T H E S I S

on

An Attempt to Hasten the After-ripening and Germination of the Sweetbrier, Rosa rubiginosa

---

Submitted to the

OREGON STATE AGRICULTURAL COLLEGE

In partial fulfillment of
the requirements for the
Degree of

MASTER OF SCIENCE

by

William Brewster Hayes

April 12, 1930

APPROVED:

Professor of Research in Horticulture

In Charge of Major

Head of Department of Horticulture

Chairman of Committee on Graduate Study.

OUTLINE

I. The problem of delayed germination.

A. Many species effected.

B. Rosa rubiginosa taken as a difficult case.

II. Review of the literature.

A. Types of delayed germination.

B. Treatment of seed to overcome delayed germination.

C. The physiology of delayed germination.

D. The rest period of buds and tubers, and methods of shortening it.

III. Proceedure.

A. Preparation of the seeds.

B. Scarification.

C. Chemical treatment.

D. Cold storage treatment.

IV. Results.

V. Discussion.

AN ATTEMPT TO HASTEN THE AFTER-RIPENING AND GERMINATION OF THE SWEETBRIER, ROSA RUBIGINOSA

Many kinds of seeds may be fully matured on the plant and placed under optimum conditions for germination, and yet fail to germinate for long periods. If left in the ground, or stored under certain conditions, eventually a large percentage of the seeds germinate. This phenomenon of delayed germination is of much interest scientifically, and of very real practical importance.

Many members of the family Rosaceae exhibit this behavior, including the different species of roses. In the latter case, the seed ordinarily germinate only after one or more years. The common sweetbrier, Rosa rubiginosa L., is grown to a certain extent as a stock for cultivated roses. In breeding experiments it is also necessary to grow this and other species from seed. It was therefore felt that an attempt to find a method of hastening germination would be of some value directly, as well as for the light it might shed on the larger problem of delayed germination in general. It was with this object that the present study was undertaken.

Review of the Literature

The question of delayed germination has been studied by numerous investigators during the present century. In some of the earlier literature there is little attempt to

distinguish between different causes of delayed germination but in 1916 Crocker (10) published a paper in which he brought out very clearly the various causes. These he classified as: 1, seeds with rudimentary embryos which must mature; 2, seeds in which the absorption of water is completely inhibited; 3, seeds in which the mechanical resistance of the enclosing structure prevents growth; 4, seeds in which the enclosing structures interfere with the absorption of oxygen and possibly with the elimination of carbon dioxide; 5, seeds in which the embryo is dormant; 6, seeds having a combination of two or more of these factors; and 7, seeds having developed a secondary dormancy. It will be seen that in types 2, 3 and 4, germination is delayed by the seed coat. Types 1 and 5 represent conditions of the embryo itself.

Seed coats which delay germination are o f frequent occurence. Crocker (9) showed that this was the case with certain water plants. Here the seed coat prevented germination by effectively excluding the water. Harrington and Crocker (21) studied the seeds of Johnson grass and Sudan grass. The latter germinates readily at any time after reaching maturity. Johnson grass, on the other hand, does not germinate immediately. They found that by removing the seed coat they could force immediate germination. Examination showed that the coat of Johnson grass was

somewhat stronger and more resistant to water than that of Sudan grass.

Atwood (4) studied germination and after-ripening of oats. He found that the rate of oxygen absorption increased during after-ripening, but that if the seed coat were removed, it reached the maximum immediately. The acidity and water holding power also increase during after-ripening. However, he concluded that the limiting factor was the oxygen supply. In this and many other cases it is probable that the two factors of the seed coat and the need for certain chemical and physiological changes within the embryo are combined.

Delayed germination due to an immature embryo is not common, but is well illustrated by holly, Ilex opaca, as reported by Ives (23). He found that the embryo is immature, and requires a long time to reach maturity. Even when mature, it is difficult for it to break through the pericarp. In nature, probably not more than one seed in ten million germinate. Ives found that by drying the seed six hours at 40 degrees, C., he could cut away the pericarp, and that this hastened germination. But even with the naked embryo kept at a temperature of 25 to 30 degrees C. and moistened with a 5% solution of dextrose, these conditions having been found optimum, germination required five months. Treatment with normal KOH for five

minutes, and normal HCl for three minutes, rendered the pericarp more permeable, and could be substituted for the removal of the pericarp.

