Plant Science Letters, 37 (1985) 265—271
Elsevier Scientific Publishers Ireland Ltd.

FREEZE SURVIVAL IN PEACH AND PRUNE FLOWERS

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(Received July 16th, 1984)
(Revision received September 21st, 1984)
(Accepted September 21st, 1984)

A laboratory study was carried out using field grown peach (*Prunus persica)* and prune (*P. domestica)* flowers. The object was to find out why prune flowers are more freeze tolerant than peach flowers. After the flowers are fully open, it was found that the ovaries may still supercool, even with ice crystals present in the flower stem. The mechanics involved were explored with a computer model of the simultaneous heat, water and solute flow in the flower and stem tissue during freezing. Water flow toward growing ice crystals may cause a discontinuity in the liquid phase between the flower stem and the ovary, creating a barrier to nucleation. It was concluded that the prune flowers survive lower temperatures than the peach because the water in their ovaries is more apt to supercool, particularly when the dew point of the air is not reached.

Key words: freeze survival; flowers; ovaries; *Prunus persica; Prunus domestica*

Introduction

The temperature at the Kimberly Idaho weather station fell to −8°C on April 19th, 1982. On April 30th, with the peach and the prune in full bloom, a low of −3.3°C occurred. This was followed by another freeze of −5°C on May 3rd. Peach trees in the area set no fruit that year, yet the prunes produced well.

Tender plant tissue may survive mild freezing temperatures in two ways: (1) the water in the tissue may supercool so that no ice forms, or (2) extracellular ice may form but leave enough unfrozen water in the cells for survival. The chances of supercooling are greatest when the air is so dry that its dew-point is not reached during the cooling period [1]. If the dew point is reached, vapor condenses on the plant leaves and forms ice crystals unless special care has been taken to eliminate nucleating agents [2,3]. External ice crystals nucleate the water inside the plant, except in some uncommon cases where the plant is so stressed for water that its leaves are wilted [1]. In general, the nucleation of water in plant tissue leads to rapid formation of internal extracellular ice and, in some cases, intracellular ice, too. In the case of severe water stress, the rate of spread of ice may be reduced [1], but once ice forms in plant tissue, the tissue's survival depends on cell hardiness. Hardiness is generally believed to be associated with high cell membrane permeabilities. The type of solutes in the extracellular space and their osmotic pressures are also important in determining the spread of ice and generally benefit tissue survival during freezing and thawing of the plant's liquid phase [4—6].

It has been shown that water in flower primordia surrounded by bud tissue may supercool even though many ice crystals form in and around the tissues near the flower [7]. Embryos may also supercool and avoid injury even though ice is present in the surrounding seed tissue [8]. This requires a discontinuity in the liquid phase between supercooled cells and the adjacent tissue containing ice. The discontinuity might be caused by a non-wetting membrane, but there is increasing evidence that the removal of water in the immediate area around the flower primordia

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creates a discontinuity that, in turn, allows the flower tissue to remain isolated and unnucleated [9,10].

Methods

Beginning April 12th, as the first flowers were starting to open, twigs were cut from the trees each morning for laboratory freezing tests. The cut ends were submerged in water and the flowers covered with plastic until they were removed from the twigs to be frozen.

Three types of freezing tests were carried out.

The Dry Freeze Test

Flowers with stems were placed on wire shelves in a watertight container. The container was submerged in a constant temperature water bath and allowed to equilibrate at a temperature below freezing. Desiccant placed in the bottom of the container prevented water vapor from condensing on the flowers. This simulated freezing conditions in which the dewpoint of the air is not reached and so favors supercooling of the plant water. After the flowers had been held at the specified freezing temperature for 4 or 5 h, they were warmed 3—4°C/h until thawed, removed from the dry container and placed in a closed chamber overnight. The ovaries were then inspected for freeze damage.

