Relation of Ethylene and Cellulase Activity to Abscission of Freeze-Injured Citrus Fruit

Roger Young

Agricultural Research Service, U. S. Department of Agriculture,
Orlando, Florida

Abstract. Freeze-injured citrus fruit produced above-normal amounts of ethylene 1 to 4 days after injury. Elevated ethylene levels were often found in fruit 3 weeks after injury. Cellulase activity in the abscission zone increased 4 to 8 days after injury and preceded abscission. Some severely injured fruit that did not abscise were responsive to abscission-inducing chemicals. High internal ethylene content did not correlate as well with abscission as did high rates of abscission-zone cellulase activity.

Evidence strongly indicates that ethylene plays a mediating role in leaf and fruit abscission (1, 2, 3, 11, 12). Cellulase synthesis is induced by ethylene, and its synthesis in the abscission zone precedes abscission (2,12). Factors which affect stress conditions in citrus have been shown to induce above-normal ethylene evolution. These include injuries by chilling (9), freezing (10), chemicals (5, 7, 8), and salt (14). Citrus grown in the United States is periodically exposed to subfreezing temp, and injury to the fruit often leads to abscission.

This report relates ethylene and cellulase synthesis in freeze-injured fruit to subsequent abscission.

Materials and Methods

Plant materials. Citrus cultivars included in this study were ‘Pineapple’, ‘Valencia’, and ‘Hamlin’ oranges [(Citrus sinensis (L.) Osb.] and ‘Temple’ orange (C. reticulata Blanco) hybrid. Fruit from 2 mature bearing trees in each orchard planting were used for analysis.

Description of freezes. Two freezes with min temp in the mid-to-low twenties severely injured citrus fruit in Central Florida in 1970 and 1971. The 1st freeze, on November 25, 1970, injured fruit in some groves, while the 2nd freeze, on January 21, 1971, caused extensive fruit injury throughout Central Florida. Fruit from trees of ‘Pineapple’ oranges, injured in both freezes, and ‘Valencia’, ‘Hamlin’, and ‘Temple’ oranges, injured only in the 2nd freeze, were sampled periodically for analysis.

Ethylene measurement. Internal ethylene content (ppb) of 10 fruits from each tree sampled was measured by gas chromatography (GC). A 2-ml internal air sample from under the abscission zone of the fruit was removed with a syringe and the ethylene content determined as previously reported (13).

Cellulase activity. Cellulase was determined by methods similar to those reported by Abeles (2). Ten fruits were used for each sample. Two-mm transverse sections containing the abscission zone of each set of 10 fruit were chopped and homogenized in 10 ml of 0.05M potassium phosphate buffer (pH 7.0). The homogenate was filtered through miracloth, and 1 ml of the homogenate (enzyme solution) and 1 ml of 1.5% carboxymethyl cellulose (CMC) solution were incubated at 40°C in viscometer heads. Readings were taken at 10 and 30 min. The results are presented as percent change in viscosity (%An/hr), compared with a blank that contained 1 ml of CMC and 1 ml buffer without enzyme.

Abscission measurements. Fruit abscission was determined by estimating the percent fruit drop and by measuring the fruit removal force, or pull force, with a Chatillion pull tester. The 10 fruits used for pull force determinations were also used for measuring internal ethylene contents.

In addition, 1 test was conducted on severely frozen fruit which had not abscised to determine if freezing had destroyed the abscission mechanism. On February 1, 1971, ‘Pineapple’ orange fruit, which had been frozen November 25 and January 21, were detached with 6-inch stems and treated at room temp (with stems in water) with 1 of the following chemicals: 10 ppm ethylene, 500 ppm ethephon, and 20 ppm cycloheximide. Ten fruits were used for each chemical treatment. Ethephon and cycloheximide were applied by dipping the fruit in a solution for 1 min. Other fruit were exposed to 10 ppm ethylene gas for 4 days in a chamber at room temp. Abscission activity was followed by the methods just described.

Results and Discussion

Internal ethylene, cellulase, pull force, and fruit drop changed in ‘Valencia’, ‘Temple’, and ‘Hamlin’ oranges frozen on January 21, 1971 (Figs. 1, 2, 3). Above-normal ethylene levels occurred in frozen fruit within 4 days of the freeze. High levels of ethylene occurred in ‘Hamlin’ oranges while low levels occurred in the ‘Valencia’ and ‘Temple’ oranges even though...
cellulase activity between 23 and 37% n/hr or a net change from unfrozen fruit of 24 to 30% n/hr. Cellulase activity of unfrozen 'Valencias' and 'Temples' did not vary greatly after the freeze.

Fruit pull force readings decreased rapidly after freeze injury and simultaneously with increased cellulase activity. Fruit drop began to occur when the avg pull force readings approached 8 lb.

An abscission response did not always occur after freezing or where ethylene formation was stimulated by freezing. Internal ethylene, cellulase, and pull force of 'Pineapple' oranges were measured subsequent to freezing on November 25, 1970, and again January 21, 1971 (Fig. 4). Ethylene, cellulase, and pull force did not change materially in 'Pineapples' after the severe injury did occur. Stage of maturity might be somewhat of a factor, as fruits of 'Valencia' and 'Temple' are higher in acid content than 'Hamlin'. Unfrozen fruit of 'Valencia' and 'Temple' oranges did not have any material change in interval ethylene during the same time period after the freeze.

Cellulase activity increased 4 to 8 days after freeze injury and on, or subsequent to, peak-ethylene formation. Abscission of 'Valencia', 'Hamlin', and 'Temple' oranges was associated with

Table 1. Effect of ethylene and ethylene-producing chemicals on abscission of severely frozen 'Pineapple' oranges on detached stems.2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial</th>
<th>4 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>15.5</td>
<td>10.7</td>
</tr>
<tr>
<td>10 ppm ethylene</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>500 ppm ethephon</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>20 ppm cycloheximide</td>
<td>2.1</td>
<td></td>
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<tr>
<td>L.S.D.</td>
<td>3.0</td>
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Ethylene, cellulase, and abscission measurements in fruit following injury by freezing are consistent chronologically with the hypothesis that freeze-induced ethylene may induce...
cellulase activity in the abscission zone which, in turn, loosens the fruit. The role of ethylene-induced cellulase in naturally and chemically induced citrus fruit abscission has been reported (12). This freeze-induced system is analogous to the proposed abscission mechanism of fruit sprayed with ascorbic acid and cycloheximide (6, 7). Cooper (4) has reported evidence suggesting that both rind and juice vesicles contain wound-stimulated ethylene-producing systems, and that freeze injury will activate ethylene synthesis (10).

The failure of severely desiccated ‘Pineapple’ fruit to abscise after injury from 2 freezes was probably due to a lack of sufficient cellulase activity in the abscission zone 4 to 10 days after the freeze. These fruit did not abscise 11 days after the freeze when the rinds were treated with the wound-ethylene-inducing wound-stimulated ethylene-producing systems, and that freeze freezing is consistent with many observations, and indicates variability in abscission response following severe injury from translocation. After the November 25 freeze, these fruit began to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a sufficient hinderance to desiccate which may have been a 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