THE RESPONSE OF APPLES TO EVAPORATIVE COOLING FOR BLOOM DELAY AND THE EFFECT OF LEACHING ON THE HARDINESS OF APPLE AND PEACH BLOSSOMS

By

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CHAPTER I

INTRODUCTION

In much of the fruit growing regions considerable fruit bud development occurs before the last frost in the spring. Freezing temperatures can injure or kill flowers and young fruit. The damage may result in reduced yields or in severe cases, total crop losses.

Growers use a variety of methods to protect the orchard from freezes when the buds are susceptible to low temperature injury. Primary methods of protection include prudent site selection and careful cultivar evaluation. Use of these methods does not insure protection from frost injury. When the danger of injury from frost is high, more active methods of protection may be practiced. Wind machines or helicopters are used to mix cold air on the floor and in the canopy of the orchard with warm air above. Orchard heaters are activated in the event of a freeze to warm the orchard. Over-tree sprinkling when danger of a frost is predicted requires careful monitoring of weather conditions while the system is running. Any system used is generally hard to manage, costly and not always effective. Evaporative cooling is a method of frost protection which attempts to prevent blooming until after the danger of a frost is past. This delay is achieved by sprinkling the tree with water during the dormant season after the rest period has been completed. Evaporation of the water cools the tree and delays its development. Evaporative cooling has been reported to delay the bloom of apple, pear, peach, apricot, sour cherry and nectarine (1,2,3,6,8,10,42). Delays in bloom have been accompanied by reduced fruit set (9), loss of flower bud viability (3,10) and changes in fruit size (9). Bauer et. al (3) suggest the water in some way weakens the flower bud. Work by Hewett (20) concluded that the increased water content of a flower bud decreases its hardiness.

The objectives of this study are:

1. Determine the effectiveness of evaporative cooling under Oklahoma conditions.
2. Evaluate an antitranspirant as a protective coating to prevent detrimental effects of evaporative cooling.
3. Examine the effect of evaporative cooling on flower bud hardiness.
4. Study the effect of water on flower bud hardiness.

CHAPTER II

REVIEW OF LITERATURE

Evaporative Cooling

In 1973, Alfaro et. al (1) tested evaporative cooling as a method of spring frost protection. Dormant trees which had completed their chilling requirement were sprinkled with water whenever the air temperature exceeded a base temperature of 5.56^{o}C. As the water evaporated from the surface of the tree, the energy lost as heat of vaporization cooled the tree (36). According to Alfaro et. al (1) the amount of cooling depends on: (1) the temperature of the surface of the tree; (2) the difference in vapor pressure between the surface of the bud and the air; and (3) the rate at which the evaporated water is removed from the boundary layer surrounding the bud.

In order to achieve the maximum amount of cooling with the least amount of water, the surface of the tree should almost completely dry before rewetting. In comparing sprinkler systems and mist systems, Wolfe et. al (9) reported mist systems use less water and provide greater cooling than sprinkler systems. Chesness et. al (8) found very little cooling early in the morning or late at night. Their system was designed to operate during daylight hours in order to reduce water application. Engineering aspects of evaporative cooling are concerned with minimizing the amount of water applied, evaluating nozzles for uniform coverage (1) and decreasing the energy consumption (35). Robertson and Stang (39) determined evaporative cooling would be economically feasible in all areas of Ohio.

Evaporative cooling has been found to delay the time of flowering for apple, 9 to 7 days (1,2,39,42,49), 6 to 12 days for pear (9,10), 10 to 15 days for peach (3,6,8), 15 days for sweet cherry (1) and 10 days for nectarine (6).

Generally, a delay in flowering results in a similar but shorter delay in harvest maturity. In one study (42) apples delayed 6 days at bloom were delayed only one day at harvest. But, bloom delays of 17 days have been reported to be accompanied by one week delays at harvest (1).

Although evaporative cooling during the dormant season can delay the bloom time, many other tree responses are also altered. Collins et. al (10) reported increased fruit set and increased number of seed per fruit with pear. Stang et. al (42) found decreased fruit set in 'Golden Delicious' apples and substantial tree losses. Increased fruit set in peaches from evaporative cooling was reported by Chesness et. al (8). The application of water seems to have adverse effects on flower bud viability. Sprinkling for bloom delay led to excessive abscission of dormant peach flower buds (3,6).

Reduction of yields have been observed (3,6,10,42). The reduction result from both loss of viable buds and/or poor fruit set. Both increases and decreases in fruit size have been reported from evaporative cooling (6,10).

Hardiness

Two stages of dormancy normally occur in temperate zone woody perennials. The first is true dormancy, or rest, which occurs in the fall after leaf abortion. During this period, the treès will not grow when placed in a normally favorable environment. Growth does not occur until chilling temperatures above freezing have accumulated in sufficient quantity. The amount of chilling required depends on the species and cultivar. The second stage of dormancy is called forced dormancy or quiescence. During this stage the tree is capable of normal growth, however, environmental conditions prevent the development of the plant.

Hardiness of a dormant plant can fluctuate during both rest and quiescence. Proebsting (37) proposed a three stage hardiness model for fruit trees. The first period of hardiness is achieved late in the fall. While the tree is in rest, hardiness may increase but it will not deharden above a minimum level, usually around -21°C. The second stage begins after the termination of rest. In response to warmer temperatures the plant may deharden to a point above the stage 1 minimum. In the same fashion, cooler temperatures can result in the rehardening but not to a point below the stage 1 minimum. The third stage occurs around flowering time and post bloom. Very little hardiness can be expected at this time. During dormancy, resistance to frost is strongly affected by the temperature preceding the frost (4,36). Temperatures resulting in deharding work much faster than do cool temperatures in increasing hardiness. Proebsting found resistance to cold can also be affected by elevation, soil type and cultural practices (37).

Richardson et. al (38) developed a model for determining the end of rest. The model is based on the accumulation of chill units (CU). In the model, one CU equals one hour of exposure at 6°C. The contribution to chilling decreases as temperatures rise above or drop below the optimum. Negative contributions occur at temperatures above $15^{o}C$ and below $0^{o}C$. A method of calculating hourly temperatures from maximum and minimum temperatures is utilized to determine CU.

Many factors are correlated with hardiness although not always in a cause and effect relationship. Zlobina (50) found hardiness was associated with accumulation of RNA, phospholipids and proteins in cherry bark. Seasonal fluctuations in the level of growth promoters and growth inhibitors are often correlated with the state of dormancy of the tree (11,16,17,18,19,26). Concentrations of growth promoters have been found to decrease as trees become dormant and increase again as bud activity begins (17). High levels of growth promoters do not, however, decrease in response to cold temperatures. The level of growth inhibitors generally build up beginning at leaf fall, reach a maximum near the end of rest and then begin to decrease (11,18,19). Hendershott et. al (19) found an increase in growth inhibitors again just prior to bud break.

