time of isotherm II was 1.8 minutes and was constant.

Variation of Freeze Curves

Average and range of temperatures in °F where isotherm I and isotherm II occurred in dormant flower buds are presented in Table 2. No temperature range indicates that either none of the six thermocouples went through the isotherms, only one of