AFTER-RIPENING AND GERMINATION OF SEED OF *JUNIPERUS VIRGINIANA* L. AND *JUNIPERUS SCOPULORUM* SARG.

AFTER-RIPENING AND GERMINATION OF SEED OF JUNIPERUS VIRGINIANA L. AND JUNIPERUS SCOPULORUM SARG.

By

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Bachelor of Science

Oklahoma Agricultural and Mechanical College

Stillwater, Oklahoma

1939

Submitted to the Department of Horticulture

Oklahoma Agricultural and Mechanical College

In Partial Fulfillment of the Requirements

For the degree of

MASTER OF SCIENCE

1941

APPROVED BY:

Chairman, Thesis Committee

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Dean Graduate School

ACKNOWLEDGMENT

The author wishes to express sincerest appreciation to Dr. Michel Afanasiev, who so willingly gave his time and interest in connection with the research study.

Grateful acknowledgment is also expressed for the assistance rendered by Dr. Frank B. Cross, other members of the Horticulture Department, and Mr. Robert Ealy.

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INTRODUCTION

Eastern red cedar, Juniperus virginiana Linneaus is one of the most widely distributed North American trees. Sargent (62) describes the range and distribution of red cedar as follows: "Dry gravelly slopes and rocky ridges, often immediately on the sea coast from southern Nova Scotia and New Brunswick to the coast of Georgia, the interior of southern Alabama and Mississippi and westward to the valley of the lower Ottawa river, eastern Dakota, eastern Kansas and Nebraska, the Indian territory*, and eastern Texas, not ascending the mountains of New England and New York, nor the high southern Alleghanies, in middle Kentucky and Tennessee and northern Alabama and Mississippi, covering great areas of low rolling limestone hills with nearly pure forests of small trees**. Red cedar is found practically throughout the state of Oklahoma. It grows on dry slopes, rocky ledges, abandoned farm lands and other lands that are unfit for cultivation. Red cedar in Oklahoma is rarely found in pure stands but is associated with other native species such as oaks and hickories. The species is of considerable economic importance in this state as well as in other parts of the country and is used extensively in both horticultural and forestry work.

Red cedar is a prolific producer of seed. Seed crops are abundant every second or third year, yet the quality of the seed is usually very low. Crops containing 20 per cent or less of viable seeds are not uncommon. Individual trees vary a great deal in the amount and quality of seed produced as well as in the regularity of production.

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* Oklahoma

** Sargent, C.S. Manual of Cultivated Trees of North America. pp.94-95. 1905.

The fruit of red cedar usually matures in one season. In Oklahoma the fruit ripens in late October or November, but remains on the tree through the winter. It should be collected as early as possible. Birds feed on the fruit and thus may greatly reduce the supply of available seed. Birds are responsible also for spreading of the seed of red cedar and the appearance of young seedlings at a considerable distance from the parent tree. Seed should be collected early also because it must be stratified for a relatively long period and yet planted rather early in the spring.

The range of Rocky Mountain red cedar, Juniperus scopulorum Sargent is rather narrow. According to Sargent (62) "it is scattered often singly over the dry rocky ridges except near the coast, usually at elevations of 5,000 feet above the sea, from the eastern foothill region of the Rocky Mountains, to Alberta to western Texas and westward to the coast of British Columbia, and Washington, and eastern Oregon, Nevada and northern Arizona".* This tree is used extensively in landscape plantings and is of great value to the nursery trade. The fruit of Juniperus scopulorum remains on the tree until the end of the second season before it reaches maturity. The quality of the seed varies. Cutting tests of the samples of seed secured from several sources have shown that in some samples 70 per cent of seed were viable and sound while in others there were no viable seeds at all.

Production of seedlings of both species of junipers presents considerable difficulties to an average commercial grower in this part of the country. No standard method of handling the seed has been accepted. Instead the nurserymen often use seed treatments they personally consider helpful (treatment of seed with acid, maceration of the berries in a weak

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* Ibid pp.96-97. 1905.

solution of lye) and which nevertheless fail to bring about assurance of a uniformly good stand of seedlings from year to year.

REVIEW OF LITERATURE

Seed Dormancy

The term "dormancy" as applied to seed is a physiological state of a viable seed in which the latter is unable to germinate when placed under conditions which are ordinarily favorable for this process. Crocker (18) analysing his own data as well as those of other investigators systematized the existing information on seed dormancy and was the first to present a complete organized picture of the causes of dormancy. According to him, the dormancy of seed can be due to one of the following causes: (1) rudimentary embryo; (2) complete restriction of water absorption by action of seed coats; (3) mechanical resistance of the enclosing structures to the expansion of the embryo and the seed contents; (4) interference with gaseous exchange, particularly oxygen by seed coats or other tissues surrounding the embryo; (5) a state of dormancy in the embryo itself or some part of it; (6) a combination of the above factors; and (7) secondary dormancy.

Rudimentary embryo

Rudimentary embryo, i.e. an embryo which is not fully developed, has been found to be the cause of seed dormancy in a relatively few cases. Ives (45) found rudimentary embryo to be responsible for the dormancy of seed of *Ilex opaca*, and observed continuous development of the embryo after the seed was harvested. Chittenden (13) reported that one of the causes of dormancy of Tilia seed is the rudimentary embryo. However, Spaeth (68) has shown that dormancy of this seed is due to other causes.

Impermeability of seed coats to water

Seed coats impermeable to water have been found to be one of the common causes of seed dormancy. They were shown to be responsible for the

dormancy of seeds in many species of the Leguminosae. Raleigh (56) reported that the impermeable character of the seed coats of Gymnocladus dioica was caused by a well-developed layer of Malphigian palisade cells. Seed coats impermeable to water were reported also in seed of red clover (53); Nelumbo lutea (48); vetch (49); basswood (68); and many others.

Various treatments of seed have been tried to modify the impermeable character of the seed coats. Mechanical scarification, ether, alcohol, bases, acids, high pressure (both air and water), and hot water are the more important treatments that have been used. J.P. Jones (49) reported that the use of both hot and cold ether for three hours failed to increase the permeability of the seed coat of vetch. He concluded that the substances responsible for the impermeability were not of a fat-like nature. Crocker (17) soaked mesquite seed in ether for several days and secured a much higher germination by this treatment than without it. Spaeth (68) working on the seed of basswood failed to increase the permeability of the seed coat with ether and with alcohol.

Several methods of mechanical abrasion of the seed coat have also been used. Rose (58) working on seed of a large number of species, used air pressure to blow the seeds against needle points. He found that seed of twenty of the 130 species were aided by this treatment. Hurst, Humphries and McKee (44) working with seed of white sweet clover, Lespedeza sericea, and Crotalaria, found scarification to be effective in making seed coats permeable to water. They reported the following differences in germination between untreated and treated seed respectively: 10.5% and 75 per cent in white sweet clover; 15 per cent and 90 per cent in Lespedeza sericea and 5 per cent and 77 per cent in Crotalaria. Chapman (12) found that scarification increased germination from 8 per cent to 88 per cent in seed of

black locust. He secured much better germination from scarified than acid-treated seed. He attributed this difference to the fact that acid treated seed are more readily attacked by fungi. The loss in viability of scarified sweet clover seed was investigated by Hurst, Humphries and McKee (44). They found that over a period of four years, the viability of scarified sweet clover seed was reduced from the original of 86 to 36 per cent while untreated seed showed 89 per cent of the original 94 per cent to be still viable. Harrington (37) noted that the per cent of impermeable seeds was lower in machine hulled than in hand threshed alfalfa seed. Davis (24) subjected Medicago sativa seed to a water pressure of 2,000 atmospheres and observed that treated seed germinated fifty per cent better than untreated seed, while Flemion (32) working with Sorbus seed found an air pressure of five tons entirely ineffective.

Freezing of seed with impermeable coats has also been tried as a method of modifying the impermeable character of the seed coat. Spaeth (68) froze Tilia seed at -80°C. and -185°C., which resulted only in a very slight decrease in the number of "hard" seeds. Freezing of clover seed by Harrington (37) helped to reduce the number of "hard" seeds but killed those which had absorbed water.

The use of acid is a very effective method of breaking seed dormancy caused by impermeability of the seed coats. Concentrated sulfuric acid (specific gravity 1.84) is probably the most commonly used. The length of treatment with the acid depends upon the species being treated. The use of acid has been found to increase the permeability of the seed coats of the following species: black medick, sulla, birds foot clover, red clover and Melilotus (72), Lathrus sylvestris (60), red clover and alfalfa (53), vetch (49), Nelumbo lutea (48), Symphoricarpos racemosus (34), Crataegus (35),

Tilia (7, 68), Kentucky coffee tree (56), Cotoneaster (36), and many others. In all of the above cases concentrated sulfuric acid was used. The seeds after being treated were washed in running water. Thornber (70) working on the seed of Kentucky coffee tree, black locust, mesquite, honey locust and Acacia used a mixture of sulfuric and chromic acids. After the treatment he neutralized the acid which might have remained in the seed coat by the use of potassium hydrate. Todaro (72) reported that the effect of acid treatment is soon lost if the seed is not planted immediately. On the other hand, Jones (48) observed that the acid treated seed of Nelumbo lutea could be stored for some time without any ill effects on their germinability. Hot water treatment for various periods of time has been found to be effective in modifying the impermeable character of the seed coats of black locust, honey locust (70, 73), Acacia (6, 70), mesquite and palo verde (70). The hot water treatment of seeds having seed coats impermeable to water is used commercially on a large scale.

Mechanical resistance of the seed coat

Mechanical resistance of the seed coat to expansion of the embryo and other seed contents has been found to be the cause of dormancy in seed of Alisma plantago (20) and Celastrus scandens (41). Crocker and Davis (20) reported that the seed of Alisma plantago failed to germinate if soaked in water for a year. They attributed the lack of germination to the mechanical resistance of the seed coat as the seed germinated readily when the seed coat was removed. Hart (41) found similar conditions to be the cause of delayed germination in seed of Celastrus scandens.

Seed coats impermeable to gases

Seed dormancy caused by the limitation of gaseous exchange is usually attributed to the character of the seed coats which prevent the movement of

oxygen and carbon dioxide between the embryo and the external atmosphere. Impermeability of seed coat to gases was found to be the cause of dormancy in seed of Xanthium (16, 27, 65, 66, 67, 71), Avena fatua (5), lettuce (11), and Ambrosia trifida (26). Schull (65, 66) studied the effects of partial oxygen pressure, alternating temperature and storage conditions upon the amount of oxygen absorbed by Xanthium seed and found that partial oxygen pressure and high temperature hastened the germination of Xanthium. Crocker (16) reported that the seed coats of Xanthium reduce the oxygen supply to such an extent as to prevent growth of the embryo. The use of increased oxygen pressure, high temperature and mechanical injury increased the permeability of the seed coat to oxygen.

Dormancy of the embryo

Physiologically dormant embryo is the most common cause of delayed germination of seeds. By dormant embryo is meant one unable to grow when placed under conditions favoring this process. A seed with a dormant embryo must pass through a process known as "after-ripening" before it becomes able to germinate. The term "after-ripening", as applied to seeds refers to those physical and chemical changes that take place in the endosperm and embryo of a viable seed after it apparently reaches maturity and before it begins to germinate.

Not all changes that take place in the endosperm and embryo during after-ripening are known. Eckerson (29), one of the pioneer investigators in the field of seed dormancy, observed that during the after-ripening of seed of Crataegus the acidity of the embryo increased. Correlated with this was the increased water-holding power and an increase in the catalase and peroxidase activity of the embryo. Flemion (33) reports that during after-ripening of the seed of Rhodotypos kerrioides there is a noticeable

increase in catalase, peroxidase, and lipase activity as well as an increase in water absorbing power, nitrogen soluble in 80 per cent alcohol, titratable acid and sucrose.

