Supercooling Young Developing Fruit and Floral Buds in Deciduous Orchards

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Abstract. Young developing fruit of apricot (Prunus armeniaca L.), peach (P. persica), sweet cherry (P. avium), plum (P. domestica), pear (P. communis), frost injury.

The potential ability to promote supercooling of plant tissues and thus enhance frost protection is of topical interest to pomologists since the recent discovery that strains of Pseudomonas syringae van Hall and Erwinia herbicola (Lohnis) Dye, commonly found in orchards, are an important source of ice nucleation (1, 4). Tender annual plants such as bean, corn and tomato can avoid frost injury down to −5°C or −7°C if they are free of ice nucleation active (INA) bacteria. The degree of frost injury is proportional to the log of the bacterial population (4).

Supercooling is known to occur in fruit tissues, although it is believed to be rare in orchards (2). Ice crystals can grow through the stem, presumably the vascular system, of lemons at a rate of 2–3 cm/min (5). In boxwood there also is evidence for the presence of internal nucleation sites in the wood (3).

This paper reports preliminary data describing certain limitations in supercooling of tissues during bud development that must be overcome if substantial frost protection through elimination of INA bacteria is to be achieved in deciduous fruit orchards.

Samples collected for freezing analysis came from bearing trees of apricot, peach, sweet cherry, prune, and pear. The orchard had very low levels of INA bacteria, ranging from not detectable to less than 100/g fresh tissue weight, in its natural flora throughout the developmental stages from first swelling to full bloom.

Freezing curves were developed by measuring tissue temperatures with inserted thermocouples connected to a 24-point recording potentiometer that recorded points at 30 sec. intervals or measuring relative temperatures using differential thermal analysis with the sensor on the tissue surface. Most curves were developed at rates of temperature decrease near 1°C per hour. Samples were brought directly from the field and placed in an insulated box which was put into a freezer with a programmed temperature controller. The nucleation temperature was defined as the lowest temperature measured before an increase in temperature was observed. INA bacteria were applied by dipping the fruits in a P. syringae strain WA426 cell suspension (average nucleation frequency at −5°C is 6 × 10^{−4} nuclei/cell) containing between 10^{6} and 10^{9} colony-forming units/ml to provide an excess.

Developing fruit detached with its pedicel from woody tissues supercooled to a lower temperature than fruit attached to shoots 30 cm long (Table 1). Each sample consisted of 12 fruits. The marked difference in ice nu-


leating temperatures of fruit attached to the stem compared with fruit detached from the stem suggests that ice nucleating particles active at higher temperatures are associated with the woody tissues.

Single peach fruit, each attached to 4 separate stem segments 2.5 cm long froze independently over a range of temperatures that averaged 2.8°C whereas the same number of fruit, all attached to the same stem segment 2.5 cm long froze at nearly the same temperatures, ranging over only 0.3°C (Table 2). On the other hand the mean values for fruit on separate segments had a range of 0.5°C compared with 3.8°C for 4 fruit on a single segment. Apparently the stem segments froze independently over a range of 3-4°C and the ice crystals that formed in the stem tissue then grew into each fruit on the stem.

The ability to supercool changed with stage of floral bud development. Peach buds supercooled equally whether attached or detached from the stems until the full bloom stage (Table 3). In the advanced stages of full bloom and later pronounced differences in supercooling between attached and detached fruit were observed.

P. syringae applied at a high population (as described above) raised the freezing temperature of fruit detached from the stem of pear and sweet cherry but did not affect that of attached fruit (Table 4). Differences among the plum samples were not significant. The effect of the INA bacteria on ice nucleation temperatures was of similar magnitude to the effect of the presence of stem tissue.

These preliminary data suggest several points that should be considered in assessing the potential for frost control by controlling INA bacteria.

Table 3. Mean temperature of ice nucleation for developing fruit of peach, cherry and pear as influenced by attachment to stem tissue 30 cm long.

