

A STUDY OF AFTER-RIPENING IN CERTAIN
FRUIT TREE SEEDS

by
Irvin C. Haut.

LIBRARY, UNIVERSITY OF MARYLAND

Thesis submitted to the Faculty of the Graduate
School of the University of Maryland,
in partial fulfillment of the
requirement for the degree
of Doctor of Philosophy.

1 9 3 3.

UMI Number: DP70113

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



UMI DP70113

Published by ProQuest LLC (2015). Copyright in the Dissertation held by the Author.

Microform Edition © ProQuest LLC.

All rights reserved. This work is protected against unauthorized copying under Title 17, United States Code



ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

TABLE OF CONTENTS

	Page
INTRODUCTION, - - - - -	1
REVIEW OF LITERATURE, - - - - -	2
GENERAL METHODS AND MATERIALS, - - - - -	6
RESULTS, - - - - -	7
Part I -- Germination Studies, - - - - -	7
Effect of Drying Prior to After-Ripening, - - - - -	7
Effect of Drying Following the After-Ripening Period, - - - - -	9
Effect of Temperature and Length of Time on After-Ripening, - - - - -	13
Moisture Content of the Medium in Relation to After-Ripening, - - - - -	16
Effect of Pulp Disintegration on Seed Viability,- Treatment with Chemicals Designed to Break the Rest Period, - - - - -	17
Conclusions from Part I - - - - -	19
Conclusions from Part I - - - - -	23
Part II -- Chemical Studies, - - - - -	25
Analytical Methods, - - - - -	25
Sampling, - - - - -	25
Crude Fat, - - - - -	25
Sugars, - - - - -	25
Nitrogen, - - - - -	26
Titrable Acidity, - - - - -	26
Chemical Changes During After-Ripening, - - - - -	26
Chemical Changes During the Drying of After- Ripened Seeds, - - - - -	30
Chemical Changes in After-Ripened Seeds Dried at Room Temperature for 35 Days and Again After- Ripened, - - - - -	31
Discussion of Chemical Studies, - - - - -	31
Part III -- Catalase Studies, - - - - -	34
Changes in Catalase Activity During After-Ripening	34
Procedure for Catalase Determinations, - - - - -	34
Discussion of Catalase Activity, - - - - -	37

* * * * *

A STUDY OF AFTER-RIPENING IN CERTAIN
FRUIT TREE SEEDS

INTRODUCTION

It is generally known that the seeds of most deciduous fruits do not germinate immediately upon reaching maturity, even though all external conditions requisite to growth may be favorable. The dormancy of these seeds, which is considered to be within the embryos, may be terminated by a period of exposure to low temperature. During this period the embryos undergo certain processes known as after-ripening, upon the completion of which germination can take place.

Certain of the fruit-tree seeds are sown in large quantities each year by nurserymen, the resulting plants to be used as the understocks for horticultural varieties. In the common nursery practice of fall seeding, the low temperature of the winter months provides the treatment whereby the after-ripening processes are completed. Under these conditions a maximum germination may or may not be secured, depending presumably on the climatic variations appertaining to a particular season. In order that conditions optimum for after-ripening may be provided and thus a maximum germination be assured, a knowledge

of the proper methods of handling the seed is of importance both to the nurseryman and the plant breeder.

This paper presents data showing the effects of various external conditions before, during, and following the after-ripening period, upon the subsequent germination in seeds of apple, peach, pear, and cherries of the Mazzard and Mahaleb types. A quantitative study of some of the chemical and physiological changes which occur within these seeds during the process of after-ripening has also been made.

REVIEW OF LITERATURE

Since the literature pertaining to the rest period in seeds is so abundant and has already been comprehensively reviewed by Howard (20), Crocker (6), and Eckerson (13), only those investigations relating closely to the present study will here be considered.

In a study of fruit seed storage and germination Tukey (30) indicates that apple seed will germinate well after subjection to eight weeks of cool, moist conditions. A high percentage germination was obtained with pear seed after two to four weeks of similar conditions. Peach seed likewise completed after-ripening in a relatively short time but germination was found to be impossible until the expanding strength of the embryo is sufficient to overcome the resistance offered by the confining stone. In studies with Rubus and Ribes seed a combination of internal

and external factors was encountered. By subjecting the impermeable seed coats to a sulfuric acid treatment for fifteen minutes, following the after-ripening, an increased germination could be effected in all cases excepting that of the gooseberry. The sulfuric acid treatment, in the case of the gooseberry, which apparently has a pervious seed coat, killed the embryos instead of aiding germination. Temperatures below 0°C. were not found to be particularly effective for after-ripening in any of the seeds studied.

Bakke, Richey, and Reeves (2) concluded from a study on the germination of apple seed that a moist storage of one to three degrees C. was most effective for after-ripening. Seeds prevented from drying out when extracted from the fruit yielded a high percentage germination whereas those air-dried germinated poorly in all cases. Seeds which had been air dried and kept for a year did not germinate. Apparently, however, these seeds were not after-ripened in a moist condition.

Harrington (18) (19) also studying after-ripening in apple seeds, secured the greatest germination when the seeds were stored moist at a temperature slightly above freezing. After-ripening of seeds did not occur in dry storage at low temperature.

The time and temperature effective for the after-ripening of a large number of seeds having a rest period has been published by Crocker (4). In another paper Crocker

and Barton (6) present data resulting from studies on the after-ripening, germination and storage of a number of rosaceous seeds. It was found that apple seed ranging from one-half to two and one-half years old germinated well when after-ripened for three and one-half months at one and five degrees C., or in a temperature range fluctuating between five and ten degrees C. A constant temperature of ten degrees, however, proved less effective. It was concluded further that apple seeds retain their after-ripened condition even following a month period of dry storage. Peach seeds were found to give a good germination following an after-ripening period of three and one-half months at five and ten degrees C. but one degree C. was^a/less favorable temperature. Removal of the endocarp increased the percent of germination. Seeds of Pyrus arbutifolia gave a good germination when after-ripened at one degree C. for two, three, or four months, and at five degrees C. for three months. At ten degrees C. no after-ripening was secured with four months of stratification.

Certain chemical and enzymatic changes taking place in seeds during the after-ripening period at low temperature have been studied. Eckerson (13) followed at weekly intervals some of the metabolic changes occurring in the embryo of Crataegus held on moist cotton at five degrees C. The initial change reported was an increase in acidity which was correlated with an increased water holding power

and an increase in catalase and peroxidase activity. Toward the end of the after-ripening period sugars appeared while fats decreased. Sherman (29) also found an increase in catalase activity during the after-ripening of Crataegus seeds. Pack (27) in studying certain changes occurring during the after-ripening and seedling development of Juniperus seeds found a decrease in total lipoids and increases in phosphatides alcohol soluble material, titratable acids, sugars, and in catalase activity. During after-ripening in seeds of Cornus floridus, Davis (8) reports an increase in starch, sugar amino acids and catalase, with little or no change in fats, acidity, or phosphatides. Flemion (14) has reported an increase in catalase and peroxidase during the after-ripening of Sorbus seeds while emulsin and peroxidase activity remain practically unchanged. In a recent paper (15) the same author presents results obtained with seeds of Rhodotypos kerrioides. It was reported that these seeds increased in catalase, peroxidase, and lipase activity as after-ripening took place. An increase in moisture content, titratable acidity, soluble nitrogen, and sucrose, and a decrease in fat was also noted.

During after-ripening of seeds of Ambrosia trifida at low temperature Davis (9) found a slight increase in acidity together with a considerable rise in catalase. It was concluded as doubtful, however, that either has any special significance in the process of after-ripening.

GENERAL METHODS AND MATERIALS

In conducting the various studies herein reported certain general procedures with reference to methods and materials were used. These are presented here, whereas the procedures specific to a given test are considered directly in connection with that phase of the investigation.

The seeds of Mazzard and Mahaleb cherries used in these experiments were collected from trees growing in the vicinity of the Bell Experimental Farm near College Park, Maryland. The McIntosh apple, Kieffer pear, and Elberta and Late Crawford peach seed were obtained from the College orchards of the Department of Horticulture, University of Maryland.

The seeds of apple, pear, and peach were removed from the fruit by hand, carefully washed and cleaned and all poorly developed or injured seeds discarded. The cherry seeds were freed from the fruit by rubbing over a screen.

A fine sharp sand giving good drainage was the stratification medium used for all the experiments described in this paper.

In making the germination tests the seeds were placed in sand contained in flats. To secure uniform spacing and coverage, each seed was placed individually with the hilum down, by means of forceps. The percentage germination was recorded at two days intervals beginning with the appearance of the first seedlings. The germination counts

were continued until the maximum germination for a given test was obtained.

RESULTS

Part I -- Germination Studies

The following experiments show the effect of various external conditions before, during, and following after-ripening upon subsequent germination in fruit tree seeds.

The Effect of Drying Prior to After-Ripening.

Four kinds of seed, namely, McIntosh apple, Elberta peach, and Mazzard and Mahaleb cherry were used to determine the effect of drying the seeds prior to after-ripening upon the subsequent germination.

Immediately upon harvesting, each kind of seed was divided into two lots. Lots I were immediately placed in sand and held moist at room temperature. Lots II were held at the same temperature but allowed to dry for a period of six weeks. The dried lots were then soaked five days, mixed with moist sand and both Lots I and II placed in a low temperature chamber and after-ripened at 1 - 2° C. This temperature may be slightly below the optimum for after-ripening. Employment of a somewhat higher temperature, however, usually results in the occurrence of some germination while the seeds remain within the stratification medium. Since samples were taken at each of these intervals for further study concerned with certain of the

chemical changes occurring during the after-ripening period, the employment of higher temperatures would have in this case been undesirable. At regular intervals throughout the after-ripening period germination tests were made. One-hundred/^{seeds}each of apple, Mazzard, and Mahaleb and fifty seeds of peach were used for each germination test. The tests were then conducted as previously described. Since it was found in preliminary studies that the germination of peach and Mazzard seeds may be delayed or entirely prevented by the mechanical resistance offered to the expanding embryos by the endocarp, one-half of the fifty seeds of peach and one-half of the one-hundred seeds of Mazzard used in each germination test were sown with the endocarp removed. The germination data for peach and Mazzard therefore affords this comparison.

Eight 10-day intervals were used for the peach and Mazzard seeds, eight 7-day intervals for the apple seed, and six 14-day intervals for the Mahaleb.

The results are presented in tables 1 - 6, and summarized in table 7. A close examination of the data in these tables shows no significant differences in germination between the lots dried and those held moist before the period of after-ripening. These results are in agreement with the findings of Crocker (6) and Harrington (7) for apple seeds, but are contrary to the conclusions of Bakke, Richey, and Reeves (2) also working with apple seeds.

TABLE VI -- Germination of Elberta Peach Seed as Influenced
by Dry Storage Prior to the After-Ripening Treatment
at 1 - 2° C.