The Wet Freeze Test

The second freezing test was the same as the first, except that the flowers were laid on moist filter paper and no desiccant was used in the freeze chamber. This simulated frost conditions that occur when the dewpoint temperature of the air is reached, with nucleation of plant water occurring from ice crystals on the surface as the temperature falls below the freezing point.

The Single Flower Freeze Test

This test included continuous temperature measurements of individual ovaries as the flowers were frozen. The base of the flower stem was placed in a few milliliters of water in a small vial that in turn rested on the surface of a thermoelectric cooling plate. A tiny thermocouple, 0.2 mm in diameter, was positioned on the surface of the ovary. All was enclosed in an inverted Dewar vacuum flask to insulate the system. The cooling rate was controlled by the voltage applied to the thermoelectric plate. The temperature was dropped rapidly to 0°C, and then adjusted to fall about 2°C/h until the ovary reached −6 or −7°C, or until the thermocouple registered an exotherm indicating the ovary had suddenly frozen. After thawing at 3—4°C per hour, the flower was transferred to a closed chamber and later inspected for freeze damage.

The osmotic pressure of unfrozen ovaries was measured using a press to crush several ovaries into a bit of filter paper, and then measuring the vapor pressure with a thermocouple psychrometer.

Results and discussion

Dewpoint effects

The results from the dry-freeze test are summarized in Fig. 1. The data show scatter characteristic of freezes that occur when the dewpoint of the air is not reached. The prune flowers survived lower temperatures than the

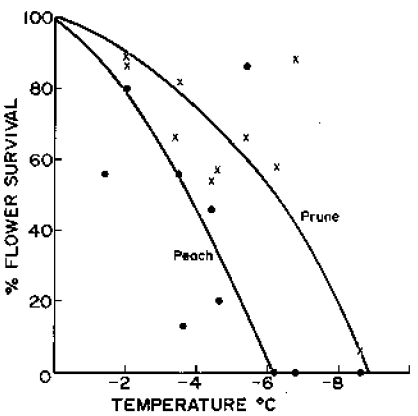

Fig. 1. The survival of peach and prune flowers subjected to various low temperatures under dry air conditions.

peaches. No doubt the predominant mechanism of survival was supercooling. The stability of liquid plant water under dry freezing conditions is affected by the temperature, plant water potential, root temperatures and duration of the freeze period; however, the exact way these factors are linked to supercooling stability remains unknown [1,11].

Results of the wet freeze tests are summarized in Fig. 2. As expected, the scatter of data was less because the plant water was nucleated by ice crystals in the moist filter paper as the temperature fell below freezing. The classical interpretation is that the surviving flower ovaries were cold hardy enough to tolerate the ice crystals. The osmotic pressures of the tissue water become an important factor in reducing ice damage under these conditions [4]. Osmotic pressures of the crushed peach ovaries varied between 1350 and 1500 kPa, while those of the prune were 1300–1700 kPa, and so the flowers were not significantly different in this respect. While the classical explanation for the survival shown in Fig. 2 is based on tissue tolerance of extracellular ice, the data obtained in the single flower freeze test suggests that discontinuities may have developed in the liquid phase of the flower, and thus some ovaries may have survived by supercooling, even with ice present in the flower stems.

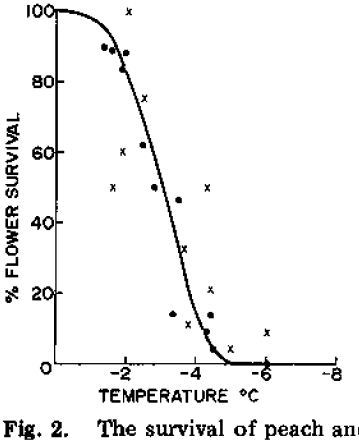

Fig. 2. The survival of peach and prune flowers subjected to various low temperatures with the flowers in contact with ice crystals. •, Peaches; ×, prunes.

