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Studies on the Role of the Seed Coat in the Germination of Rhodotypos Kerrioides

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STUDIES ON THE ROLE OF THE SEED COAT IN THE GERMINATION OF RHODOTYPOS KERRIOIDES

by
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INTRODUCTION

The propagation of plants by seeds is often made difficult by dormancy in seeds. A dormant seed is one that will not germinate when it is exposed to conditions favorable for germination. Such seeds will begin growth, however, after they have been subjected to the proper after-ripening conditions. Therefore, the grower must wait several months in many cases before his seeds will germinate. Since it is often desirable to determine the viability of seeds at an early date, seed dormancy has presented a major problem to the farmer. The testing of dormant seeds (4,6,44,56,64,) is widely practiced and rules for seed testing have been formulated (55).

Crocker (21) listed the following factors as causes of seed dormancy: (1) a rudimentary embryo; (2) inhibition of water absorption by the seed coat; (3) mechanical resistance of enclosing structures; (4) interference with oxygen and carbon dioxide diffusion by the seed coat; (5) dormancy of the embryo or part of it; (6) a combination of the above factors; and (7) secondary dormancy. Other investigators have added to this list inhibiting substances in the seed coat and in the embryo (1,2,11,14, 18,36,50,51,57).

A great many seeds may be after-ripened by a low temperature treatment (22,23,33,34,40,45). The length of

treatment may vary with the amount of incident light for seeds that are light-sensitive (12,37,42,43,63,66,71,72), although the effect of light on other seeds is not temperature-dependent (3,29,62,67). The red portion of the solar spectrum has the greatest effect on germination (3,12,75,76). Studies indicate that light initiates or inhibits germination by affecting compounds in the seed (62,67). Still other seeds will germinate only after being stored for various periods at a relatively high temperature (58,68).

Many of the chemical changes that take place during the after-ripening period (30,34,60,72,73) are reversible and secondary dormancy can occur if seeds are subjected to unfavorable conditions (10,15,21,27,38,63).

The studies reported here concern the effect of the seed coat on the germination of Rhodotypos kerrioides (jet bead).

HISTORICAL

The seed coat may be: (1) impermeable to oxygen or carbon dioxide (20); (2) impermeable to water (21,49); (3) offer mechanical resistance to the expanding embryo (24); or, (4) contain inhibiting compounds (11,51). It is possible that a combination of these factors may be expressed. Removal of the seed coat from the embryo brings about germination of a great many dormant seeds (22,28,31,33,34,35,44,54). Inhibition of germination by the seed coat may be determined by the genotype of the seed (61,66,77).

One of the earliest studies on the effect of the seed coat on germination was made by Crocker (22) in 1906. Crocker found that cocklebur seeds would take up from 1.6 to 2.4 times as much oxygen with the coats removed as they would when the coats were left intact. The oxygen was found to diffuse faster through dry seeds than it would through seeds soaked in water. Freshly gathered cocklebur seeds failed to germinate, but 100 percent germination occurred when the seed coats were removed.

Since the embryo is enclosed by seed coats, and perhaps by other structures, it does not have ready access to oxygen. The dormancy of some seeds, such as wild oats and other cereals, can be broken by increased oxygen concentra-

tion (18,48). The germination of Prosopis stephaniana, a woody shrub, can be brought about by a sulphuric acid treatment which probably increases the rate of oxygen exchange (49). The seed coat has been shown to limit the oxygen supply to Tilia americana (American basswood) embryos (70). Avena fatua (oats) germinates with less delay if the seed coats are removed (5). When the seeds of Medicago sativa (lucerne, alfalfa) are subjected to increased oxygen pressure, germination increases; short periods of high oxygen pressure seem to be more effective in breaking the dormancy of these seeds than long periods of low oxygen pressure (26).

The impermeability of the seed coat to carbon dioxide may also be a factor in dormancy, and treatment with carbon dioxide will break the dormancy of some seeds (7,59).

