AFTER-RIPENING AND GERMINATION OF APPLE SEEDS

By George T. Harrington, formerly Scientific Assistant, and Bertha C. Hite, Scientific Assistant, Seed-Testing Laboratories, United States Department of Agriculture

When first matured and in intact condition, apple seeds are wholly incapable of germinating. Furthermore, as will be shown later, they do not acquire the power to germinate under the ordinary conditions for the storage of dry seeds, or under germination conditions at moderate or warm temperatures.

It is the custom of nurserymen to layer apple seeds in sand and put them outdoors over winter. While this practice probably brings about good germination the following spring, Eckerson (8) has shown that exposure to freezing temperatures is not at all necessary, as the seeds germinate well after being kept moist for a few months at 5° to 6° C. The present paper deals further with the effect of storage conditions, including the presence or absence of the seed coats and inclusion within or removal from the fruit, on after-ripening and germination.

In the germination tests reported in this paper, usually 25 or 50 seeds, and sometimes 100 or 200 seeds, were used either in single tests or in duplicate tests, according to the number of seeds which were available. The tests were made in Petri dishes with moist blotting paper or absorbent cotton as seed bed.

AFTER-RIPENING AT LOW TEMPERATURES

1. On October 28, 1918, Black Ben Davis apple seeds, which had never been allowed to become air dry, were thoroughly washed, sterilized by treating for two or three minutes with 1 per cent silver nitrate, washed again in running water, which carried chlorids enough to precipitate the silver still remaining on the coats, placed on moist blotting paper in a large Petri dish, and put away in an ice box where the temperature varied between about 5° and about 10° C. By January 15 (two and one-half months) they had begun to germinate in the ice box. Ungerminated seeds from this lot in the ice box were then put to germinate at 20° and at 25°. At each of these temperatures over 50 per cent of the seeds germinated in the next few days. The rest were used for catalase or respiration studies, so that complete germination tests were not obtained.

2. On January 29, 1919, seeds of a mixture of varieties which had been removed from a cider press mash two months earlier and kept in dry storage during the intervening period were washed, sterilized with silver nitrate, and put to germinate at 20° and 30° C. Seeds from the same lot were washed, sterilized, and put away in the ice box to after-ripen.

In the ice box those seeds which did not decay on account of injury in the press after-ripened completely in three or four months, and many germinated during this time, while still in the ice box. At the higher temperatures, however, about 90 per cent of those seeds which did not decay were

1 Accepted for publication July 2, 1921.
2 Reference is made by number (italic) to "Literature cited," pp. 160-161.
still dormant after six months, during which time they had received the following treatments: (1) The outer seed coats were removed from all the seeds at the end of 50 days; (2) for the next 68 days those previously at 30°C were given a daily alternation between 20° and 30°, while those previously at 20° remained at 20°; (3) both lots were then used in a respiration experiment which lasted two months, and in which the temperature was frequently changed, using temperature intervals of 19° to 30°, 30° to 19°, 19° to 10°, 10° to 0°, 0° to 13°, 13° to 30°. Besides these temperature changes the seeds were washed in cold water between each two periods of the respiration experiment, or 20 times in all. None of these treatments had induced after-ripening or germination as had incubation in the ice box.

3. Seeds of the same original lot as the foregoing, which were incubated for 50 days at 20°C in intact condition, and then 68 days in the ice box with the outer seed coats removed, after-ripened completely during this time in the ice box, so that all germinated in 4 days at 19°C. As shown in the preceding paragraph, seeds which were given identical treatment except that they were at 20° while these were in the ice box remained dormant.

Seeds of the same original lot kept in dry storage for one year and then put to germinate at 16°C, 20°C, and 23°C in intact condition, with outer coats removed and with both coats removed, failed to germinate. With both seed coats removed, all embryos decayed within a week without showing any of the signs of life which were exhibited by living embryos that were not after-ripened.3

In the preceding paragraphs, apple seeds have been shown to after-ripen when kept moist in the ice box after three conditions of previous treatment:

(1) Seeds incubated in the ice box soon after removal from the fruit and without previously being allowed to dry out. (2) Seeds stored dry for two months before the ice-box incubation. (3) Seeds stored dry two months and then incubated two months at 20°C previous to ice-box incubation. In each case concurrent germination tests showed that the seeds had not after-ripened under any other circumstances than storage in moist condition in the ice box. Dry storage previous to the germination test, removal of the outer seed coats, and alteration of temperatures during the germination test all failed to induce germination. Furthermore, attempts to force germination by etherizing the seeds were not successful.

We have found that seeds of another species of Pyrus, which were received from the Federal Horticultural Board of the United States Department of Agriculture, incapable of germinating when received by us, after-ripen in the ice box very much the same as the seeds of our cultivated apples.