Spaeth (68) has summarized the observations of a number of investigators on the changes taking place in the embryo and endosperm of fatty seeds during after-ripening. His summary is as follows:

> "Absorbtion of water and marked swelling; increase in activity of catalase and peroxidase; increase in acidity (both hydrogen-ion concentration and titratable acid), particularly in the embryo; increase in amounts and activity of many enzymes, particularly lipase, resulting in the rapid disappearance of oils and increase in free fatty acids; increase in proteolytic enzymes, decrease in proteins, and increase and translocation of amino acids; appearance of oxidase; appearance and rapid increase of sugars; a sudden increase in acidity and water content, followed immediately by germination."*

As mentioned in the above summary, catalase activity in fatty seeds increased during the after-ripening. Rhine (57) found that catalase could only be used as an indicator of metabolism where there is no rapid change in respiration. Sherman (64) observed that catalase activity and respiration were fairly stable in Amaranthus and Chenopodium, while in the seeds of the Rosaceae they varied a great deal.

Stratification at low temperature is the most effective practical way to break this type of dormancy. It provides an abundant supply of moisture which together with low temperature and an adaquate supply of oxygen make up the conditions favoring the process of after-ripening. Stratification at low temperatures (1° - 10°C.) has been reported effective in breaking dormancy of seed of Crataegus (16, 21, 28, 29, 35), Tilia (7, 59), Pyrus (19, 40, 42), peach and cherry (42), Acer (47), Juniperus virginiana (54), Cornus florida (23), Sorbus aucuparia (32), Benzoin aestivale L. (63), red, green, and white ash (69), birch (50), Carya ovata, Juglans cinerea and J. nigra (8), and many others. Seed of some species do not respond to

* Spaeth, Nelson J. Cornell Agri. Exp. Sta. Memoir 169. p. 13. 1934.

stratification at a continuous low temperature but respond to a storage in which moisture and temperature conditions are varied. Seed of black ash were reported by Steinbauer (69) to require a few weeks of stratification at 20°C. before the stratification at a low temperature, so that the enlargement of the embryo could take place. Flemion (32) found that the seed of Sorbus aucuparia requires a much shorter period of stratification if previously stored dry at room temperature for six months. She found also that the seed of Rhodotypos kerrioides (33) germinate better if during the first month of stratification the temperature is maintained at 25° or 30°C. Schroeder (63) working on seed of Benzoin aestivale, reported that the after-ripening period in these seeds was greatly reduced if normal stratification at low temperature is preceded by at least one month of stratification at 25°C.

Numerous attempts to force germination of seed requiring after-ripening by means of physical and chemical stimulation have been mostly unsuccessful. Eckerson (29) working with seed of Crataegus found that treatment of embryos with dilute solutions of hydrochloric, butyric and acetic acids shortened somewhat the period needed for after-ripening. Evans (31) tried many chemicals (dilute solutions of hydrochloric and acetic acids, butyric acid, manganese sulphate, zinc sulphate, potassium chloride, acid mono-potassium phosphate, glucose, Knop's nutrient solution, potassium thio-cyanate, chloral hydrate, thiourea, dioxogen, and chloroform) in an attempt to force germination of seed of Magnolia grandiflora but found that none of them were effective. Howard (43) etherized, froze and soaked in water seeds of many plants growing around Columbia, Missouri, but failed to obtain any germination. Barton (9) studied the effect of growth promoting substances on seeds of several species. She used napthaleneacetic, indoleacetic,

phenylacetic, and indolebutyric acids and found them ineffective on seeds having dormant embryos.

In seed of a few species dormancy may be caused by a combination of factors just discussed. Some of these are as follows: Sorbus aucuparia (32), Rhodotypos kerrioides (33), Symphoricarpos racemosis (34), cherry and peach (42), Ilex opaca (45), Tilia (68), redbud (3) and black ash (69). Seed of Rhodotypos kerrioides, Sorbus aucuparia, and Symphoricarpos racemosis (32, 33, and 34) require modification of the seed coat and stratification at a low temperature before good germination can be secured. Seed of cherry and peach (42) were reported to have dormant embryos, also mechanically resistant seed coats. Ives (45) found that Ilex opaca was unable to germinate even after the rudimentary embryo had reached normal size, because of the mechanical resistance of the seed coat. Flemion (32) found that seed of Sorbus aucuparia were prevented from germination by the seed coat even after the seed had been after-ripened. Steinbauer (69) found that seed of black ash has an embryo which requires first, a temperature of 20°C. for enlargement, and only then needs a period of stratification at 5°C. to after-ripen.

Secondary dormancy

The so-called secondary dormancy has been found to be the cause of delayed germination in seed of many species. Certain external factors and some chemicals have been found to be effective in forcing an after-ripened seed back into dormancy. Some of the seeds reported to be capable of reverting to secondary dormancy are those of Alisma plantago (20), Xanthium (27, 71), apple (40), Sorbus aucuparia (32), Cornus florida and Sambucus canadensis (23).

Carbon dioxide seems to be one of the chemicals particularly effective in forcing seeds into secondary dormancy. Kidd (51) has demonstrated the dormancy inducing property of this gas on seeds of peas, beans, barley and cabbage. With these seeds the gas was found to be effective only while an increased pressure of carbon dioxide is maintained in the germinator. On the other hand, the dormancy thus induced in the seed of white mustard is of considerable duration. Kidd attributes the prolonged effect of this gas to a much lower permeability of the seed coat to gases. He has demonstrated also that carbon dioxide is one of the means by which secondary dormancy of some seeds may be induced in nature. Davis (23) found that high temperatures under germinative conditions would force after-ripened seed of both Cornus florida and Sambucus canadensis into secondary dormancy. Flemion (32) reported similar conditions in the seed of Sorbus aucuparia. Davis (27) and Thornton (71) found that by the reduction or complete elimination of oxygen the seed of Xanthium could be forced back into dormancy. Pack (54) observed that the after-ripened seed of Juniperus virginiana would revert back to dormancy if placed in a germinator where the temperature was 12°C. or higher. Harrington and Hite (40) reported that high temperature unfavorable for germination caused secondary dormancy in apple seed. However, more recent work by Haut (42) indicates that such conditions result in the loss of seed viability rather than in the reversion to secondary dormancy.

Germination

Temperature has a pronounced effect on germination of seeds. Coffman (15) stated that, "seeds of different species germinate very differently at different temperatures". He worked on the minimum germination temperature of the small grains and found that they germinated at the temperature of melting ice. Kotowski (52) reported that the optimum germination temperatures

of spinach, cabbage and beets are 4°C., 8°C. and 11°C. respectively. He reported also, that the seed of tomato, eggplant, pepper, melons and garden beans would not germinate at a temperature of 18°C., but the germination of these seeds would increase with rise of temperature up to 30°C. Cochran (14) found that pepper seed failed to germinate in 45 days at 50° to 60°F. but when transferred to a temperature of 90° to 100°F. they germinated in five days. Borthwick and Robbins (11) working with lettuce seed observed that the latter germinated at temperatures from 4° to 25°C. while 30°C. was inhibitive to germination. Seeds of eighteen species of flowers were tested by Harrington (38), who found that the optimum germination temperature for these seeds was between 17.5°C. and 22.5°C. Joseph (50) observed that the optimum constant germination temperature for dry birch seed was 32°C. and that by stratification at a low temperature for 5 or 6 months, the minimum germination temperature could be lowered to 0°C. Pack (54) found that by stratification at low temperature for a long period of time the seed of Juniperus will eventually germinate at a temperature of 0°C. to 1°C. In contrast, Borthwick and Robbins (11) found that by subjecting lettuce seed to stratification at a temperature of 4°C. for 4 to 6 days, good germination could be secured at 30°C. Harrington (39) found that the seeds of carrot, parsley, timothy, awnless brome grass, perennial and annual Italian rye grasses, meadow fesque, and of several kinds of flowers germinated practically as well at a favorable constant temperature as at alternation of temperatures. Edward (30) gives an excellent review of literature on the effect of temperature on germination. His report covers the seed of oats, wheat, maize, rice, barley, peas, bean, alfalfa, clover, melon, beet, radish and lettuce.

Juniper Seeds

Investigations on the seeds of junipers are few. Studies of the seed of Juniperus virginiana were made by Pack (54, 55) and Jelley (46). Seed of Juniperus communis, J. communis depressa, and J. prostrata have been investigated by Pack (54). Turner (74) studied after-ripening and germination of seed of J. pachyphloea. No work on Juniperus scopulorum, one of the species covered in this paper has yet been reported. The works of Turner (74) and Jelley (46) were chiefly confined to determination of the requirements of seed for after-ripening. Turner (74) found that after-ripening is essential before germination of the seed of Juniperus pachyphloea could take place. He observed that the length of period needed to bring about complete after-ripening varies with the season: after-ripening during the spring of the year proceeds much faster than during the winter. Since stratified seeds of J. pachyphloea begin to germinate May 1, Turner suggests early planting. Jelley (46) working on the theory that passage of red cedar seed through the digestive tract of birds stimulates germination, treated clean red cedar seed with solutions containing the active ingredients found in the digestive tract of birds. After this treatment the seed were divided into two lots and stratified; one lot at a temperature of 41°F. and the other at 71°F. After 60 days of stratification, 20 per cent of seeds held at 41°F. were fully after-ripened and those stored at 71°F. germinated to the extent of 33 per cent. Jelley did not attempt to explain whether germination was due to the treatment or to stratification.

The most complete and thorough investigation of seeds of red cedar was done by Pack (54, 55). He found that non-after-ripened seed of red cedar will not germinate unless stratified for some time at a temperature of between 0° and 10°C. Stratification for 100 days at 5°C. was found to be the

most effective one. Seeds stratified at 5°C. for a sufficiently long period of time were able to germinate at temperatures as low as 0° and 1°C; however, the optimum germination temperature for these seeds was observed to be 5°C. Seed after-ripened at 5°C. failed to germinate when the temperature in the germinator was raised to 15°C. Pack (54) claims that exposure of seed after-ripened at 5°C. to a temperature of 12°C. forces the seed into secondary dormancy. Jelley (46) has also observed the need for after-ripening in red cedar seed. Stratification of seed preceded by freezing of fruit for 21 days resulted in abundant germination. Jelley does not state whether he attributed germination to freezing or stratification or both. Pack (54) on the other hand, makes a definite statement to the effect that freezing and thawing has no forcing action on germination of red cedar seed. He states also that "Seeds ready to germinate (after the coat is cracked and their water content increased 52%) are killed by an exposure to -5°C."* Of all investigators working on the seed of juniper, Baldrati (6) was the only one to report germination of seed which was neither stratified prior to planting nor left in the soil over the winter. No information is found in Baldrati's article on the exact identity of the seed investigated.

Pack (54) found that the seed coat plays no part in after-ripening or germination of red cedar seed. However, it restricts the expansion of the imbibed embryo and prevents it from rupturing the nucellus before after-ripening has taken place. The seed coat is semipermeable. Water, bases, and salts pass through the seed coat rapidly while acids enter it slowly.

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* Pack, Dean A. After-ripening and germination of Juniperus seeds Bot. Gaz. 71:p. 58. 1921.

Pack's attempts to secure germination of non-after-ripened seed by use of forcing agents such as acids, bases, salts, high and alternating temperatures, mechanical injury, warm bath, dry air, or removal of the seed coat were not successful.