Lasheen et. al (28) compared the biochemical make-up of peach cultivars differing in hardiness. He found that reducing and total sugars from flower buds were relatively constant during the winter and peaked in concentration in the spring. The least hardy cultivar consistently showed less total sugars throughout the season. El-Mansy and Walker (17) found the level of amino acids and simple sugars increased after the completion of rest. Exogenously applied growth regulators may also affect hardiness. Sprays of abscisic acid increased the hardiness of seedling apples (21). Hardiness was increased in citrus by sprays of maleic hydrazide (43).

Increases in hardiness are often accompanied by changes in the nitrogen constituents of the plant. Total N was found to increase strongly at the beginning of flowering when the plants are least hardy (27). Lasheen (28) reported a tender peach cultivar showed higher amino acid levels than hardier cultivars. The killing point of apple seedlings treated with ABA has been correlated with amino acids and water soluble protein content (21,22). However, Pieniazek et. al (33) reported no relationship between hardiness enhanced by ABA treatment and the water soluble protein content. Siminovitch (40) concluded that of several N fractions, only the water soluble protein fraction increased along with increased hardiness. In black locust bark, treatments that increase hardiness increase water soluble protein (5). The same relationship has been reported for American arborvitae (12), winter rape (24), American dogwood (30 and bermudagrass (14). Water soluble proteins may act as cryoprotectants (25,46).

Water Relations and Hardiness

The hardiness of a plant has been reported to be inversely related to its moisture content (20,23,29). Levitt (29) discusses the hardiness of plants as a drying process. Work by Hewett (20) led to the conclusion that wet buds of peach, apricot, grape and apple were more susceptible to freezing injury than dry buds. Cain and Anderson (7) found "Siberian C" peach, noted for cold hardiness, to have a naturally lower water content than 'Redhaven'. However, they concluded superior hardiness was not entirely due to the lower moisture content. The moisture content of peach flower buds was negatively correlated with hardiness in a study by Johnston (23). Strasbough (44) examined moisture relations in three cultivars of plum differing in hardiness. He found semi-hardy cultivars fluctuate in moisture content in response to temperature changes. The moisture content of the hardiest cultivar remained constant through dormancy. In addition the hardy cultivar had the highest moisture content.

Morgan et. al (32) found that amino acids, organic acids, sugars and polysaccarides can be leached from plant foliage by the action of rain or mist. The leaching of substances from plants has been reviewed (45). Plant parts other than foliage are susceptible to losses from leaching. Stems and branches of woody plants are subject to losses by leaching during both the dormant and growing seasons (13,31).

CHAPTER III

MATERIALS AND METHODS

Evaporative Cooling

The study was conducted at the Fruit Tree Research Station near Perkins, Oklahoma. A block of mature 'Delicious' apple (Malus domestica Borkh.) trees on seedling rootstock were selected as the experimental site. Early in January of 1980, the trees were pruned and topped to height of approximately three meters. A Rainbird mist nozzle (2400 FLT) with a flat fan spray patern was selected on the basis of water distribution tests conducted by the Agricultural Engineering Department of Oklahoma State University. Nozzles to be tested were placed on a riser in the center of a 41 by 41 meter area. Collection cans were placed every meter over the entire area. Weather conditions and water pressure were recorded. At the end of a 3 hour test period water in the cans was collected and measured. Water distribution maps were drawn from the data (Figure 1). The nozzle discharged 11 liters per minute at a pressure of 275.76 KPa. Nozzles were mounted on the top of three meter risers. The risers were placed upright and anchored near the center of each tree.

Evaporative cooling is most efficient when the surface of the tree almost completely dries before rewetting (1). Visual observation of the system operating in the field showed a 30 second on and 4.5 minute off cycle was the most efficient. A thermostat controlled time clock turned

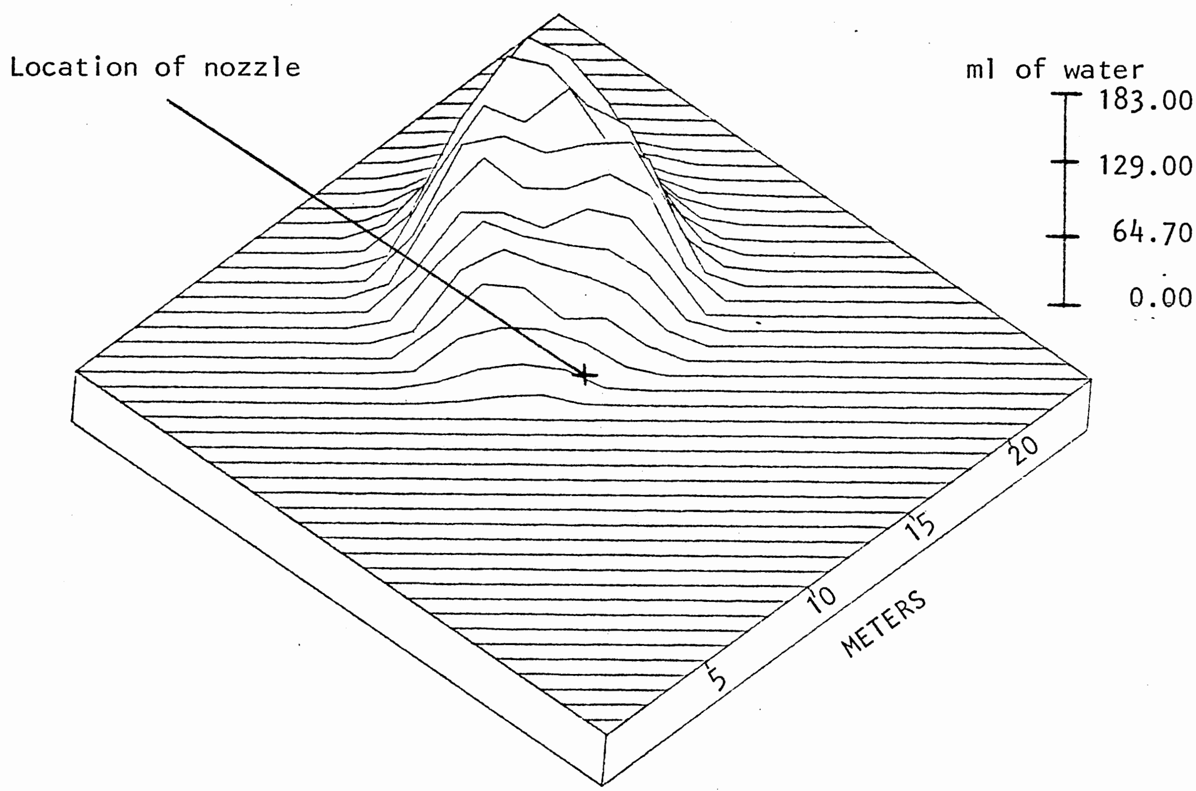

Figure 1. Water Distribution Map for Rainbird 2400 FLT Mist Nozzle.
Wind Speed: 3.33 kph; Humidity: 68.5%

the system on whenever the ambient air temperature exceded 4.5°C. A 24 hour time clock turned the entire system on at 8:00 am and off at 8:00 pm. These hours are similar to those of other workers (8).