AFTER-RIPENING IN THE FRUIT DURING COLD STORAGE

In the cases discussed hitherto, the seeds were removed from the fruits and after-ripened in the ice box under good conditions of moisture supply and aeration, the Petri dish covers being frequently removed to facilitate gaseous exchanges. But this was also true of those seeds which were incubated at higher temperatures, at which they failed to after-ripen.

3 For description of the seed coats and the effect of their removal see p. 157.
The effect of the low temperature upon after-ripening, as previously reported by Eckerson (8) and now verified by us and as reported by Appleman (1), Crocker (5), Davis and Rose (7), and Davis,1 and Jones (2), for other kinds of seeds, suggested that apple seeds might after-ripen also in cold storage within the intact fruit. Ascherson (2), in fact, found a germinated apple seed in an undecayed fruit near the end of June. It seems certain that, in order to have remained sound so late in the year, this apple must have been kept where it was very cool most of the time. At any rate, the seed had become capable of germinating while within the fruit.

Through the kindness of Dr. Brooks and Dr. Cooley of the Office of Fruit Pathology of this Department, material was secured for a study of the germination of seeds from cold-stored apples of several varieties. Table I summarizes the results.

A very small percentage of the seeds of varieties stored at 0°C until February 5, 1919, and a somewhat higher percentage (not more than 3 per cent) of Newtown Pippin seeds stored at 0°C until May 7, 1919, had begun to germinate intracarpially by the time they were taken out of the fruit a few days after taking the apples out of cold storage. When put to germinate at 25°, a few days after removal from the fruits, about one-fifth to one-half of the seeds germinated promptly and vigorously. In every case, however, there was very little germination at 25° after the very incomplete germination of the first few days. In the ice box, on the contrary, germination started rather slowly but progressed gradually until practically complete in about three weeks. The temperature of the ice box occasionally rose considerably above 10° for a short time but was usually between 5° and 10°. At 20° germination was nearly complete in two weeks.

Table I.—Germination of apple seeds after-ripened in cold storage and ice box

<table>
<thead>
<tr>
<th>Variety</th>
<th>Previous storage, successive periods.</th>
<th>Germination.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room temperature.</td>
<td>Ice box.†</td>
</tr>
<tr>
<td>Newtown Pippin</td>
<td>(Oct. 29 to May 7, 190 days.)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>(Oct. 29 to May 7, 150 days.)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(Nov. 28 to May 7, 190 days.)</td>
<td>7</td>
</tr>
<tr>
<td>York Imperial</td>
<td>Oct. 1 to Feb. 5, 127 days.</td>
<td>10</td>
</tr>
<tr>
<td>Grimes Golden</td>
<td>To Feb. 10</td>
<td>5</td>
</tr>
<tr>
<td>Jonathan</td>
<td>Oct. 1 to Feb. 5, 127 days. 3</td>
<td>10</td>
</tr>
</tbody>
</table>

1 Some seeds had already germinated in the ice box before the beginning of the germination tests. Only those not yet germinated were included in the tests.
2 Most of the time at room temperature the seeds were within the fruits; sometimes dry-stored a few days after taking out. In the ice box the seeds, freed from the fruits, were incubated as for germination.
3 During the last two or three weeks of this time the Jonathan apples were kept at 5°C.
The germination of Newtown Pippin seeds at 25° C. decreased with increase in the length of time the seeds were kept at room temperature after cold storage in the fruit. Also York Imperial and Jonathan seeds which had been at room temperature for 10 days germinated only half as well at 25° as Grimes Golden seeds which had been at room temperature only 5 days. During the period at room temperature the seeds were either still inclosed in the fruit, or, if removed for a part of the time, were not allowed to become entirely air-dry. While the data at hand are not conclusive, the behavior of these seeds suggests that they were acquiring the condition of secondary dormancy which has been induced in a number of kinds of seeds by keeping the fully germinable seeds under conditions unfavorable for germination. Atwood (3), Crocker (5), Crocker and Harrington (6), and Zade (23, 24) have called attention to the assumption of secondary dormancy by various seeds in which the embryo is always capable of germination and dormancy is imposed by coat structures. Davis (5) and Jones (13) also have demonstrated the occurrence of this phenomenon in seeds in which, as in the apple seed, the dormant embryo itself is incapable of germination, and in which coats play only a secondary rôle in dormancy.

In January, 1920, seeds from Snow apples, which had been kept in a cold cellar in Vermont until early in January and were then sent by express to Washington, D. C., were put to germinate at 16°, 20°, 23°, and 27° C., and in an ice box where the temperature was about 4° to 5° for a few days and then increased gradually to about 10° at the end of the second week and 12° at the end of the third week. Table II shows their germination.