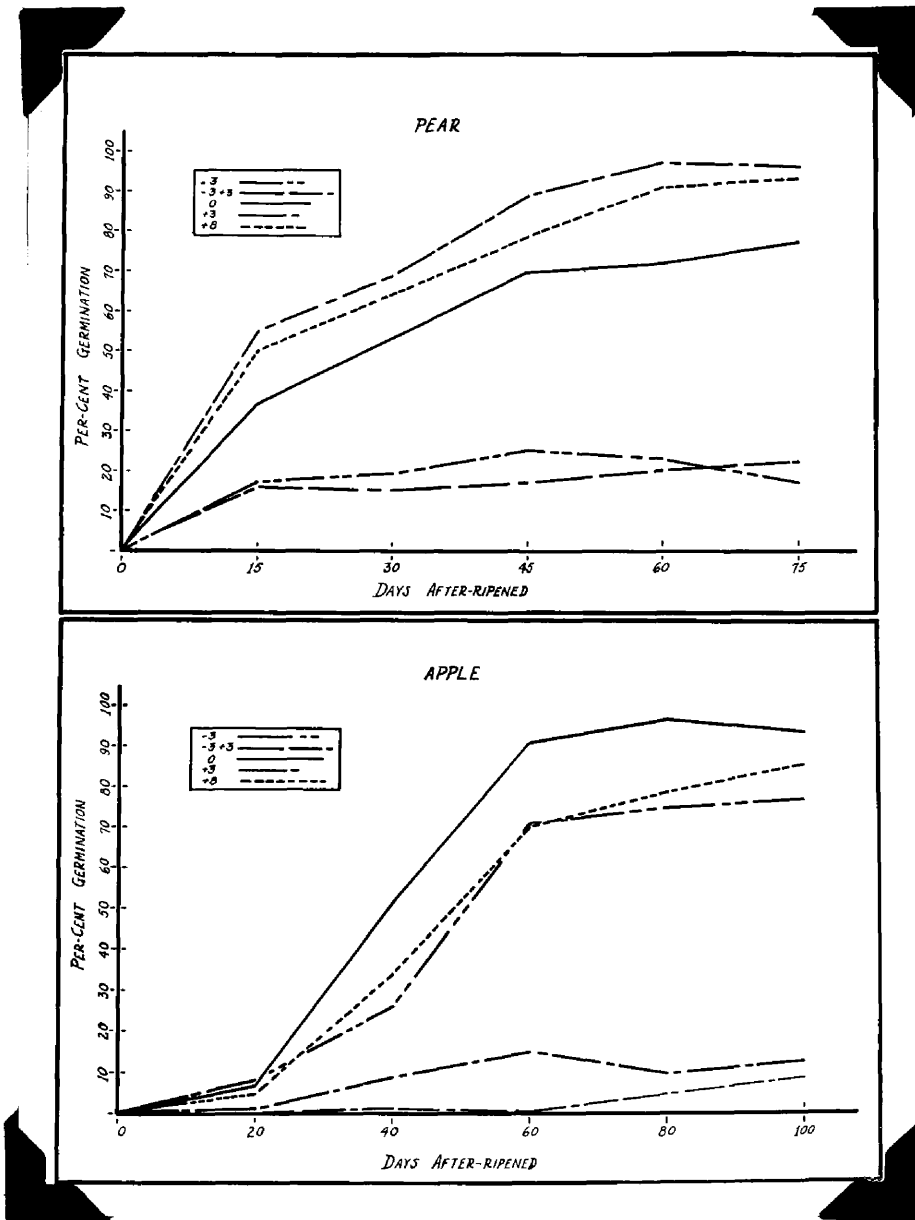
Days after- ripened: 1-2° C.	Endocarp	Percent germination after days indicated													Total percent germina- tion		
		8	10	12	14	16	18	20	22	24	26	28	30	32		over 34	
Check (dry)	Removed																0
	Intact																0
Check (soaked)	Removed																0
	Intact																0
10	Removed										4						4
	Intact																0
20	Removed					4	8										8
	Intact																0
30	Removed			8	20	28											28
	Intact						4										4
40	Removed		8	16	20		24	28	32								32
	Intact						4										4
50	Removed		8	20	28												40
	Intact				4			8									8
60	Removed		4	20	39	32		36	40	44							44
	Intact							4									4
70	Removed	8	16	28	36		48	52									52
	Intact				4	8											8
80	Removed	12	20	32	48	52	56										56
	Intact			4		8				12							12

TABLE VII -- Summarized Table Showing Influence of Drying
Seeds Prior to After-ripening upon Subsequent Germination

Seed	Lot No.	Treatment previous to after-ripening	Endocarp	No. seed to each test	Percent germination when after-ripened at 1-2° C. for days indicated								
					(Ck)	10	20	30	40	50	60	70	80
Elberta peach	I	Moist	Removed	25	0	0	20	16	20	40	32	40	56
			Intact	25	0	0	0	0	0	0	4	8	16
	II	Dried	Removed	25	0	4	8	28	32	40	44	52	56
			Intact	25	0	0	0	4	4	8	4	8	12
Mazzard cherry	I	Moist	Removed	50	0	0	2	8	6	8	8	48	50
			Intact	50	0	0	0	0	0	0	6	10	12
	II	Dried	Removed	50	0	0	2	4	10	8	18	50	56
			Intact	50	0	0	2	0	0	2	4	4	6

TABLE VII (continued)

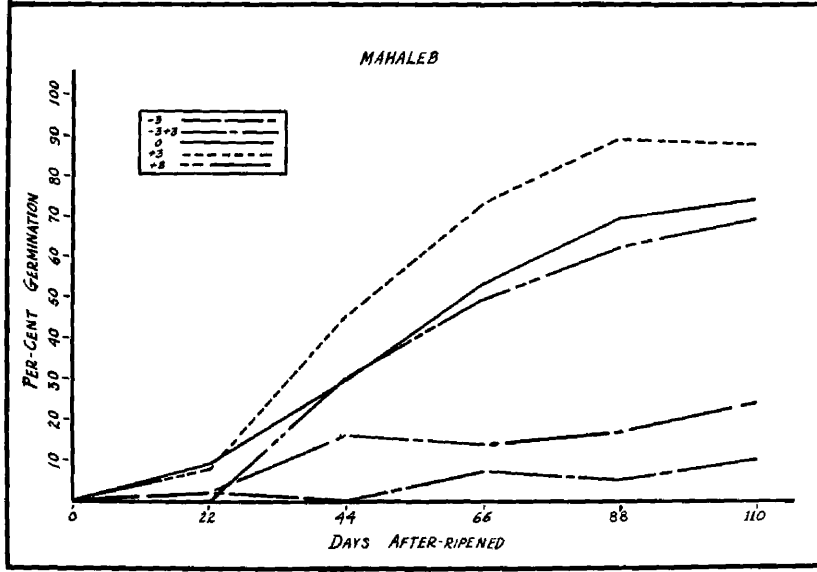
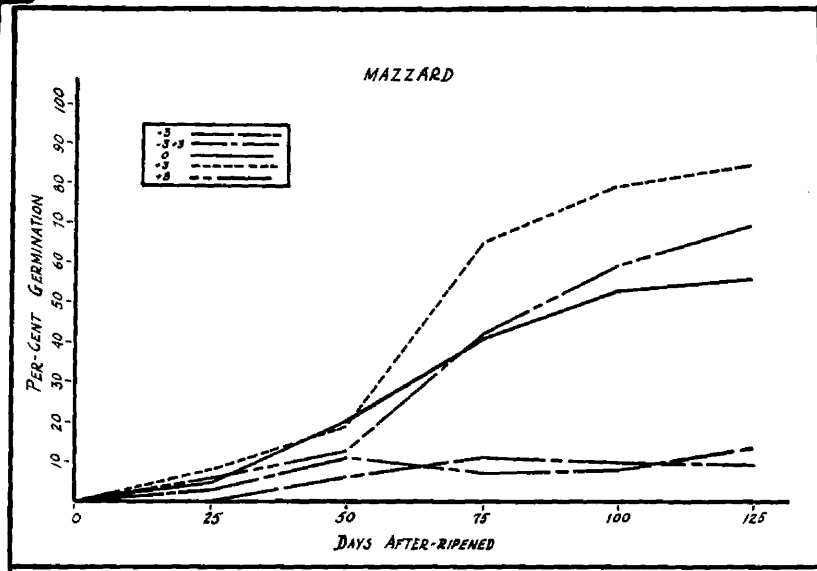
Seed	Lot No.	Treatment previous to after-ripening	No. seed to each test	Percent germination when after-ripened at 1-2° C. for days indicated											
				(ck)	7	14	21	28	35	42	49	56	63	70	77
McIntosh apple	I	Moist	100	0	3	11	29	44	43	68	75	90			
	II	Dried	100	0	2	1	1	13	27	50	64	98			
Mahaleb cherry	I	Moist	100	0		2		10		36		41		65	84
	II	Dried	100	0		3		12		14		52		71	81



The effect of temperature and length of time on after-ripening.

Figure 1 -- Pear

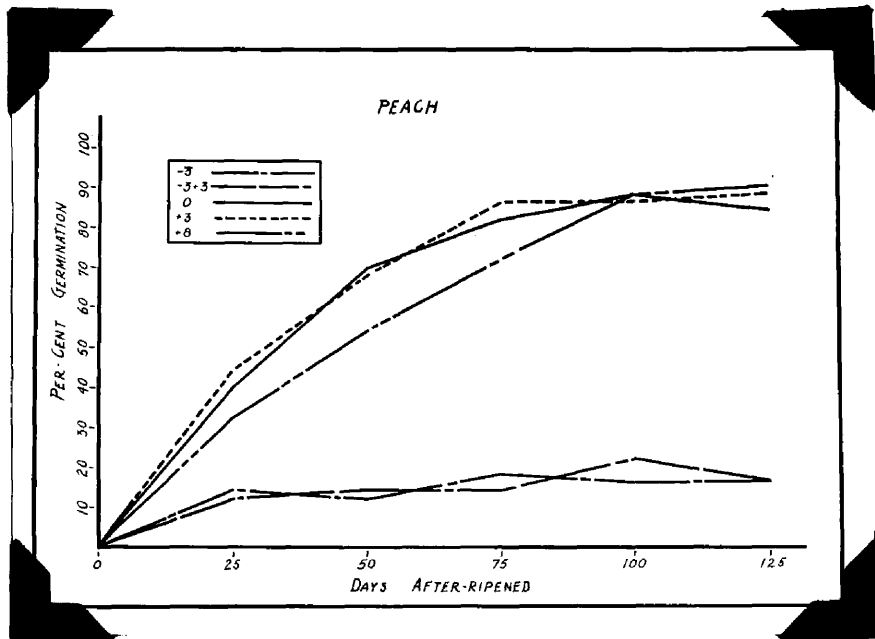
Figure 2 -- Apple



The effect of temperature and length of time on after-ripening.

Figure 3 -- Mazzard

Figure 4 -- Mahaleb



The effect of temperature and length of time on after-ripening.

Figure 5 -- Peach.

In the latter work, however, the seeds were apparently not after-ripened in a moist condition.

The data presented in these tables also indicates that as the after-ripening period progressed the time required for germination following removal from the stratification medium was considerably shortened. Not only do the seeds begin to germinate more quickly, but the maximum germination is obtained in a shorter period of time. This is of importance in that it results in a much more uniform group of seedlings.

Because the endocarp of peach and Mazzard seeds greatly decrease the germination, even though the embryos may be after-ripened and capable of germination, the percentage germination for those seeds with the endocarp removed has been used for subsequent comparisons in the case of these two kinds of seeds.

The Effect of Drying Following the After-Ripening Period. It has been found by a number of investigators (3), (9), (23) that should the after-ripened embryos of certain seeds be placed under external conditions unfavorable for germination, they will after a time revert to a dormant condition, which in turn requires a second period of after-ripening, before germination can be secured.

Under field conditions, or during the handling of artificially after-ripened seeds before they are sown, the possibility exists that a certain loss in moisture may occur following after-ripening. Seeds are often per-

mitted to dry somewhat before sowing to facilitate handling, or may become partly dry from delay in sowing as the result of unfavorable weather conditions. The possible effect of this loss in moisture upon the induction of a second period of dormancy is here considered.

For this study seeds of McIntosh apple, and Mazzard and Mahaleb cherries were after-ripened at 1 - 2°C. They were then removed from the stratification medium and allowed to dry at room temperature, for periods of 2, 5, 10, 15, 25, and 35 days. Upon the termination of each drying period seeds of the three kinds were soaked 48 hours to restore the moisture content and the germination tests then conducted as previously described. One-hundred seeds of each kind were used for each germination test. The results are reported in table VIII.

The data presented in table VIII shows a marked reduction in germination to be associated with the drying of the seeds. A loss of germination approximating 78, 14, and 10 percent for Mazzard, Mahaleb, and apple, respectively, resulted from only two days of drying. After five days of drying a very large percentage of the after-ripened embryos of Mazzard and Mahaleb were incapable of germination; likewise in the apple after 25 days of drying.

These findings are not in agreement with the statement of Crocker and Barton (6) that the after-ripened condition in Baldwin apple seed is retained even following

TABLE VIII — Effect of Drying Seeds Following After-ripening upon
Subsequent Germination. (Expressed in Percent.)

Seed	Days after- ripened 1-20 C	No. seed to each germ. test		Seeds dried for days indicated						
				Check	2	5	10	15	25	35
Mazzard cherry	90	100	Germination	94	21	8	1	0	0	0
			Moisture	39.8	17.9	4.8	4.4	3.9	4.1	4.0
Mahaleb cherry	90	100	Germination	76	68	14	6	5	0	0
			Moisture	49.0	26.2	4.7	4.6	4.5	4.6	4.4
McIntosh apple	63	100	Germination	99	86	70	46	37	21	6
			Moisture	40.9	15.4	9.0	7.9	6.2	5.8	4.4

one month in dry storage. Since this point is of considerable practical importance their work will be briefly reviewed. Baldwin seeds were removed from cold storage fruit and immediately following removal the seeds divided into two lots. The first lot was placed at once in moist peat at 5°C. However, after two weeks of this additional low temperature treatment only a 9 percent germination was obtained. This may indicate that these seeds were not completely after-ripened while the fruit was in cold storage. Unfortunately a germination test was not made immediately following removal from the fruit to definitely determine the capacity of these seeds to germinate. The second lot of seeds was allowed to dry for one month in the laboratory, and then placed in moist peat at 5°C. After four weeks of this treatment a 9 percent germination resulted. A 67 percent germination was obtained after five weeks. These results seem to indicate that the seeds after-ripened during the exposure at 5°C., instead of the after-ripened condition being retained for a one month period of dry storage. Had these seeds been placed at room temperature conditions favorable for germination following this period of drying, instead of at a low temperature effective in after-ripening as well, a better status of their after-ripened condition and resultant capacity to germinate would have been obtained.

The results here presented appear to have practical significance. Under conditions in the seed bed or in the field, a soil moisture deficiency in early spring when the

seeds have become after-ripened and are prepared to germinate may result in this induced inability to grow. With artificially after-ripened seed, any treatment which permits the seed to partly or entirely dry prior to sowing, or following sowing, as for example, sowing in a dry soil, or poor coverage permitting exposure to a drying atmosphere, may result in reduced germination.