Wilt-pruf[1], a commercially available antitranspirant, was used in an attempt to protect the trees against the adverse effects of water while still retaining the cooling effect. The antitranspirant was applied with a handgun sprayer to the point of runoff on February 20, 1980, one day prior to activation of the mist system. Rates were 0, 10 and 20% Wilt-pruf.

A split plot design was arranged over two rows of trees with mist treatments as main plots and antitranspirant treatments as subplots. Each tree was divided into east and west quadrants to provide two experimental units per tree for subplots.

Mist cooling began February 21, 1980 and terminated April 7, 1980. According to the Utah Model (38) the trees had accumulated 987 chill units (CU) when misting began. Approximately 29,000 liters/hectare of water was applied to the trees during evaporative cooling.

Fruit Bud Hardiness

During the spring, fruit buds from misted and non-misted apple trees were collected to determine hardiness. Since misted trees were delayed in development, fruit buds from misted trees were sampled when their stage of development matched that of previously sampled non-misted trees.

Fruit buds were collected in the field, placed in plastic bags and stored at 4°C for no more than 24 hours prior to the freezing tests.

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[1] Nursery Specialty Products, Division of J.A. Hartman Corp., Greenwich, CT

A chest type freezer was modified for use as a freezing chamber with a temperature drop of approximately 1.8°C per hour. A small fan was placed inside the freezer to avoid temperature differences due to air stratification. Fruit buds were separated into bundles and placed inside the freezer on an elevated wire rack. Temperatures for removal of the buds were chosen on the basis of critical temperature charts (48). When the chamber reached a preselected temperature, buds were removed and placed inside chilled thermos bottles. The thermos bottles were stored at 4°C for at least 24 hours to allow for browning of injured tissue. Buds were then dissected and recorded as dead or alive. Since hardiness curves are generally sigmoidal in nature probit analysis was used to determine the lethal temperature required to kill 50% of the buds (LT_{50}) (34).

Bloom Delay and Fruiting Factors

Prior to anthesis, limbs were selected on each experimental unit and tagged. As the trees began to bloom the flowers on the tagged part of the limb were counted daily until 80% of the flowers were open sufficiently for pollination. This was considered full bloom. The same portion of a limb was recounted again after fertilization to determine the fruit set. The number of fruit remaining after the June drop period were counted for percent June drop. The number of seed per fruit was determined at harvest by dissecting 40 fruit per experimental unit.

Water Soluble Protein Content

Extraction of water soluble protein (WSP) followed the procedure of Davis and Gilbert (14) with slight modifications. Lyophilized apple fruit buds were ground in a Wiley mill to pass a 20 mesh screen. An 0.25 g sample was homogenized in 15 ml of cold extracting solution (0.1 M tris-glycine buffer and 0.06 M cystiene adjusted to pH 8.0 with 0.1 N NaOH) for 30 seconds. An additional 5 ml of solution was used to wash down the sides of the test tube. The homogenate was shaken for 30 minutes and filtered through Whatman #1 filter paper. The filtrate was stored at 4°C. Protein content was determined by the coomassie blue procedure. Coomassie blue dye reagent[2] was diluted 1:5 and 5 ml was added to 0.1 ml of filtrate. Absorbance was read at 595 nm on a Bausch and Lomb Spectrophotometer 20.

Additional laboratory studies were conducted to further examine the effect of water on fruit bud hardiness. Two separate experiments were conducted on fruit buds of mature peach and apple buds not included in the evaporative cooling study.

1. Flower buds were excised from trees in the field. The buds were divided into two groups. One group received a 6 hour leaching treatment in tap water at room temperature. At the end of the leaching, both leached and non-leached buds were placed in a humidity box. To achieve 100% relative humidity, several containers of water were placed in the bottom of a glass aquarium which was then covered with plastic film. The buds were set on wire racks in the bottom of the box. Buds were incubated in the himidity box at 4°C until visual observation indicated both groups were nearly equal in moisture content. A sample of 20 buds from each group was removed and fresh weights recorded. After drying at 90°C for 48 hours, dry weights were recorded and percent moisture calculated. Hardiness was determined on the remaining buds as described

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[2]Bio Rad Laboratories, Richmond, CA.

earlier. 'Delicious' apple buds were tested at the half-inch green stage. 'Velvet' peaches were tested at red calyx and 'Redskin' peaches at first pink.

2. Excised buds were leached with tap water at room temperature for 16 hours. After leaching buds were air dried for 0, 4, 8, or 16 hours prior to hardiness tests. A non-leached control was included in the freezing tests. Drying times were synchronized so drying periods ended at the same time in order that freezing tests could begin on all samples at the same time. 'Jonared' apple buds were tested at half-inch green and 'Redskin' peach buds tested at first pink.

CHAPTER IV

RESULTS AND DISCUSSION

Bloom Delay

Evaporative cooling delayed the time of full bloom of apples appoximately three days (Figure 2). The height of each bar represents the time of 80% full bloom for each treatment using the date the first tree in the study reached full bloom as day zero. Antitranspirants applied at 10 and 20% concentrations did not affect the time of bloom on either misted or non-misted trees.

Delays in bloom for apple of 8 to 17 days have been reported (1,2, 39). Therefore a greater delay in flowering was anticipated than the three day delay reported in this study. Earlier in the season a greater difference existed between the development of misted and non-misted trees. At greentip through first pink stages of development, the misted trees were approximately seven days behind the non-misted trees. Temperatures during the blooming period of the non-misted trees were relatively cool, averaging 18.3^{o}C during the day and 3.3^{o}C at night. During the same developmental stages of the misted trees both day and night temperatures were elevated, 26.2^{o}C and 7.2^{o}C, respectively. Since metabolic processes are very temperature dependent, the increased temperatures favored a rapid development of the misted trees. This may account for a part of the short delay in flowering.

