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Ice Formation in *Prunus persica* under Field Conditions

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Abstract. **Ice formation was initiated between −0.6° and −2.6°C in mature *Prunus persica* (L.) Batsch trees growing in the field. Trees supercooled very little. Ice formation was initiated at several locations in the tree and subsequently spread throughout. The release of the latent heat of fusion following ice formation in the tissue maintained tissue temperatures 1° to 3° above air temperature for several hours and mitigated the tissue's response to ambient temperatures.**

Recent studies on the role of ice nucleation active (INA) bacteria in frost injury in plants have led to renewed interest in establishing the temperature at which ice formation is initiated in plant tissues (2, 4, 7, 8, 9). Studies utilizing small plant parts and tissue homogenates have led to proposals that intact plants lack intrinsic ice nuclei active above −5° to −10°C (8, 9). Ice nucleation above these temperatures was believed to occur via INA bacteria. Recently, we demonstrated that ice formation was initiated at about −2° in 1-year-old potted peach trees and dormant shoots from mature trees (4, 5). Once initiated, ice spread throughout the tissue. Ice formation appeared to be initiated by intrinsic nuclei associated with the woody tissue and was not correlated with the presence of INA bacteria (4, 5). The purpose of this study was to determine the temperature at which ice formation was initiated in mature trees growing in the field, whether significant supercooling occurred, and whether portions of the tree could remain supercooled when ice was present in another portion of the tree.

Materials and Methods

Thermal analyses of trees in the orchard. The freezing of water was monitored in two 6-year-old 'Columbia' nectarine trees growing at the Appalachian Fruit Research Station, Kearneysville, W. Va. Thirteen, 30 gauge copper-constantan thermocouples (45 cm long) were used to monitor temperature within each tree. The junctions were placed in the cambial layer in September of 1983 by cutting an L-shaped flap and peeling back the bark. After the thermocouples were inserted, the bark was put back in place and the incisions were wrapped with plastic tape. In late fall, the incisions had closed and the wrappings were removed.

A single thermocouple was placed in the trunk of each tree at 30 cm above the soil surface. In addition, thermocouples were placed in 4 scaffold limbs arising from the trunk (100 cm above the soil surface), 4 laterals from the scaffold limbs (170 cm), and 4 twigs of the previous season's growth (200 cm). The junctions of additional thermocouples were taped to the tree limbs on some nights. A 4 × 9 × 220 cm board, perpendicular to the soil and secured in the center of the canopy, served as a reference. Thermocouple junctions were inserted 1 cm deep into the board at heights of 30, 100, 170, and 200 cm and sealed with carpenter's glue. Air temperature was monitored at 150 cm with an exposed thermocouple. Thermocouples from the tree and the reference were connected to a datalogger (CR5 Digital Scanner, Campbell Scientific, Logan, Utah) using 750 cm of shielded 20 gauge thermocouple wire and shorter lengths (30 to 400 cm) of 24 gauge wire.

Temperatures were scanned at 1 min intervals in the field and data analyzed using a computer-interfaced method of thermal analysis (3). The data were recorded on cassette tape and subsequently transferred to a computer and graphics terminal. Temperature profiles of tissue, reference, and air were plotted and the temperature and time of ice nucleation were determined by comparing the plots of tissue and reference temperatures. Observations were made on 11 nights under a variety of environmental conditions, including rapid and slow cooling and both advective and radiative conditions.