According to Pack (54) non-after-ripened red cedar seeds contain abundant stored food in the form of fats and proteins, with traces of sugars, but no starch. The changes observed by Pack in seeds undergoing the process of after-ripening are as follows: "The accumulation of cell building materials; acids, phosphatides, active reducing substances, soluble sugars, pentoses, amino acids, soluble proteins, and other nitrogenous compounds; the accumulation of enzymes; the dispersion of materials; and the transformation of storage materials. This rapid accumulation of simple plastic cell materials coupled with minimum respiration and combustion of materials probably forces the dormant organs to activity. One thus sees the awakened active organ as a very unstable structure made up of many unstable compounds. If these changes are not the basis of the after-ripening process, they are found to accompany after-ripening process."*

Pack (54) studied also the effect of temperature on growth of seedlings and chlorophyll formation. He found that the optimum temperature for growth of red cedar seedlings was 15° C. Chlorophyll development is independent of light, but greatly affected by the temperature; chlorophyll fails to form in seedlings held at either 0° C. or 30° C.

Pack's investigation included also a study of the anatomy of seed. The following is a brief summary of Pack's discussion of the structure and composition of seed of red cedar.**

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* Pack, Dean, A. Chemistry of after-ripening, germination, and seedling development of Juniper seeds. Bot. Gaz. 72: p. 149, 1921.

** Pack, D.A. After-ripening and germination of Juniperus seeds. Bot. Gaz. 71: 37. 1921.

The seed of Juniperus virginiana consists of four easily distinguishable parts: (1) the seed coat; (2) nucellus; (3) the endosperm, and (4) the embryo. The seed coat constitutes about seventy-five per cent of the weight of the seed and is made up of three layers: the outer fleshy (Figure 1, a), the stony (b), and the inner fleshy (c). The stony layer is heavily lignified and contains some calcium pectate, while the fleshy inner layer is made up mostly of suberin and of a small amount of cellulose. The nucellus (d), a very thin layer of cells, is composed of cellulose. The endosperm (g) and the embryo (h) make up only about one-fourth of the weight of the seed yet are about one-half of the diameter of the seed in thickness. The endosperm serves as a place of food storage, its cells being filled with fats and proteins. The walls of the endosperm cells are rather heavy and are composed of cellulose and some pectic substances. The outer walls of the outer endosperm cells (k) are heavily suberized. The walls of the embryo cells are thin and composed of cellulose and some pectic substances. Cells of the embryo contain fats and proteins.

The writer was unable to find any reference on the anatomy of seed of Juniperus scopulorum. His own study revealed a similarity between the structure of the seed of Juniperus scopulorum and that of red cedar. The most pronounced difference between the structure of the seeds of the two species is in the relative thickness of the seed coat. The outer fleshy layer of the seed coat of Juniperus scopulorum is comparable to the same layer in the seed of red cedar. However the stony layer in general is much thicker than the corresponding layer in Juniperus virginiana, and may vary in thickness because of the asymmetrical location of the embryo. Similar difference is noted in the thickness of the inner fleshy layer of the seed coat of the two species. As in the case of the stony layer, the inner fleshy

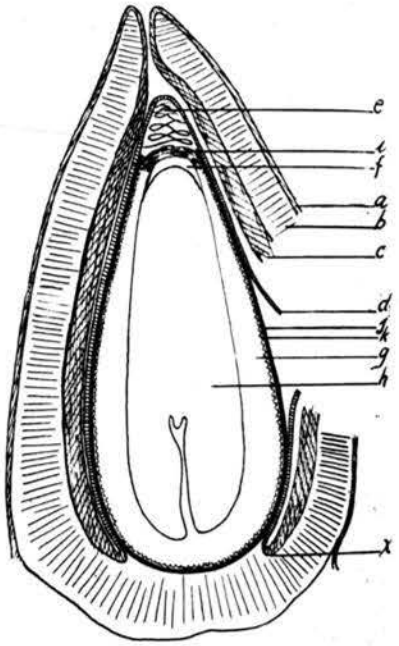

FIG. 1.—Longitudinal section of seed of *Juniperus virginiana* with part of nucellus and integument removed from one side: *a*, outer fleshy; *b*, stony; *c*, inner fleshy; *d*, nucellus; *e*, distorted tissue; *f*, hypocotyl cap; *i*, protective cap; *j*, megaspore membrane; *k*, endosperm wall; *g*, endosperm; *h*, embryo.

(by Pack)

layer may not be uniform in thickness. There is no essential difference in the structure of the embryos of the two species.

MATERIAL AND METHODS

All seed of Juniperus virginiana L. used during this investigation were collected locally, (Stillwater, Oklahoma, and vicinity), in 1939 and 1940. Since many red cedar trees produce a high proportion of empty seeds, collection of seed was always preceded by a cutting test. Seeds used in this study were of better than average quality: from 50 to 60 per cent of all seeds were well filled and apparently sound. (Table 1)

Table 1 -- Origin, date of collection and original quality of seed of Juniperus virginiana

Lot No.	Date of Collection	Place of Collection	Per cent of filled sound seed
39 C	Nov. 6, 1939	Vicinity of Perkins, Oklahoma	51%
39 D	Nov. 11, 1939	"	60%
39 E	Dec. 2, 1939	"	53%
40 B	Nov. 1940	Stillwater, Oklahoma	50% +

Seed of Juniperus scopulorum Sarg. was purchased from commercial seed dealers: lot number 38 from Barteldes Seed Company, Denver, Colorado, in December of 1938; lots 39 and 40 from Moran Seed Company, Medora, North Dakota in 1939 and 1940 respectively. When clean seed of either of the two species were needed, berries were soaked in tap water for three or four days and then macerated on a cement block. The macerated berries were placed in a large volume of water: most of the good seeds sank to the bottom while pulp and the majority of the empty seeds were floated off. The clean seed were then removed from the water and either air dried, stratified or otherwise made ready for use.

Petri dishes with moist cotton were used as germinators in most in-

stances. The cotton was moistened with distilled water in all tests, unless otherwise indicated. Some of the germination tests were carried on in pots filled with soil and some in the nursery bed. Most of the germination tests were set in duplicate. During the tests the petri dishes were opened only when germination count was made or when water had to be added.

Granulated peat moss* was used as the standard stratification medium. For stratification the peat was prepared as follows: first, it was put through a ¼" mesh wire screen and then allowed to soak in water for several hours. When the peat was thoroughly saturated, the excess water was removed. Glass jars with paper over the mouth of the jar were used for stratification of small lots while larger lots were placed in half bushel baskets lined with paper. Stratified seed were kept in a cold storage room, in which the temperature was maintained at between 36°F. and 46°F. (Ave. 41°F.)

Most of the germination tests were carried on in the laboratory at temperatures varying from 70°F. to 85°F. Whenever constant temperatures were required, insulated cabinets with thermostatically controlled temperatures were used. Some of the tests were also set in the greenhouse and outdoors in the nursery. In the course of the investigation approximately 50,000 Juniperus virginiana and 20,000 J. scopulorum seed were used.

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* Horticultural grade (pH 5) secured from Pearson-Ferguson Chemical Company, Kansas City, Missouri

CAUSES OF DORMANCY

Mature seed of Juniperus virginiana and J. scopulorum are dormant. The basic cause of the inability of ripe seed of these two species to germinate is the dormancy of the embryo. In seed of J. scopulorum the seed coat is also probably responsible to some extent for the delay in germination. This conclusion was arrived at after a few experiments designed to check on all possible causes of seed dormancy as listed by Crocker (18).

Seed coats of both Juniperus virginiana and J. scopulorum are permeable to water: dry seed of both species absorbed water very freely when soaked in cold water at room temperature. During 72 hours of soaking, air dry seed of Juniperus virginiana absorbed water in an amount equal to 18.52 per cent of the dry weight of the seed. Water absorbed by the seed of Juniperus scopulorum during the same period amounted to 20.44 per cent of the dry weight of the seed. Seventy-two hours of soaking dormant seed brought the total moisture content of seed of Juniperus virginiana and J. scopulorum to 27.09 and 30.97 per cent respectively.

Free absorption of water and the fact that removal of the seed coat did not promote germination of non-after-ripened seed suggest that the seed coat is not of primary importance in seed dormancy in seed of red cedar. However there are some indications that the seed coat may play some part in the delayed germination of seed of Juniperus scopulorum. These are discussed later in this paper.

Failure of the excised embryos of non-after-ripened seed of both species to grow and of the increased oxygen pressure to force germination indicates that deficiency of oxygen is not responsible for dormancy. The

fact that the removal of the seed coat failed to bring about germination eliminates also the mechanical resistance of the seed coat as a likely cause of continued dormancy. The need for after-ripening and easy germination of after-ripened seed show very definitely that the chief cause of dormancy lies in the embryo itself. This conclusion is in line with that of Pack (54) who reported the dormancy of the embryo to be responsible for delayed germination of seed of Juniperus virginiana, J. communis, J. communis depressa, and J. prostrata.

The writer has not observed secondary dormancy in seed of Juniperus scopulorum but, as shown elsewhere in this paper after-ripened seed of red cedar under certain conditions can revert to secondary dormancy.

AFTER-RIPENING

Stratification

Seed of Juniperus scopulorum as a rule do not germinate the first spring after collection irrespective of whether they were stratified over winter at low temperature or planted in the fall or stored dry. In commercial practice, germination of this seed occurs during the second spring after planting, which means that the seed remains in the seed bed for at least one full year. Before germination of the seed occurs the latter normally remains at a low temperature for two periods (two winters) separated by a period of relatively high temperature (summer). On the assumption that these three sets of conditions are necessary for the normal process of after-ripening, an attempt was made to determine the shortest periods of storage under the above conditions, that would result in satisfactory germination.

A sample of approximately 10,000 seed was divided into 24 lots and each placed in stratification for various periods at low and high temperatures. Each lot went through three distinct periods of stratification: during the first and third periods the seed was kept at an average temperature of 41° F., during the second at room temperature fluctuating between 70° and 85° F. The complete schedule of stratification of various lots is presented in Table 2. Since drying of soil and seed during the summer is of common occurence in nature, it was decided also to investigate its effect if any on after-ripening. In connection with this, lots 20, 21, 22, and 23 were allowed to dry during the stratification at high (room) temperature, but remoistened before the seed was returned back to low temperature. In lot 24 the seed were removed from the peat moss and air dried until it attained a constant weight.

The percent of after-ripened seed in each lot was determined by means of germination tests at the conclusion of the third period of stratification. The tests were carried on in the laboratory at a temperature varying from 70° to 85° F. and also in the icebox at a temperature of 41° F. The results of the tests conducted at 41° F. were in line with those set at room temperature, insofar as the relative germination percentages are concerned. However, germination at 41° F. required a much longer period of time.