According to the Utah model for chill unit accumulation the trees

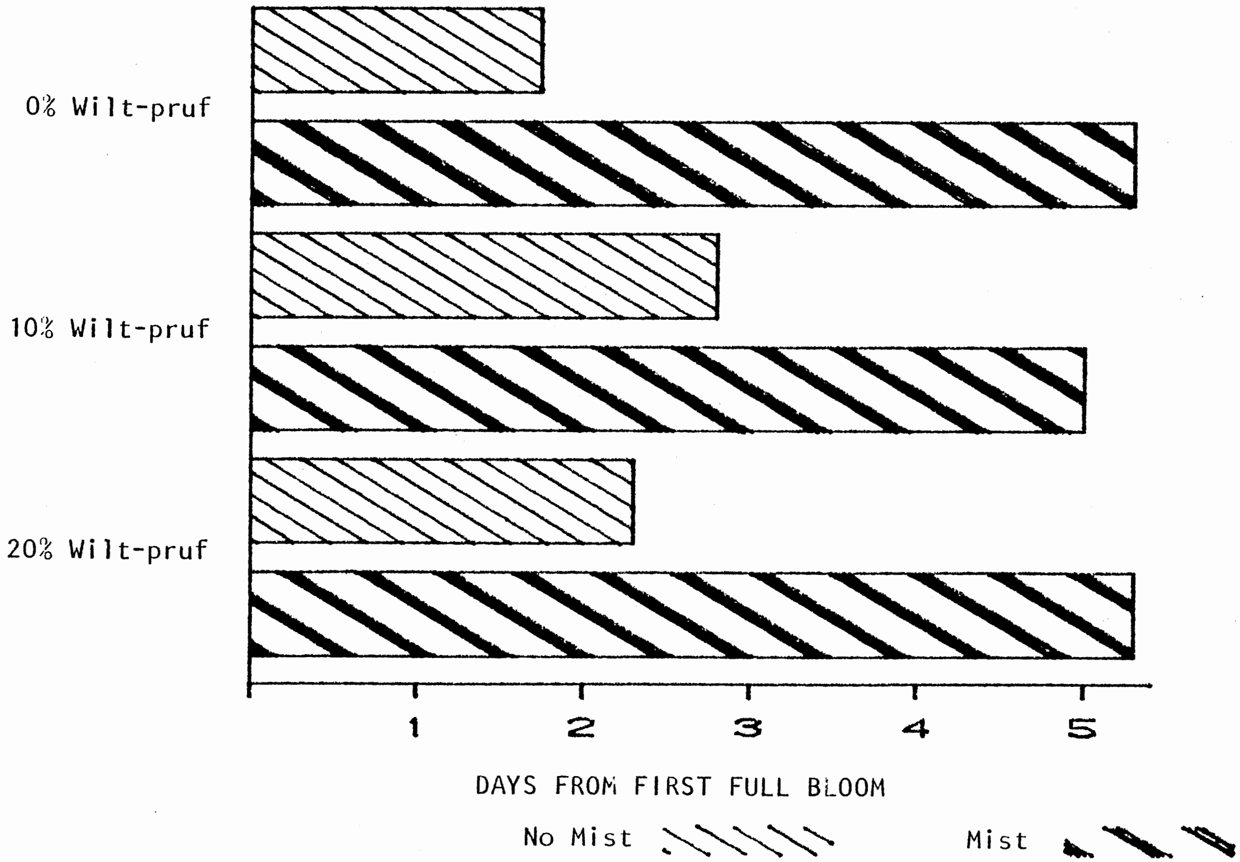

Figure 2. The Interaction of Evaporative Cooling and an Antitranspirant for Bloom Delay of 'Delicious' Apples

had not received the total 1200 chill units required for the cultivar 'Delicious' (38). Ideally, evaporative cooling should begin as soon as rest is completed. The effect of cooling before the chilling requirement has been met is unknown. However, it does not seem very probable that early cooling would reduce the amount of bloom delay.

Flower Bud Hardiness

Delaying the development of apple flower buds did not increase hardiness of the buds. Misted and non-misted buds on the same day did not differ significantly in hardiness even though the stages of development were different (Figure 3). The antitranspirant treatments did not affect the hardiness of misted or non-misted trees (data not shown). Therefore, the horizontal bars in figure 3 represent averages for main plots. When the hardiness of misted and non-misted trees were compared at the same developmental stage, the misted trees were significantly less hardy.

The lethal temperature (LT) required to kill 10, 50, or 90% of the flower buds is well documented for apple (48). As flower buds advance in development in the spring, the buds are killed at progressively higher temperatures. The degree of hardiness exhibited for a particular stage of development at a specific time depends in part on the environmental conditions just prior to that time (4,36,37). With adequate coverage from the mist system, the trees should have been cooled near the wet bulb temperature (49). In theory trees delayed in their development should retain most of the hardiness associated with each stage of development. From Figure 3 it can be seen that this was not observed in this study. Misted trees reached the same phenological stages as non-misted trees