A number of seeds fail to germinate promptly, even with the coats removed, due to dormancy of the embryo. Harrington and Hite (25) have reported that the naked embryos of the apple never grow normally when fresh, although they sometimes grow feebly. Other seeds requiring after-ripening, as listed by Crocker and Harrington (11), are Crataegus, peach, Ambrosia trifida, Ambrosia artemisiaefolia, and basswood, Tilia americana.

Many ways of treating seed to overcome delayed germination have been worked out. Investigators have not always differentiated between different types of dormancy, although it is obvious that methods effective in one case would be useless in another. The practice of stratifying seeds, such as the peach, has long been used by nurserymen and farmers. Weiss (34) found that the germination of the gray birch, Betula populifolia, could be accellerated by prolonged soaking in sulfuric acid in dilute solution, or using this to smoisten the substratum; by soaking in one tenth normal KNO_3 for 24 hours; by soaking 24 hours in .1% solution of $HgCl_2$ or in Uspulun (chlorophenol mercury), .25%. Storing for two months in moist granulated peat at from 0 to 10 degrees C. also

greatly improved germination.

Further work was done on the birch by Joseph (25), who applied the cold storage treatment to three additional species, Betula lenta, B. papyrifolia and B. lutea. In all cases he found the most rapid after-ripening at temperatures between 0 and 5 degrees C. At 10 degrees the process still went on, but more slowly than at the lower temperatures. From four to 10 weeks were required.

Some of the earliest work in overcoming dormancy was done on seeds of Crataegus. Davis and Rose (15) reported that seeds of C. mollis failed to germinate until the second or third year. With the testas removed there was a very small percentage of seeds in which the hypocotyl grew, not more than 4%, without after-ripening. With the carpels removed, and the embryos held in a moist condition at 5 to 6 degrees C. for 96 days, there was 50 to 80% germination in 10 to 20 days. Even with the seed coat not removed, there was considerable after-ripening. Seeds treated dry, or under water, did not after-ripen. After-ripening was less at 0 and did not occur at -2 or -3 degrees, C. Temperatures above 7 degrees were not as favorable as 5 and 6 degrees. There was some germination at temperatures slightly above freezing, but when after-ripening is complete, germination is more rapid at the temperature of a greenhouse.

Further work on Crataegus was done by Eckerson (20), who found that the time of after-ripening at 5 degrees C. was greatly reduced by previous treatment with dilute hydrochloric, acetic or butyric acid. When the entire seed was treated, germination occurred in 45 to 53 days, while with the testa removed, only 16 to 18 days were required.

Davis (12) worked with three species. Cornus florida was found to after-ripen best when stored under moist conditions at from 5 to 10 degrees C. It was not aided by acid treatment, nor by ether or ethylene. Some seeds of Sambucus canadensis were found dormant. They would grow after 85 to 100 days at 0 to 5 degrees C., or after 14 to 30 days at alternating temperatures of 10 and 27 degrees. Berberis thunbergii also responded to alternating temperatures.

Rose (30) also worked with Sambucus, and found that a number of different chemical treatments gave better results than the untreated checks. The seeds were held at 4 to 6 degrees C. for 63 days, and then at room temperature for from four to 14 days. Of the chemicals, $\frac{n}{20}$ $ZnSO_4$, $\frac{n}{200}$ $NaNO_3$ and $\frac{n}{20}$ KNO_3 seemed to produce the best results. The naked embryo gave about the same percentage of germination as the better chemical treatments, but stratification in moist sand over winter gave about

twice as high germination. This would indicate that dormancy was caused by both the seed coat and the condition of the embryo, and that chemicals effected only the former.

In the same paper, Rose reports on seeds of Tilia and of Rubus. Chemical treatments of the former were unavailing, but the seeds were found to after-ripen at temperatures just above freezing, though they would not germinate until placed in a warmer atmosphere. On the other hand, seeds of Rubus respond to vigorous chemical treatment. When the endocarp was removed by soaking for two hours in concentrated sulfuric acid, washing and neutralizing with 5% sodium bicarbonate, rinsing and rubbing on filter paper, the embryos grew in from four to 20 days. In this case the effect of the coat was not in keeping out water, as the whole seed absorbed more water than that with the endocarp removed. It may be that the coat is too strong to be readily broken by the embryo.