Sufficient seed of each kind was after-ripened and dried with the preceding lots so that a quantity remained following the 35 day interval of drying. Upon the completion of this period these were soaked for 48 hours, placed in moist sand at low temperature, and again after-ripened. Germination tests were made at six successive 10-day intervals with Mazzard and Mahaleb at 7, 15, 25, 35, 45, and 55 day intervals with the apple. The results obtained are given in table IX.

Only very low germination as indicated by the results in table IX were obtained following restratification. There are definite indications of injury to the seeds, becoming increasingly evident as this second period of after-ripening progressed, until at the close a very large percentage of seed was noticeably injured. Apparently injury occurred during the drying period preceding this second after-ripening.

It is well known that tissues dried too rapidly may be injured. Conceivably, drying at room temperature

TABLE IX -- Germination of After-ripened Seeds Dried at Room Temperature
for Thirty-five Days and Again After-ripened.

Seed	Days	Percent germination	Days dried	No. seed to each germ. test	Percent germination upon second after-ripening at 1-2° C for days indicated											
	first after-ripened 1-2° C				Check	7	10	15	20	25	30	35	40	45	50	55
Mazzard cherry	90	94	35	100	0	7	2	3	0	1	3					
Mahaleb cherry	90	76	35	100	0	9	3	1	1	3	0					
McIntosh apple	63	99	35	100	6	19	17	16	15	11	13					

is sufficiently rapid to injure the embryos. Table VIII discloses, however, that after two days of exposure approximately 54, 46, and 38 percent of the original moisture of the embryos was retained in Mahaleb, Mazzard, and apple respectively. Not until after five days of drying did the moisture content of the seed become approximately constant. Therefore, the loss of moisture seems not to have been unduly rapid.

Furthermore, drying seed in a similar manner immediately following harvest and prior to after-ripening, has not proved detrimental to the embryos, and in fact is common practice with fruit tree seeds. The embryos in a completely after-ripened condition are, therefore, apparently much more susceptible to injury from desiccation.

The results obtained upon restratifying again direct attention to the necessity for careful handling of after-ripened seed. This point may be important not only to those engaged in commercial propagation, but also to workers engaged in fruit breeding who often after-ripen seeds under controlled conditions in an effort to obtain a maximum germination. A complete germination is obviously desirable in breeding work.

Effect of Temperature and Length of Time on After-Ripening. McIntosh apple, Kieffer pear, Late Crawford peach and Mazzard and Mahaleb cherries were used in a study to determine the effect of different low temperatures upon

after-ripening. Immediately upon harvesting, the seeds were dried at room temperature and allowed to remain in an air dried condition for approximately two months. The seeds were then soaked for 48 hours after which each kind was divided into five lots and mixed with sand. With the peach and Mazzard the endocarp was removed immediately prior to stratification. Lots I were stored at a temperature of -3°C ; Lots II were alternated between a temperature of -3°C ; and $+3^{\circ}\text{C}$; Lots III were stored at 0°C ; Lots IV at $+3^{\circ}\text{C}$ and Lots V at $+8^{\circ}\text{C}$. In the case of Lots II ten days alternations between the -3°C and $+3^{\circ}\text{C}$ temperatures were used for the apple and pear seed, and fifteen day alternations of these two temperatures for the seeds of peach, Mazzard and Mahaleb. The -3°C temperature preceded the $+3^{\circ}\text{C}$ temperature at the beginning of these alternations.

At intervals during the storage periods seeds of each kind were removed from the various lots and germination tests were made. Five 15-day intervals were used for the pear seed; five 20-day intervals for the apple, five 22-day intervals for the Mahaleb; and five 25-day intervals for the Mazzard and the peach. Fifty seeds of peach and one-hundred seeds of apple, pear, Mazzard and Mahaleb respectively were used for each germination test. The tests were then conducted as previously described.

The results are reported in tables X to XIV and are graphically presented in figures 1 to 5. A study of table X shows that for the seeds of Kieffer pear either

TABLE X -- Effect of Temperature and Length of Time
on the After-Ripening of Kieffer Pear Seed

Lot No.	After-ripening temp. ° C.	No. seeds for each test	Percent germination when after-ripened for days indicated					
			Check	15	30	45	60	75
I	-3	100	0	17	19	25	23	17
II	-3+3	100	0	16	15	17	20	22
III	0	100	0	37	53	70	72	77
IV	+3	100	00	55	69	89	97	96
V	+8	100	0	50	64	79	91	93

TABLE XI -- Effect of Temperature and Length of Time
on the After-Ripening of McIntosh Apple Seed.

Lot No.	After-ripening temp. ° C.	No. seeds for each test	Percent germination when after-ripened for days indicated					
			Check	20	40	60	80	100
I	-3	100	0	0	1	0	5	9
II	-3+3	100	0	1	9	15	10	13
III	0	100	0	8	26	71	75	77
IV	+3	100	0	7	52	91	97	94
V	+8	100	0	5	34	70	79	86

TABLE XII -- Effect of Temperature and Length of Time
on the After-Ripening of Mazzard Cherry Seed.

Lot No.	After-ripening temp. ° C.	No. seeds for each test	Percent germination when after-ripened for days indicated					
			Check	25	50	75	100	125
I	-3	100	0	0	6	11	10	9
II	-3+3	100	0	3	11	7	8	13
III	0	100	0	5	20	41	53	56
IV	+3	100	0	8	19	65	79	84
V	+8	100	0	6	13	42	59	69

TABLE XIII -- Effect of Temperature and Length of Time
on the After-Ripening of Mahaleb Cherry Seed.

Lot No.	After-ripening temp. ° C.	No. seeds for each test	Percent germination when after-ripened for days indicated					
			Check	22	44	66	88	110
I	-3	100	0	2	0	7	5	10
II	-3+3	100	0	2	16	14	17	24
III	0	100	0	9	29	53	69	74
IV	+3	100	0	8	45	73	89	88
V	+8	100	0	0	30	49	62	69

TABLE XIV -- Effect of Temperature and Length of Time
on the After-Ripening of Late Crawford Peach

Seed

Lot No.	After- ripening temp. ° C.	No. seeds for each test	Percent germination when after-ripened for days indicated					
			Check	25	50	75	100	125
I	-3	50	0	14	12	18	16	16
II	-3 1/3	50	0	12	14	14	22	16
III	0	50	0	40	70	82	88	84
IV	+3	50	0	44	68	86	86	88
V	+8	50	0	32	54	72	88	90

0°, 3°, or 8°C is an effective temperature for after-ripening, while both the alternating and -3°C temperatures proved much less effective.

These same general conclusions may be drawn from the results obtained for apple, Mazzard, Mahaleb and peach. However, with these seeds the -3° temperature proved more ineffective than in the case of the pear, which apparently will after-ripen over a somewhat wider range of low temperature.

Both Tukey (30) and Crocker (4) (5) have reported that freezing, or freezing and thawing temperatures, are relatively ineffective for the after-ripening of certain of these fruit-tree seeds.

From the standpoint of nursery practice 8°C is not a desirable temperature for these seeds for, although after-ripening occurs, germination can also readily take place during the storage period at this temperature. Unavoidable injury may attend the handling and sowing of germinated seeds.

A further study of the data in tables X to XIV discloses that a high percentage germination was obtained following 45 days of after-ripening at 3°C with the Kieffer pear, and following 60 days for the McIntosh, 100 days for the Mazzard cherry, 88 days for Mahaleb cherry, and 75 days for the Late Crawford peach. Although with each of these seeds a good percentage germination was obtained following after-ripening at 0° and 8° C. in most cases the time required

was somewhat longer than 3°C.

Moisture Content of the Medium in Relation to After-Ripening. Differences of opinion are prevalent among nurserymen with respect to the moisture content of the medium which should be maintained in order to secure optimum after-ripening in the fruit tree seeds.

Three kinds of seed, namely, Kieffer pear, McIntosh apple and Late Crawford peach were used in a study to secure information on this point. Immediately upon harvesting the seeds were dried at room temperature and allowed to remain in an air dried condition for approximately two months. Following this period of dry storage each kind of seed was divided into four lots. Lots I were mixed with air dry sand. Lots II, III, and IV were mixed with sand to which sufficient water was added to bring the moisture content to 6, 10, and 16 percent respectively, the latter percentage representing about the maximum water holding capacity for the type of sand used. These lots were further designated as "dry," "moist," "very moist," and "wet." Lots II, III, and IV were weighed at the beginning and at weekly intervals thereafter in order that the moisture content of each lot might be maintained. All lots were allowed to remain at room temperature for two weeks after which they were stored at 3°C.

At intervals during the storage periods seeds of each kind were removed from the various lots and germination

tests were made. Five 15-day intervals were used for the pear seed; five 20-day intervals for the apple; and five 25-day intervals for the peach. Fifty seeds of peach and one-hundred seeds each of apple and of pear were used for each germination test. The tests were conducted as previously described. At the time of each germination test a sample of seed was also removed and a moisture determination made.

The results obtained are shown in tables XV to XVII. It is clearly evident that the seeds of Lots I, stored at the low temperature in an air-dried condition, did not after-ripen.

The seeds in Lots II (moist) and III (very moist) yielded a high percentage germination subsequent to after-ripening for 45, 60, and 75 days in the case of the pear, apple, and peach respectively. With the seeds of Lot IV (wet) a percentage germination equal to that in Lots II and III was not obtained with any of the seeds. It may be possible that the high moisture content of the wet medium did not provide optimum aeration.

Effect of Pulp Disintegration on Seed Viability.

It has been reported that stratifying the seed of certain stone fruits without removing the pulp may result in decreased germination (31). Nurserymen frequently secure peach pits for the propagation of seedling stocks from canning factories where the pits are allowed to remain in piles for varying lengths of time. Pits secured from culls

TABLE XV -- Moisture Content of the Medium in Relation to the After-ripening of Kieffer Pear Seed. (Expressed in Percent)

Lot No.	Condition of medium	No. seed to each germ. test		Seed after-ripened at 30° C. for days indicated					
				Check	15	30	45	60	75
I	Dry	100	Germination	0	0	0	1	0	1
			Moisture	5.2	5.2	5.2	5.4	5.2	5.3
II	Moist	100	Germination	0	48	62	81	97	98
			Moisture	39.3	41.3	41.0	40.8	41.9	42.1
III	Very Moist	100	Germination	0	43	65	88	100	99
			Moisture	40.9	41.7	42.8	42.5	43.2	43.9
IV	Wet	100	Germination	0	41	60	72	82	84
			Moisture	42.4	43.8	43.5	44.8	45.1	45.3

TABLE XVI -- Moisture Content of the Medium in Relation to the After-Ripening of McIntosh Apple Seed (Expressed in Percent).

Lot No.	Condition of medium	No. seed to each germ. test		Seed after-ripened at 3° C. for days indicated					
				Check	20	40	60	80	100
I	Dry	100	Germination	0	0	0	0	0	0
			Moisture	4.6	4.7	4.7	4.6	4.7	4.8
II	Moist	100	Germination	0	5	47	84	93	96
			Moisture	38.1	39.7	39.9	39.9	39.3	40.1
III	Very Moist	100	Germination	0	9	46	89	99	95
			Moisture	39.8	41.1	41.5	41.3	42.0	42.9
IV	Wet	100	Germination	0	11	39	76	73	75
			Moisture	38.9	42.3	42.9	43.6	43.1	42.9

TABLE XVII -- Moisture Content of the Medium in Relation to the After-Ripening of Late Crawford Peach Seed (Expressed in Percent).

Lot No.	Condition of medium	No. seed to each germ. test		Seed after-ripened at 3° C. for days indicated					
				Check	25	50	75	100	125
I	Dry	100	Germination	0	0	0	0	0	0
			Moisture	3.2	3.3	3.4	3.3	3.3	3.4
II	Moist	100	Germination	0	38	60	80	84	86
			Moisture	34.9	37.8	37.9	38.5	38.1	38.6
III	Very Moist	100	Germination	0	46	68	86	88	90
			Moisture	36.6	39.1	40.9	41.4	42.6	42.1
IV	Wet	100	Germination	0	40	58	62	66	60
			Moisture	36.1	41.7	42.0	43.2	43.6	44.0

from which the pulp has been permitted to disintegrate, are also frequently utilized. In fact it is common practice in securing the seeds of peach, plum, and cherry, to place the fruit in piles until a convenient time for removal of the pits. In some cases pit removal is purposely delayed until sufficient disintegration of the pulp has occurred to allow an easier separation from the seed.

The possibility that such treatment may cause injury to the seeds of peach has been here considered.

Approximately twenty bushels of Elberta peaches were placed in a pile and allowed to disintegrate. One lot was removed at the beginning of the experiment (check lot), a second lot five days thereafter, and a third seven days following the second lot. A thermometer was placed in the center of the pile and the temperature recorded. The highest temperature recorded was 34°C, and was attained on the sixth day of disintegration.