The following discussion refers to the tests carried on in the laboratory. The best germination (31.5 per cent in 14 days) was obtained from seed which was stratified one month at 41° F., eight months at room temperature and again two months at 41° F., or for a total period of eleven months. (Lot 5, Table 2) The length of the first period at low temperature does not seem to be directly correlated with the extent of the final germination. Some of the seed lots kept at 41° F. for one month (lots 5 and 6) germinated better than those which at first were stored at low temperature for three months. Neither is there any definite correlation between the germination and the length of the last period of stratification at the low temperature. Germination appears to be correlated somewhat with the length of the stratification period at high temperature. However, whether it is due to the effect of the storage at high temperature or to the total length of stratification period is not clear; seed which were kept longest at high temperature had also the longest total period of stratification. Apparent lack of correlation between the individual periods of storage and germination might be due, at least in part, to the fact that germination tests were set at room temperature which varied as much as 15° F. It will be

Table 2. Effect of stratification at alternate low, high, and low temperatures on the after-ripening of seed of Juniperus scopulorum

Lots	Period of stratification			Total	Per cent germination
	At 41°F.	At room temperature	At 41°F.		
	MONTHS				
1	1	1	1	3	1.0
2	1	2	3	6	1.0
3	1	4	3	8	8.0
4	1	9	1	11	*
5	1	8	2	11	31.5
6	1	8	3	12	22.0
7	2	1	2	5	1.0
8	2	2	2	6	2.0
9	2	4	2	8	2.0
10	2	6	2	10	9.0
11	2	8	2	12	2.5
12	2	8	3	13	1.0
13	3	1	3	7	20.0
14	3	2	3	8	9.5
15	3	4	3	10	9.5
16	3	6	3	12	21.0
17	3	8	1	12	15.5
18	3	8	2	13	21.0
19	3	8	3	14	21.0
20	3	8	3	14	25.5
21	3	6	3	12	15.5
22	3	3	3	9	4.5
23	3	4	4	11	17.5
24	3	**5 days	3	6	0.0

* Accidently destroyed.

** Stored dry

shown later that germination temperature has a pronounced effect on the germination of the seed of juniper.

In the lots which were allowed to dry after the first three months of stratification at 41° F. there is about the same degree of correlation between germination and the length of the total storage period as in the seed kept continuously moist; the longer the total storage period the higher the germination (table 2). Seed tested 14 months after the initiation of the experiment germinated to the extent of 25.5 per cent, whereas seed tested after six months of storage did not germinate at all. The results of germination tests of the last four lots (20 - 24) are significant also because they show that drying of partly after-ripened seed neither interferes with the process of after-ripening nor throws the seed into a state of secondary dormancy. Pack (54) found that dessication of partly after-ripened Juniper seed for a short period not only was harmless but actually shortened the period required for the completion of after-ripening. Possibility of drying partly after-ripened seed without markedly affecting its germinability was also observed by Crocker and Barton (19) who found that partly after-ripened seed of apple, dried for one month, germinated nearly as well as did the seed which was not dried.

Requirements for after-ripening of seed of Juniperus virginiana were thoroughly investigated by Pack (54). He found that stratification for one hundred days at 41°F. provided the optimum conditions for after-ripening. In the present investigation it was observed that this period varies not only with individual lots and from season to season but with individual seeds as well. Stratification for one hundred days insures after-ripening of most seeds. Seed of lot 39E was used to determine the required length of stratification for the after-ripening of seed of red cedar. The seed had been

cleaned, dried, and stored at 41°F. on February 1, 1940 and stratified on July 6 of the same year. The stratified seed was placed in an electric ice-box at an average temperature of 41°F. Germination tests, each involving 200 seeds, were made at intervals of two weeks. The results of these tests are presented in Table 3. The original intention was to continue the tests for fourteen weeks, but due to an abundant germination of seed in the ice-box, the tests had to be stopped earlier. At the end of the tenth week of stratification, germination in storage had reached 45 per cent.

Table 3. Effect of length of stratification at 41°F. on after-ripening of seed of Juniperus virginiana

Stratification period (weeks):	2	4	6	8	10
Per cent germination	0.0	0.5	12.0	33.0	77.0

The figures in the above table represent the percentages of after-ripened seed and are based on the total germination in the laboratory and in the ice-box while the seed was still stratified. Germination of 77.0 per cent of seeds in the case of lot 39E is very close to the maximum that could possibly be attained because the lot, despite separation of good and poor seed by floating, still contained a number of empty seed.

The data show that very little germination can be expected from seed stratified for a period of six weeks or less, but there is a rapid increase in the per cent of after-ripened seed if they are held in stratification longer. The period required for after-ripening of seed of red cedar may vary, as can be seen from comparing the results of the above experiment with those of Pack (54) who suggests 100 days at 41°F. as the optimum pre-treatment of red cedar seed. If the seed begins to germinate in storage

long in advance of planting, as would be the case with some lots of seed, it is entirely possible to check the growth of radicles without destroying the germinative capacity of the seed is shown elsewhere in this paper.

Forcing Agents

Many attempts have been made by various workers to break dormancy of the embryo or to speed up after-ripening by means of forcing agents (11, 24, 29, 31, 44, 53, 54). These investigations were discussed in the review of literature. In most cases the use of forcing agents proved to be ineffective. During this investigation pretreatments with increased oxygen pressure and with a solution of vitamin B_1 were used in an attempt to hasten after-ripening of seed of Juniperus virginiana.

Oxygen

It has long been known that oxygen requirements of seed of different species varies. In some species as in cocklebur and lettuce the oxygen supply is the controlling factor in germination of the seed (11, 27, 65), while in others the oxygen requirements for germination are very low. The purpose of subjecting dormant red cedar seed to increased oxygen pressure was: (1) to find out whether deficiency of oxygen is responsible for the dormancy, and if so, (2) whether increase of oxygen pressure would break the dormancy or at least speed up after-ripening.

About 2,000 dry dormant seed of red cedar (lot 39C) were kept in pure oxygen for twenty-four hours, and then placed in stratification at a temperature of 41°F. A duplicate lot of the same seed was soaked in tap water for twenty-four hours and then stratified and kept at the same temperature as the oxygen treated seed. The results of the bi-weekly germination tests of the treated and untreated seed are presented in Table 4.

Table 4. Effect of pretreatment with oxygen and vitamin B_1 on after-ripening of seed of Juniperus virginiana

Treatment	Stratification period (weeks) at 41° F.					
	0	2	4	6	8	10
	Per cent germination in 14 days					
Oxygen: 100 per cent:	0.0	0.0	0.5	10.5	29.0	62.0
Vitamin B_1: 4 mg. per: liter for 24 hours	0.0	0.0	0.0	9.5	25.5	62.2
Check: seed soaked in: tap water for 24 hours:	0.0	0.0	0.5	12.0	33.0	77.0

Subjecting the seed to increased oxygen pressure for twenty-four hours prior to stratification did not hasten after-ripening of dormant seed. On the contrary, after ten weeks of stratification the final germination of oxygen treated seed was lower (62.2 per cent) than that of the untreated seed (77.0 per cent). This is in line with the findings of Pack (54), who reported that increased oxygen supply retarded after-ripening and had no forcing action on germination of seed of red cedar.

Vitamin B_1

The effect of vitamin B_1 on the growth of roots of plants has been investigated by many workers (1, 10). However the writer has no information on the effect of vitamin B_1 on after-ripening and germination of seed, except that appearing in popular magazines and advertisements.

To determine the effect of vitamin B_1 on after-ripening, about 2,000 red cedar seed (lot 39C) were placed in a solution containing 4 mg. of the vitamin per liter. After twenty-four hours of soaking, the seed without being rinsed were stratified and placed at a temperature of 41°F. The results of the bi-weekly germination tests of these seed appear in Table 4.

Pretreatment of red cedar seed with vitamin B_1 not only failed to hasten after-ripening but apparently either slowed it down or reduced the viability of the seed. After ten weeks of stratification 62 per cent of the treated seed germinated while germination of the untreated seed at that time was 77.0 per cent.

Sulfuric acid

The use of sulfuric acid to increase the permeability of the seed coat and to remove the seed coat that mechanically resists expansion of the seed content, has been investigated by a number of workers (6, 35, 53, 56, 60, 68, 70, 72). Flemion (35) working on Crataegus spp. found that by a treatment of the seed with concentrated sulfuric acid, preceding stratification, germination of the seed could be secured during the first spring. Without such treatment, seed of Crataegus germinate normally only during the second spring after collection, which is true also of the seed of Juniperus scopulorum. Because of this similarity in the normal behavior of the two species, it seemed desirable to investigate the effectiveness of sulfuric acid on the after-ripening of seed of Juniperus scopulorum.

The seed used in this experiment were of the 1940 crop, cleaned and stored dry for two weeks. One sample of the seed was treated with concentrated sulfuric acid for thirty minutes and another for one hour. Upon removal from the acid the seed were thoroughly washed in running water, divided into nine parts, stratified and placed in that state at temperatures or combination of temperatures as indicated in Table 5.

Many of the seed treated for one hour and a few treated for thirty minutes were definitely injured. After being stratified for about fifty days, the cotyledons in many seeds enlarged and broke through the seed coat. This was particularly common among the seed treated one hour. This abnormal

growth of cotyledons, preceding that of the radicle has been found to be characteristic of seed either having a very dormant embryo or of those in which dormancy of various parts of the embryo is not of the same intensity (2, 71). This abnormality occured also in the seed of red cedar, being caused by scarification of seed during the process of cleaning (Fig. 3).

Table 5. Effect of stratification on after-ripening of acid-treated seed of Juniperus scopulorum

Sample	Treatment			Per cent germination
	Concentrated H_2SO_4 (minutes)	Stratification period		
		At 60° F. (days)	At 41° F. (days)	
1	0	0	0	0
2	0	0	120	0
3	30	0	0	0
4	30	0	106	1
5	30	0	126	3
6	30	53	0	1
7	30	53	61	38
8	60	0	0	0
9	60	53	0	0
10	60	120	0	1
11	60	53	61	15

Seed treated for thirty minutes, stratified for fifty-three days at 60° F. and then kept for sixty-one days at 41° F. gave the most promising results, 38 per cent of these seed being fully after-ripened, (sample 7). Seed similarly handled but treated for one hour germinated to the extent of 15 per cent (sample 11). None of the other combinations of acid treatments and stratification gave significant results comparable to those obtained with samples 7 and 11. In view of the rather encouraging results obtained by the combined treatment of seed with concentrated sulfuric acid and stratification, the problem should be investigated further.

Properties Of After-ripened Seed

Effect of drying

The purchase of after-ripened seed by nurserymen would improve their chances of producing a good crop of seedlings. It was thought desirable to investigate the possibilities of holding after-ripened seed in such a state in which it would not germinate and yet remain ready for germination. One of the possibilities of attaining this end appeared to be drying. In some instances drying of after-ripened seeds will lower their viability. Haut (42) found this to be the case with after-ripened apple seed. On the other hand, cases are known where after-ripened seed could be dried without appreciable reduction in their ability to germinate (3).

To determine the effect of drying on after-ripened seed of red cedar, two samples of seed (lot 39C) were dried to a constant weight at room temperature and stored dry for various periods of time. One of the samples prior to drying was stratified 66 days and the other 91 days. The effect of the drying on germination of the seed in these two samples is summed up in Table 6.

Table 6. Effect of drying on germination of after-ripened seed of Juniperus virginiana.

Length of stratification period prior to drying (days)	Period of drying (weeks)	Per cent germination: At room temperature: Per cent	At room temperature: in days	At 41°F.: Per cent	At 41°F.: in days
66	0	66.0	14	66.0	31
66	1			49.0	54
66	5	5.5	18	48.5	47
66	9	0.0	18	25.5	46
91	0	74.5	25	58.5	18
91	1	9.0	20	5.5	20
91	4	.5	20	68.5	50
91	10	10.5	16	59.5	61
91	12	9.5	30	45.0	56

Drying of after-ripened seed of red cedar at room temperature generally reduced germination. The reduction of germinability was greater in tests set at room temperature than in those set at 41° F. which, at least in part, should be attributed to the effect of the temperature in germinators. However the chief immediate effect of drying appears to be the secondary dormancy which, as will be shown later, can be overcome by providing again the conditions favorable to after-ripening.