In the ice box all of the seeds germinated in three weeks, although there was no germination in the first 10 days when the ice box was very cool. The percentage of germination decreased regularly with increase in germination temperature. No seeds germinated after the first 7 days at temperatures above 20° C. and only a small percentage even at 16° C.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>7 days</th>
<th>10 days</th>
<th>13 days</th>
<th>14 days</th>
<th>16 days</th>
<th>19 days</th>
<th>21 days</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice box</td>
<td>0</td>
<td>0</td>
<td>42</td>
<td>36</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>16° C</td>
<td>52</td>
<td>12</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>74</td>
</tr>
<tr>
<td>20° C</td>
<td>48</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>56</td>
</tr>
<tr>
<td>23° C</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>27° C</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

1 Temperature 4° to 5° C. for about a week, then gradually rising to about 10° at the end of the second week and 12° at the end of the third week.

RELATION OF OXYGEN SUPPLY TO AFTER-RIPENING

The fact that apple seeds after-ripen and sometimes even begin to germinate within the apples while these are still sound raises the question of the relation of oxygen to the processes of after-ripening. Atwood (3) has shown that the access of free oxygen in abundant supply is necessary

---

for the after-ripening of wild oats, while Kondo (18) has shown that abundant oxygen accelerates the after-ripening of rice, which, when fully after-ripened, will, according to Takahashi (22), germinate in entire absence of oxygen. Oxygen has also been shown by Kiessling (17) to accelerate after-ripening in cereals under certain conditions, and Hoffman (12) has put forth the hypothesis that after-ripening of the cereals consists essentially of a storage of oxygen. Hiltner (11) and Kiessling (17) have also shown, however, that cereals after-ripen, though sometimes rather slowly, in closed vessels in almost entire absence of oxygen. If free oxygen is essential for after-ripening in this case, the seeds must be capable of using it when present in very low partial pressures, and very small quantities must suffice. Recent and earlier work by one of us (9, 10), as well as Atwood’s work on wild oats, indicated that the embryos of the cereals and some other grasses are never essentially dormant, the dormancy of the caryopses being imposed by coat structures which, in the wild oat at least, limit oxygen supply. Crocker (4) found a similar condition in Xanthium seeds.

The case is different with apple seeds, in which the embryos are dormant and require time-consuming changes before they can germinate, even if freed from all surrounding structures. But with apple seeds we have found very low respiratory quotients at low temperatures which favor their after-ripening. There is, therefore, in apple seeds a storage of oxygen during after-ripening. Jones (13) has shown that the after-ripening of the similarly dormant embryos of sugar maple seeds is retarded, though not prevented, by inclosing them in desiccators, so that oxygen is rapidly replaced by respired carbon dioxide. Recent measurements by Magness (19) show that the concentration of oxygen within the core of the apple is not much below that of the surrounding atmosphere. The oxygen supply available to the seeds while inclosed within the fruit cannot, therefore, be considered physiologically deficient for their after-ripening processes, even if these depend upon a much greater abundance of oxygen than seems to be necessary for the after-ripening of sugar maple seeds.

EFFECT OF REMOVAL OF THE SEED COATS

The coverings of the embryos in a mature apple seed consists of: (1) A thick, brown, fibrous outer seed coat with open hilum; (2) a thin, translucent inner seed coat of very dense structure and without openings; and (3) a delicate, whiteish, cellular tissue, somewhat thicker than the inner coat, and apparently endospermous. Layer 3 is closely adherent to layer 2, and it is impossible to remove the two separately, though the embryo is easily bared by removing them together.

Removal of the outer seed coat has no effect on the germination of apple seed previously dry-stored or incubated for a short time at temperatures unfavorable for after-ripening. When the inner seed coats and adhering endosperm tissue are removed from such seeds, baring the embryos, many of the cotyledons enlarge slowly and, if in the light, become green. After a week or two some of the radicles show geotropic bending and slow elongation, but normal growth does not occur.

When one or both of the seed coats are removed from after-ripened seeds germination is accelerated even under conditions under which the seeds would germinate in a few days if the coats were left intact. An example of this is furnished by Newtown Pippin seeds which were put
to germinate at 19°C. after cold storage in the fruit until May 7, 1919, followed by removal from the fruit and incubation in the ice box for about a week. With the outer coats removed 67 per cent germinated in 3 days and 100 per cent in 6 days; with the outer coats left on 26 per cent germinated in 3 days and about 50 per cent in 6 days.

The effect of the removal of the seed coats from seeds which have previously been incubated for a considerable time at temperatures unfavorable for after-ripening, and which are still incapable of germinating in intact condition is of special interest. Seeds of the cider press lot, which had remained dormant under germination conditions for 6 months—4 months with outer coats removed—all germinated in 3 days after removal of the inner seed coats.