Crocker (19) suggested in his study in 1907 that failure of some seeds to germinate was due to effects of the seed coat. His study (25) in 1918 shows that the embryo itself may sometimes be dormant. Seeds with coats that inhibit water absorption may remain viable for many years (19). As an example, seeds of Nelumbo (sacred bean) may germinate after 200 years of damp storage (20).

The seed coats of many species are made permeable to water by the action of sulphuric acid (18,49,69) or by heating (20,47). Water enters through the hilar fissure or eroded pits of the testa of treated seeds (16). Khudairi (49) demonstrated that the seeds of Prosopis

stephaniana (a woody xerophytic shrub of Iraq) are dormant when collected because of a hard seed coat. A sulphuric acid treatment cracks the seed coat making water absorption possible. Spaeth (69) showed that the impermeability of the testa of Tilia (basswood) seeds increases with dry air storage. Permeability of these seeds increases during stratification, but water resistance may persist for several years. The dormancy of Medicago sativa can be broken by freezing in liquid air. The seed coat is probably cracked in the process, allowing greater penetration by water (16).

Inhibition of germination by the seed coat may be determined by the genotype of the seed, but meteorological conditions also influence the nature of the coat (3,9,53). White (77) showed that only 2 percent of the seeds produced by a single Melilotus alba (white sweet clover) plant were of the same hardness as the parent. He produced hard and soft varieties by selective breeding.

Certain seed coats may restrict mechanically the expanding embryo making germination impossible. Crocker and Davis (24) have shown that Alisma plantago (water plantain) seeds are able to remain in water for years without germinating because of mechanical restraint of the achenes.

Many compounds have been discovered that stimulate seeds to germinate (8,53). The dormancy of Amygdalus persica (Lovell peach) seeds can be broken by treatment with thiourea solutions (74). These seeds germinate only

if the stony pericarp and the inner seed coats are removed, and they develop into dwarf seedlings. Peach seeds with only the pericarp removed will not germinate unless they are stored at a temperature of five degrees centigrade for about three months in a moist medium. Treatment with thiourea will bring about germination with the seed coats intact. Gray (41) shows that dormant peach seeds will germinate if they are treated with gibberellins. Lawns treated with 100 ppm of gibberellins for a period of two weeks will show a fifty percent increase in Digitaria sanguinalis (crab grass) germination. This could be an aid in irradicating the weed. Gibberellin will also improve the germination of the noxious weed Fagopyrum tatarical (tartary buckwheat) (9) and Douglas-fir (66). The seeds of Pennisetum setosum, a Hawaiian range grass, can be stimulated to increased rate of germination by a treatment of thiocyanate, potassium or potassium nitrate. Potassium nitrate breaks dormancy to some extent in Agropyron smithii (western wheatgrass), but it will not overcome the adverse effect of light. If these seeds are soaked in an ethylene cholorphydrin solution, increased germination will result regardless of light conditions (29). Two ways of breaking the dormancy of Trifolium (subterranean clover) seeds have been presented by Ballard (7). Imbibed seeds are treated with low concentrations of carbon dioxide or with activated carbons. The activated carbons apparently do not absorb inhibitors, but they produce carbon dioxide which initiates

germination. Other legumes also are stimulated by carbon dioxide treatment (59).

Some substances have been discovered in the seed coat and in berry juice that stimulate germination. The washed seeds of Fragaria fragrans (strawberry) will not germinate. If the berry juice is added to the seeds 90 percent germination results. Many seeds, such as Muntrugia calabura (a berry of China), are inhibited by their fruit sap (39).

Many compounds have been discovered in the seed coat and/or the embryo that will inhibit germination (46,52). Kockemann (51) found that the compounds that inhibit germination are: ammonia, hydrocyanic acid, essential oils, alkaloids, glycosides and an unknown compound called "blastokilin." According to Barton and Solt (11) there are three groups of chemical inhibitors in seeds (1) essential oils, (2) alkaloids, and (3) glucosides. Free ammonia is also inhibitory but can be washed off easily with water. Many of these compounds that inhibit germination diffuse away in soil (14).