Pack (54) reported that after-ripened seed of red cedar was thrown into a state of secondary dormancy when exposed to a temperature of 12° C. From the data on germination temperature (Table 10 and Figure 4), it can be concluded that after-ripened seed of Juniperus virginiana does not revert to secondary dormancy under the influence of high temperatures only. However air drying of after-ripened seed of red cedar at room temperature caused the seed to revert to secondary dormancy. Seed in a state of secondary dormancy usually require an additional period of stratification before their germination can be expected. In the above experiment dried after-ripened seed placed in germinators at room temperature remained in a dormant state while those placed in germinators at 41° F. were under conditions favorable for after-ripening and apparently the process of after-ripening was repeated. The extended period of time that was required to reach maximum germination indicates that the seed did after-ripen under the conditions of the germinators at 41° F. Fully after-ripened seed required 18 days to germinate to the extent of 58.5 per cent whereas dried seed had to remain at 41° F. for from 50 to 61 days to complete germination. This second period needed for after-ripening was much shorter than the initial stratification period.

The effect of air drying after-ripened seed of Juniperus scopulorum also was investigated. Samples of after-ripened seed of this species (lot 38) were dried for 2, 7, and 14 days after which they were tested for germination at room temperature. The germination of these seed was as follows: seed not dried, 30 per cent; seed dried two days, 38 per cent; seed dried one week, 30 per cent; and seed kept dry one week at room temperature and one week at 90° F., 24 per cent. In other words germination of these seed was not reduced by one week of drying and only slightly reduced when drying period was extended to two weeks. After-ripened seed of Juniperus scopulorum, in contrast to those of J. virginiana, do not revert to secondary dormancy due to drying for two weeks but are able to germinate at room temperature after such treatment.

Effect of freezing

In order to insure complete after-ripening of all newly acquired seed of red cedar, it is desirable to extend the period of stratification to 100 or more days (54). On the other hand, since many seeds complete the process of after-ripening in a course of 60 or 75 days (Table 3) extension of stratification to 100 days may in some instances be undesirable: as shown elsewhere in this paper, fully after-ripened seed of red cedar germinate easily at the temperature of stratification (41°F.). Considerable variation in the stratification requirements of individual lots of seed and difficulties created by this variation, suggested the need of finding a method which would prevent germination of after-ripened seed and yet would allow the seed to maintain their germinative power. In connection with this, an investigation of the effect of freezing on the viability and germinability of after-ripened seed was undertaken. Two lots of seed, after being stratified for from 66 to 100 days were frozen in blocks of ice and left in that state in the freezing unit of

an electric ice box. Samples of these seeds were removed from time to time, thawed out and placed in germinators: one-half of each sample at room temperature and the other at 41° F. The results of germination tests of these seed are presented in Table 7.

Germination of the two lots of seed tested at two different temperatures varied considerably. Tests at 41° F. should be considered more representative of the viability of these seed than those set at room temperature. Uneven germination at room temperature was unquestionably due to considerable variations in the conditions under which the tests were conducted. As shown elsewhere in this paper (Fig. 4, Table 10), temperature has a marked influence on germination. To the same effect of external conditions might be attributed the increase in germination after ten weeks of freezing (53.0 per cent and 33.5 per cent) as compared to germination after six weeks of similar treatment (20.0 per cent and 17.5 per cent respectively).

It is of interest to note that almost one-half of the originally viable seed still remained viable after twenty-four weeks of freezing which is contrary to Pack's findings (54), who reported that fully after-ripened red cedar seed (seed cracked open and the water content increased to 52 per cent) were killed by exposure to -5° C. It is significant that there was relatively small loss of viability in seed frozen for one and four weeks. This may permit a few weeks delay in planting should there be a need for it, even if the seed is completely after-ripened and ready to germinate.

A practical application of the property of after-ripened seed to withstand freezing without losing its ability to germinate was demonstrated on a sample of seed of lot 40, which had been stratified 73 days and completely after-ripened somewhat in advance of planting season.

Table 7. Effect of freezing after-ripened seed of *Juniperus virginiana* on seed viability

Lot	Number of weeks the seed were frozen							
	0	1	4	6	7	10	12	24	52
	Per cent germination in 36 days								
	at 41°F.								
39C(a)	58.5	72.0	70.0		69.0		75.0	34.5	0.0
39C(b)	66.0	49.0		36.5		26.5			
39D	44.0	44.5		30.5		14.5			
	at room temperature								
39C(a)	74.5	56.0	65.0		49.5		16.5	23.5	0.0
39C(b)	66.0	42.0		20.0		53.0			
39D	40.0	40.0		17.5		33.5			

The seed was removed from storage and frozen in blocks of ice. About a month later the seed was removed from the ice, planted in the nursery, and produced a good stand of seedlings.

GERMINATION

The process of germination in the seed of Juniperus virginiana and J. scopulorum is essentially the same as in all other seeds requiring after-ripening. There is no definite separation between the end of after-ripening and the beginning of germination. In this paper breaking of the embryo through the endosperm is considered as the beginning of germination. Normal germination of Juniper seeds begins with the growth of and protrusion of the radicle through the endosperm (Figure 2). The cotyledons elongate slightly but remain enclosed within the seed and continue to absorb the stored food from the endosperm. Only when this food is exhausted are they pulled out and appear above the soil. In certain cases and under certain conditions the growth of the cotyledons precedes that of the radicle (Figure 3). This abnormality in germination is discussed elsewhere in this paper.

Germination of a well after-ripened seed may occur on the first day after the conditions favoring this process are provided. In some seed however, as many as 28 days may elapse between the time the seed are placed under germinative conditions and the time when actual germination takes place. Emergence of seedlings from soil usually occurs in three or four weeks after planting, depending upon the degree of after-ripening, the depth of planting, and the temperature and moisture conditions of the soil.

Effect of the reaction of the germination medium

It is known that the reaction of the medium may have a pronounced effect on the germination of seed even if the range of pH values under which the seed will germinate is rather wide. For instance Salter and McIlaive (61) working with seed corn, wheat, soybeans, red clover, and

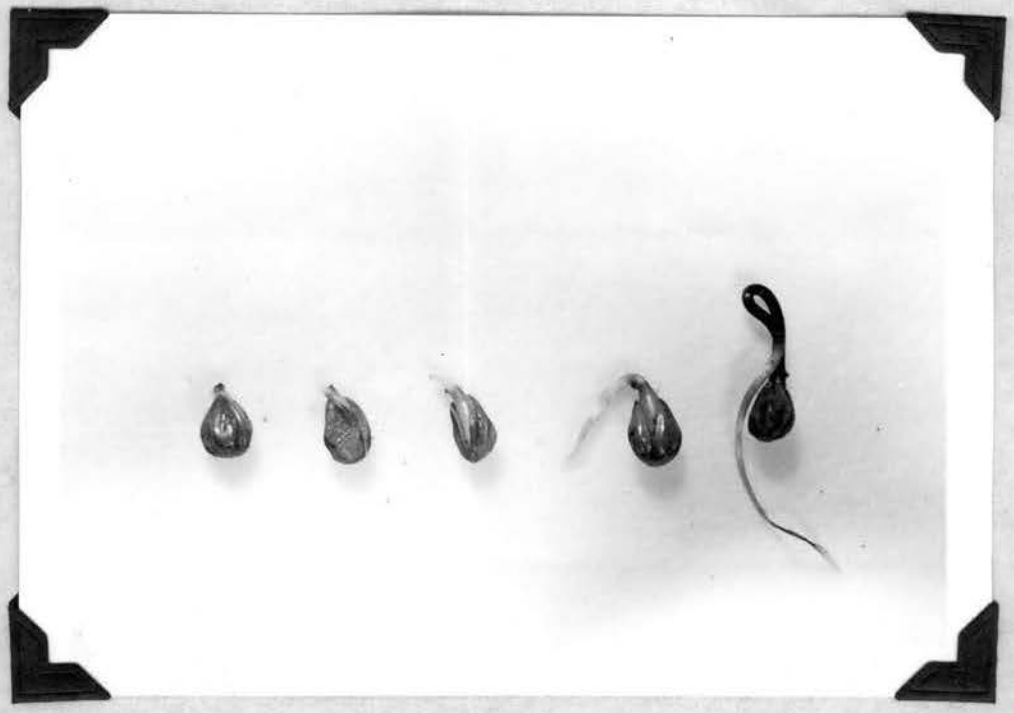

Fig. 2. Several stages of normal germination of seed of Juniperus virginiana.

Fig. 3. Abnormal germination of seed of red cedar showing the emergence and growth of the cotyledons and the hypocotyl preceding that of the radicle.

alfalfa found that they all germinated in media ranging in reaction from 2.96 to 7.71 pH units, while the optimum reaction for these seeds was of pH of 4.11.

Since germination of some seeds is affected by the reaction of the soil, it seemed desirable to investigate the effect of the pH of the medium on the germination of seed of Juniperus virginiana and J. scopulorum. After-ripened seed of Juniperus virginiana were placed to germinate on cotton moistened with phosphate buffer solutions of various pH values. Germination tests were carried on at a constant temperature of 70° F. A constant supply of buffer solutions was maintained in germinators by adding to the cotton proper solutions whenever necessary. The tests were set in duplicate, each sample containing 100 seeds. Germination was recorded for fifteen days following the setting of the test, after which time the germination in all samples was completed.

Germination in all samples began at the same time and progressed at the same rate irrespective of the reaction of the media. The final germination per cent was the highest at pH of 6.2, and the lowest at pH of 5.28 (Table 8). However, the difference in total germination was too small to be significant. The harmful effect of alkaline buffer solutions (pH of 7.8 and 8.4) on the growth of radicles was evident in two samples in which the radicles turned brown upon coming into contact with the solution. Similar injury to the growing embryo caused by high alkalinity of the solution was observed also on seed of Magnolia acuminata by Afanasiev (2). Highly acid reactions might also be unfavorable for the growth of seedlings: Salter et al (61) reported severe injury caused by the germination medium of pH of 2.96 to seedlings of corn, alfalfa, wheat, red clover, and soybeans. Growth of red cedar seedlings seemed best on the slightly acid medium (pH 6.2). This combined with the

Table 8. Germination of seed of Juniperus virginiana as affected by the reaction of the germination media.

Reaction of the germination media (pH)	Per cent germination					
	Days					
	1	3	6	9	13	15
4.40	35.5	63.0	66.5	67.5	68.0	68.5
5.28	35.0	59.0	62.5	63.0	63.5	64.5
6.20	40.5	69.5	72.6	73.0	73.0	73.0
7.00	35.0	60.0	63.0	64.5	65.5	66.0
7.80	43.5	60.5	64.0	65.0	65.0	65.0
8.40	38.0	60.0	64.5	66.0	66.0	66.0

somewhat higher germination at this reaction than in the other samples, suggests pH of 6.2 as the optimum for the germination of red cedar seed.

A similar experiment was started with the seed of Juniperus scopulorum. It was carried on under the same conditions as that on seed of red cedar. The reactions of the solutions tried ranged in pH from 5.91 to 8.04. Fifty seeds were used in each sample. The highest germination was obtained at pH of 5.91 and the lowest at pH of 8.04, (Table 9).

Table 9. Germination of seed of Juniperus scopulorum as affected by the pH of the medium.

Reaction of the medium (pH)	Per cent germination in 26 days
5.91	52
6.98	38
8.04	20
Distilled water	30

Contrary to the case of Juniperus virginiana there was a marked difference in germination at the extremes of the range tried. Strongly alkaline medium (pH 8.04) was detrimental not only to germination but to the viability of the seed as well: 46 per cent of the seed which did not germinate were found to be dead at the end of the experiment. Although only relatively small number of seeds were used in this experiment, striking differences between the germination at different reactions justify the conclusion that fairly acid reaction is more favorable for germination of seed of Juniperus scopulorum than a reaction which is either neutral or basic.