When removed from the pile the pits were washed, allowed to dry in a cool place and stored dry until planted in the nursery on November 2nd.

Eight hundred pits were planted from each lot, the pits being placed in rows and covered at a depth of about two inches. The following spring germination counts were made. A germination of 27.7 percent was obtained for the check lot, .88 percent for the second lot having five days of disintegration, and .13 percent for the third lot

allowed to decay for twelve days. Even though the percentage germination obtained with the check lot was relative low, (apparently characteristic of Elberta) the results clearly indicate that even five days of disintegration caused considerable injury to the seeds.

Considering the fact that in this experiment a temperature of 34°C was the highest attained, it may suggest that some factor other than temperature causes injury during the fruit breakdown. Possibly an excessive accumulation of carbon dioxide associated with a deficiency of oxygen, or an accumulation of alcohol from fermentation should receive greater consideration as factors causing injury.

The hitherto unexplainable failure sometimes experienced with these seeds may possibly be accounted for on this basis. It is recommended that the fruit not be allowed to decay even though recovery of the seeds is facilitated thereby.

Treatment with Chemicals Designed to Break the Rest Period. Considerable experimental evidence exists to show that in addition to exposure to low temperature, various agents including warm baths, nutrient salts, narcotics, anaesthetics, and wounding have been successfully employed to shorten the normal rest period of plants. The after-ripening period of seeds has apparently been shortened by treatment with sugars, acids, etc. Eckerson (13) has reported that the after-ripening period can be shortened in Crataegus by treating the embryos with

dilute hydrochloric, butyric and acetic acids. Ives (22) found a five percent glucose solution to be a successful forcing agent for seeds of Ilex opaca.

Recently Deuber (12) has reported that treatment with ethylene chlohydrin and thiourea shortened the normal rest period in seeds of the sugar and Norway maples, and in acorns of the black and red oaks. Flemion (14), however, used a wide variety of chemical treatments on seeds of Sorbus aucuparia without shortening the normal after-ripening period.

Should it be possible to after-ripen fruit tree seeds by any means other than low temperature, it would obviously be of distinct practical value as well as suggesting the mechanism involved in growth releasal. To this end the following agencies were used in an attempt to terminate the rest period in seeds of French pear and apple:

Warm Baths.

- 40° C. for 24, 48, and 72 hours,
- 45° C. for 24, 48, and 72 hours,
- 50° C. for 12, 18, 24, and 36 hours,
- 50° C. for 12 hours + 57° C. for 18 hours,
- 50° C. for 18 hours + 57° C. for 18 hours,

Warm Baths + Ether (vapor)

- 45° C. for 72 hours + ether 0.5 cc. per liter for 3, 6, 12, 18, and 24 hours.
- 45° C. for 72 hours + ether 1 cc. per liter for 3, 6, 12, 18, and 24 hours.
- 45° C. for 72 hours + ether 2 cc. 24*hours.
- 50° C. for 12 hours + ether 1.5 cc. 10 hours.

* Perceptible injury.

Ether (vapor)

0.5 cc. per liter for 1, 3, 6, 9, and 12 hours.
1 cc. per liter for 1, 3, 6, 9, and 12 hours.
1 cc. per liter for 18 and 24 hours.
2 cc. per liter for 3, 6, 9, 12*, 15* hours.

Ether (immersed)

(same periods of time as for vapor treatments)

Ethylene chlorhydrin (vapor)

.25 cc. per liter for 1, 6, 9, 12 and 24 hours.
.50 cc. per liter for 1, 9, 12, 18, and 24 hours.
1 cc. per liter for 1, 6, 12, 18, and 24 hours.
2 cc. per liter for 1, 12, 18, and 24 hours.
3 cc. per liter for 1, 3, 6, 12, and 24 hours.
5 cc. per liter for 1, 3, 6, and 18 hours.

Ethylene chlorhydrin (immersed)

(same periods as for vapor treatments).

Carbon tetrachloride (vapor)

.5 cc. per liter for 1, 12, and 24 hours.
1 cc. per liter for 1, 12, and 24 hours.
2 cc. per liter for 1, 12, and 24 hours.
3 cc. per liter for 1, 12, and 24 hours.
5 cc. per liter for 1, 12, and 24 hours.

Carbon tetrachloride (immersed)

1, 6, 9, 12, 18, and 24 hours.

Acids

Sulphuric (conc)

For 5, 10, 15, 20*, and 30* minutes.

Hydrochloric

0.5 percent for 24, 48, and 72 hours.
1 percent for 24, 48, and 72 hours.
2 percent for 24, 48, and 72* hours.

* Perceptible injury.

Acetic

- 0.5 percent for 48 hours.
- 1 percent for 48 hours.
- 2 percent for 48 hours.
- 3 percent for 48 hours.

Sugars

Glucose

- .5, 1, 2, 3, and 5 percent each for 48 hours.

Sucrose

- .5, 1, 3, 5, and 7 percent each for 48 and 72 hours.

Fifty seeds of each kind were used for each test. Seed soaked in water at room temperature for the period of a specific test were used for the checks.

None of the treatments proved effective in terminating the state of rest or in shortening the length of time required for after-ripening, when certain of these tests were subsequently followed by periods of low temperature.

Since the factors causally related to the breaking of the rest period in seeds and plants are not known, these various treatments are necessarily of the trial and error class. Even though the above attempts were unsuccessful it should not be concluded that this study has exhausted all the possibilities in this field or that no treatment, except low temperature, will be effective in breaking the state of rest in these seeds. Despite the fact, however, that a number of treatments other than low temperature have been successfully employed by different investigators in shortening the rest period in some seeds and buds, in most cases no abrupt cessation of the rest period has been thus brought about.

Conclusions from Part I

From a study of a number of factors which may affect the after-ripening and subsequent germination in fruit tree seeds, the following conclusions of important practical value seem justified:

1. Drying seeds at room temperature prior to after-ripening does not adversely affect the percentage germination provided these dried seeds are subsequently after-ripened at low temperature, in a moist condition. When stratified dry at low temperature, however, after-ripening does not take place.

2. Drying at room temperature following after-ripening results in a very marked reduction in viability. Apparently seeds completely after-ripened are much more susceptible to injury by dessication.

3. Temperatures of 0° , 3° , and 8°C . were found effective for the after-ripening of the seeds which have been considered. However, slightly higher germination followed after-ripening at 3°C . A freezing temperature of -3°C and an alternating temperature of $-3 + 3^{\circ}\text{C}$. proved to be relatively ineffective for after-ripening.

4. At 3°C a high percentage germination was obtained following an after-ripening period of 45 days with the pear, 60 days with the apple, 75 days with peach, 88 days with Mahaleb, and 100 days with the Mazzard.

5. Allowing the pulp to disintegrate while the fruit remains in piles results in considerable injury to the seeds of peach, It is recommended that the fruit not be allowed to thus disintegrate even though recovery of the seeds is facilitated thereby.

6. The percentage germination of Elberta peach and Mazzard cherry seeds may be considerably reduced by the mechanical resistance offered to the expanding embryo by the endocarp.

7. Attempts to after-ripen French pear and apple seeds by various methods of stimulation other than cold temperature were unsuccessful.

Part II -- Chemical Studies.

The data here presented are the results of a quantitative study to determine some of the chemical changes occurring during the after-ripening period in fruit tree seeds.

Analytical Methods

Sampling. In the preparation of the material for chemical analyses the enveloping structures were removed from the embryos. The endocarp of the peach was removed by means of a hammer whereas a small pair of pliers was employed to remove the endocarps from the Mazzard and Mahaleb seeds. With the apple seeds the integuments were removed with the thumb nail. The embryos were preserved by drying in a fan ventilated oven at 70°C. All determinations have been made in duplicate and the averaged values reported on the dry weight basis.

Crude Fat. Samples of three grams each were weighed out, freed of fat by extraction with anhydrous ethyl ether, and re-weighed. The difference in weight is recorded as crude fat.

Sugars. The residues from the ether determinations were placed in Soxhlet extractors with eighty per cent alcohol and extracted for four and one-half hours. The alcoholic extract was freed from alcohol on a sand bath by means of an air blast (16), cleared with neutral lead acetate and delead with potassium oxalate. The

reducing value was determined directly on an aliquot of the cleared solution by the Bertrand-Walker-Munson method and reported as free reducing substances. A second aliquot was hydrolyzed with 2.5 percent hydrochloric acid for twenty-four hours at room temperature and then neutralized with sodium hydroxide. The reducing power was determined and reported as total sugars. The difference between total sugars and free reducing substances is recorded as sucrose.

Nitrogen. Total alcohol insoluble nitrogen was determined directly on the alcohol extracted residue by the Arnold-Gunning modification of the Kjeldahl method. Determinations of total nitrogen were made, and the difference recorded as alcohol soluble nitrogen.

Titrateable Acidity. The crude fat extract was freed of ether, twenty-five cc. of 95 percent alcohol added, and the titrateable acidity recorded as the number of cc of .02 normal sodium hydroxide required to bring this solution to neutrality. Phenolphthalein was used as the indicator.

Chemical Changes During After-Ripening.

Four kinds of seed were used for this study, namely, McIntosh apple, Elberta peach, and Mazzard and Mahaleb cherries. Immediately upon harvesting each kind of seed was divided into three lots and the following treatments given. Lots I was placed in moist sand and allowed to remain at room temperature throughout the experiment (check lot). Lots II and III were after-ripened at a low temperature (1 - 2°C.) but were given different storage

conditions for a period of six weeks prior to this after-ripening. Lots II were stored in moist sand at room temperature, whereas Lots III were allowed to air dry at room temperature. Following this storage period the air dried lots were soaked for five days to reestablish their moisture content and placed in moist sand. Both Lots II and III were then placed in the low temperature chamber and together after-ripened at 1 - 2°C. Although this temperature may be slightly below the optimum for after-ripening it was purposely used since the employment of a somewhat higher temperature usually results in the occurrence of some germination while the seeds remain within the stratification medium. Since in these studies, only changes during the after-ripening period were being considered, this would have been obviously undesirable.

At regular intervals throughout the after-ripening period seed was taken from each of these lots, the enveloping structures removed, and the embryos preserved for chemical analyses. Eight 10-day intervals were used for the peach and Mazzard seeds; eight 7-day intervals for the apple seed; and six 14-day intervals for the Mahaleb seed. At each interval of sampling a germination test/^{was}made to determine the progress of the after-ripening process. One-hundred seeds each of apple and Mahaleb and 25 and 50 seeds, respectively, for peach and Mazzard were used for each germination test. The tests were conducted as previously described.

The results of the chemical analyses are presented in tables XVIII, XIX, XX, and XXI. Although minor changes from one interval of sampling to another occurred in the various fractions it is evident that no significant changes resulted which can be associated with growth release. However, the data discloses certain interesting facts relating to the chemical composition of these seeds.

The change from starch to sugar is of frequent occurrence in plants and has been associated with after-ripening by certain investigators. However, Appleman (2) considers the increase in sugars resulting from exposure to low temperature, to be no essential part of the after-ripening process in potato tubers. It is of interest to know that in the seeds here studied starch was at no time found, and no significant changes in sugars occurred during after-ripening.

Of considerable interest is the high percentage of fats, indicating that they constitute one of the chief storage reserves. Apparently, however, there is no significant change to sugars during after-ripening, although such transformation no doubt occurs rapidly after growth starts.

No marked change in total nitrogen would be expected unless an actual loss in dry matter by respiration changed the relative amount. Apparently such loss did not occur for no significant change in total nitrogen took

TABLE XXI -- Chemical Changes in Relation to
After-ripening in Mahaleb Cherry Seed.

Lot I (Room Temperature)								
Days of treatment.	Percent water	Crude fat	Total* sugar	Titratable acid	Nitrogen			Total percent germination
					Total	Alc. Insol.	Alc. Sol.	
Check	39.58	35.11	4.02	5.67	3.64	3.18	.46	0
14	39.58	35.13	4.04	5.74	3.66	3.14	.52	0
28	43.38	35.19	4.05	5.69	3.62	3.16	.46	0
42	43.40	34.83	4.11	5.78	3.68	3.12	.56	0
56	45.49	35.09	4.17	5.84	3.65	3.19	.46	0
70	43.09	35.23	4.19	5.83	3.70	3.20	.50	0
84	44.31	35.52	4.25	5.90	3.68	3.18	.50	0
Lot II (After-ripened at 1-2° C.)								
14	41.49	35.05	4.20	5.75	3.63	3.20	.43	2
28	43.48	35.83	4.43	5.81	3.61	3.17	.44	10
42	42.65	35.27	4.08	5.82	3.63	3.15	.48	36
56	43.19	35.69	4.17	5.90	3.65	3.19	.56	41
70	43.07	35.89	4.09	5.86	3.59	3.17	.42	65
84	43.89	35.39	4.58	5.92	3.65	3.24	.41	84
Lot III (Dried Prior to After-ripening at 1-2° C.)								
Check (dry)	3.87	36.44	4.63	5.95	3.58	3.19	.39	0
Check (soaked)	37.14	36.91	4.47	5.83	3.64	3.22	.42	0
14	36.16	36.52	4.47	5.89	3.60	3.21	.39	3
28	-	-	-	-	-	-	-	12
42	38.55	35.80	4.57	5.94	3.57	3.20	.37	41
56	39.50	35.77	4.71	5.97	3.61	3.23	.38	52
70	-	-	-	-	-	-	-	71
84	41.05	35.14	4.64	5.90	3.63	3.18	.45	81

* Free reducing substances were found only in traces.