Results and Discussion

The freezing of water in intact trees in the orchard was detected readily. When ice formation was initiated, the release of the heat of fusion raised tissue temperature. Freezing was noted as either a distinct exotherm (Fig. 1) or as a divergence of the tissue and reference temperatures (Fig. 2). Mature trees were observed to supercool very little with freezing initiated between

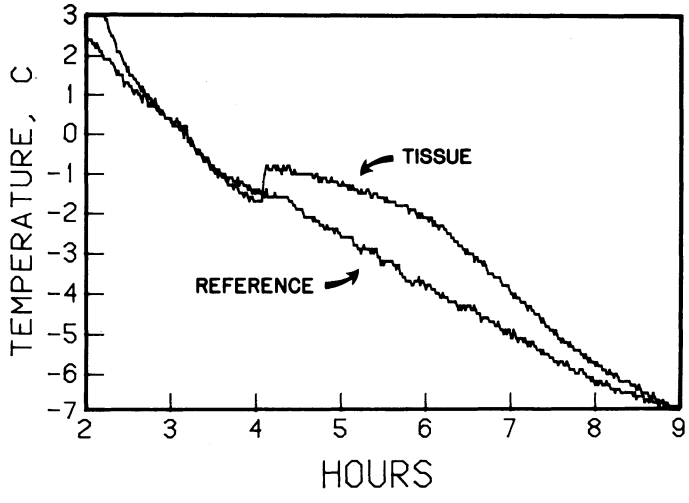

Fig. 1. Thermal analysis of an intact nectarine tree growing in the orchard. The plot is a comparison of reference temperature and the temperature of a scaffold limb. A distinct exotherm was observed due to the freezing of water within the scaffold limb. Measurements were begun at dusk, and the elapsed time indicated on the X axis.

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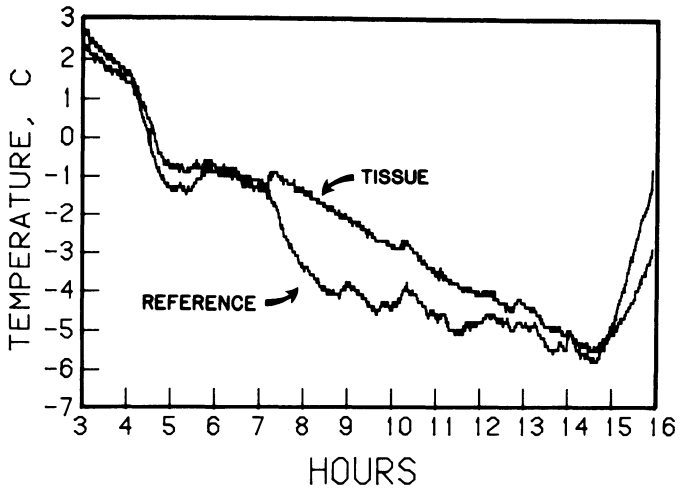

Fig. 2. Thermal analysis of an intact nectarine tree growing in the orchard. The plot is a comparison of reference and branch temperatures. The freezing of water within the tissue was detected by the divergence in the tissue and reference temperature plots. Measurements were begun at dusk, and the elapsed time indicated on the X axis.

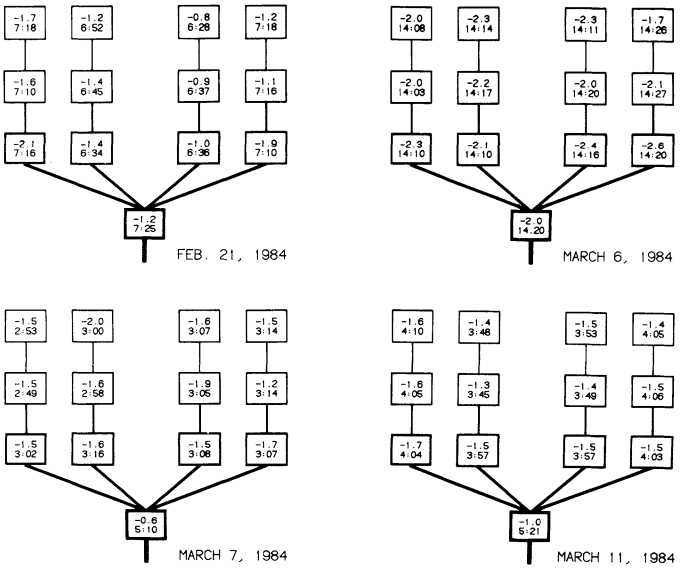

Fig. 3. Four schematic drawings illustrating the elapsed time and temperature when ice formation was initiated in a nectarine tree on four separate occasions. Observations were made on 21 Feb. and 6, 7, and 11 Mar. 1984.