Harrington and Hite (23) found that apple seeds germinated after cold storage in the fruit, or when held at 5 to 10 degrees C. for a few months. After-ripening did not take place in apple seeds stored at temperatures above 20 degrees.

Seeds of Ambrosia trifida are believed by Davis (13) to be delayed in germination by both the dormancy of the

embryo and the nature of the seed coat. In dry storage, after-ripening is slow and unequal, requiring from a few months to several years. At 5 degrees C., seeds kept moist will germinate in 70 to 90 days, and show greater vigor than those after-ripened under warm dry conditions. However, previous dry storage decreases the period of cold which is necessary. Following after-ripening, the seeds germinate slowly at 20 degrees C., more rapidly at 30 degrees, and most rapidly when the temperature alternates between 20 and 30 degrees. However, naked embryos will germinate immediately after a period in cold storage. It is thought that germination is delayed by the slow intake of oxygen. The nucellar membrane was found more effective in preventing the exchange of gasses than the involucre and cell wall combined.

A number of horticultural fruits were investigated by Tukey (33). Peach kernels were held 11 months under conditions favorable to germination, and absorbed water, but did not grow. This seed after-ripens in a short time, but does not germinate until it can break the seed coat. On the other hand, the Palatine plum did not germinate until after a long period, even when cracked. Seeds of the currant, gooseberry, raspberry and blackberry responded to a combination of stratification and acid treatment which reduced the coat.

Many coniferous seeds require after-ripening. The seeds of junipers seem very unresponsive to treatment. Pack (27,28) tried the removal of the coat, various chemical treatments, and storage at varied temperatures. Only the latter gave positive results. Seed kept in running water at 10 to 12 degrees C. gave about 10% germination between the fourth and sixth months. Moist storage at 5 degrees gave the most rapid after-ripening, but still required a long period. Drying and moistening at the 45th day reduced the period by five to 10 days. After-ripening was slower at 10 degrees, and at a temperature of 12 degrees secondary dormancy occurred.

Barton (6) found that several species of pine germinated well when stratified one or two months at 0, 5, 10 or 15 degrees C. He quotes Larsen as believing that in the Western White pine, delayed germination was due to the impermeability of the seed coat. In a later paper (5) he reports experiments in which he stored the seeds of many species of conifers in acid peat for from one to three months, at 0, 5 and 10 degrees C. In most cases germination was hastened by the treatment, especially at 5 degrees, and in the majority, the total percentage of germination was also increased.

Bittersweet, Celatrus scandens, also responds to similar treatment, according to Joseph (26). In this

case the seed must first be dried at least two weeks at room temperature. It was stratified in sand, soil or peat for three months at 0 to 10 degrees C. or for five months in the open, with good results.

The only work bearing directly on the problem of after-ripening of rose seed is that of Crocker, who reported on four species in 1926 (7). The best temperature for after-ripening was found to be 41 degrees F. although temperatures fluctuating between 32 and 50, and averaging about 41 degrees were also suitable. A considerable percentage of the seed germinated at 41, but when germination began the seed was placed at a temperature of 68 degrees. In order to give 60% germination after three to five days in the warmer atmosphere, it was found necessary to keep Rosa multiflora 60 days in cold storage, and R. rugosa and R. rubiginosa three to four months. R. marreti required a much longer period of after-ripening. In a later paper, Crocker (8) added that R. canina required nearly a year. Hybrids with southern roses, on the other hand, do not require as low temperatures.

In the same paper, Crocker observes that the best temperature for after-ripening seeds of the Rosaceae was about 5 degrees C. That temperature was given as best for the damson plum, and slightly better for the peach than 10 degrees, while the pear was said to do about

equally well at 5, 10 or 15 degrees. More recent, unpublished work by Crocker indicates that each species has its own optimum temperature and that variations of much less than five degrees are significant.

Secondary dormancy often develops in seeds which have been after-ripened, if they are held at higher temperatures under conditions unsuitable for germination, as, for instance, in ordinary storage. Harrington and Hite (23) referred to this in the case of the apple, and Davis (13) found that if after-ripened seeds of Ambrosia trifida failed to germinate, secondary dormancy set in, necessitating another period of after-ripening as long as the first.