It was noted that 10–20% of the peach flowers on the tree had already lost their ovaries before the flowers opened. This was likely caused by an air temperature of −8°C on April 6th. Of course, these flowers were not used in the freeze trials, but they did indicate that the ovary is the most freeze sensitive part of the peach flower and that the other parts of the peach flower can develop enough cold hardiness to survive ice at temperatures a few degrees below 0°C while in the bud stage (see Ref. 12). No damaged ovaries were found among the flowers on the prune tree.

Supercooling with ice crystals present

All of the ovaries were killed in the single flower freezing test unless an exotherm occurred and the freeze was ended above −2°C. The presence of ice below −2°C likely caused intracellular freezing leading to tissue death. When the ovaries cooled all the way to −6 or −7°C without showing an exothermic heat release, I assumed that ice had formed in the ovaries as the temperature reached the freezing point of their water, that is, slightly below 0°C. On the other hand, when an exotherm did occur, it indicated that the water in the ovary had supercooled and then abruptly nucleated. The whole system was being cooled by the thermoelectric plate on the bottom of the freezing chamber. Therefore, the cooling and freezing started in the water around the cut end of the flower stem and slowly proceeded upward toward the petals and ovary. Under these particular conditions, water in the ovary could only supercool if a discontinuity in the liquid phase developed outside of the ovary preventing the continued extension of ice crystals.

A total of 30 peach and 38 prune flowers were frozen individually. The percentage of the total flowers that produced exotherms between −0.1 and −1°C was plotted as a single data point at −0.5°C in Fig. 3. Exotherms between −1 and −2°C were likewise grouped and shown as a single point at −1.5°C. Summarizing the data in this way shows the

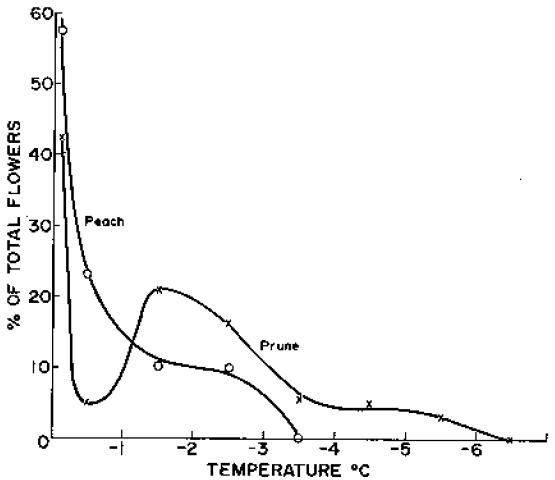

Fig. 3. The distribution of temperatures at which peach and prune ovaries froze when ice crystals were present in the flower stems, i.e. 42% of the prune ovaries froze as the temperature reached the freezing point of the water in the ovary, 5% more froze before the temperature reached −1°C, 22% froze between −1 and −2°C, 18% between −2 and −3°C, etc.

supercooled cell water was more stable in the prune than in the peach flowers down to −6°C. It follows that the anatomy and/or solutes in the prune flowers were more conductive to the formation of a discontinuity in the liquid phase of the prune flower than in the peach. The greater stability of the liquid phase in the prune, shown in Fig. 3, is less obvious in Fig. 2 because nucleation was not as well controlled.

Since the single flower freeze tests all produced badly-damaged ovaries when exotherms occurred below −2°C, it is likely that most of the survival shown in Fig. 2 at temperatures below −2°C was due to supercooling stability in the ovaries. Some of the survival above −2°C may have resulted from enough cold hardiness of the ovaries to tolerate ice crystals between 0 and −2°C and so could conform to the more classical concepts of tissue survival when ice crystals are present.

The freezing response of a few violet flowers (*Viola beckwithii*) were compared with peach and prune. The violets bloom 2 or 3 weeks earlier and are quite cold hardy. Their ovaries produced exotherms at temperatures as high as −1.1°C and as low as −6.5°C. Unlike the peach and prune, the violet ovaries did not appear to be killed following exotherm responses between −2 and −6.5°C, suggesting that intracellular ice did not form.