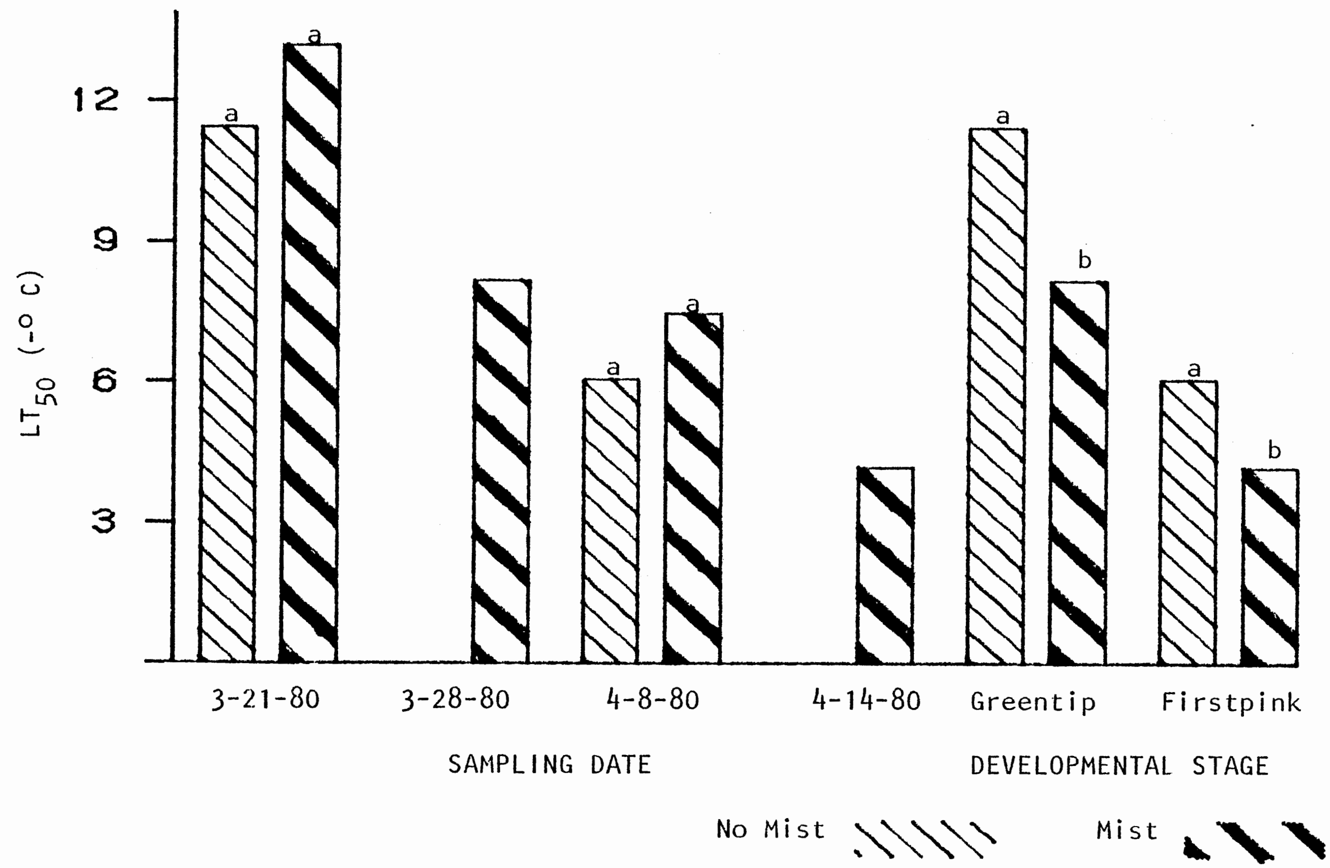

Figure 3. The Effect of Evaporative Cooling on 'Delicious' Apple Flower Bud Hardiness. Treatments within the same date or stage with the same letter are not significant at the 5% level by Fishers F-test

but under warmer temperatures. Cooling from the mist system should have negated some of the effects of warmer environmental conditions. Development under warmer conditions would account for some of the loss of hardiness, particularly in the later stages of development when the tree is very active in its growth. However, freezing tests revealed hardiness was lost with misting even in the earlier stages of development.

Fruit Responses

Trees which were delayed in development by mist cooling set less fruit than non-misted trees (Table 1). A decrease in fruit set has been reported by Collins et. al (9). The decrease probably was not due to pollination problems since misted trees set more seed per fruit. The percent of fruit dropping in the June drop period was not affected by mist cooling. The number of seed per fruit increased with mist cooling. During the pollination period of the delayed trees, temperatures were warmer and bee activity was increased. Warmer temperatures may also have favored pollen tube growth (48).

Water Soluble Protein

Evaporative cooling neither increased or decreased the amount of water soluble protein (WSP) of misted and non-misted flower buds sampled on the same day (Figure 4). The WSP content did not differ when phenological stages of development were compared. A general trend of increasing WSP content as the developmental stage advances can be seen from figure 3. Although increased total WSP and increased hardiness are correlated in some plants (5,12,14), the relationship does not seem to exist in apple (33). Some workers have reported difficulty in the

TABLE I

THE EFFECT OF MIST COOLING ON FRUIT SET, JUNE DROPS, AND NUMBER OF SEEDS PER FRUIT OF 'DELICIOUS' APPLES

Treatment	% Fruit Set	% June Drop	Number of Seed/Fruit
Mist	31.0	45.7	6.55
No Mist	41.6	49.5	5.59
Significance	*[z]	NS	**

[z]Non-significant (NS), significant at 5% (*), significant at 1% (**).

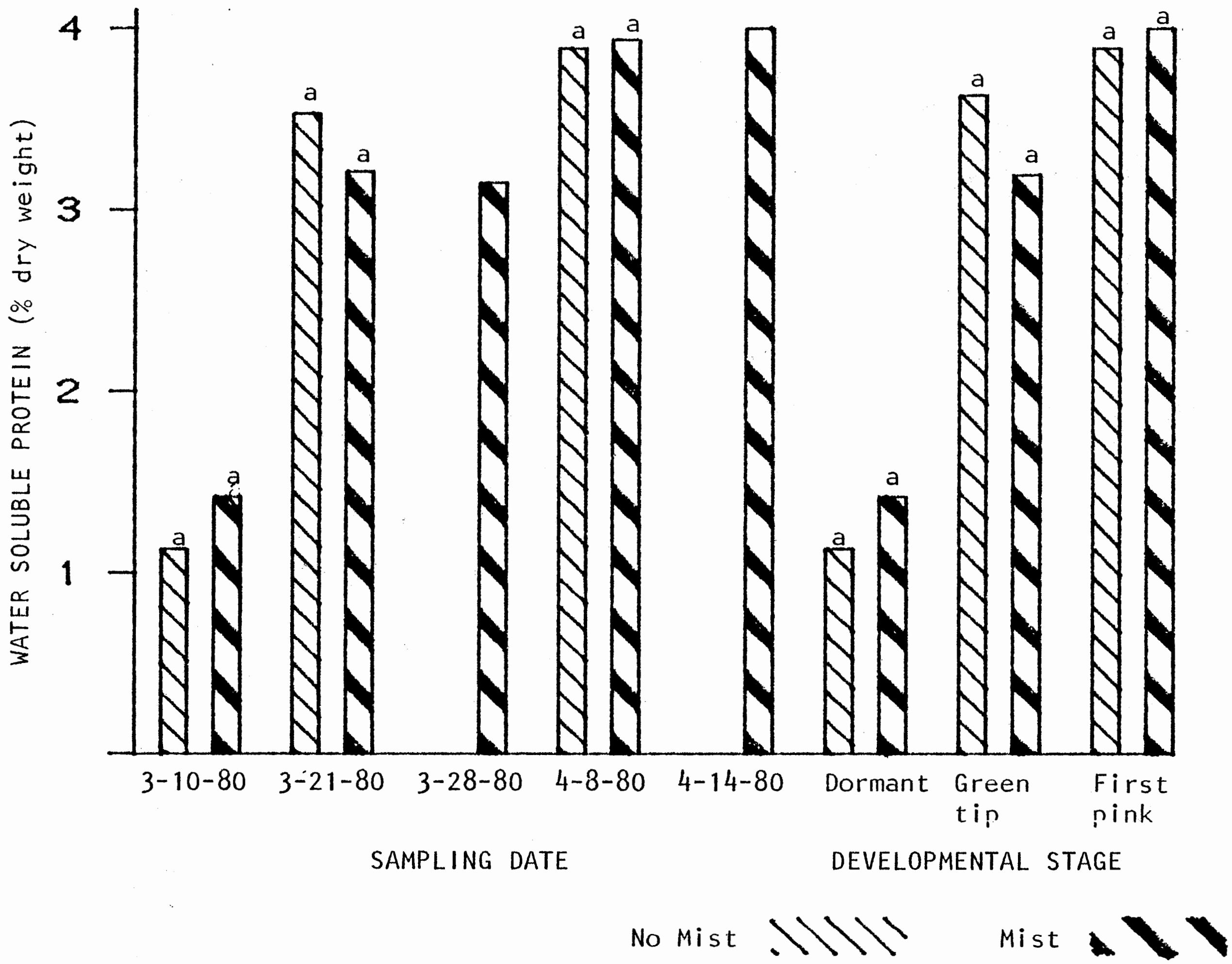

Figure 4. The Effect of Evaporative Cooling on the Water Soluble Protein Content of 'Delicious' Apple Flower Buds. Treatments within the same date or stage with the same letter are not significant at the 5% level by Fishers F-test.

extraction procedure. Possibly a different extraction procedure would have yielded different results (41). In addition, the total WSP may not be as important as changes in selected proteins or even specific amino acids. Lasheen (28) and El-Mansey and Walker (17) have reported changes in the amino acid make-up of peaches that is correlated with hardiness. Electrophoresis has been used to detect changes in levels of certain proteins in relation to cold hardiness (5).