The cocklebur, Xanthium, is inhibited from germination by its seedcoat, but the naked embryo will ordinarily grow at once under favorable conditions. However, Davis (14) was able to induce dormancy by imbedding the hypocotyl end of the seed in clay, or the entire seed in agar for eight to 10 weeks. Seeds treated in this way failed to grow when the naked embryo was subjected to favorable conditions. Several weeks of storage at 5 degrees C. removed this induced dormancy. Ungerminated upper seeds were dug in March, and the naked embryos germinated immediately, while those dug in July did not, having become dormant. It is thought that the development

of dormancy during the summer, and after-ripening in winter are rather general among seeds in the temperate zone.

A number of investigators have studied the physiological changes which go on during after-ripening. The work of Eckerson (20) on Crataegus is classical. She found that during after-ripening there is a gradual increase in acidity and in enzymes. After 80 or 90 days at 5 degrees C., the fats begin to break up and sugar appears. A little later oxidase appears. At about the 75th day HCN is found, and increases until germination, after which it decreases. The water holding capacity of the hypocotyl also increases.

Treatment with hydrochloric, butyric and acetic acids not only decreased the period of after-ripening, but increased the catalase, oxidase and peroxidase, while butyric and acetic acids also increased the acidity.

Crocker and Harrington (11) confirmed this work and extended it to other species, finding increased oxidase and catalase activity in after-ripened seeds. Davis (12) reported increased catalase activity in dogwood seeds during after-ripening, and also an increase in starch, sugar and soluble protein. He found no change in the acidity. Pack (27,28) found that conditions which favored after-ripening also favored constructive metabolism, and

increased catalase activity. Davis (13) observed that during the after-ripening of Ambrosia trifida, both acidity and catalase activity increased, but that acidity also increased at storage at higher temperatures, while catalase activity did not. Appleman (3) has pointed out that catalase activity shows a striking correlation with respiration.

It seems probable from these investigations that enzyme activity is more a result of metabolism than a cause of after-ripening, but that it, and particularly catalase activity, may be useful as an indication of the progress of processes which accompany after-ripening. The question of how different temperatures and chemical treatments effect the metabolism of the seed remains unanswered.

A summary of the more important treatments of delayed germination is given in the accompanying table.

In plant tissue, dormancy occurs in buds as well as in seeds, and it seems reasonable that conditions effecting dormancy in one case might do so also in the other. It is well known that potato tubers will not grow immediately after reaching maturity. Appleman (1,2) has shown that storage at 0 degrees C. shortens the rest period. Aiding the absorption of oxygen is also effective.

Rosa (29) tested several chemical treatments to

Summary of Experiments on

Seed	Type of Dormancy	Investigator
Water plants	Exclusion of water	Crocker
Johnson grass	Exclusion of water	Harrington and Crocker
Oats	Exclusion of oxygen and embryo dormancy	Atwood
Holly	Immature embryo	Ives
Apple	Dormant embryo	Harrington and Hite
Betula populifolia	?	Weiss
Betula spp.	?	Joseph
Cornus florida	?	Davis
Sambucus canadensis	?	Davis
Berberis thunbergii	?	Davis
Sambucus spp.	Seed coat and embryo dormancy	Rose
Tilia sp.	?	Rose
Rubus spp.	Coat too strong (?)	Rose
Ambrosia trifida	Exclusion of oxygen and embryo dormancy	Davis

Removal of Dormancy in Seeds

Method	Results
Removal of coat	Complete germination
Removal of coat	Complete germination
Removal of coat	Hastened germination
Naked embryo kept at low temperature with dextrose solution	Germination in five months
After-ripening at 5-10 degrees	Good germination
After-ripened seed held at room temperature	Secondary dormancy
Chemicals and low temperature	Hastened germination
Low temperatures. 0-5 best	Hastened germination
Held at 5-10 degrees	Hastened germination
Chemical treatment	Negative
0-5 degrees Alternating 10-27 degrees	Germinated in 100 days Germinated in 30 days
Alternating temperatures	Hastened germination
Chemicals at 4-6 degrees	Hastened germination
After-ripened just above freezing	Good germination
Chemical treatment	Negative
Severe chemical treatment	Growth in 4-20 days
Held at 5 degrees	Slow growth in 90 days
Naked embryos at 5 degrees	Immediate growth
After-ripened, then warm	Secondary dormancy