With intact York Imperial and Newton Pippin seeds, which had been dormant under germination conditions for more than 5 months and more than 2 months, respectively, the respiratory intensity was increased more than four-fold in 24 hours by removal of the outer seed coats. The inner seed coats were then removed and all of the seeds germinated in the next 2 days. It is evident that these embryos were in a very different condition when freed from the coats than were the embryos of the cider press lot which were freed from the coats soon after putting to germinate 6 months earlier, and which then failed to germinate. Yet as far as ability to germinate with the coats on is concerned they showed no difference. There is here a joint action of the coats and of the condition of the embryo in imposing dormancy, whereas in the dry-stored seeds the same result is produced without the coats. It seems, therefore, that some part of the complex of processes which constitute after-ripening must go on both at the higher temperatures and at the after-ripening temperatures. Apparently other processes, perhaps consisting essentially in the removal of inhibitors to germination (which can also be dissipated or oxidized upon removal of the coats) take place only at the lower temperatures.

In this connection Kidd's work (14) on CO₂ and Maze's (20, 21) on acetic aldehyde as inhibitors to germination are of interest. Experiments show that there is a free interchange of oxygen and CO₂ responding in characteristic ways to differences in temperature, in the respiration of dormant apple seeds. This makes it seem unlikely that inhibiting concentrations of CO₂ accumulate within their coats. That some other inhibiting substance is produced at higher temperatures and is held in by the coats, or else, being initially present, is removed at lower temperatures, seems more plausible; but no work was done to test the validity of such an assumption.

An alternative hypothesis is Kidd and West's conception (15) of a mechanical stimulus according to which dormant white mustard embryos are considered as in a state of very delicate equilibrium from which they are aroused to activity by the mechanical shock resulting from removal of the coats, without reference to any causal chemical change.

The inner seed coats are very effective in preventing decay of the embryos during the after-ripening, whereas the thick hard outer seed coats with their open hila are of little value in this respect. This was shown by seeds of the cider press lot, many of which had been so injured that it was impossible to prevent severe decay in the incubator even after carefully discarding all seeds showing visible injury and surface sterilization of the remaining seeds. After 50 days' incubation at 20°C, 25°C, and
30° C., about 50 per cent of these seeds were wholly decayed, and the rest were entirely obscured by a dense mass of all sorts of micro-organisms. Observations a few days after first putting these seeds into the incubators indicated that in general only those seeds decayed in which the inner coat was injured. At the end of 50 days the outer coats of all the seeds were so softened and decayed that they were easily rubbed off between the thumb and fingers in water. After thorough washing, these seeds, with the outer coats removed but the inner coats intact, were returned to the incubators where almost all of them remained in good condition during the following 4 months, although during a part of that time they were on moist blotters thoroughly infected with organisms of decay.

SUMMARY

(1) Apple seeds, when taken from the apples at their maturity, are incapable of germination. Etherization or the use of alternating temperatures does not bring about germination.

(2) The dormancy is resident in the embryo. Naked embryos fail to germinate normally.

(3) Apple seeds acquire the power to germinate—that is, they after-ripen, in a few months when kept moist at a temperature between 5° and 10° C. They also after-ripen within the fruit in commercial cold storage (0° C.) or in a cold cellar. They do not after-ripen in dry storage or when kept moist at 20° C. or at a higher temperature.

(4) The relation of oxygen to after-ripening was not determined, but apparently a good supply of oxygen is always present within the core of the apples when they are kept at low temperatures.

(5) After-ripened seeds will germinate completely in a few weeks in an ice box if the temperature is not too low. They germinate fairly well at 20° C., but not as well at 25°. The optimum temperature for their germination seems to lie somewhere between 10° and 20° and to vary according to the condition of the seed or possibly according to the variety of apple.

(6) There seems to be a tendency for the after-ripened seeds to go into a state of secondary dormancy when kept under conditions which prevent their germination.

(7) The commercial practice of layering apple seeds out of doors over winter is not necessary in order to bring about their complete after-ripening and germination.

(8) Removal of the outer seed coat has no apparent effect on completely dormant apple seeds. Removal of both seed coats causes some of the dormant embryos to make feeble growth, but these do not produce normal seedlings.

(9) Removal of the outer coat or of both coats accelerates the germination of after-ripened seeds.

(10) Removal of the coats from seeds which have been incubated for a long time under germination conditions, but at a temperature too high for complete after-ripening, may induce prompt and vigorous germination. Some phases of after-ripening must therefore take place at these higher temperatures, while others are dependent upon a lower temperature, or upon the removal of the coats.

(11) The inner seed coat is very efficient in preventing decay of the seeds, but the outer seed coat, with its open hilum, is of little use in this respect.
LITERATURE CITED


(19) **Magne, J. R.**
1920. **Composition of gases in intercellular spaces of apples and potatoes.** *In* Bot. Gaz., v. 70, no. 4, p. 308-316, 1 fig. Literature cited, p. 316.

(20) **Mazé, P.**

(21) **Takahashi, T.**

(22) **Zade, Adolph.**