TABLE XX -- Chemical Changes in Relation to After-ripening
in Mazzard Cherry Seed.

Lot I (Room Temperature)

Days of treatment	Percent water	Crude fat	Free red. sub.	Sucrose	Total sugar	Titratable acid	Nitrogen			Total percent germination
							Total	Alc. Insol.	Alc. Sol.	
Check	37.43	39.05	.71	4.46	5.17	6.73	4.90	4.06	.84	0
10	37.00	40.03	.66	4.51	5.17	6.94	4.97	4.24	.73	0
20	38.71	39.04	.50	4.33	4.83	6.79	5.00	4.24	.76	0
30	37.24	39.03	.61	4.31	4.92	6.93	4.98	4.13	.85	0
40	37.61	39.16	.55	4.39	4.94	6.89	4.99	4.16	.83	0
50	37.60	38.88	.67	4.25	4.92	6.95	4.97	4.12	.85	0
60	38.45	39.71	.56	4.35	4.91	6.92	4.93	4.15	.78	0
70	38.11	38.53	.57	4.20	4.77	7.00	4.96	4.14	.82	0
80	37.93	38.07	.46	4.31	4.77	7.07	4.92	4.13	.79	0

Lot II (After-ripened at 1-2° C.)

10	36.82	40.06	.51	4.24	4.75	6.91	4.99	4.14	.85	0
20	39.23	39.59	.53	4.22	4.75	6.96	5.01	4.17	.84	2
30	38.31	40.30	.54	3.98	4.52	7.08	4.98	4.19	.79	8
40	38.69	40.02	.54	4.18	4.72	6.99	4.97	4.13	.84	6
50	38.61	40.44	.50	4.30	4.80	7.10	4.97	4.16	.81	8
60	40.64	39.45	.48	4.25	4.73	7.13	4.99	4.13	.86	8
70	39.38	39.58	.49	4.23	4.72	7.19	4.94	4.13	.79	48
80	38.47	39.24	.44	4.25	4.69	7.28	4.96	4.12	.84	50

Lot III (Dried Prior to After-ripening at 1-2° C.)

Check (dry)	4.28	40.04	.63	4.44	5.07	7.25	4.76	4.12	.64	0
Check (soaked)	40.27	40.58	.59	3.39	3.98	7.07	4.81	4.18	.63	0
10	39.91	40.91	.59	3.38	3.97	7.01	4.77	4.12	.65	0
20	41.01	40.21	.51	3.28	3.79	7.14	4.79	4.18	.61	2
30	40.52	40.92	.56	2.94	3.50	7.14	4.79	4.16	.63	4
40	41.17	39.92	.50	3.30	3.80	7.25	4.81	4.19	.62	10
50	41.99	40.50	.49	3.32	3.81	7.39	4.78	4.11	.67	8
60	42.37	41.13	.51	3.37	3.88	7.30	4.75	4.14	.61	18
70	42.33	40.71	.43	3.30	3.73	7.32	4.78	4.18	.60	50
80	42.54	40.68	.39	3.64	4.03	7.40	4.74	4.09	.65	56

TABLE XIX -- Chemical Changes in Relation to After-ripening

in Elberta Peach Seed.

(Lot I (Room Temperature))

Days of treatment	Percent water	Crude fat	Free red. sub.	Sucrose	Total sugar	Titratable acid	Nitrogen			Total percent germination
							Total	Alc. Insol.	Alc. Sol.	
Check	42.66	50.30	.69	3.29	3.98	9.32	4.82	4.12	.70	0
10	48.31	50.55	.75	3.46	4.21	9.29	4.86	4.09	.77	0
20	44.05	50.02	.77	3.05	3.82	9.49	4.84	4.15	.69	0
30	46.28	50.82	.71	3.15	3.86	9.37	4.82	4.14	.68	0
40	47.57	50.69	.74	3.09	3.83	9.51	4.80	4.18	.62	0
50	43.03	50.85	.73	3.16	3.89	9.50	4.79	4.16	.63	0
60	43.82	51.24	.77	2.75	3.52	9.43	4.82	4.21	.61	0
70	42.27	-	.68	2.88	3.57	9.54	4.78	4.12	.66	0
80	44.45	49.29	.75	2.85	3.60	9.49	4.77	4.17	.60	0

Lot II (After-ripened at 1-2° C.)

10	48.16	50.59	.69	3.00	3.69	9.27	4.75	4.16	.59	0
20	43.36	50.28	.65	3.42	4.07	9.31	4.76	4.18	.58	20
30	40.64	51.00	.74	2.78	3.52	9.20	4.69	4.24	.45	16
40	44.63	49.76	.65	3.63	4.28	9.30	4.75	4.24	.51	20
50	43.40	50.24	.70	3.54	4.24	9.43	4.76	4.18	.58	40
60	44.20	49.93	.52	3.68	4.20	9.48	4.78	4.17	.61	32
70	42.27	50.65	.61	3.23	3.84	9.59	4.73	4.09	.64	40
80	48.20	49.67	.60	3.30	3.90	9.56	4.75	4.20	.55	56

Lot III (Dried Prior to After-ripening at 1-2° C.)

Check (dry)	4.98	49.45	.69	2.90	3.59	10.01	4.76	4.21	.55	0
Check (soaked)	34.21	50.05	.52	3.14	3.66	9.57	4.83	4.14	.69	0
10	38.05	50.46	.75	2.82	3.57	9.68	4.79	4.22	.57	4
20	38.74	50.42	.71	3.01	3.72	9.74	4.76	4.23	.53	8
30	40.95	49.91	.74	2.96	3.70	9.59	4.79	4.28	.51	28
40	40.55	50.44	.78	3.11	3.89	9.70	4.77	4.17	.60	32
50	41.03	51.30	.76	2.72	3.48	9.80	4.77	4.09	.68	40
60	40.65	50.99	.79	2.73	3.52	9.72	4.80	4.11	.69	44
70	41.33	51.34	.58	3.23	3.81	9.89	4.78	4.22	.56	52
80	41.93	50.45	.37	4.03	4.40	9.86	4.75	4.10	.65	56

TABLE XVIII — Chemical Changes in Relation to After-ripening
in McIntosh Apple Seed.

Lot I (Room Temperature)

Days of treatment	Percent water	Crude fat	Free red. sub.	Sucrose	Total sugar	Titratable acid	Nitrogen			Total percent germination
							Total	Alc. Insol.	Alc. Sol.	
Check	39.42	33.81	.40	3.03	3.43	3.40	7.82	7.31	.51	0
7	41.46	33.20	.36	3.53	3.89	3.52	7.85	7.33	.52	0
14	38.37	32.70	.43	3.15	3.58	3.43	7.89	7.29	.60	0
21	42.84	33.05	.40	3.73	4.13	3.50	7.82	7.19	.63	0
28	42.55	32.64	.31	3.62	3.93	3.55	7.86	7.21	.65	0
35	42.80	32.76	.28	3.85	4.13	3.65	7.83	7.22	.61	0
42	41.50	32.49	.34	3.69	4.03	3.50	7.84	7.37	.47	0
49	42.90	33.22	.44	3.55	3.99	3.67	7.81	7.36	.45	0
56	43.76	32.62	.40	3.30	3.77	3.60	7.82	7.30	.52	0

Lot II (After-ripened at 1-2° C.)

7	41.56	33.21	.40	3.66	4.06	3.48	7.88	7.35	.53	3
14	42.24	32.64	.44	3.63	4.07	3.55	7.85	7.38	.47	11
21	42.15	33.65	.47	3.68	4.15	3.69	7.82	7.32	.50	29
28	42.10	32.92	.43	3.79	4.22	3.66	7.80	7.34	.46	44
35	42.30	32.67	.48	3.88	4.36	3.73	7.84	7.35	.49	43
42	44.06	32.93	.40	3.90	4.30	3.72	7.83	7.34	.49	68
49	42.81	33.27	.35	3.94	4.29	3.78	7.85	7.32	.53	75
56	41.99	33.51	.32	4.14	4.46	3.85	7.79	7.32	.47	90

Lot III (Dried Prior to After-ripening at 1-2° C.)

Check (dry)	4.78	34.53	.42	3.01	3.52	3.80	7.88	7.35	.53	0
Check (soaked)	36.16	34.77	.36	2.97	3.33	3.67	7.78	7.20	.58	0
7	38.39	34.62	.40	2.78	3.18	3.77	7.81	7.36	.45	2
14	38.24	34.56	.41	2.76	3.17	3.82	7.81	7.23	.58	1
21	39.83	34.03	.44	2.88	3.22	3.69	7.79	7.36	.43	1
28	39.28	34.31	.46	2.66	3.12	3.78	7.82	7.20	.62	13
35	39.13	34.15	.48	2.67	3.15	3.74	7.82	7.33	.59	27
42	40.37	34.49	.41	3.09	3.50	3.90	7.78	7.39	.39	50
49	40.48	34.63	.35	2.98	3.33	4.00	7.82	7.40	.42	64
56	40.21	34.39	.27	3.15	3.42	3.91	7.80	7.41	.39	98

place during these treatments. There was also no significant change in the soluble nitrogen fraction. The latter constitutes but a comparatively small percentage of the total nitrogen, ranging from approximately 7 percent in the apple seed to 15 percent in the case of Mazzard. Since the only partition of total nitrogen was into the alcohol soluble and insoluble groups, however, it does not preclude the possibility of important changes within these two groups.

A close examination of the data discloses a definite trend in certain fractions as the treatment continues. For example, the titratable acidity for McIntosh apple seed increased slightly as the after-ripening progressed at low temperature in Lots II and III. Yet such changes are not consistently related to after-ripening for these trends are just as perceptible in the room temperature lots where after-ripening did not take place.

Although the lots were kept uniformly moist throughout the after-ripening period, no special effort was made to keep the moisture content of the medium absolutely constant. Yet it is seen that these embryos showed little change in moisture content during the after-ripening period.

With the moisture content within the various lots of seed relatively constant, and evidently no significant loss in weight of the embryos occurring during the after-ripening period, percentages of the various constituents based on dry weight would seem justifiable as a com-

parable basis of expression.

Chemical Changes During the Drying of After-
Ripened Seeds.

Since it was found that the process of drying seeds following after-ripening results in a rapid reduction of their capacity to germinate, it was hoped that a study of the changes during this drying period might disclose a retention or reversion of certain conditions which it was believed would be attained in the process of after-ripening, and thus might serve to indicate which changes, if any, are necessary for growth release.

For this study seeds of apple, Mazzard, and Mahaleb were after-ripened at 1 - 2 degrees C. They were then removed from the stratification medium and allowed to dry at room temperature, for periods of 2, 5, 10, 15, 25, and 35 days. Upon the termination of each drying period seeds of the three kinds were soaked 48 hours to restore the moisture content, the enveloping structures then removed and the embryos preserved for analysis. Following each period of drying germination tests were conducted as previously described, using 100 seeds for each test.

The results are reported in tables XXII, XXIII, and XXIV. It is evident from a study of these tables that although marked reduction in germination was found to be associated with the drying of these after-ripened seeds no significant changes occurred in any of the fractions which were determined.

TABLE XXII -- Chemical Changes During the Drying of
After-ripened McIntosh Apple Seed.

Days of treatment	Percent water	Crude fat	Free red. sub.	Sucrose	Total sugar	Titratable acid	Nitrogen			Total percent germination
							Total	Alc. Insol.	Alc. Sol.	
Check (no after-ripening)	41.3	32.90	.37	2.95	3.32	3.49	7.79	7.34	.45	0
Check (after-ripened)	40.9	32.72	.38	2.47	2.85	3.69	7.83	7.40	.43	99
2	15.4	32.85	.38	3.04	3.42	3.68	7.80	7.41	.39	86
5	9.0	33.61	.39	3.39	3.78	3.72	7.84	7.36	.48	70
10	7.9	33.01	.43	3.49	3.92	3.74	7.84	7.40	.44	46
15	6.2	33.27	.46	3.39	3.85	3.67	7.82	7.43	.39	37
25	5.8	33.46	.48	3.30	3.78	3.72	7.86	7.39	.47	21
35	5.5	33.40	.52	3.23	3.75	3.76	7.87	7.46	.41	6

TABLE XXIII --- Chemical Changes During the Drying of
After-ripened Mazzard Cherry Seed.