Summary of Experiments on Removal

Seed	Type of Dormancy	Investigator
Crataegus mollis	Seed coat and embryo dormancy (?)	Davis and Rose
Crataegus spp.	Seed coat and embryo dormancy (?)	Eckerson
Coniferous seeds	?	Pack
Pinus spp.	Impermeable coat (?)	Barton
Conifers	?	Barton
Bittersweet	?	Joseph
Rosa spp.	Embryo dormancy	Crocker
Rosaceae	?	Crocker
Xanthium spp.	Not dormant	Davis

of Dormancy in Seeds (Continued)

Method	Results
Held at 5 degrees with carpels removed	80% germination in 20 days
Chemical treatment at 5 degrees Same with testa removed	Germination in 53 days Germination in 18 days
Removal of coat	Negative
Held at 5 degrees	Hastened germination
Held at 12 degrees	Secondary dormancy
Chemical treatment	Negative
Held at 0-15 degrees	Germination after one month
Held at 0-10 degrees	Germination hastened and increased
Dried, then held at 0-10 degrees	Germination after 3 months
Held at 5 degrees, or fluctuating between 0 and 10 degrees	60% germination in 2-12 months
Held at 5 degrees	Hastened germination
Imbedded in clay or agar 8-10 weeks	Induced dormancy

break the rest period, and reported considerable success. Nitrates and other oxidizing agents broke the rest, a .5 mol solution of sodium nitrate being very effective.

Denny (16,17) also investigated the rest period of potatoes, and found other chemicals even more effective than sodium nitrate. Ethylene chlorhydrin and the thiocyanates of sodium and potassium were found most effective. The tubers were either soaked in solutions or exposed to the vapors. Ethylene dichlorid, thiourea and other organic compounds also hastened emergence from dormancy.

Similar work was done by Denny and Stanton (18,19) on woody plants. It was found that a number of organic compounds were effective in shortening the rest period. Ethylene dichlorid and ethylene chlorhydrin proved most effective. The remarkable effect which these compounds were shown to have in breaking the rest period of buds, suggests the possibility that they would bring about similar results with dormant embryos. The dormancy of the embryo, like that of buds, is probably a device for preventing growth during the winter, and seems to be a similar phenominon. It would not be surprising if treatment which effects one would also effect the other. In the present experiment, the wild rose was chosen to test this theory, as it is one of the few plants in which delayed germination seems to be purely a matter of

embryo dormancy.

Proceedure

Fruits of the sweetbrier, Rosa rubiginosa, were gathered from the neighborhood of Corvallis late in January and in February, 1930. An unusually cold winter had subjected them to freezing and thawing a number of times in the field. The first lot of fruits were dried, crushed, and the seeds separated by screening and winnowing. The second lot were put through a food chopper with the knives removed, and washed and screened, a method which proved more satisfactory. In both cases, seeds which were light enough to float on water were discarded.

Part of the seeds were scarified by being struck lightly with a small bundle of needles, soldered together. Others were treated for 15 minutes in concentrated sulfuric acid, neutralized and thoroughly washed. A small batch were kept in the acid for 50 minutes, but this treatment seemed to be much too severe.

The seeds were treated in lots of 100 each, placed on three thicknesses of filter paper and kept moist. Except for checks which were not chemically treated and which were stored at a temperature which fluctuated but little from 65 degrees F., the seeds were kept in a cold store, where the temperature fluctuated between 32 and 50 degrees F. but was generally around 40.

Forty eight different chemicals, mainly organic compounds, were used, most of them in two or three concentrations or for different periods. In the liquid treatments, the seeds were placed in small beakers and covered with aqueous solutions of the chemicals. Vapor treatment was given by placing the dry seed in two-liter jars and putting the chemical on cotton at the top. In the case of ethylene, the gas was introduced directly into such a jar.

With each concentration, one set of seeds was used which had had no treatment of the coat, and one which had been scarified. Seeds which had been treated with concentrated sulfuric acid were used in a number of cases, in addition to the plain and scarified seed. Checks of plain and scarified seed, not chemically treated, were also kept in cold storage.