Effect of Temperature

The desirable practice of planting after-ripened seed of red cedar in nursery beds in late February or early March is not always possible. The seed may be slow in after-ripening and require the extension of stratifi-

cation until late spring. Delay in planting may also be caused by unfavorable weather and soil conditions. Late plantings would mean that germination of seed must take place at rather high temperatures which according to Pack (54) are extremely unfavorable if not entirely prohibitive to this process. For this reason an experiment was set up to determine more definitely the effect of temperature on germination and find the maximum temperature at which germination of after-ripened seed of red cedar would take place. The range of temperatures selected for trial represents that of the outdoor temperatures prevailing in Oklahoma from February to May. The constant temperatures used were as follows: 32° , 40° , 50° , 60° , 70° , 80° , 90° , and 100° F. Sixty-eight hundred seed were used in this test. The results of the germination tests of after-ripened seed of red cedar at these temperatures are presented in Table 10 and Figure 4. The test at 32° F. was not included in the graph because no germination was observed at this temperature in a period of thirty days.

Germination of red cedar seed is definitely and markedly affected by temperature. Within the range of 32° to 70° F. there is a strong correlation between the initial rate of germination and the temperature at which germination takes place. At 80° and 90° F. germination is somewhat slower than at 70° F., while at 100° F. the depression on germination rate becomes very pronounced.

In regard to the effect of temperature on the total germination of the seed at the end of thirty days, seed set at 50° F. gave the best results. Germination at 60° and 70° F. was almost as good as that at 50° F., but at 80° and 90° F. the final germination per cent fell off considerably. At 100° F. germination was the lowest with the exception of that at 32° F., at which temperature no germination took place at all.

Table 10. Effect of temperature on germination of after-ripened seed of Juniperus virginiana.

Temp. of germinator	No. of seeds	Per cent germination * (days)											
		2	3	5	7	9	12	15	17	19	24	26	30
32°F.	900	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40°F.	900	4.2	5.0	15.2	23.5	28.0	35.4	39.1	41.2	43.8	48.9	49.7	54.6
50°F.	900	9.1	15.0	40.2	59.7	63.1	69.5	71.0	71.4	71.8	72.9	73.4	74.3
60°F.	700	35.7	44.8	58.2	62.3	64.8	67.0	67.8	68.0	68.0	68.0	68.0	68.0
70°F.	900	43.8	53.8	64.8	67.2	68.4	69.3	69.3	69.3	69.7	69.8	69.8	69.8
80°F.	900	35.2	47.7	55.9	57.3	57.9	58.7	58.7	58.7	59.0	59.0	59.0	59.0
90°F.	900	37.4	46.7	57.2	58.0	58.2	58.3	58.4	58.5	58.5	58.6	59.0	59.0
100°F.	700	13.8	14.5	18.7	19.9	20.3	20.5	20.5	20.5	20.5	20.5	20.5	20.5

* Each number represents the average of three tests.

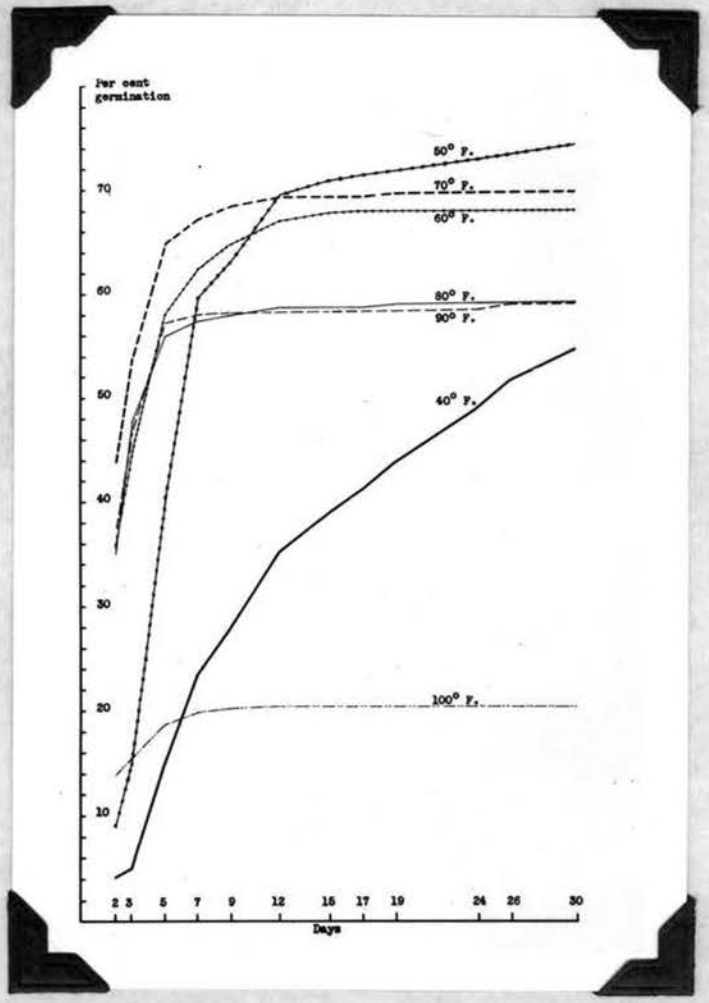

Fig. 4. Effect of temperature on germination of after-ripened seed of Juniperus virginiana.

Sharp decrease in germination at 100° F. is probably due to the death of the embryos in some of the seed. Many seeds at that temperature showed definite signs of injury. High temperature affected not only the germination but growth of the seedlings as well. Embryos in seed which germinated between 90° and 100° F. failed to grow normally and died shortly after the radicle broke through.

Results closely similar to those just discussed were obtained also with seed planted outdoors in a nursery bed. In this case factors other than temperature have undoubtedly affected the final outcome of the experiment. Yet the results of the experiment are significant because the work was done under conditions during the normal time of planting. Several samples containing from 100 to 600 seed each were removed from stratification and planted in the nursery bed on different dates between March 30th and May 4th of the same year. The information concerning these plantings is summed up in Table 11.

Table 11. Effect of time of outdoor planting on germination of after-ripened seed of Juniperus virginiana.

Lot	Period of stratification (days)	Number of seeds	Date planted	Per cent germination in 4 weeks*
39C	84	300	3/30/40	54.6 +
39C	98	175	4/13/40	48.0
39C	112	300	4/27/40	58.0
39C	119	600	5/4/40	45.8
39E	102	300	3/30/40	45.3 +
39E	131	100	4/27/40	44.0
39E	138	200	5/4/40	31.5

* These percentages also represent the total germination.

The figures marked with a + are somewhat lower than the actual percentage of germinated seed because of the destruction of a few seedlings by cutworms.

The per cent of seed which produced seedlings fell off gradually with the delay in planting. This reduction in the proportional number of seedlings is well correlated with the rise in temperature at the time the germination took place (Figure 5).

Taking into account the results of this experiment and considering the normal stratification requirements of the seed of red cedar it becomes clear that the postponement of planting for a few weeks is a better practice than early planting of non-after-ripened seed. The reduction in germination due to higher temperature (late planting) is not as large as that caused by incomplete after-ripening (Table 3). This statement should not be taken as a suggestion that late planting (April and May) is generally a desirable practice. Late planting should be resorted to only in exceptional cases when the seed were not stratified early enough to become completely after-ripened before April.

Late planting is undesirable not only because of the probable reduction in germination but also because of the shortening of the growing season and the dwarfing effect of high temperatures on young seedlings. It can be seen in Figure 5, that at the end of the first growing season the size and vigor of seedlings produced in May were decidedly inferior to those of the seedlings which were produced earlier.

Initial growth of seedlings is markedly affected by the temperature. This became apparent from the results of the following experiment: fifty fully after-ripened seed were planted one-half inch deep in each of three pots of soil. The latter was composed of two parts of garden soil and one part of sand. The pots were placed at temperatures of 50° , 70° , and 90° F. After fourteen days practically all seeds placed at 50° and and 70° F. had germinated, while those held at 90° F. germinated only to the extent of 10 per cent. At this time thirty-six seedlings kept at 70°F.

Fig. 5. One year old seedlings of red cedar. The seed were planted on the following dates: a, March 30; b, April 13; c, April 27; and d, May 4, 1940.

appeared above the soil yet none of the seedlings kept at 50° and 90° F. reached the soil surface. The few seeds which germinated at 90° F. failed to produce normal seedlings (Figure 6).

Preliminary work on the effect of temperature on germination of seed of Juniperus scopulorum was also carried out. The seed had been planted in the fall of 1938 and were fully after-ripened when removed from the nursery bed in January 1940. About fifty-five per cent of the seed were viable at that time. The germination temperatures tried were more or less the same as used in the similar experiment with seed of red cedar, namely: 34° , 40° , 60° , 70° , 90° , and 100° F. One hundred seed were used in each test. The results of these tests are presented in Table 12.

Within the range of temperatures between 34° and 90° F. there was a definite correlation between the rate of germination and the total germination on one hand,and the germination temperature on the other. At 100° F. germination was definitely reduced. Seedlings grew best at a temperature of about 60° F. Seedlings produced at temperatures of 90° and 100° F. failed to grow normally and soon died.

Effect of vitamin B_1

Vitamin B_1 was found to have no effect upon after-ripening as discussed elsewhere in this paper (page 30). To determine if this substance had any effect on germination of after-ripened seed, the following experiment was performed: duplicate samples of red cedar seed stratified 77 days were soaked for 24 hours in a solution containing 4 mg. of vitamin B_1 per liter, and placed in germinators. Seed so treated germinated to the extent of 73.0 per cent while germination of the untreated seed during the same period was 77.0 per cent. In other words vitamin B_1 neither increased nor prevented the germination of after-ripened seed of red cedar.

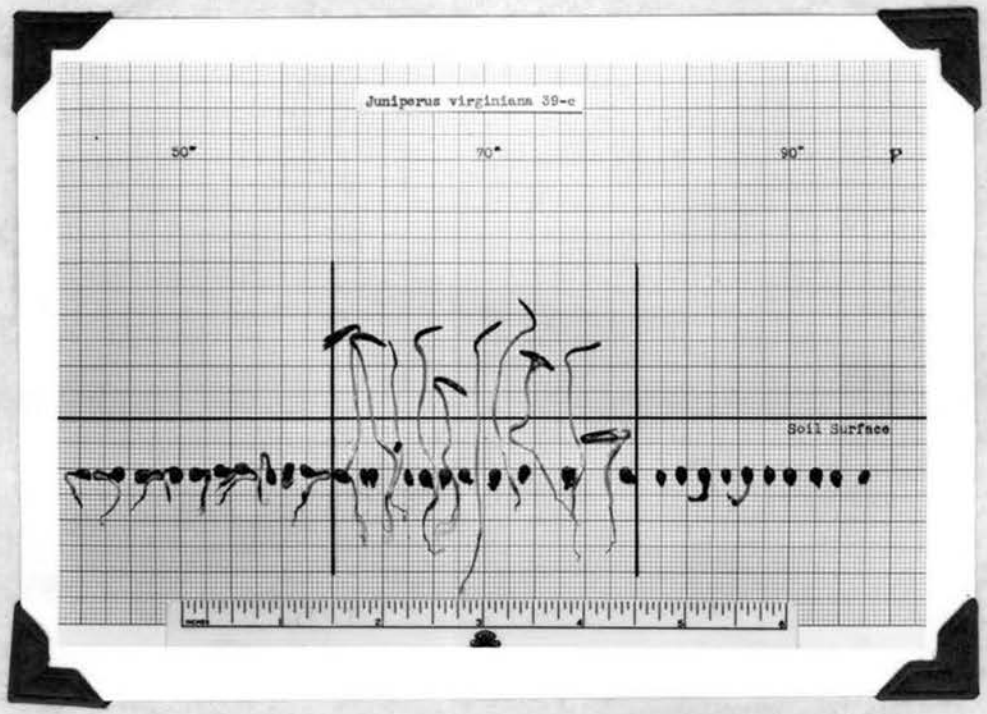

Fig. 6 Growth of seedlings of Juniperus virginiana as affected by temperature.