Days of treatment	Percent water	Crude fat	Free red. sub.	Sucrose	Total sugar	Titratable acid	Nitrogen			Total percent germination
							Total	Alc. Insol.	Alc. Sol.	
Check (no after-ripening)	38.9	37.69	.67	4.38	5.05	6.87	4.89	4.09	.80	0
Check (after-ripened)	39.8	37.50	.72	4.09	4.81	6.99	4.97	4.16	.81	94
2	17.9	37.92	.83	3.87	4.70	6.94	4.91	4.14	.77	21
5	4.8	37.76	.77	3.66	4.43	7.01	4.88	4.17	.71	8
10	4.4	38.43	.81	3.66	4.47	7.07	4.92	4.19	.73	1
15	3.9	37.35	.83	3.53	4.36	7.04	4.81	4.12	.69	0
25	4.1	37.61	.97	3.27	4.24	7.09	4.83	4.13	.70	0
35	4.0	37.20	.98	3.27	4.25	7.16	4.76	4.10	.66	0

TABLE XXIV -- Chemical Changes During the Drying of
After-ripened Mahaleb Cherry Seed.

Days of treatment	Percent water	Crude fat	Total sugar *	Titratable acid	Nitrogen			Total percent germination
					Total	Alc. Insol.	Alc. Sol.	
Check (no after-ripening)	43.9	35.23	4.08	5.77	3.67	3.22	.45	0
Check (after-ripened)	49.0	35.04	4.41	5.89	3.62	3.16	.46	76
2	26.2	35.19	4.40	5.87	3.64	3.19	.45	68
5	4.7	35.31	4.42	5.95	3.59	3.16	.43	14
10	4.6	35.46	4.29	5.97	3.57	3.17	.40	6
15	4.5	35.20	4.39	5.92	3.61	3.21	.40	5
25	4.6	35.54	4.02	5.96	3.55	3.10	.45	0
35	4.4	35.63	4.23	5.99	3.59	3.20	.39	0

* Free reducing substances were found only in traces.

Chemical Changes in After-Ripened Seeds Dried At Room
Temperature for 35 Days, and Again After-Ripened.

A study of certain changes which may occur when after-ripened seeds which have been allowed to dry, are again after-ripened, has been made. The purpose was to afford a comparison between the composition of these seeds during the first after-ripening period and the composition during the second period of after-ripening, which might thus disclose whether certain changes were common to both periods.

Sufficient seed of each kind was after-ripened with the preceding lots so that a quantity of seed remained following the 35/^{day} interval of drying. Upon the completion of this period these seeds were soaked for 48 hours, placed in moist sand at the low temperature and again after-ripened. The lots of Mazzard and Mahaleb were sampled as previously described on four successive 10-day intervals, and the McIntosh at intervals of 7, 15, 25, 35, and 45 days.

The results are presented in table XXV. An inspection of the data shows that no significant changes in the various fractions have occurred as a result of this second after-ripening treatment.

Discussion of Chemical Studies

From the chemical studies herein reported it is evident that for the constituents which have been analyzed no significant changes have occurred which may be associated with the termination of the rest period. Contrary to these

TABLE XXV -- Chemical Changes in After-ripened Seeds Dried at Room Temperature
for Thirty-five Days, and Again After-ripened.

Mazzard Cherry Seed.										
Days of treatment	Percent water	Crude fat	Free red. sub.	Sucrose	Total sugar	Titratable acid	Nitrogen			Total percent germination
							Total	Alc. Insol.	Alc. Sol.	
10	39.2	37.20	.55	4.45	5.00	7.27	4.81	4.19	.62	7
20	38.9	37.74	.59	4.92	5.51	7.51	4.69	4.14	.55	2
30	39.1	37.80	.58	4.91	5.49	7.46	4.74	4.20	.54	3
40	38.7	37.52	.56	4.83	5.39	7.60	4.87	4.27	.60	0
Mahaleb Cherry Seed.										
10	42.8	35.13	—*	—	3.91	5.84	3.59	3.17	.42	9
20	43.1	35.41	—	—	4.21	5.91	3.66	3.25	.41	3
30	43.9	35.09	—	—	3.89	5.96	3.68	3.21	.47	1
40	42.6	35.56	—	—	3.74	5.90	3.63	3.15	.48	1
McIntosh Apple Seed.										
7	39.5	33.86	.37	2.83	3.20	3.69	7.81	7.34	.47	19
15	40.1	34.30	.35	2.91	3.26	3.77	7.87	7.36	.51	17
25	39.8	34.65	.35	2.98	3.33	3.83	7.80	7.39	.41	16
35	41.2	34.57	.29	2.94	3.23	3.74	7.84	7.40	.44	15
45	41.7	34.43	.29	3.08	3.37	3.84	7.76	7.32	.44	11

* Free reducing substances found only in traces.

results with fruit tree seeds, changes in some of these same fractions have been reported in the literature for certain other seeds which also require an after-ripening period at low temperature. (8) (13) (15) (27).

Before engaging further in discussion, it should again be emphasized that the seeds used in these studies were after-ripened at a temperature sufficiently low to preclude germination within the stratification medium. The fact that the seeds were after-ripened without germination in the medium is considered of importance.

A critical review of the literature concerned with chemical changes occurring in seeds during the after-ripening period at low temperature discloses that in much of the work a definite distinction has not been made between the changes which occur during after-ripening and those during the earlier stages of subsequent germination. However, if we adhere to the conception of after-ripening as explained by Eckerson (13), it refers to those changes or processes occurring within the resting embryo which eventually terminates the state of rest and makes growth possible.

Thus, in so far as showing the causal relationship between chemical changes and after-ripening, those changes undergone during the after-ripening period should not be confused with the changes occurring after the seed is capable of germination, or after germination has begun. In other words, as soon as germination can occur, the rest period is over, and

the changes which have made germination possible have already taken place.

This factor has not received sufficient consideration by certain investigators (13) (15) who have made comparisons of the composition of seeds before and following after-ripening. When such comparisons are made without careful consideration of this point, some doubt may exist as to whether the changes in composition which are found related to the breaking of the rest period, or are changes accompanying germination. In studies designed to follow the processes of after-ripening, it is therefore desirable to employ a temperature which does not permit germination within the stratification medium.

Part III -- Catalase Studies

Catalase activity has been used by numerous investigators as a measure of relative physiological activity in plant and animal tissues. It was the object of this study to determine whether changes in catalase activity occur during after-ripening in certain of the fruit tree seeds, and to what extent these changes may be associated with growth release.

Changes in Catalase Activity During After-Ripening.

At each interval of sampling for chemical analyses samples were also removed from each lot of seed for determination of catalase activity. Thus, as in the case of the chemical studies during after-ripening, the catalase determinations are reported for seeds of apple, peach, Mazzard, and Mahaleb under three treatments designated in Lots I, II, and III. Lots I, it will be recalled, were held moist at room temperature under which conditions after-ripening did not occur. Lots II and III were after-ripened at low temperature with Lot III air-dried previous to the low temperature exposure. The germination percentages reported at the various intervals in the chemical studies also serve in these studies as a criterion of the progress of after-ripening.

Procedure for Catalase Determinations. Following the removal of the enveloping structures the embryos were prepared for the catalase determinations as follows:

A definite weight of the sample was placed in a mortar together with an equal weight of calcium carbonate. One gram of sand and 5 cc. of water were added and the tissue was macerated for three minutes. If the quantity of sand is not constant for each sample the same degree of fineness will not be obtained in a definite period of grinding. Where volumetric flasks are employed to obtain the desired dilution a volume error will also be introduced. One gram of tissue was used in each determination for Mazzard, apple, and Mahaleb, and two grams for peach. Sufficient water was added to give final dilutions of 1 - 50 for Mazzard and Mahaleb, 1-100 for apple, and 1-25 for peach.

Immediately following dilution the catalase activity of the preparations were determined by means of an apparatus similar to that used by Knott (1) but modified to permit simultaneous shaking of duplicate samples. Because of the high catalase activity with these seeds, it was necessary to use certain precautions without which comparable results could not have been obtained. The sampling procedure will thus be here briefly reviewed.

Five cc of the prepared solution and 5 cc of hydrogen peroxide were placed in the opposite arms of a Bunzel reaction tube. It is recognized that, with tissues low in activity, errors which may accrue during the sampling procedure may not be sufficiently magnified to become readily observable. However, with tissues high in catalase activity

a closely standardized procedure of sampling is necessary for a high degree of accuracy. Therefore, in the withdrawal of a sample aliquot, a standardized procedure, based on the results of preliminary studies, was made.

The bottle containing the sample was first thoroughly shaken and after fifteen seconds standing 5 cc of the solution were withdrawn from the bottle at the rate of 1 cc per second. Sample bottles of the same size were used and the pipette was marked in such manner that it would be inserted to the same depth in the solution during the withdrawal of each sample.

The heavier material in the solution settles to the bottom rather quickly. It also settles out of the pipette. Preliminary studies indicated, therefore, that to obtain close checks between duplicate determinations, as well as to obtain results from one period to another which have been sampled on a comparable basis, it is essential that the length of time allowed for settling, the depth to which the pipette is inserted into the solution, and the time in which the solution is withdrawn into the pipette, are based upon a standardized procedure. The reaction tubes were connected with the burettes, and then submerged in the water bath which was maintained at 30 degrees C. Five minutes were allowed for the reaction tubes and their contents to come to equilibrium with the temperature of the bath. The reaction was then begun and the time seconds required to dis-

place 3, 5, 6, 7, 8, 9, 10, and 12 cc of water, successively recorded with results reported in tables XXVI to XXIX.

Discussion of Catalase Activity.

The results with each kind of seed indicate that as the after-ripening period progressed at the low temperatures in lots II and III, very pronounced increases in catalase activity occurred. Furthermore, comparison of the percentage germination with the catalase activity at each interval in the low temperature treatments indicate a fairly close association between increases in germination and increases in catalase activity.

In the catalase studies, as with the chemical studies, the results of Lots III are fundamentally the same as those of Lots II, thus serving to corroborate the findings in these studies that a period of dry storage after harvest does not interfere with the subsequent after-ripening processes.

In contrast to the increased catalase activity at low temperatures there was no increase in the seeds of Lot I held at room temperature. In these seeds after-ripening did not take place as evidenced by their complete lack of germination.

It is of interest to note that with each kind of seed a distinct difference in catalase activity exists between the dried and soaked checks of Lots III. Although the only difference in treatment between these two checks is that the one was soaked in water for five days the rate of activity is

TABLE XXVI-- Catalase Activity in Relation to After-Ripening

in McIntosh Apple Seed.

Lot I (Room Temperature)

Days of treatment	Seconds for the evolution of the following cubic centimeters of oxygen								Total percent germination
	3 cc.	5 cc.	6 cc.	7 cc.	8 cc.	9 cc.	10 cc.	12 cc.	
Check	28	45	54	68	82	97	121	170	0
7	32	47	56	71	84	102	128	175	0
14	29	45	56	67	82	95	118	162	0
21	30	47	58	69	86	98	125	173	0
28	28	45	54	62	80	97	120	168	0
35	31	48	59	66	82	97	123	164	0
42	29	46	55	68	82	95	119	169	0
49	28	45	54	66	80	95	116	162	0
56	30	47	57	66	83	96	121	164	0

Lot II (After-ripened at 1-2° C.)

7	29	46	57	66	82	92	122	164	3
14	28	44	54	65	81	97	113	157	11
21	27	42	50	61	75	90	103	150	29
28	22	39	50	57	70	81	93	146	44
35	22	38	49	55	66	76	89	138	43
42	20	35	45	53	62	71	85	131	68
49	19	32	43	48	57	61	78	115	75
56	15	29	35	44	52	54	70	97	90

Lot III (Dried Prior to After-ripening at 1-2° C.)

Check (dry)	39	69	87	103	113	124	146	202	0
Check (soaked)	29	47	58	71	87	94	125	165	0
7	32	53	65	77	90	101	129	171	2
14	29	48	58	70	82	97	113	149	1
21	26	40	48	59	71	85	101	138	1
28	24	36	44	53	64	75	90	123	13
35	23	35	46	53	64	76	90	124	27
42	20	33	44	49	58	69	83	119	50
49	17	28	40	46	53	62	73	107	64
56	13	25	33	39	46	52	61	98	98

TABLE XXVII-- Catalase Activity in Relation to After-ripening in

Elberta Peach Seed.