The following chemicals were used as indicated:

1. Hydrochloric acid, n/1000, n/3000, n/5000, 18 hours.
2. Butyric acid, n/100, n/200, n/800, 17⅓ hours.
3. Acetic acid, n/500, n/1000, n/2000, 15⅓ hours.
4. Sulfuric acid, n/500, n/1000, n/100,000, 18 hours.
5. Potassium nitrate, n/10, n/20, n/100, 24 hours.
6. Mercuric chlorid, .1%, ⅓, 3 hours.
7. Thiourea, 5%, 3 hours, 15 hours.
8. Ethylene chlorhydrin, .4%, 3, 6 hours; .8%, 3,6 hours.

9. Propylene chlorhydrin, .4%, 3, 6 hours; .8%, 3, 6 hours.

10. Chloral hydrate, 2%, 5%, 2 hours.

(In the following, the amounts given are for a 2-liter container.)

11. Ethylene chlorhydrin, .2 cc, 48 hours;.4 cc 24, 48 hours.

12. Propylene chlorhydrin, .2 cc, 48 hours; .4 cc, 24, 48 hours.

13. Uspulun (chlorophenol mercury) .25% 3 hours; 1%, ½, 3 hours.

14. Ethylene chloride, .2 cc, 48 hours; .4 cc, ½, 3 hours.

15. Ethyl citrate, .2 cc, 48 hours; .4 cc, 24, 48 hours.

16. Propyl chloride, .2 cc, 48 hours; .4 cc, 24, 48 hours.

17. Ethylidine chloride, trace, 48 hours.

18. Trimethylene chlorohydrin, .2 cc, 48 hours; .4 cc, 24 hours.

19. Ethylene, 2, 4 cc (95%), 24 hours; 2 cc (95%) 168 hours.

20. Tetrachloroethylene, .2 cc, 48 hours; .4 cc, 24, 48 hours.

21. Octylene, .2 cc, 48 hours; .4 cc, 24, 48 hours.

22. Dichloroethylene, .2 cc, 48 hours; .4 cc, 24, 48 hours.

23. Ethylene bromohydrin, .2 cc, 48 hours; .4 cc, 24

hours.

24. Trimethylene chlorohydrin, .4 cc, 48 hours.

25. Ethylene bromide, .2 cc, 48 hours; .4 cc, 24, 48 hours.

26. Ethyl formate, .2 cc, 48 hours; .4 cc, 24, 48 hours.

27. Allyl chloride, .2 cc, 48 hours; .4 cc, 24, 48 hours.

28. Piperidine, .1 cc, 48 hours; .4 cc, 17 hours; .05 cc, 48 hours.

29. Trimethyline chloride, .2 cc, 48 hours; .4 cc, 24, 48 hours.

30. Propylene chloride, .2 cc, 48 hours; .4 cc, 24, 48 hours.

31. Tetrachloroethane, .2 cc, 48 hours; .4 cc, 24, 48 hours.

32. Chloromethyl ether, .2 cc, 48 hours; .4 cc, 24, 48 hours.

33. Propionyl chloride, .2 cc, 48 hours; .4 cc, 24, 48 hours.

34. Allylamine, .2, .4 cc, 48 hours.

35. Ethyly proprionate, .2, .4 cc, 48 hours.

36. Ethyl propane alpha alpha beta gamma tetracarboxylate, .2, .4 cc, 48 hours.

37. Butylene bromide, .2, .4 cc, 48 hours.

38. Pyridine, .2, .4 cc, 48 hours.

39. Methyl chlorocarbonate, .2, .4 cc, 48 hours.

40. Bromoethylbenzene, .2, .4 cc, 48 hours.

41. Methylene chloride, .05 cc, 48 hours.

42. Thioacetic acid, .05 cc, 48 hours.

43. Propionaldehyde, .05 cc, 48 hours.

44. Methylene chloride, .1 cc, 48 hours.

45. Thioacetic acid, .1 cc, 48 hours.

46. Ethyl acetate, .5 cc, 48 hours.

47. Methyl acetate, .5 cc, 48 hours.

48. Ethyl carbonate, .5 cc, 24 hours.

The total number of chemical treatments, therefore, is 118. With the different treatments of the coat, more than 250 lots of seed were used.