Water Relations and Hardiness

Apple flower buds receiving 6 hours of leaching were less hardy than non-leached buds (Figure 5). The moisture content of leached and non-leached flower buds were nearly equal. Peaches, at both the red calyx and first pink stages of development reacted in the same manner as apples (Figures 6 and 7). The differences in moisture content are slight. There seem to be an adverse effect of the water on hardiness that is not linked to increased moisture levels.

Figures 8 and 9 illustrate the lack of a relationship between moisture content and hardiness after leaching with water. Differences in drying time resulted in varying moisture content without affecting the mortality of the buds. These data indicate high moisture content is not related to low hardiness. The adverse effect of water is not associated with water content of the flower buds but with its action in removing some factor(s) needed for flower bud hardiness.

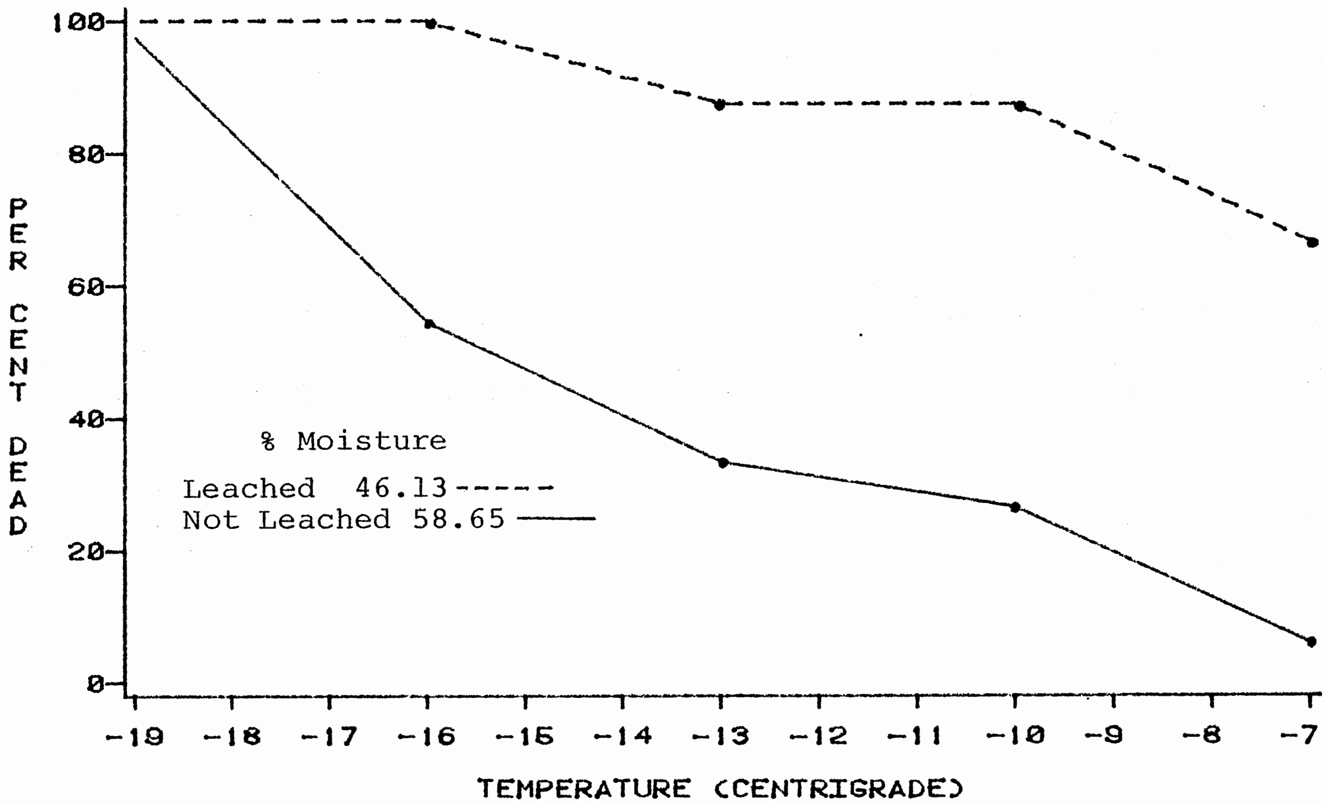

Figure 5. The Effect of a Six Hour Leaching Period on the Hardiness of 'Delicious' Apple Flower Buds at the Greentip Stage of Development