A model of supercooling in floral tissue with ice present

How do liquid phase discontinuities develop so that bits of tissue with supercooled water are isolated from ice crystals? When ice starts to form in moist porous material, water flows toward the ice crystals [13]. This results in drying of the area that the water leaves, possibly creating a discontinuity in the liquid phase. The amount of water that flows to the growing ice crystals is larger when the hydraulic conductivity of porous material is high. The amount of water flow depends on the cooling rate and becomes less as the ice phase begins to grow through the liquid-filled pores faster than water can move toward the freezing front. The system's behavior is controlled by the temperature distribution in the porous material because the vapor pressure of ice is fixed by the temperature. The vapor pressure, in turn, controls the amount of unfrozen water as modified by the matric and osmotic potentials in the system, i.e.

$$-\Upsilon + OP = -1200\ T \qquad (1)$$

where Υ is the matric potential or negative capillary water pressure, OP is the osmotic pressure, and 1200 kPa is the change in water potential per °C as required by the change in vapor pressure of ice as the temperature, T, changes. The flow of water, J_w, in such a system assuming no semi-permeable membrane effects is:

$$J_w = -k \nabla \Upsilon = -k \nabla (1200\ T + OP) \qquad (2)$$

where k is the water conductivity. The flow of solutes, J_s, may be described as

$$J_s = J_w OP - D \nabla OP \qquad (3)$$

where the term $J_w OP$ accounts for the solutes carried along by the flow of the extracellular solution and D is a coefficient that describes the diffusion of solutes along the osmotic pressure gradients in this same solution. The flow of heat, J_q, can be described as

$$J_q = -K \nabla T + H \nabla J \qquad (4)$$

where K is the thermal conductivity, ∇T is the temperature gradient, H the latent heat released by freezing water, and J the freezing rate of water.

Equations 1--4 show that the flow of heat, water and solutes are all temperature coupled during the freezing or thawing processes in moist porous materials. Because the water conductivity and capillary pressure depend on water content and pore size distribution, there is no general exact simultaneous solution for the three transport equations. However, approximate solutions for special cases can be developed using a computer and a finite difference approach. Let us consider the single flower freezing study. Ice formed at the cut end of the stem and began to move up the stem toward the flower as the chamber cooled. At the same time, water flowed out of the upper parts of the flower toward the growing ice crystals in the stem. If the water conductivity across some interface, for example, between the ovary and the receptacle, is less than the conductivity in the stem, it is possible that the xylem vessels will cavitate, leading to a discontinuity in the liquid phase near that interface. This can be modeled beginning with the simple diagram in the upper part of Fig. 4. Here the receptacle and two sections of the flower stem are represented by the three small squares which were taken as 1 mm^3 elements in a finite difference solution of Eqns. 1--4. The curves in Fig. 4 show the theoretical distributions of temperature, total volumetric water fraction, θ_T, the volumetric liquid phase water θ_l, and osmotic pressures in the ovary, receptacle and two sections of stem at 5, 10 and 30 min after the ice phase, moving up the stem toward the flower, reached the