		Lot I (Room Temperature)								Total percent germina- tion
Days of treat- ment	:	Seconds for the evolution of the following cubic centimeters of oxygen								
	:	3 cc.:	5 cc.:	6 cc.:	7 cc.:	8 cc.:	9 cc.:	10 cc.:	12 cc.:	
Check	:	95	172	---	260	308	363	421	551	: 0
10	:	88	168	212	254	298	350	410	532	: 0
20	:	98	180	223	268	319	373	439	578	: 0
30	:	107	188	229	280	328	375	439	573	: 0
40	:	110	191	235	283	334	391	456	597	: 0
50	:	109	197	242	290	341	396	462	602	: 0
60	:	112	204	257	311	368	429	499	674	: 0
70	:	120	224	282	347	415	485	559	755	: 0
80	:	119	211	268	330	398	468	544	744	: 0
Lot II (After-ripened at 1-2° C.)										
10	:	88	162	198	245	294	347	405	539	: 0
20	:	89	163	206	253	301	354	416	544	: 20
30	:	80	149	182	223	268	320	380	520	: 16
40	:	75	142	178	214	251	294	341	436	: 20
50	:	56	96	118	141	164	189	218	282	: 40
60	:	64	105	130	154	178	210	243	316	: 32
70	:	57	99	118	140	166	194	226	281	: 40
80	:	49	88	106	129	152	176	203	266	: 56
Lot III (Dried Prior to After-ripening at 1-2° C.)										
Check (dry)	:	121	226	273	328	378	430	504	640	: 0
Check (soaked)	:	87	161	194	236	296	342	406	533	: 0
10	:	90	170	206	249	298	350	410	542	: 4
20	:	87	164	201	243	293	348	409	539	: 8
30	:	83	153	187	224	271	324	388	529	: 28
40	:	79	147	179	213	254	302	356	475	: 32
50	:	68	109	133	165	203	244	274	367	: 40
60	:	59	101	122	145	170	196	224	284	: 44
70	:	53	93	111	135	155	179	206	270	: 52
80	:	48	84	101	122	146	170	197	254	: 56

TABLE XXVIII--- Catalase Activity in Relation to After-ripening in
Mazzard Cherry Seed.

Lot I (Room Temperature)									
Days of treatment	Seconds for the evolution of the following cubic centimeters of oxygen								Total percent germination
	3 cc.	5 cc.	6 cc.	7 cc.	8 cc.	9 cc.	10 cc.	12 cc.	
Check	51	88	113	134	163	189	219	296	0
10	56	92	114	138	164	195	231	321	0
20	53	86	110	132	159	187	218	281	0
30	54	89	105	135	158	194	226	286	0
40	55	84	104	130	153	184	221	304	0
50	57	87	106	137	158	191	225	303	0
60	52	91	111	134	156	185	220	287	0
70	55	97	115	150	186	210	244	327	0
80	59	101	128	157	187	224	262	362	0
Lot II (After-ripened at 1-2° C.)									
10	52	93	116	140	166	191	224	314	0
20	46	81	102	124	147	174	199	261	2
30	45	80	101	120	142	167	193	244	8
40	46	77	94	114	136	162	184	245	6
50	39	69	85	105	123	140	160	215	8
60	34	64	76	89	101	116	134	182	8
70	30	55	66	77	89	101	120	166	48
80	34	59	71	82	94	108	126	174	50
Lot III (Dried Prior to After-ripening at 1-2° C.)									
Check (dry)	72	122	161	190	218	245	267	346	0
Check (soaked)	52	89	113	133	159	186	216	289	0
10	51	90	115	137	163	189	220	301	0
20	53	88	112	134	157	185	218	293	2
30	50	91	110	132	154	181	214	288	4
40	51	89	112	135	160	188	219	291	10
50	47	80	102	125	148	176	204	272	8
60	41	73	89	109	127	147	169	227	18
70	33	64	80	95	113	123	139	187	50
80	29	59	73	88	102	116	129	171	56

TABLE XXIX--Catalase Activity in Relation to After-ripening in

Mahaleb Cherry Seed.

Lot I (Room Temperature)

Days of treatment	Seconds for the evolution of the following cubic centimeters of oxygen								Total percent germination
	3 cc.	5 cc.	6 cc.	7 cc.	8 cc.	9 cc.	10 cc.	12 cc.	
Check	31	57	70	87	101	125	153	225	0
14	31	57	73	90	104	124	153	226	0
28	29	58	71	88	102	122	153	220	0
42	30	61	73	95	108	130	162	232	0
56	32	60	73	96	107	130	160	227	0
70	31	60	76	96	110	131	163	231	0
84	29	58	70	89	100	122	151	221	0

Lot II (After-ripened at 1-2° C.)

14	31	56	71	87	102	125	153	224	2
28	29	50	64	78	94	113	138	196	10
42	26	46	57	69	84	104	125	172	36
56	24	40	49	60	77	99	118	155	41
70	20	35	41	55	69	94	102	140	65
84	15	27	34	39	48	57	75	101	84

Lot III (Dried Prior to After-ripening at 1-2° C.)

Check (dry)	36	68	84	101	122	145	173	238	0
Check (soaked)	25	44	56	72	90	114	140	210	0
14	27	45	57	72	86	110	133	184	3
28	25	43	56	67	81	101	123	173	12
42	23	40	48	62	76	96	116	162	41
56	22	38	46	58	72	90	109	153	52
70	19	31	40	52	65	82	102	132	71
84	14	25	30	37	48	53	68	94	81

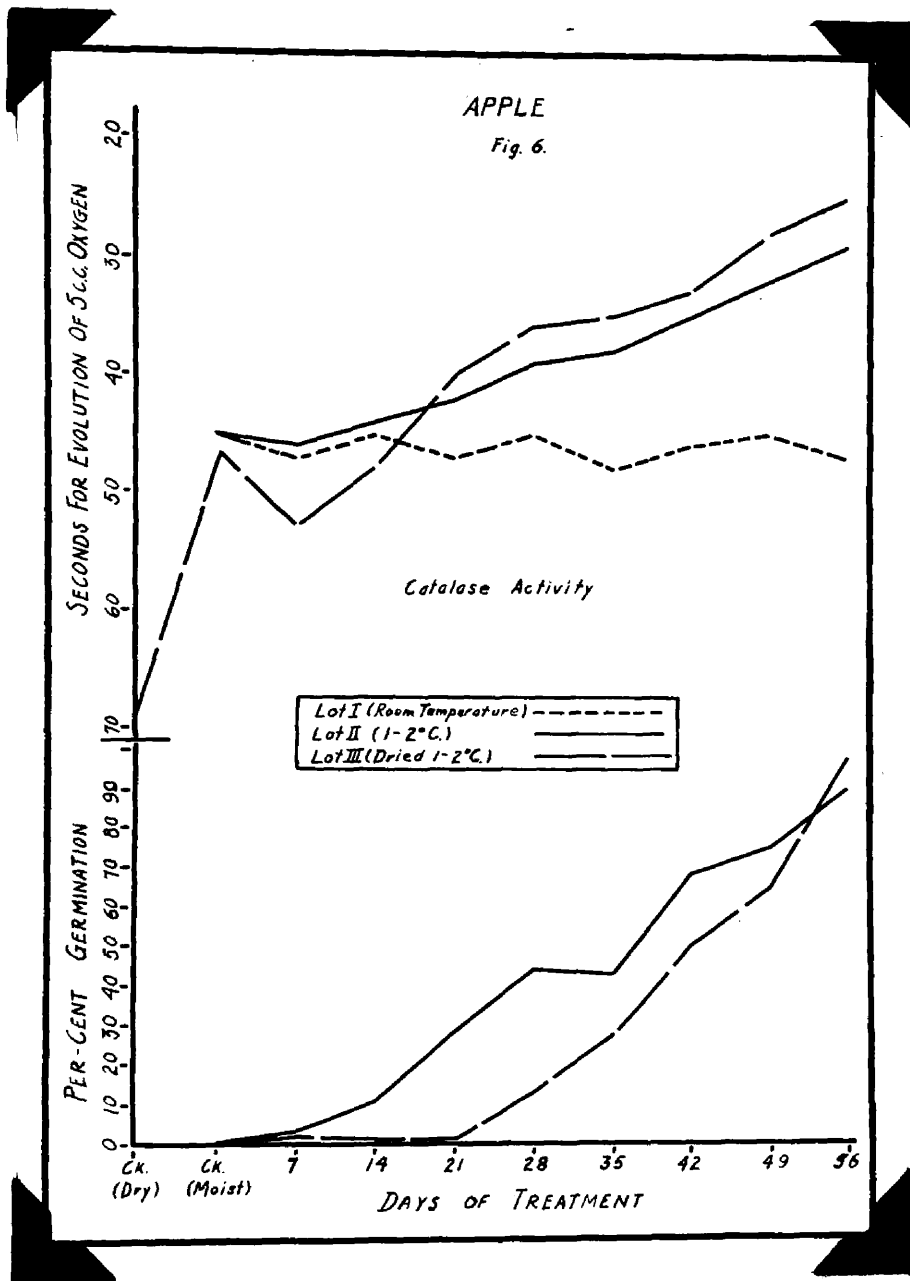


Figure 6 -- Catalase activity in relation to after-ripening.

McIntosh apple seed.

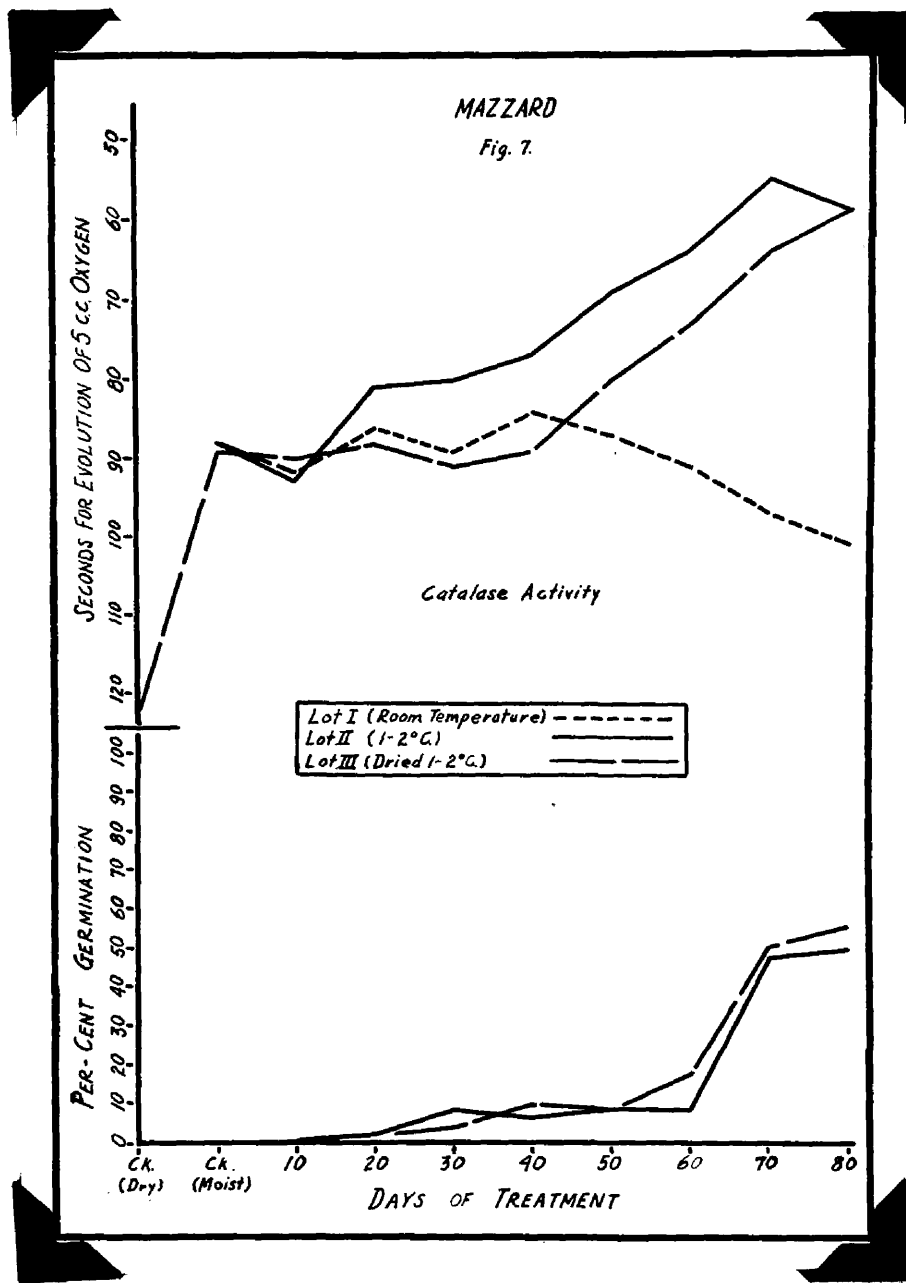


Figure 7 -- Catalase activity in relation to after-ripening.

Mazzard cherry seed.