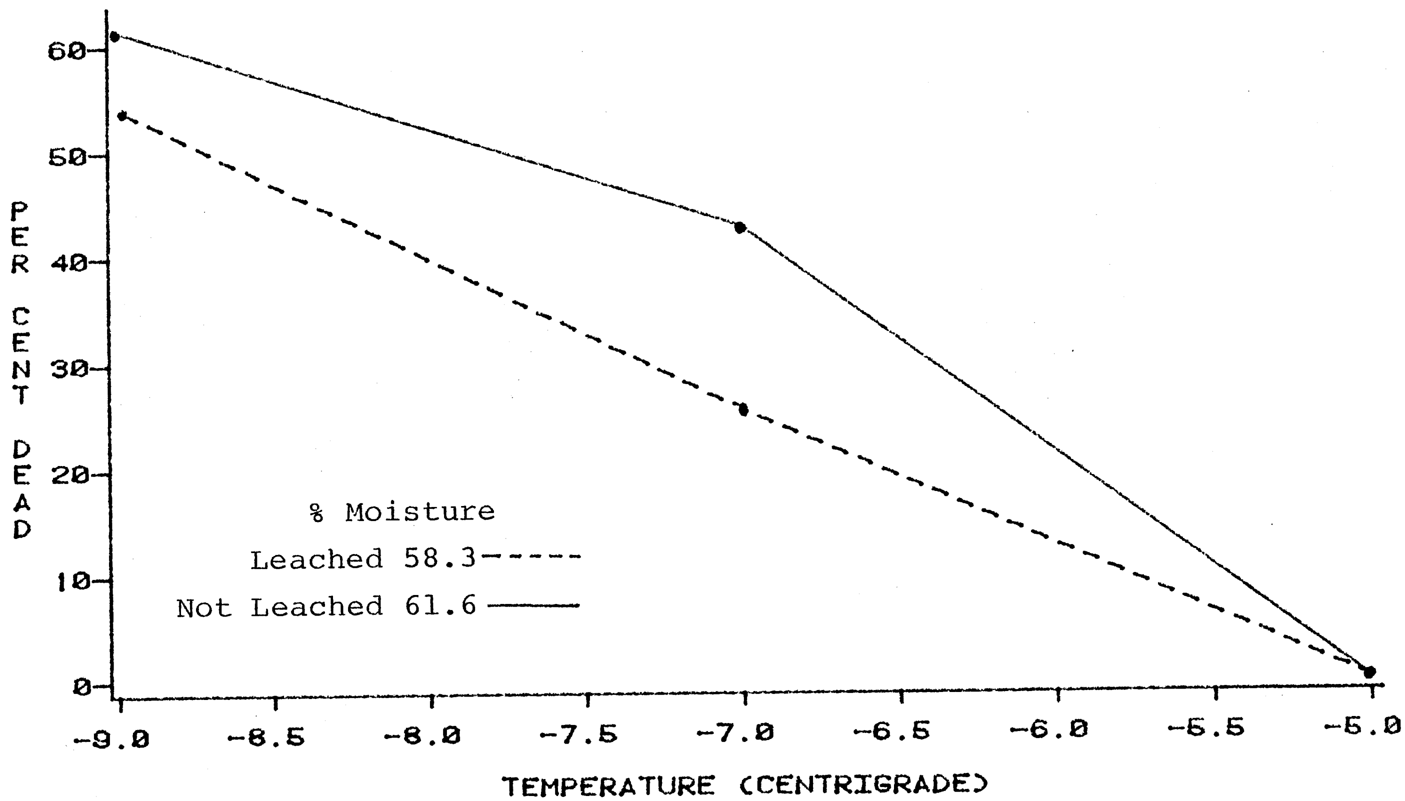

Figure 6. The Effect of a Six Hour Leaching Period on the Hardiness of 'Velvet' Peach Flower Buds at the Red Calyx Stage of Development

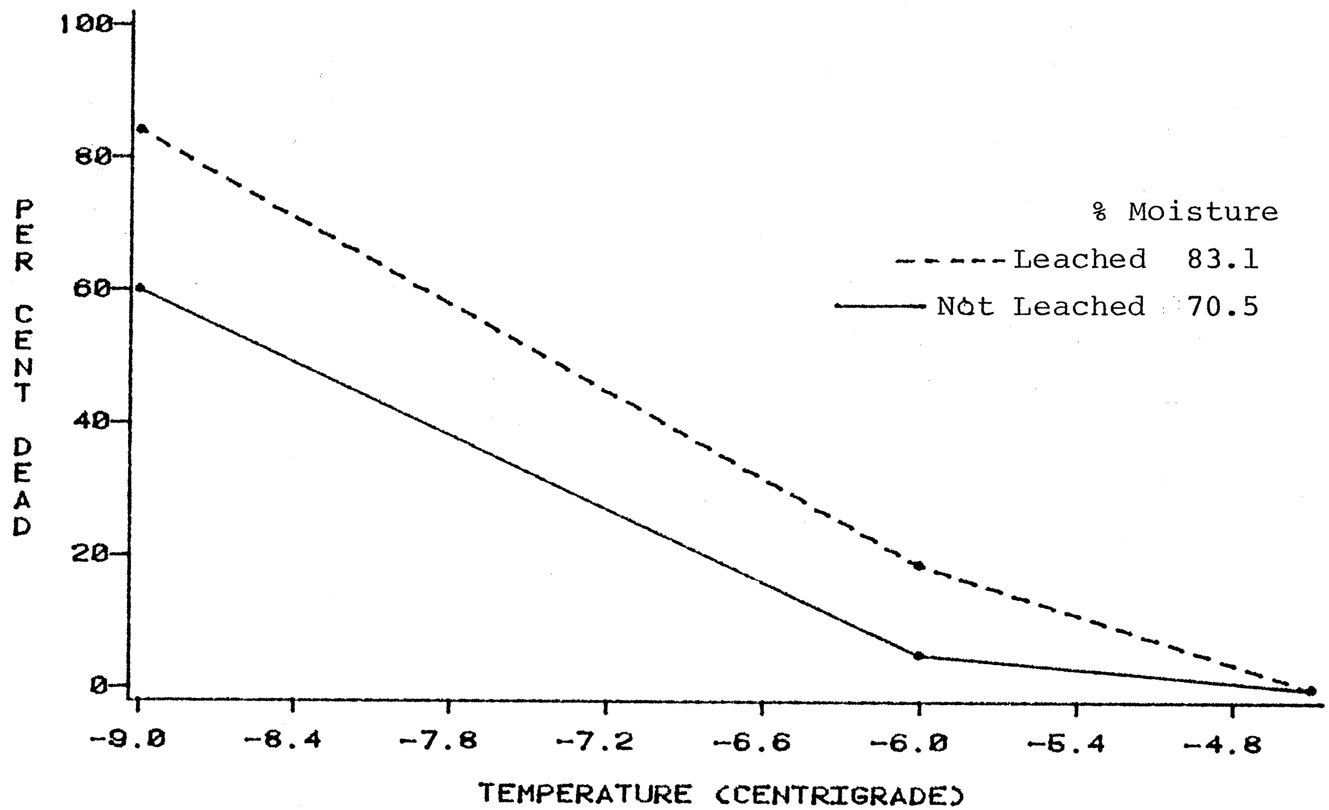

Figure 7. The Effect of a Six Hour Leaching Period on the Hardiness of 'Redskin' Peach Flower Buds at the First Pink Stage of Development