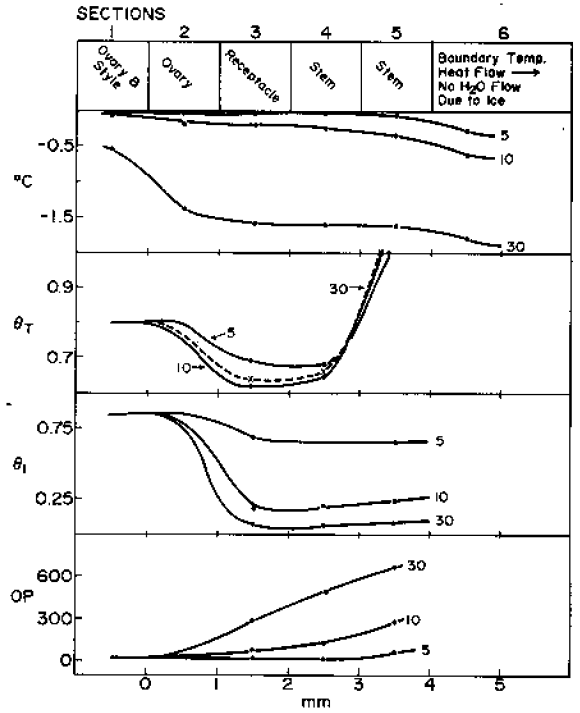

Fig. 4. A diagram of the simple model used to study freezing of a flower, including the results of one test case showing the predicted changes in the distribution of temperature, total water content, θ_T, liquid water content, θ_l, and the extracellular solute concentration expressed in units of kPa osmotic pressure, OP, at 5, 10 and 30 min after ice formed in stem element, No. 6. The values of OP, θ_l and θ_T are shown over a 4 mm distance extending from the lower part of the ovary through the receptacle and including 2 mm of flower stem.

imaginary boundary between elements 5 and 6. It was assumed that the initial water conductivity across the interface between the ovary and the receptacle was 0.01 that of the stem.

The predicted decrease in total water content θ_T that occurs so rapidly is particularly interesting. This may be what initiates the formation of a dry layer between the stem and the ovary. The model shown at the top of Fig. 4 was used to obtain an idea of how changes in the rate of cooling, the initial osmotic pressure, and the ratio of ovary to receptacle conductivity might affect drying in

Table I. Predictions of the lowest water content (θ_T, Fig. 4) that occurs in the first 30 min after freezing starts. Comparisons show the effects of: initial extracellular osmotic pressure; rate of temperature drop; and the ratio of conductivity at the ovary-receptacle interface to the conductivity in the stem.

Case	Lowest H_2O content vol. fraction	Hydraulic conductivity ratio	Rate of temp. decrease (°C h^{-1})	Osmotic pressure (kPa)
1	0.70	0.1	3.6	25
2	0.56	0.1	3.6	0
3	0.77	0.1	3.6	100
4	0.52	0.1	10.8	25
5	0.74	0.1	1.8	25
6	0.79	1.0	3.6	25
7	0.62	0.01	3.6	25

the stem and receptacle. Typical results are shown in Table I where the initial volumetric water fraction was 0.8. Case 7 is the same shown in detail by the curves in Fig. 4. Increasing initial osmotic pressure decreased the drying rate, cases 1, 2 and 3. Increasing the cooling rate increased the drying, cases 1, 4 and 5. Decreasing the relative water conductivity between the ovary and the receptacle also increases the drying, cases 1, 6 and 7. When sepals and petals are added to the model with the assumption that ice is nucleated in them at the same time it starts moving up the stem, the rate of drying at the ovary-receptacle interface increases. The xylem vessels may begin to cavitate at capillary pressures of −400 kPa in this type of tissue [14]. When the extracellular osmotic pressure is small, this pressure can be created by ice crystal temperatures of 0.4°C below freezing (Eqn. 1).

The results from this model, shown in Table I and Fig. 4, are qualitative because the conductivity and other physical properties of the flower's stem, receptacle and ovary are not well known. The results are intended only to show how redistribution of water can occur during freezing and to suggest a starting point for more quantitative studies. Exponential functions previously developed for soil conductivity and matric potential dependence on soil water content were used in the calculations [13], along with estimates of solute and thermal diffusivities obtained from handbook tables. Details concerning the finite difference computer program written in basic and the values used for the various physical constants are available upon request.

