EFFECTS OF SUB-FREEZING TEMPERATURES ON THE
VIABILITY OF CITRUS SEED

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The low temperatures during the winter of 1957-58 have caused citrus nurserymen and seed dealers to wonder how much reduction of viability may occur in citrus seeds collected after a severe freeze. Batchelor and Webber (1) state that "the seeds from frost-injured fruits are evidently as good to use as those from unfrosted fruits. This was found to be true with frozen fruit in Florida after the very severe freeze of 1894-95." This very general statement does not delineate the extremes of low temperature that citrus seeds can withstand, and it is certain that there is some limit. Damage might take place in two ways: (1) direct injury to the seeds by low temperatures, and (2) injury due to fermentation within freeze-damaged fruit. In the latter case membranes and cell walls are ruptured by freezing, allowing the fruit juice to come into direct contact with the seeds. The fermenting juice could, conceivably, cause injury to the delicate citrus seed embryo.

It is well known that seeds of stone fruits, particularly those of peaches, are easily injured by even short exposures to the fermenting juices of their fruits. Such seeds may appear to be sound and bright in color and have normal-appearing cotyledons for many weeks after the fermentation exposure. They simply do not germinate and eventually rot in the seedbed. We have experienced one similar disaster with a valuable lot of Carrizo citrange seeds allowed to remain in a bucket over the weekend, wet and surrounded by their gelatinous coating. After 3 days of fermenting in this state they were washed and carefully stored, but upon planting only a few seeds of many thousands germinated. Apparently this is not a common experience with citrus seeds generally, for Hume (2) writes regarding methods of securing seeds from fruit, "Another method is to place the fruit in barrels and allow it to rot, after which the seed is washed free from the pulpy mass."

PROCEDURES

To gain information on the effect of low temperatures and of fermentation on citrus-seed viability, we devised a number of simple experiments. Seeds of the commonly used root-stock sorts—Rough lemon, Cleopatra mandarin, sour orange, and sweet orange (chiefly Pineapple variety)—were used. Seeds of the various sorts were extracted from sound fruit, washed, and surface-dried for the several experiments. In each test 100 seeds were used, and the germination results were expressed as percentages.

The germination tests were made by planting the treated seeds in flats containing a peat moss-vermiculite mixture in the greenhouse. The seeds were carefully spaced so that an accurate germination count could be made for each seed regardless of the number of nucellar embryos which sprouted. At this point it should be mentioned that seeds germinating with multiple embryos were also counted, but the degree of polyembryony was not influenced by any of the treatments and is therefore omitted from the data presented.

Direct effect of low temperatures. Three lots of 100 seeds each of Pineapple orange were put at once in polyethylene bags to prevent moisture loss. Three other lots of 100 seeds each were allowed to dry on the laboratory desk for 24 hours, during which period they lost 25.3% of their moist weight. A third group of 3 lots were similarly dried for 48 hours, and thereby they lost 32.7% of their moist weight. A third group of 3 lots were similarly dried for 48 hours, and thereby they lost 32.7% of their moist weight. These seeds were then also placed in polyethylene bags and one lot of each treatment placed in carefully controlled temperature
chambers for 24 hours at 15°, 20°, and 25°F., respectively. Upon removal from the freezing chambers the seeds were planted for germination counts. The results are shown in Table 1.

It is evident that 25°F. did not harm the fully moist seeds nor those dried beforehand for 24 hours. Seeds in both of these prior treatments, however, were considerably reduced in viability by 20°F. and were killed by 15°F. As for the seeds dried for 48 hours, it is not clear from the data whether the marked impairment in germination after exposure to 20 and 25°F. is the result of cold injury or drying injury or both. Although it is well known that citrus seeds lose viability rapidly with increased desiccation, it seems unlikely that the additional 24 hours of drying would in itself affect germination so adversely.

Effect of juice fermentation. Sweet orange seeds (var. Pineapple) were extracted from the fruit, placed in beakers, and covered with juice of their fruits. The respective germination percentages after fermentation for 0, 2, 4, and 6 days were 95, 96, 89, and 88. A slight decrease in germination from the 4- and 6-day treatments is indicated. It does not follow that other kinds of citrus seeds would be similarly affected.

Seeds from frozen fruits. To determine the effect of low temperatures on seeds still in the fruit, as well as the effect of subsequent fermentation within the frozen fruit, sound fruits of Cleopatra mandarin, Rough lemon, sour orange, and sweet orange were placed in cold storage rooms at 28° and 18°F. for 12 hours. At these storage temperatures there was, of course, a progressive temperature drop in the interior of the fruits, which was observed by means of thermometers inserted in the fruit. At 28°F. the interior of the fruit reached the storage temperature within a few hours and remained at 28 ± 1° for the rest of the storage period. The interior of the fruit was much slower in attaining the storage temperature of 18°. Cleopatra mandarin, which is a small, thin-skinned fruit, was below 20°F. for 5 hours. Rough lemon, larger and thick-skinned, did not reach 20°F. until the last 2 hours of storage. Thus the seeds of the several kinds of fruit were not all subjected to the same length of exposure. At both 18° and 28°, however, the fruits were frozen and broke down when removed from the cold rooms and held on the laboratory desk. Those from the 18°F. storage broke down faster and more completely than those from 28°F. One hundred seeds to be used as controls were removed from each variety of fruit prior to placement in the low-temperature rooms. Additional lots of 100 seeds were removed from the fruits immediately upon thawing and also after 7 days and 14 days on the laboratory desk. The germination results from these treatments are presented in Table 2.