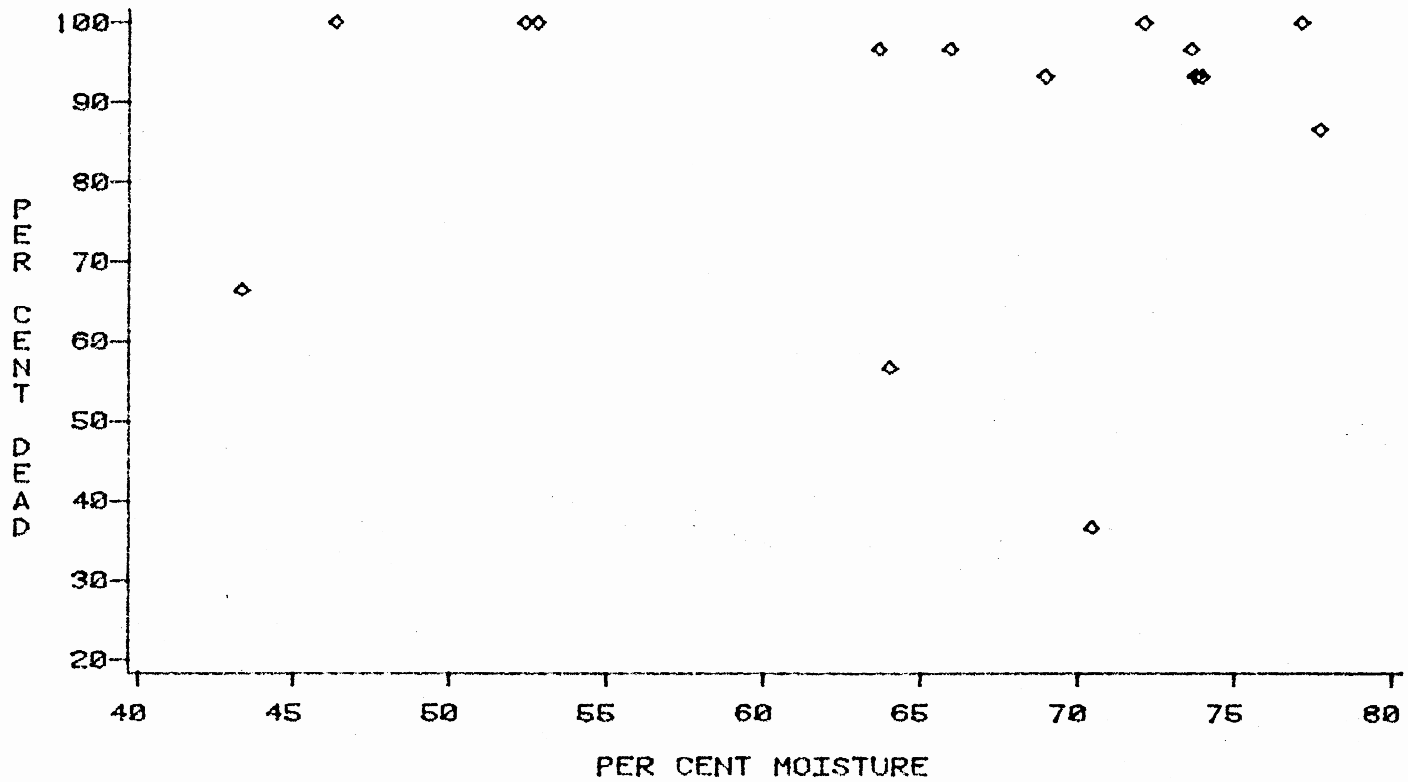

Figure 8. The Relationship Between Percent Moisture and Hardiness of 'Jonared' Apple Flower Buds at -9°C, r = .49 ns

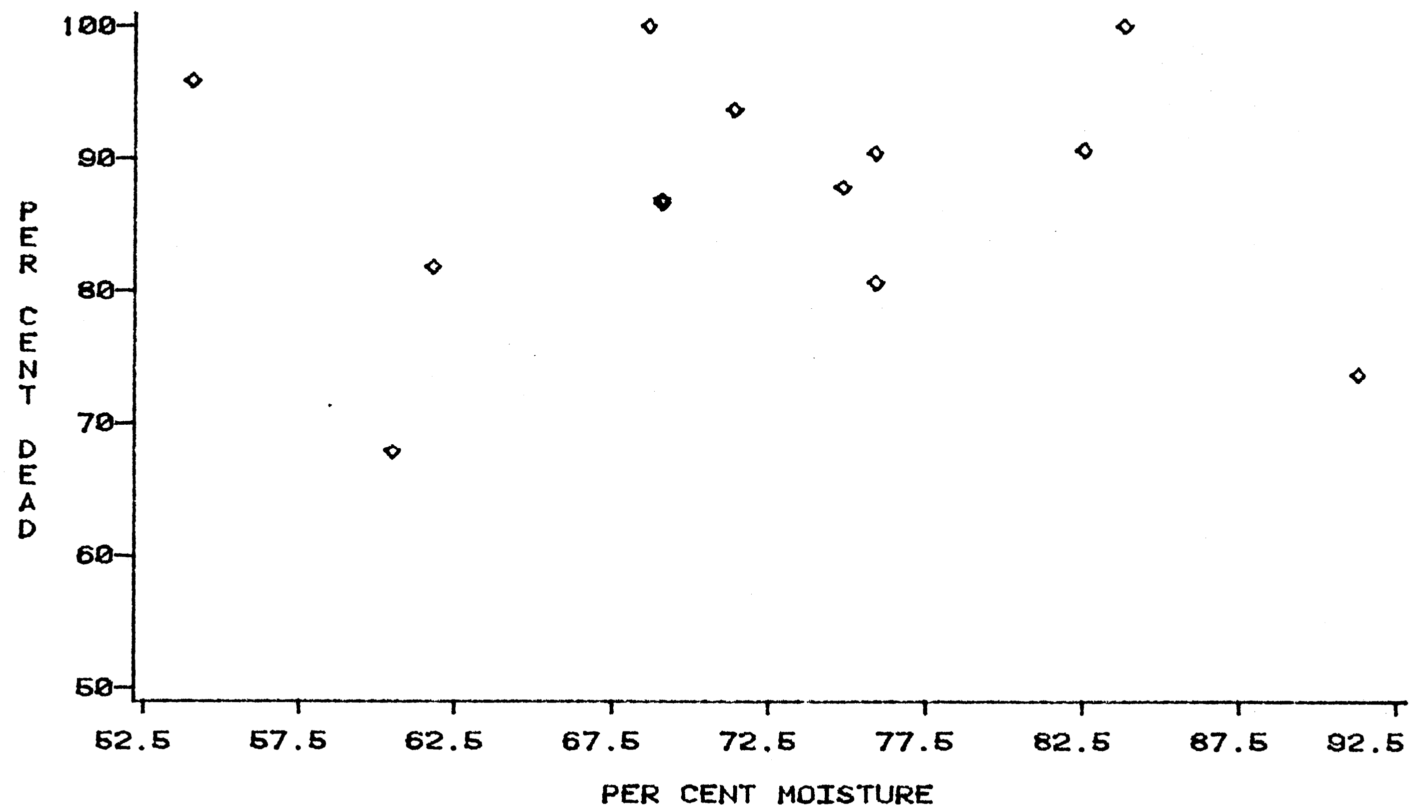

Figure 9. The Relationship Between Percent Moisture and Hardiness of 'Redskin' Peach Flower Buds at -8°C, r = .15 ns

CHAPTER V

SUMMARY AND CONCLUSIONS

Evaporative cooling is a method of spring frost protection for decidious fruit crops. Instead of protecting open blooms or young fruit, this method attempts to delay bloom, thus, reducing the chance of frost injury.

The objectives of this study were:

1. Determine the effectiveness of evaporative cooling under Oklahoma conditions.
2. Evaluate an antitranspirant as a protective coating to prevent the adverse effects of evaporative cooling.
3. Examine the effect of evaporative cooling on flower bud hardiness.
4. Study the effect of water on flower bud hardiness.

Evaporative cooling delayed the bloom of apple by approximately three days. The antitranspirant treatments had no effect on the time of flowering for either misted or non-misted trees. Delayed trees set less fruit, but June drop was not affected. The number of seed per fruit was increased with evaporative cooling. The water soluble protein level was not affected by misting. No hardiness was gained from cooling. The degree of hardiness associated with phenological stages of development was increased by evaporative cooling.

Leaching studies indicated that moisture content of the flower buds was not associated with hardiness. However, leaching decreased hardiness of apple flower buds and peaches to a lesser extent.

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