Effects of solutes on ice crystals and supercooling

As noted, cases 1, 2 and 3 in Table I suggest that solutes decrease the rate of drying at the ovary-receptacle interface. Nevertheless, solutes may be important in other ways for they tend to change the pattern of ice crystal growth. Figure 5 shows ice advancing into and over the cut edge of sugarbeet (*Beta vulgaris*) seedling stems, The photographs were made through a microscope during the course of a previous study [4]. Part A shows a distilled water system with a straight boundary between the ice and liquid phase with a few air bubbles trapped in the ice. The feathered edge of the ice crystals in part B shows the advance into a long-chained carbowax solution with an osmotic pressure of about 800 kPa and a molecular weight of 200. The feathering is presumably caused by the increasing concentration of carbowax as liquid water freezes out of the solution. Part C shows ice crystals growing into a 0.1 molar KCl solution. The different shapes of the ice crystals in B and C probably result from the difference in diffusion coefficients of KCl and carbowax. The simple model in Fig. 4 does illustrate how solutions concentrate during freezing, but

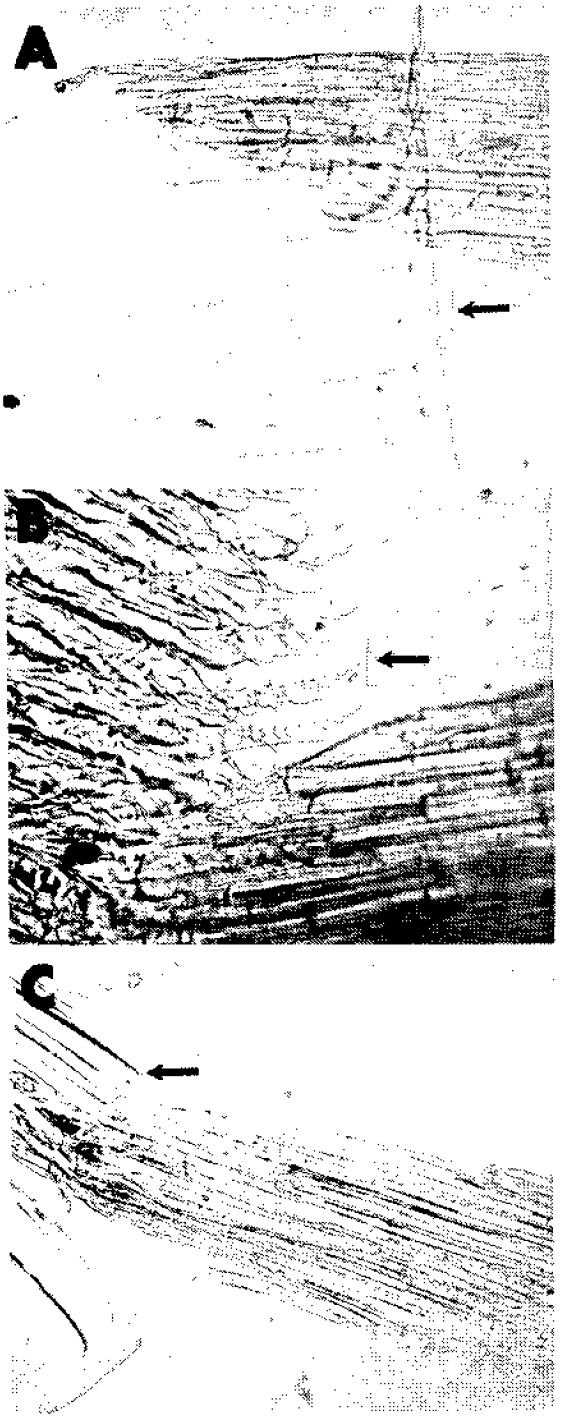

Fig. 5. Ice crystals (arrows) growing from left to right into and over the sliced ends of sugarbeet seedling stems submerged in: A, distilled water; B, a long chain carbowax solution; and C, a 0.1 N KCl solution.

their effects on supercooling in the ovary remain unknown (see also Ref. 13 and 15 on solute concentrating). It is possible that the concentrated liquid phase impedes ice spreading because it increases the unfrozen water content and, consequently, raises the hydraulic conductivity.

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