Table 1. Percent germination of sweet orange seeds after exposure of 24 hours at the temperature indicated

<table>
<thead>
<tr>
<th>Pre-freezing treatment</th>
<th>15°F.</th>
<th>20°F.</th>
<th>25°F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>70</td>
<td>94</td>
</tr>
<tr>
<td>Dried 24 hours</td>
<td>0</td>
<td>74</td>
<td>93</td>
</tr>
<tr>
<td>Dried 48 hours</td>
<td>1</td>
<td>9</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 2. Percent germination of citrus seed extracted 0, 7, or 14 days after fruit had been 12 hours in 18° and 28°F. storage

<table>
<thead>
<tr>
<th>Variety</th>
<th>Control (no freezing)</th>
<th>Immediately after thawing after</th>
<th>Holding for 7 days after</th>
<th>Holding for 14 days after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28°</td>
<td>18°</td>
<td>28°</td>
<td>18°</td>
</tr>
<tr>
<td>Cleopatra mandarin</td>
<td>98</td>
<td>96</td>
<td>79</td>
<td>98</td>
</tr>
<tr>
<td>Rough lemon</td>
<td>96</td>
<td>95</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>Sour orange</td>
<td>95</td>
<td>92</td>
<td>84</td>
<td>87</td>
</tr>
<tr>
<td>Sweet orange</td>
<td>97</td>
<td>90</td>
<td>94</td>
<td>93</td>
</tr>
</tbody>
</table>
In comparing the germination of the controls (seeds from untreated fruit) with that of seed extracted immediately after thawing, it is evident that at 28°F. there was no impairment of germination. At 18°F., however, the Cleopatra mandarin and sour orange seeds suffered some cold damage, whereas the Rough lemon and sweet orange seeds were not injured. The Cleopatra mandarin fruits, as already noted, were small, and their interior temperature drop was more abrupt than that of the larger fruited varieties. Difference in fruit sizes, however, does not explain the seeming damage to the sour orange seeds.

Any impairment of germination of seeds removed from the fruit 7 and 14 days after freezing would be attributed to a fermentation effect in the decomposing fruit. Sour orange and sweet orange seeds in both the 18° and the 28° lots apparently suffered damage from this effect after 14 days in the injured fruit. Cleopatra mandarin and Rough lemon seeds appear not to have been affected by the fermentation process (extracted immediately compared with 14 days after thawing).

**Summary and Conclusions**

Seeds of sweet orange extracted from sound fruit and placed at controlled temperatures of 25, 20, and 15°F. for 24 hours were not injured at 25°, partially damaged at 20°, and practically all killed at 15°.

Seeds taken from fruit which had been severely frozen had reduced viability if the temperature within the center of the fruit reached a critical temperature for a sufficient period. These limits were not definitely established in this study because of the differences in the times for fruits of the several varieties to reach the minimum exposure temperature at the center. However, with fruit exposed to 28°F. for 12 hours none of the varieties suffered seed damage. Seeds of Cleopatra mandarin and sour orange from fruit exposed for 12 hours at 18°F. showed some reduction of viability, but those of Rough lemon and sweet orange did not. Presumably this varietal difference was largely due to differences in rate of fruit cooling. From a practical standpoint the experiment demonstrated that citrus seeds can be cold-injured in the fruit by a severe freeze but that for this to happen temperatures of 20°F. and lower would have to be of considerable duration.

It was also demonstrated that seed viability can be reduced in some varieties by fermentation within fruit which has been badly frozen even though the actual temperatures are not low enough to injure the seed. In the work reported here the viability of both sweet and sour orange seed was lowered, but not that of Cleopatra mandarin or Rough lemon, by 14 days of fermentation in badly frozen fruit.

**LITERATURE CITED**


**INFLUENCE OF VARIOUS ROOTSTOCKS ON THE COLD RESISTANCE OF THE SCION VARIETY**

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Numerous observations in citrus plantings support the general belief that the rootstock frequently influences the cold resistance of the scion variety. In general these observations place the cold hardiness effect of the commonly used stocks in the descending order as follows: *P. trifoliata*, sour orange, Cleopatra mandarin, sweet orange, and Rough lemon. It is well known that this is the same order in which the un budded stocks themselves tolerate low temperatures, and this agreement might lead one, perhaps erroneously, to the conclusion that a stock which is itself cold-resistant would automatically confer resistance on the scion variety. The relationship is probably not so simple. If, as generally agreed, cold resistance of the scion variety is determined chiefly by its degree of dormancy at the time of the low temperatures, then any rootstock which confers dormancy on the scion should thereby