Table 12. Effect of temperature on germination of after-ripened seed of Juniperus scopulorum

Germination temperature	Per cent germination (days)							
	2	4	6	8	10	14	18	20
34°F.	0	0	0	0	0	1	1	2
40°F.	0	2	4	10	18	20	24	24
60°F.	11	27	33	33	34	34	34	34
65°F.	16	32	38	38	38	38	38	38
70°F.	17	34	40	40	40	40	40	40
90°F.	28	41	45	45	46	47	47	47
100°F.	5	11	17	20	20	20	20	20

Effect of variations in oxygen supply

To determine the effect of variations in the oxygen content of the air on germination of red cedar seed, the following experiment was carried out: before being tested for germination the seeds, previously stratified for 77 days, were placed for twenty-four hours in an atmosphere containing various amounts of oxygen. One sample was thus held in an atmosphere containing 5 per cent of oxygen, another 10 per cent, third 20 per cent (normal air), and fourth in pure oxygen. Twenty days after the beginning of germination tests the seed germinated to the extent of 48.5 per cent, 53.5 per cent, 77.0 per cent, and 73.5 per cent respectively.

Reduction of oxygen content of the air markedly reduced germinability of the seeds while an increase in oxygen did not affect germination.

After somewhat similar treatment of after-ripened seed of Juniperus scopulorum it was observed that increase of oxygen from normal of 20 per cent to 100 per cent for 48 hours reduced germination from 32.5 per cent to 22.0 per cent.

MICROCHEMICAL TESTS

Observations on stored food and oxidizing enzymes in seed of Juniperus scopulorum were made by means of the following tests: Sudan iv (Scharlach R, Scarlet Red) for fats; Millon reaction (nitric acid and mercury), Biuret reaction (copper sulphate and sodium hydroxide) and xanthoproteic reaction (nitric acid and ammonium hydroxide) for proteins; Fluckiger reaction (copper tartrate and dilute sodium hydroxide) for glucose and fructose, phenylosozone formation (phenylhydrazine hydrochloride and sodium acetate) for glucose and methyl phenylosozone formation for fructose. Sucrose was inverted with dilute solutions of citric acid and Fluckiger reaction was used on the inversion products. Starch was tested by the use of iodine potassium iodide solution. In the test for oxidase alcoholic solution of gum guiac was employed. Peroxidase was tested by means of alcoholic solution of gum guiac and 3 per cent solution of hydrogen peroxide.

The food reserve in dormant seed of Juniperus scopulorum is in the form of fats and proteins. Neither starch nor sugar were detected. Peroxidase was found in small quantities while oxidase was absent or inactive. Catalase activity was found to be very pronounced even in dormant seed. Water content of the air dry seed ranges from six to twelve per cent of the oven dry weight of the seed. The kernel of the seed is slightly acid, ranging in pH from 6.4 to 6.9.

The changes observed in the seed of Juniperus scopulorum during the period of stratification are as follows: rapid initial absorption of water, after which the water content decreases slightly; this is followed by an increase in moisture as the seed becomes after-ripened; decrease in the amount of fats and proteins in the endosperm and increase in the

amount of these substances in the embryo; appearance of sugar in the endosperm near the embryo; slight increase in peroxidase content; appearance of oxidase; increase in catalase activity and slight growth of the embryo. The summary of the above changes is presented in Table 13. The number of crosses in this table represent the relative amounts of various substances at different stages of stratification and after-ripening. These changes in the seed of Juniperus scopulorum are essentially the same as found in after-ripening seed of Juniperus virginiana by Pack (54, 55), except that they progress at a much slower rate.

The changes that take place in an after-ripened seed of Juniperus scopulorum just before and during germination are rapid increase in moisture content, slight increase in sugar, and oxidase, increase in peroxidase and marked increase in catalase activity. During germination fats and proteins decrease in quantity, the amount of sugar and the activity of oxidising enzymes increase markedly, starch appears in the growing embryo and the moisture content of the seed decreases slightly.

Table 13. Summary of the changes taking place in the seed of Juniperus scopulorum during stratification.

Materials	Stratification period at 41°F. Months							After-ripened seed	Germinating seed
	0	1	3	5	7	9	12		
Fats	XXXX	XXXX	XXXX	XXXX	XXX	XXX	XXX	XXX	XX
Proteins	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XX
Sugars					trace	trace	trace	X	XX
Starch									X
Oxidase							trace	X	XX
Peroxidase	X	X	X	X	X	X	X	XX	XXX
Catalase**	32.6		40.0				64.2	342.5	445.6
Moisture content*	6.1	25.8	20.0				26.5	43.3	40.0

* Per cent oven dry weight.

**Figures representing catalase activity indicate the number of cubic centimeters of oxygen evolved from 5 cc of hydrogen peroxide in 10 minutes per one gram of oven dry weight of seed.

CATALASE

Because of the apparent correlation between catalase activity and some physiological processes in plant tissues, many workers have attempted to use the former as an indicator of the progress of after-ripening. Similar attempts were made during the present study.

The standard method described by Davis (25)(Figure 7) was employed. The actual determination of the activity of catalase was as follows: samples of seed to be tested were weighed and placed in a small mortar. To this was added an equal weight of calcium carbonate, a little pure quartz sand, and 2 cc of distilled water. This mixture was ground for two minutes. After grinding was completed, the material was washed into a shaking bottle with an additional 10 cc of distilled water and placed in a water bath at 25° C. The bottle was stoppered with a rubber stopper containing the gas delivery tube and a small separatory funnel. Five cubic centimeters of hydrogen peroxide* freshly neutralized with a little calcium carbonate were placed in the separatory funnel. When the temperature of the ground material in the shaking bottle had reached that of the bath, the hydrogen peroxide in the separatory funnel was allowed to flow into the shaking bottle and the shaking device started. The volume of oxygen released was recorded at the end of one, three, five and ten minutes of shaking. The values found in this paper and representing catalase activity are the averages of at least two determinations: if the results of the first two runs varied by more than ten per cent, additional determinations were made until the results checked within the allowed variation. The catalase activity was determined in dry (dormant),

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* Fisher Scientific Company. Pittsburg, Pa. (3 per cent solution).

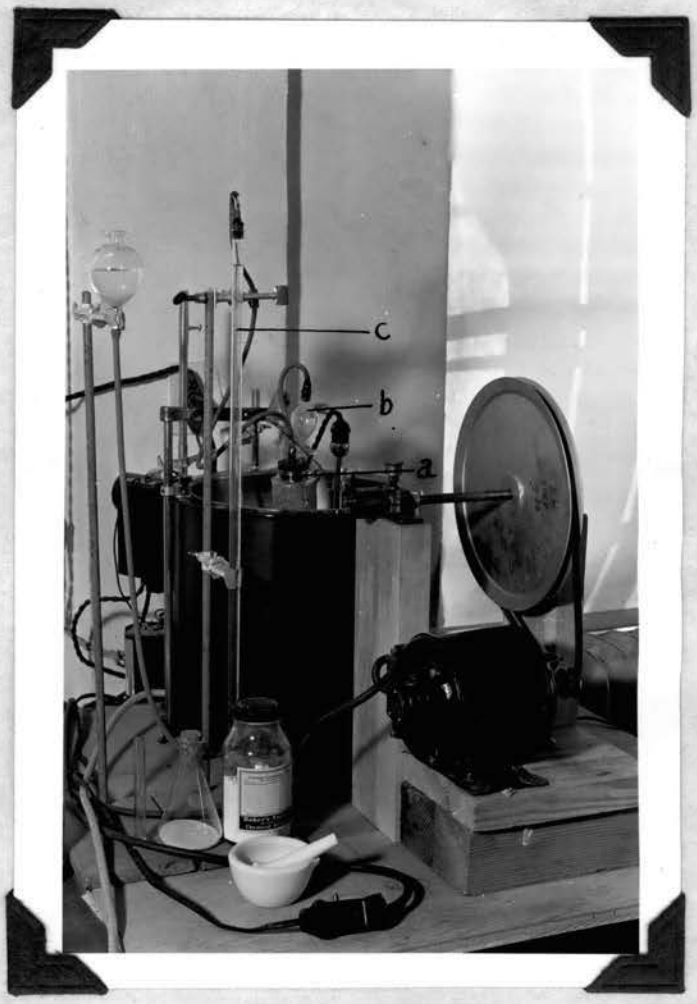

Fig. 7. Apparatus for determining the catalase activity in plant tissue. a, shaking bottle; b, separatory funnel; c, volumetric burette.

stratified, after-ripened and germinating seeds. In the following discussion the figures representing catalase activity indicate the number of cubic centimeters of oxygen evolved from 5 cc of hydrogen peroxide in 10 minutes per one gram of oven dry weight of seed.

Dry seed of Juniperus scopulorum stored at room temperature for a year exhibited more or less constant catalase activity throughout the entire period of storage, the original being 32.65 and that after one year 28.25 (Table 14). Seed stratified continuously for a year at 41° F. showed in one set of tests a gradual rise in catalase activity. In a course of a year it was practically doubled (tests8, 10, Table 14). However, in a similar set involving the determination of catalase activity of stratified seed (lot 38) every five days, no marked change in catalase activity caused by stratification was noted; after 88 days of stratification the catalase activity was practically the same as at the beginning of the experiment (Table 15). Transfer of seed stratified for forty days at 41° F. to a temperature of approximately 75° F. resulted in a gradual decrease of catalase activity in a period of 41 days (Table 15). Seed stratified at 41° F. for forty days and at room temperature for twenty-eight days, and then transferred back to cold storage, showed a loss in catalase activity in a course of seven days but when the last period of cold storage was extended to twenty days the catalase activity rose very markedly (Table 15).

In view of the above, it appears that stratification as such has no definite effect on catalase. However if stratification results in bringing the seed toward complete after-ripening catalase activity increases markedly (Tests16, 17, 18, Table 14).

To determine the effect of complete after-ripening and germination on catalase activity the following experiment was performed: a sample

Table 14. Effect of stratification and after-ripening on the catalase activity of seed of Juniperus scopulorum. Lot 39.

Test	Stratification period: 41°F.	Stratification period: Room temp.	Stratification period: 41°F.	Amt. oxygen evolved from 5 cc H_2O_2 per gram of dry matter in 10 minutes	Remarks
1	Seed stored dry			32.65 (0)	The length of dry storage (days) is indicated by the figures in parentheses.
2				26.85 (147)	
3				30.88 (189)	
4				31.21 (224)	
5				28.25 (372)	
6	0			32.65	
7	45			41.10	
8	65			48.46	
9	93			40.01	
10	365			64.21	
11	30	30	30	42.25	1 per cent germination
12	60	30	60	43.66	1 " " "
13	90	dry*	90	44.91	No germination
14	60	60	60	43.88	2 per cent germination
15	30	60	90	56.28	1 " " "
16	90	30	90	82.06	20 " " "
17	90	120	90	63.72	9 " " "
18	90	120	120	68.35	17 " " "
19		60	90	445.60	Radicle emerged
20		60	90	342.50	After-ripened seed
21		60	90	48.46	Non-after-ripened seed
22		53**		148.60	Cotyledons growing ***
23		53**		78.73	Seed intact ***

* Air dried and then restratified immediately.