<table>
<thead>
<tr>
<th>Species</th>
<th>Fruit attached</th>
<th>Fruit detached</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet cherry</td>
<td>2.2 a</td>
<td>8.0 b</td>
</tr>
<tr>
<td>Peach</td>
<td>3.8 a</td>
<td>8.3 b</td>
</tr>
<tr>
<td>Pear</td>
<td>3.0 a</td>
<td>6.2 b</td>
</tr>
</tbody>
</table>

1. The young detached developing fruit supercooled to low temperatures in the absence of high populations of INA bacteria.
2. The same fruit, attached to woody tissues, supercooled very little.
3. INA bacteria inoculated onto detached fruit raised the ice nucleation temperature to near that of the non-inoculated fruit attached to stems.

It seems likely that there are ice nucleating sites associated with stems. The nature of these sites has not been identified. Pseudomonas syringae tends to colonize younger tissues such as the developing fruit, yet in the absence of INA bacteria the developing fruit supercooled readily if detached from the stem. If surface bacteria are controlled there may still be ice nucleating sites in the wood that are active at —2° to —3°C. Furthermore, the evidence is strong that ice crystals, once initiated, can grow through the vascular system into the developing fruit. If this is the case, obtaining frost protection by promoting supercooling will require that the ice nucleating sites in the wood be neutralized at the same time INA bacteria are controlled.

The ability to supercool detached fruit by as much as 5 to 8°C suggests that highly effective frost control could be achieved if ice nucleation could be completely controlled. A more realistic goal would be to obtain 1° of supercooling. This would provide a useful amount of protection from late spring frosts for the highly susceptible young developing fruits. At this stage in mild frosts, fruit exposed to the sky may cool below freezing while stem tissue remains above freezing. One would expect reduced frost marking in this case from controlling INA bacteria. Earlier in the spring, before full bloom for most species, some freezing can be tolerated without crop loss. One degree of supercooling would be of little help at these earlier stages when considerable frost injury is incurred in central Washington orchards. Furthermore if nucleation occurs, supercooled tissues may result in more serious injury than non-supercooled tissues because the rate of freezing would be faster.

Frost control by promoting supercooling deserves further intensive research to solve problems that are inherent in deciduous fruit production.

Table 4. Effect of topically applied INA bacteria on ice nucleation temperatures (°C) of attached and detached fruits.

<table>
<thead>
<tr>
<th>Species</th>
<th>Attached</th>
<th>Detached</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet cherry</td>
<td>-2.5°C a</td>
<td>-8.7°C b</td>
</tr>
<tr>
<td>Pearson</td>
<td>-2.9 a</td>
<td>-6.2 b</td>
</tr>
<tr>
<td>European plum</td>
<td>-1.9 a</td>
<td>-3.3 a</td>
</tr>
</tbody>
</table>

Mean separation within species by Duncan’s multiple range test, 5% level.

Table 2. Mean temperatures and range for ice nucleation in 4 peach fruit attached to a single stem segment (2.5 cm) compared with 4 fruit attached to 4 separate segments (2.5 cm).

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit attached to a single segment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-2.2°</td>
<td>-6.0°</td>
<td>-5.0°</td>
<td>-4.2°</td>
<td>-4.3°</td>
<td>3.8°</td>
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<tr>
<td>Range</td>
<td>0°</td>
<td>0.5°</td>
<td>0°</td>
<td>0.8°</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Fruit attached to 4 segments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-3.3°</td>
<td>-3.8°</td>
<td>-3.8°</td>
<td>-3.3°</td>
<td>-3.6°</td>
<td>0.5°</td>
</tr>
<tr>
<td>Range</td>
<td>3.4°</td>
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<td>3.4°</td>
<td>2.8°</td>
<td>2.8°</td>
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</tr>
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</table>

Mean separation within species by Duncan’s multiple range test, 5% level.

Literature Cited