After chemical treatment, the seeds were placed in the petri dishes and moistened with distilled water, and then placed in the cold store. More distilled water was added as needed, two or three times a week, in order to prevent the seeds from becoming dry.

When the seed had been in the cold room for a month it was removed, examined for germination, and kept three days at a temperature of about 65 degrees. It was then examined again and returned to the cold store. The proceedure was repeated at the end of the second month.

Results

Results are entirely negative, as no seed has germinated. Were it possible to continue the experiment

until the seeds in the check lots germinated, some differences might appear. As seeds of Rosa rubiginosa are known to after-ripen at the temperatures used, in three or four months, it is probable that if any of the chemical treatments were able to hasten germination, this would have occurred within the two months.

In a private communication, Crocker has informed the writer of similar experiments carried on with several other species of Rosa. A large variety of chemicals was used, but in no case was germination hastened.

Discussion

The large number of chemicals tried included those which have given such startling results in breaking the rest period of buds and tubers, as well as the few which have been used successfully in treating what may have been embryo dormancy. From the uniformly negative results, both in the present experiment and in those conducted by Crocker, it seems very probable, therefore, that after-ripening of seeds of Rosa cannot be hastened by chemical treatment. This genus is one of the very few in which delayed germination is known to be entirely a matter of embryo dormancy. Naked embryos germinate no more readily than the whole seed.

The question has been raised by Crocker as to whether it is possible to hasten the after-ripening of

any dormant embryo by chemical treatment. In most, if not all, cases in which chemical treatment has proved effective, there is at least the possibility that its action was entirely upon the seed coat. As far as a review of the literature shows, there is no case on record of which it is possible to say positively that chemical treatment has overcome embryo dormancy. There are cases in which chemical treatment has overcome dormancy, but these may be explained by its action on the seed coat. If the difference between dormancy of the embryo and that of buds were understood, and the nature of the action of the chemicals which are effective with dormant buds, it would probably be possible to explain why the embryos have failed to respond. It might also appear whether there is hope that further experiments with dormant embryos would result in effective chemical treatment. With present knowledge it is only possible to conclude that there is no evidence that chemical treatment of dormant embryos is of any value.

Summary

1. Many investigators have studied the question of delayed germination of seed.

2. A number of factors were found to cause delayed germination.

3. Delayed germination in the case of the rose is

caused by dormancy of the embryo.

4. Chemical treatments have hastened the germination of some seeds.

5. Storage at temperatures around 5 degrees C. have hastened the germination of many species, including several species of roses.

6. Enzyme activity, especially catalase activity, increases during after-ripening, but is probably not a cause of it.

7. Chemical treatments have been very successful in breaking the rest period of buds and tubers.

8. In the present experiment, treatment with 48 chemicals was used in addition to scarification of the seed coat and moist storage at 32 to 50 degrees F. with seeds of Rosa rubiginosa. Results are entirely negative.

9. There is no evidence that germination of Rosa rubiginosa or of other seeds with dormant embryos is hastened by chemical treatment.

Acknowledgment

It is a pleasure to acknowledge with gratitude the aid and helpful suggestions of Dr. E. M. Harvey, Professor of Research in Horticulture at the Oregon State Agricultural College, upon whose suggestion and under whose supervision the work was carried out.

LITERATURE CITED

1. Appleman, C. O.
Biochemical and Physiological Study of the Rest Period in Tubers of Solanum Tuberosum. Md. Ag. Ex. Sta. Bul. 183. 1914.

2. ———
Physiological Behavior of Enzymes and Carbohydrate Transformations in After-ripening of the Potato Tuber. Bot. Gaz. 52:306-315. 1911.

3. ———
Relation of Catalase and Oxidase to Respiration in Plants. Md. Ag. Ex. Sta. Bul. 191. 1915.

4. Atwood, W. M.
A Physiological Study of the Germination of Avena Fatua. Bot. Gaz. 17:386-414. 1914.

5. Barton, L. V.
Hastening the Germination of Some Coniferous Seeds. Am. Jour. Bot. 17:88-115. 1930.

6. ———
Hastening the Germination of Southern Pine Seeds. Jour. of Forestry 26:774-785. 1928.