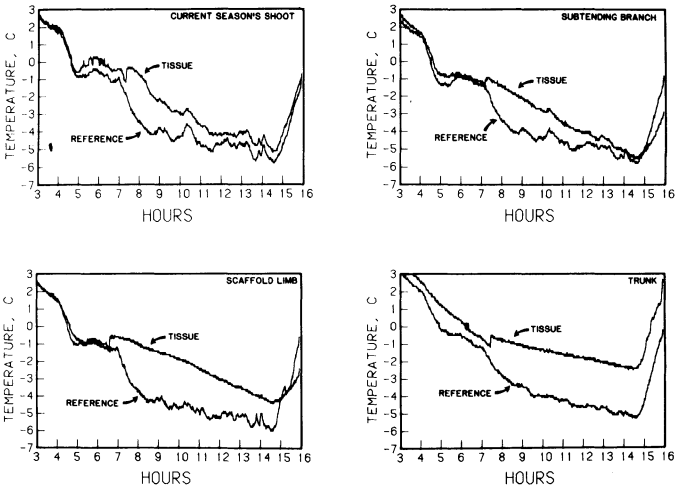

Fig. 4. A comparison of the response of current season's shoots, subtending branches, scaffold limbs, and trunk to ice formation and the dissipation of the heat of fusion under natural conditions. Measurements were begun at dusk, and the elapsed time indicated on the X axis.

$-0.6°$ and $-2.6°$C. Once initiated, ice spread throughout the tissue and, on the average, tissues froze at $-1.6°$.

Monitoring the temperature of an inanimate reference was critical for the detection of freezing within the tree. Under natural conditions, air temperature fluctuated rapidly. Although the extent of the fluctuations varied with weather conditions, rapid changes (1° to 2°C/ 5 min) were not uncommon. Although many of the transient temperature fluctuations were damped out by the mass of the tree tissue, the only way to distinguish an exotherm positively from a response to changing air temperature was to compare tissue and reference temperatures. The presence of the reference was especially critical when freezing was initiated just below 0°. In these instances, only the divergence of the reference and tissue temperatures indicated freezing.

Using the present monitoring system, we were unable to pinpoint where ice formation was initiated within the tree. However, by noting the time when individual locations froze, it was concluded that tree freezing was not the result of a single nucleation event followed by crystal growth. Instead, ice formation was initiated at several locations within the tree (Fig. 3). In the course of our observations, we observed freezing to be initiated in the current season's wood, subtending branches, and the scaffold limbs (Fig. 3). The location of ice nucleation appeared to be influenced by the prevailing weather conditions. On nights when the current season's wood cooled faster than the larger branches, ice was initiated in the shoots and spread downward, whereas, on other occasions, tissue temperature was more uniform and freezing was initiated in the lower branches.

The time required for an entire tree to freeze varied. In one instance, ice spread throughout the tree in 16 min; however, time intervals longer than 1 hr also were observed. The trunk generally froze last (Fig. 3). The time required for ice to spread throughout the tree was influenced by the prevailing weather conditions and the extent of supercooling prior to ice formation (data not presented). Accelerated cooling rates and increasing supercooling appeared to increase the rate at which ice spread throughout the tissue (data not presented), probably because of the rapid dissipation of the heat of fusion under these conditions.

The response of tissues to ambient temperatures was mitigated by the formation of ice in the tissue. Tissue temperatures were warmer than reference or air temperatures until the heat of fusion was dissipated. The rate at which the heat of fusion dissipated depended on both the prevailing environmental conditions and the mass of the tissue. Large tissues, such as the trunk and scaffold limbs, remained 2° to 3°C warmer than air temperature for several hours after freezing was initiated (Fig. 4).