** At 60° F.

*** Treated with sulfuric acid for 30 minutes prior to stratification.

Table 15. Catalase activity in seed of Juniperus scopulorum as affected by stratification. Lot 38.

Stratification period (days)			Amount of oxygen evolved per 1 gram of dry material in ten minutes. (cc)
41°F.	Room temperature	41°F.	
0			48.5
5			41.3
10			36.0
15			42.7
21			54.0
25			40.5
30			47.5
35			45.0
40			51.5
45			49.5
50			48.0
56			52.5
60			54.0
65			40.5
71			50.5
75			36.5
81			50.0
88			50.5
40	5		53.0
40	10		45.0
40	16		54.0
40	20		49.5
40	25		48.0
40	31		37.5
40	35		39.5
40	41		38.0
40	28	7	33.0
40	28	13	45.5
40	28	20	43.0

of seed stratified for a total period of 240 days was divided into three groups: one containing the seed which had begun to germinate (Test 19, Table 14), another consisting of fully after-ripened seed (Test 20), and third, of seed still dormant (Test 21). Catalase activity of each group was then determined. The completion of after-ripening and germination caused an increase in catalase activity from the original of 48.46 to 342.50 and 445.60 respectively.

Seed treated with concentrated sulfuric acid and stratified at 60° F. for 53 days showed a marked increase in catalase activity suggesting rather rapid progress in after-ripening. The completion or near completion of after-ripening in many of these seeds was indicated by the growth of cotyledons (Table 14, Test 22). Intact seed treated in the same manner showed much less increase in catalase activity (Test 23).

Correlation between the progress of after-ripening and the catalase activity has been reported by many workers (16, 22, 29, 32, 64). Pack (54) reported similar correlation in seed of red cedar and suggests that the catalase activity can be used as a measure of the rate of after-ripening of stratified seed.

STORAGE

Seed of some plants retain their viability after being stored dry for a number of years. Crocker and Barton (19) found that rose seed not only remained viable after two years of dry storage but actually germinated much better after being thus stored. Crocker et al (19) and Haut (42) reported that viability of apple seeds is not reduced after two years of dry storage at room temperature. Sweet clover seed was found to remain viable at least four years after collection (44). It is commonly known that seed of certain legumes retain their viability for long periods of years.

Although it is generally accepted that juniper seed loses viability with age rather slowly, no data to support this contention are available. It was decided therefore to get more specific information on the effect of dry storage on the viability of red cedar seed. A sample of seed of lot 39C was cleaned and stored dry in an uncovered glass jar for seven months at 41° F., while another sample of the same lot consisting of dry berries was left at room temperature where it remained for a period of one year. After the completion of the above periods, the seed were stratified and their viability determined by germination tests. Judging by the results of these tests (Table 16) the loss of seed viability, if any, caused by dry storage of seed, either cleaned or still enclosed in fruits, was negligible. Germination of the seed stored for seven and twelve months (66.0, 72.5 and 75.0 per cent) was about the same as that of the seed which were stratified soon after collection (73.5 per cent).

Table 16. Effect of dry storage on viability of seed of Juniperus virginiana

State of seed and place of storage	Period of dry storage	Stratification period (days)	Germination at 70°F. (per cent)
Stratified as soon as collected.	none	106	73.5
Dry seed at 41°F	7	73	66.0
" " " " "	7	73	72.5*
Dry fruit at room temperature	12	75	75.0

* Germination at 80°F.

RECOMMENDATIONS FOR HANDLING SEED

In view of findings reported and discussed in this paper, the following procedure of handling seed prior to planting is suggested: the seed should be collected as soon as ripe which in this State means late October or early November. Because of frequent failures of seed to develop properly, a cutting test should always precede collection. A lot containing from 50 to 70 per cent of well filled sound seed may be considered to be of a good quality. As soon as brought in, fruits should be spread on the floor or table and allowed to dry for a week or ten days.

The seed before acquiring the ability to germinate must complete the process of after-ripening. The latter takes place at a low temperature in presence of a supply of moisture. Such conditions can be provided by means of stratification. Before being stratified the seed must be cleaned. Soaking berries for three or four days in water facilitates cleaning of the seed. After the berries become soft they should be macerated, care being taken not to crack the seed. After the berries are thoroughly macerated, they should be placed in a large volume of water: most of the good seeds sink so that the pulp and the majority of the empty seeds can be floated off. The common practice of soaking the berries in a weak solution of lye seems to be of no value in seed cleaning.

Peat moss is a good stratification medium for seed of red cedar and Juniperus scopulorum because it has a very large water holding capacity and yet does not interfere with aeration. The peat must be thoroughly moist but not too wet. The seed is mixed with at least four times its volume of peat and placed in any convenient container. A bushel basket lined with wrapping paper serves very well. For small amounts of seed glass jars or coffee cans may be used.

Storage of stratified red cedar seed at a temperature of 41°F. was found very effective in promoting after-ripening but temperatures ranging from 36° to 45°F. have given good results also. Stratified seed should remain at low temperature until it is completely after-ripened. Some seeds after-ripen much more rapidly than others. A period of from 100 to 120 days is sufficiently long to allow after-ripening of most seeds. Seed should be stratified before the middle of December to insure the completion of after-ripening by planting time. The stratified seed should be inspected occasionally to check on the supply of moisture in the peat and to note the progress of after-ripening. The completion of after-ripening is marked by the splitting of the seed coat. The seed should be planted as soon as after-ripening is completed because fully after-ripened seed of red cedar germinate freely at the normal stratification temperature. If the after-ripened seed cannot be planted for some time and yet begins to germinate in stratification, it can be removed from the peat moss, frozen and kept in that state for a few weeks. This method of preserving after-ripened seed cannot be recommended as a standard practice but should be resorted to only in case of emergency.

Since germination is favored by a relatively low temperature (50°- 70°F.), planting should be done early, preferably in March. According to the records of 1940, soil temperatures in April on many occasions were higher than 80°F. However good germination may also be secured at a temperature somewhat higher than 70°F. and if seeds are not fully after-ripened in March, it is preferable to lengthen the stratification period rather than plant non-after-ripened seed. Fall planting of dormant seed is a common practice among growers in this State. The success of such plantings depends upon three factors: (1) the temperature must remain low for a sufficiently long period

to promote after-ripening of the seed, (2) the seed bed must be kept moist, and (3) the seed must be protected against rodents and against danger of being washed off. Warm winters are rather common in Oklahoma and are probably the cause of frequent failures to secure a good stand of seedlings from fall-planted seed.

The seed should be planted about one-half inch deep and preferably in rows five or six inches apart. Broadcasting the seed is easier but the distribution of seedlings in the seed bed is seldom uniform. It takes more time and labor to care for ununiformly spaced seedlings than for those growing in evenly spaced rows. Spacing of seed depends on the potential productivity of seed, on the purpose for which the plants are grown, and on the care the plants are going to receive. Productivity of any lot of seed depends upon (1) the viability of seed and (2) on the germinative energy of the lot. Unless measures are taken to prevent or reduce damping-off, one should consider mortality from this source in spacing seeds in the seed bed.

If the preceding recommendations on the handling of red cedar seed are followed, there should be no reason why a good stand of seedlings cannot be grown every year.

Raising seedlings of Juniperus scopulorum from seed presents a much more difficult problem than does the seed of red cedar. Juniperus scopulorum seed planted during the first spring after collection does not germinate until the second spring. Seed should be purchased or collected, cleaned and planted in the nursery bed as soon as they become available. The method of cleaning the seed is the same as suggested for the seed of red cedar. The seed must be well protected against rodents because they must remain in the seed bed for over a year before they will germinate. If properly handled, a good stand of seedlings can be secured and by

planting seed every year a continuous supply of seedlings can thus be assured. Preliminary work has shown that there is a possibility of developing a method by which germination of Juniperus scopulorum seed can be forced during the first spring after collection. Treatment of seed with concentrated sulfuric acid followed by stratification proved to be successful in after-ripening some seed in a course of four months. This problem deserves further investigation.

SUMMARY

1. The cause of delayed germination in seed of Juniperus virginiana is a dormant embryo. In seed of Juniperus scopulorum delayed germination is also due to the dormancy of the embryo. However the seed coat may also be responsible for it to some extent.

2. Red cedar seed requires stratification at low temperature to complete after-ripening. Individual seeds and different lots of seed vary in the length of period required to complete the process of after-ripening. Seed used in this phase of the investigation were completely after-ripened after 70 days at a temperature of 41° F.

Complete after-ripening of seed of Juniperus scopulorum was secured only after twelve months of stratification at a combination of high and low temperatures. Attempts to shorten the period of after-ripening to five or six months have proved ineffective.

3. Treatment of dormant seed of Juniperus virginiana with pure oxygen for 24 hours or with a solution of vitamin B_1 for the same period not only failed to hasten the after-ripening but actually slowed down this process.

4. Treatment of dry (dormant) seed of Juniperus scopulorum with concentrated sulfuric acid for thirty minutes, followed by stratification, was effective in reducing the length of the after-ripening period. Thirty-eight per cent of seeds thus treated germinated after four months of stratification while untreated seed normally requires 12 months of similar stratification.

5. Drying of after-ripened seed of red cedar generally reduced the germination and caused reversion to secondary dormancy. The period of stratification at low temperature required to overcome the secondary

dormancy was much shorter than the original stratification period. Seed dried for 28 and 35 days and set in germinators at 41° F. germinated in fifty and forty-seven days respectively.

6. Freezing of after-ripened seed of red cedar for four weeks did not reduce germinability to any appreciable extent.

7. Temperature had a marked effect on germination of after-ripened seed of both species of juniper. The optimum germination temperature for red cedar seed was 50° F. However at 60° and 70° F. germination per cent was only slightly lower than at 50° F. The rate of germination was directly correlated with the temperature within the range of 40° to 70° F. but above 70° F. germination was severely retarded. Temperatures of 80° F. and higher affected unfavorably the extent and the rate of germination. A temperature of 100° F. was almost prohibitive to germination of seed and growth of seedlings.

Germination of seed of Juniperus scopulorum proceeded most rapidly at 90° F. However at this temperature seedlings failed to grow normally. A temperature of 70° F. favored germination of seed as well as growth of seedlings.

8. Germination of red cedar seed was not affected markedly by the reaction of the medium when pH of the latter varied between 4.4 and 8.4. The highest germination was obtained at a pH of 6.2.

The optimum reaction of the medium for seed of Juniperus scopulorum was pH of 5.91. Neutral and basic reactions of the media reduced germination markedly.

9. Soaking after-ripened seed of red cedar in a solution of vitamin B_1 for 24 hours had no effect upon germination.

10. Subjecting after-ripened seed of Juniperus scopulorum to increased oxygen pressure (100 per cent) for 48 hours not only failed to

help germination but actually lowered it. Increased oxygen pressure failed also to increase germination of after-ripened seed of Juniperus virginiana. Reduction of oxygen pressure to 5 or 10 per cent for 24 hours immediately preceding germination test markedly lowered the germination.

11. One year of storage of cleaned red cedar seed at low temperature and of dry berries at room temperature did not reduce viability.

12. Practical suggestions for raising seedlings of Juniperus virginiana and J. scopulorum, based on the results of this investigation are offered.

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