7. Crocker, W.
After-ripening and Germination of Rose Seeds. Am. Rose Annual 1926. pp.34-39.

8. ———
Dormancy in Hybrid Seeds. Mem. Hort. Soc. N. Y. 3:33-38. 1927.

9. ———
Germination of Seeds of Water Plants. Bot. Gaz. 44: 375-380. 1903.

10. _____ Mechanics of Dormancy in Seeds. Am. Jour. Bot. 3: 99-120. 1916.

11. _____ and Harrington, G. T. Catalase and Oxidase Content of Seeds in Relation to their Dormancy, Age, Vitality and Respiration. Jour. Ag. Res. 15:137-174. 1919.

12. Davis, O. H. Germination and Early Growth of Cornus Florida, Sambucus Canadensis and Berberis Thunbergii. Bot. Gaz. 84:225-263. 1927.

13. Davis, W. E. Primary Dormancy, After-ripening, and the Development of Secondary Dormancy in Embryos of Ambrosia Trifida. Am. Jour. Bot. 17:58-86. 1930.

14. _____ The Development of Dormancy in Seeds of Cocklebur (Xenthium). Am. Jour. Bot. 17:77-87. 1930.

15. _____ and Rose, R. C. The Effect of External Conditions upon the After-ripening of the Seeds of Crataegus Mollis. Bot. Gaz. 54:49-62. 1912.

16. Denny, F. E. Hastening the Sprouting of Dormant Potato Tubers. Am. Jour. Bot. 13:118-125. 1926.

17. _____ Second Report on the Use of Chemicals for Hastening the Sprouting of Dormant Potato Tubers. Am. Jour. Bot. 13:386-396. 1926.

18. _____ and Stanton, E. N.
Chemical Treatments for Shortening the Rest Period of Pot-grown Woody Plants. Am. Jour. Bot. 15:327-336. 1928.

19. _____
Localization of Response of Woody Tissues to Chemical Treatments that Break the Rest Period. Am. Jour. Bot. 17:58-76. 1928.

20. Eckerson, S.
A Physiological and Chemical Study of After-ripening. Bot. Gaz. 55:286-299. 1913.

21. Harrington, G. T.
Respiration of Apple Seeds. Jour. Ag. Res. 23:117-130. 1923.

22. _____ and Crocker, W.
Structure, Physical Characteristics and Composition of the Pericarp and Integument of Johnson Grass Seed in Relation to its Physiology. Jour. Ag. Res. 23: 193-222. 1923.

23. Harrington, G. T. and Hite, B. C.
After-ripening and Germination of Apple Seeds. Jour. Ag. Res. 23:153-161. 1923.

24. Ives, S. A.
Maturation and Germination of Seeds of Ilex Opaca. Bot. Gaz.76:60-77. 1923.

25. Joseph, H. C.
Germination and Vitality of Birch Seeds. Bot. Gaz. 87:127-151. 1929.

26. _____
Growing Bittersweet (Celastrus Scandens) from Seed.

Flor. Exchange and Hort. Trade World, June, 1928.

27. Pack, D. A.
After-ripening and Germination of Juniperous Seeds. Bot. Gaz. 71:32-60. 1921.

28. _____
Chemistry of After-ripening, Germination and Seedling Development of Juniper Seeds. Bot. Gaz. 72: 139-150. 1921.

29. Rosa, J. T.
Abreviation of the Dormant Period in Potato Tubers. Am. Soc. Hort. Sci., 1923. pp.180-186.

30. Rose, R. C.
After-ripening and Germination of Seeds of Tilia, Sambucus and Rubus. Bot. Gaz. 67:281-308. 1919.

31. Shull, C. A.
The Oxygen Minimum and the Germination of Xanthium Seeds. Bot. Gaz. 52:453-477. 1911.

32. _____ and Davis, W. B.
Delayed Germination and Catalase Activity in Xanthium. Bot. Gaz. 75:268-281. 1923.

33. Tukey, H. B.
Studies of Fruit Seed Storage and Germination. N. Y. State Ag. Ex. Sta. (Geneva) Bul. 509. 1924.

34. Weiss, F.
Seed Germination in the Gray Birch, Betula populifolia. Am. Jour. Bot. 13:737-742. 1926.