On many nights, trunk temperature never reached the minimum air temperature. The time required to dissipate the heat of fusion completely from the current season's shoots, subtending branches, and scaffold limbs averaged 3, 5, and 6 hr, respectively. These differences reflect the differences in tissue mass and the rate of heat transfer. The formation of ice within the tree stabilized tissue temperature. Tissue temperature fluctua-

tions were damped out compared to those of the reference (Fig. 4). Once the heat of fusion was dissipated, this effect no longer was observed.

Recently there have been several reports that the woody tissues of peach trees contained intrinsic ice nuclei, active at −2°C (1, 4, 5, 6, 10). These intrinsic nuclei limited the extent of supercooling. Proebsting and coworkers (1, 6, 10) have demonstrated that detached flower buds and fruitlets of pear and several *Prunus* sp. supercool readily. These tissues did not appear to contain ice nuclei active at warm temperatures; however, when the flowers and developing fruit were left attached to the woody stem, little supercooling was observed. It appeared that once ice formation was initiated within the woody tissues, it spread from the woody stem into the developing flowers and fruit. Based on this evidence, it would appear that under most conditions ice formation would be initiated within the woody tissues of peach trees and spread into the flowers.

One possible exception to this scenario may occur during a radiation frost. If developing blossoms and open flower buds cooled faster than adjacent woody tissues, they may become significantly colder than the shoot tissue. In this instance, ice formation may be initiated within the blossoms first. We had hoped to examine this possibility by monitoring blossom, shoot, and air temperatures during a radiation frost. However, freezing conditions did not occur during the bloom period. We did monitor tissue temperatures on several evenings. Generally, blossoms and shoot temperatures were very similar. Transient differences of 1° to 2°C were observed; however, these were not sustained, and differences of less than 0.5° were the norm (data not presented). These observations were preliminary, and additional measurements under a variety of natural conditions will be required.

Literature Cited

1. Andrews, P.K., E.L. Proebsting, Jr., and D.C. Gross. 1983. Differential thermal analysis and freezing injury of deacclimating peach and sweet cherry reproductive organs. J. Amer. Soc. Hort. Sci. 108(5):755–759.
2. Arny, D.C., S.E. Lindow, and C.D. Upper. 1976. Frost sensitivity of *Zea mays* increased by application of *Pseudomonas syringae*. Nature 262:282–284.
3. Ashworth, E.N., G.W. Lightner, and D.J. Rowse. 1981. Evaluation of apricot flower bud hardiness using a computer-assisted method of thermal analysis. HortScience 16(6):754–756.
4. Ashworth, E.N. and G.A. Davis. 1984. Ice nucleation within peach trees. J. Amer. Soc. Hort. Sci. 109(2):198–201.
5. Ashworth, E.N., J.A. Anderson, and G.A. Davis. 1985. Properties of ice nuclei associated with peach trees. J. Amer. Soc. Hort. Sci. 110(2)287–291.
6. Gross, D.C., E.L. Proebsting, Jr., and P.K. Andrews. 1984. The effects of ice nucleation active bacteria on temperatures of ice nucleation and freeze injury of Prunus flower buds at various stages of development. J. Amer. Soc. Hort. Sci. 109(3):375–380.
7. Lindow, S.E., D.C. Arny, C.D. Upper, and W.R. Bartchet. 1978. The role of bacterial ice nuclei in frost injury to sensitive plants, p. 249–263. In: P.H. Li and A. Sakai (eds.). Plant cold hardiness and freezing stress. Academic Press, New York.
8. Lindow, S.E., D.C. Arny, and C.D. Upper. 1982. Bacterial ice nucleation: A factor in frost injury to plants. Plant Physiology 70:1084–1089.
9. Marcellos, H. and W.V. Single. 1979. Supercooling and heterogeneous nucleation of freezing in tissues of tender plants. Cryobiology 16:74–77.
10. Proebsting, Jr., E.L., P.K. Andrews, and D. Gross. 1982. Supercooling young developing fruit and floral buds in deciduous orchards. HortScience 17(1):67–68.