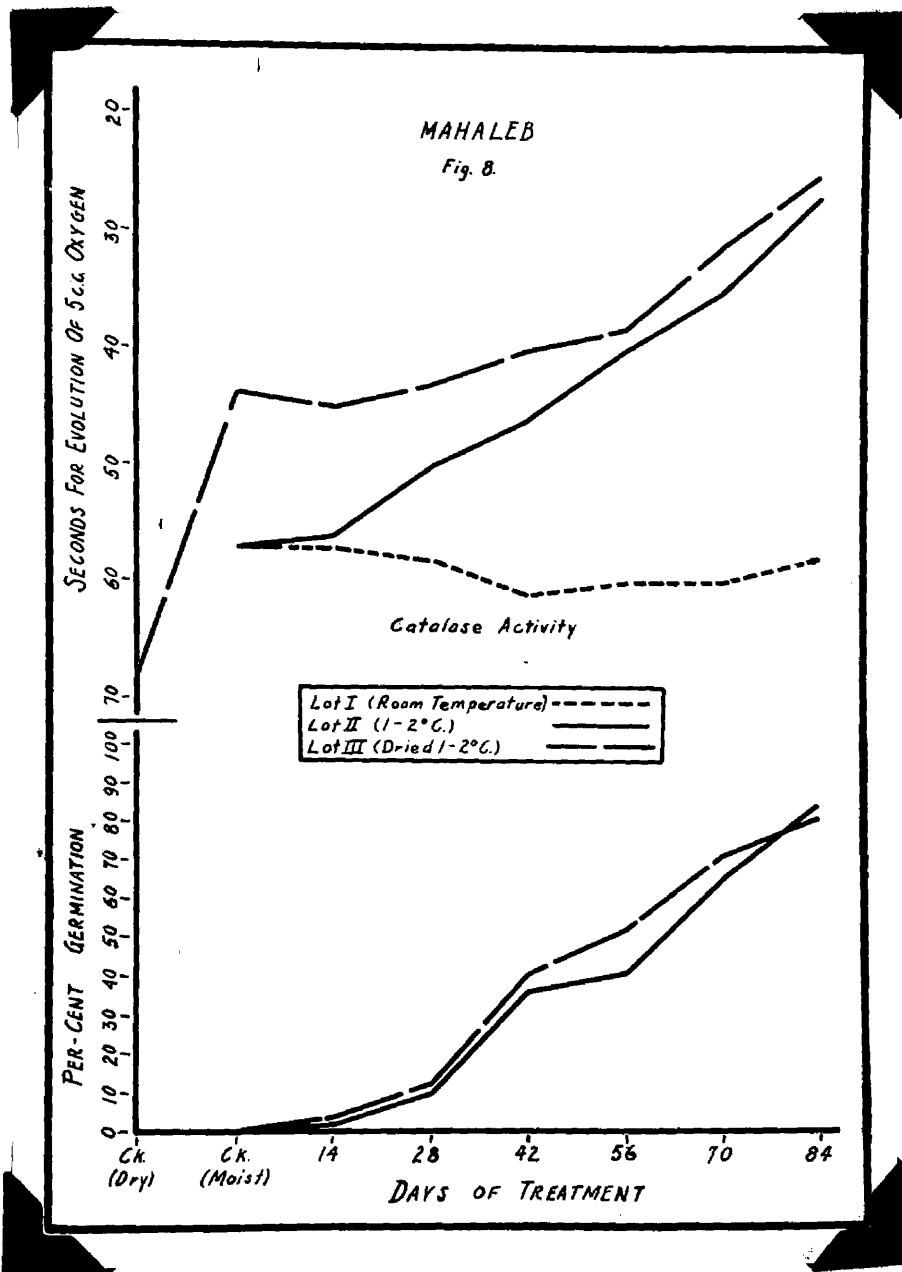


Figure 8 -- Catalase activity in relation to after-ripening.

Mahaleb cherry seed.

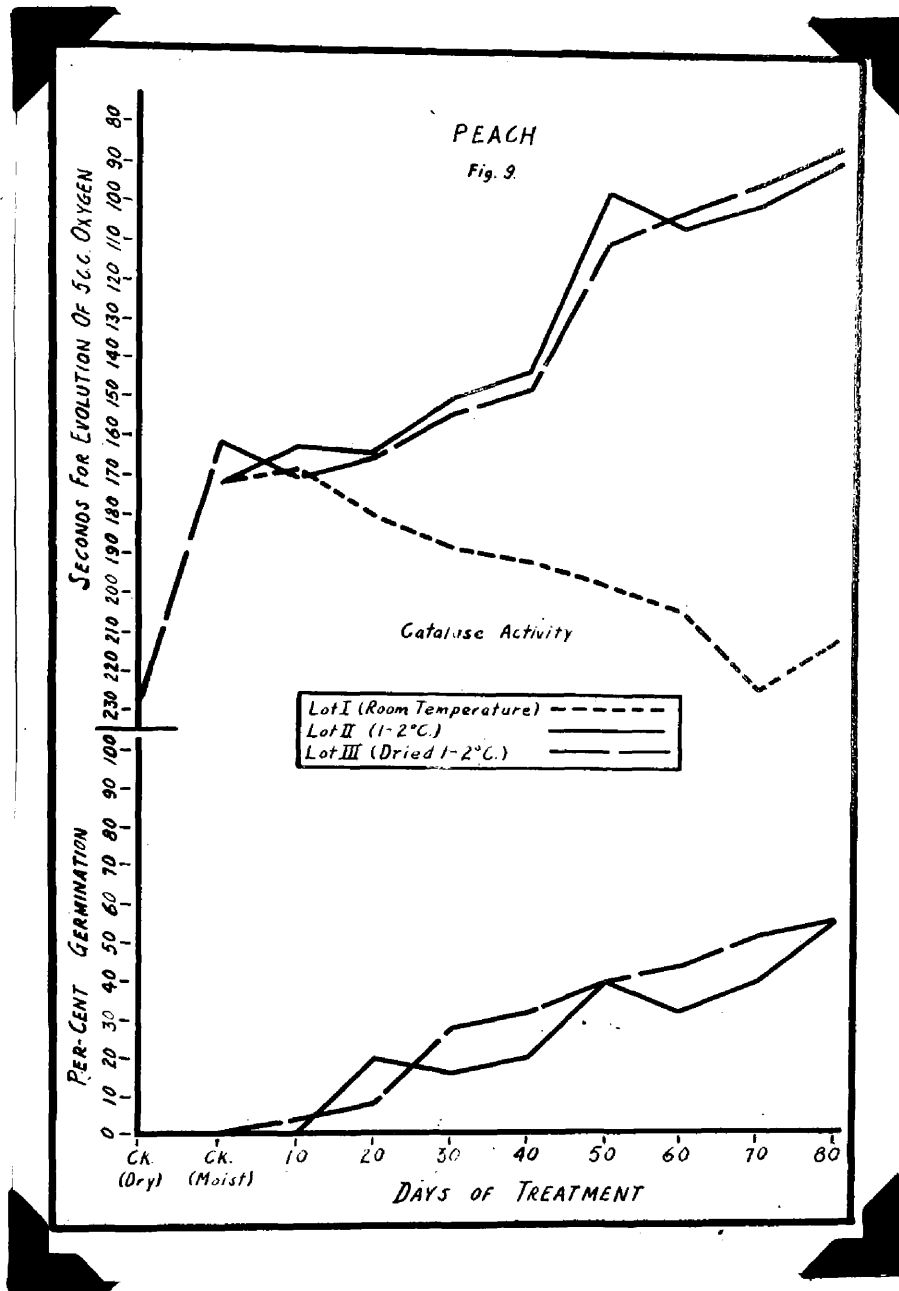


Figure 9 -- Catalase activity in relation to after-ripening.

Elberta peach seed.

considerably slower in the dried seeds. This is true despite the fact that the aliquot sample represented considerably more living tissue in the dried seeds than does a similar aliquot in the case of those seeds in a moist condition. This suggests that greater attention should be given in catalase studies in general to the relative moisture contents of given tissues when sampled for a comparison of different treatments.

Although the results of the studies of various factors which influence after-ripening and germination are of immediate practical value to horticulture, the biochemical phases, in which considerable hope had been entertained as a key to the secret of the rest period, have failed to disclose any relationship between after-ripening and chemical composition. While this study has shown that the constituents here determined are not involved, at least by variations in their amounts, with the processes of after-ripening, it should not necessarily be concluded that nutritional changes are non-essential, or that future investigations in this direction will prove fruitless. The possibility of important changes in constituents other than those which have been here considered is not precluded.

The close relationship of catalase activity with after-ripening suggests, however, that further related studies of this nature should be made.

LITERATURE CITED

1. Appleman, C. O. Biochemical and physiological study of the rest period in the tubers of *Solanum tuberosum*. Bot. Gaz. 61: 265-294. 1916.
2. Bakke, A. L., Richey, H. W., and Reeves, Kenneth. Germination and storage of apple seeds. Iowa Agric. Exp. Sta. Res. Bull. 97:241-255. 1926.
3. Crocker, William Secondary dormancy in seeds. Bot. Gaz. 67:269-270. 1919.
4. Crocker, William Harvesting, storage, and stratification of seeds in relation to nursery practice. Boyce Thompson Inst. Prof. Pap. I (15): 114-120. 1930.
5. Crocker, William Dormancy in hybrid seeds. Boyce Thompson Inst. Prof. Pap. I (6): 36-41. 1927.
6. Crocker, William and Barton, V. After-ripening, germination, and storage of certain rosaceous seeds. Contrib. Boyce Thompson Inst. 3:385-404. 1931.
7. Crocker, William and Harrington, G. T. Catalase and oxidase content of seeds in relation to their dormancy, age, vitality, and respiration. Jour. Agr. Research 15: 137-174. 1918.
8. Davis, O. H. Germination and early growth of *Cornus florida*, *Sambucus canadensis*, and *Berberis thunbergii*. Bot. Gaz. 84:225-263. 1927.
9. Davis, W. E. Primary dormancy, after-ripening, and the development of secondary dormancy in embryos of *Ambrosia trifida*. Amer. Jour. Bot. 17:58-76. 1930.
10. Davis, W. E. The use of catalase as a means of determining the viability of seeds. Boyce Thompson Inst. Prof. Pap. I (2): 6-12. 1926.

11. Deleano, N. T. Recherches chimiques sur la germination. Centralbl. Bakt. 24: 130-147. 1909.
12. Deuber, G. G. Chemical treatments to shorten the rest period of red and black oak acorns. Jour. Forestry, 30: (6) 674-679. 1932.
13. Eckerson, S. H. A physiological and chemical study of after-ripening. Bot. Gaz. 55: 286-299. 1913.
14. Flemion, Florence After-ripening, germination, and vitality of seeds of *Sorbus aucuparia*, Contrib. Boyce Thompson Inst. 3:413-439. 1931.
15. Flemion, Florence Physiological and chemical studies of after-ripening of *Rhodotypos kerrioides* seeds. Contrib. Boyce Thompson Inst. 5:143-159. 1933.
16. Gardner, F. E. Useful device for evaporating alcohol from plant extracts. Plant Physiol. 5:(4) 1930.
17. Green, J. R. On the germination of the castor-oil plant. Proc. Roy. Soc. 48:370-392. 1890.
18. Harrington, G. T. Respiration of apple seeds. Jour. Agr. Research 23:117-130. 1923.
19. Harrington, G. T. and Hite, B. C. After-ripening and germination of apple seeds, Jour. Agr. Research 23:153-161. 1923.
20. Howard, W. L. An experimental study of the rest period in plants. Mo. Res. Bull. 17. 1-50. 1915.
21. Ivanow, Sergius Über den Stoffwechsel beim Reifen ölhaltiger Samen mit besonderer Berücksichtigung der Ölbildungsprozesse. Beih. Bot. Centralbl. 28:159-191. 1912.
22. Ives, S. A. Maturation and germination of seeds of *Ilex opaca*. Bot. Gaz. 76:60-77. 1923.

23. Kidd, F. and West, C. On the production of secondary dormancy in seeds of *Brassica alba* following treatment with carbon dioxide and the relation of this phenomenon to the question of stimuli in growth processes. *Annals. Bot.* 31:457. 1917.
24. Knott, J. E. Catalase in relation to growth and to other changes in plant tissue. *Cornell Memoir* 106. 1927.
25. Miller, E. C. A physiological study of the germination of *Helianthus annuus*. *Ann. Botany* 24:693-726. 1910.
26. Müntz, M. Sur la germination des graines oleagineuse. *Ann. de Chimie* IV. 22: 472. 1871.
27. Pack, D. A. Chemistry of after-ripening, germination, and seedling development of Juniper seeds. *Bot. Gaz.* 72:139-150. 1921.
28. Rhine, L. E. Divergence of catalase and respiration in germination. *Bot. Gaz.* 78:46. 1924.
29. Sherman, Hope Respiration in dormant seeds. *Bot. Gaz.* 72:1. 1921.
30. Tukey, H. B. Studies of fruit seed storage and germination. *New York Agr. Exp. Sta. Bul.* 509. 1924.
31. Tukey, H. B. Seedling fruit stocks. *New York Agr. Exp. Sta. Bul.* 569. 1924.

ACKNOWLEDGMENT

The writer expresses his deepest appreciation to Dr. F. E. Gardner, who suggested the problem and has given many helpful suggestions and kindly criticisms throughout the course of the work and in the preparation of this paper.

Grateful acknowledgment is due Dr. H. B. Cordner for many valuable suggestions in laboratory methods and technique; and to Doctors J. H. Beaumont and F. B. Lincoln for the many helpful suggestions received.

The writer also expresses his appreciation to the Graduate School of the University of Maryland for the fellowship which permitted this investigation to be undertaken.

APPROVED F. E. Gardner

DATE May 13, 1933