Cox (18) reported that there is an inhibitor in the seed coats of certain varieties of Brassica (cabbage). This inhibitor becomes inactive when the seeds are soaked for one minute in sulphuric acid and washed with water (18). An inhibiting substance is present in the scutellum and endosperm of Zea mays (corn) that retards growth of coleoptile cylinders (36). Borriss (13) noted that when Vaccaria pyramidata (cow-herb) seeds were planted in soil they grew

well, but they failed to germinate on blotting paper. He believes that an inhibiting compound in the seed coat is absorbed by the soil. Coumarin was found to be the most active inhibitor in Trigonella arabica (a legume) and Zygophyllm dumonum (a member of the Caltrop family). Lerner (57) showed that much of the inhibition caused by extracts of these seeds is a result of osmotic effects which are accentuated by the other soluble inhibitor. A sterilization treatment with calcium hypochlorite almost completely inhibits the germination of Magnolia acuminata (magnolia). Akamine (2) showed that poor germination of Pennisetum ciliare, a range grass, is due to an inhibitor in the hull as well as to impermeability of the seed coat. This inhibiting compound is thermostable but is made inactive by sulphuric acid. Knowles (50) found that dormancy of Viburnum trilobum (arrow-wood) is caused by a water-soluble inhibitor. Two inhibitors are present in the hulls of Avena fatua which do not affect exchange of gases. Treatment with carbon dioxide reduces the inhibitor while lack of oxygen brings about a marked increase of the inhibitors (13).

The dormant seeds of Rhodotypos kerrioides require an after-ripening period of about three months at five degrees centigrade. Better germination results if the temperature is alternated between one degree and ten degrees centigrade daily or weekly during the after-ripening period. A higher percentage of germination will take place if the seeds

are mixed in moist peat moss and held at 25 to 30 degrees centigrade for one month before the after-ripening period begins. The best production of seedlings results when the seeds are planted in flats and placed in board-covered cold frames. If partially after-ripened seeds are placed in a situation unfavorable for after-ripening, a second dormancy develops and the seeds then require a second after-ripening period. Both the embryo and the seed coat are involved in dormancy (34).

Analysis during the after-ripening period shows an increase in catalase, peroxidase, and lipase activity. Water absorbing power increases as well as titrable acid and sucrose, but the ether-soluble fraction decreases (34).

When the dormant seeds of Rhodotypos are excised, the seeds will germinate but may fail to grow. Seeds that grow may develop into "physiological dwarfs" (31,32,34). Failure of growth following germination of dormant seeds may be overcome by removal of the cotyledons (35,50). Seeds treated in this way will not develop into "physiological dwarfs" as do embryos with the cotyledons intact.

MATERIALS AND METHODS

Five lots of Rhodotypos kerrioides seeds were used in this investigation during the fall, winter and spring of 1959 and 1960.

Lot 1 (1 year seeds) seemingly produced in the summer of 1959, was collected from the shrubs the following fall (1959).

Lot 2 (2 year seeds), seemingly produced in the summer of 1958, and which remained on the shrubs throughout the winter of 1958-1959, was collected in the spring of 1959. These seeds were stored in open containers at room temperature during the summer of 1959. According to a study made by Flemion (31), storage under these conditions will induce dormancy in R. kerrioides seeds.

Lot 3 (refrigerated seeds) was taken from lot 2 and stored in the cold for approximately 4 months.

Lot 4 (shrub seeds), seemingly produced in the summer of 1959, and which remained on the shrubs during the following winter, was collected in the spring of 1960.

Lot 5 (ground seeds), seemingly produced in the summer of 1959, then fell and remained on the ground throughout the following winter, was collected in the spring of 1960.

Certified Avena (oat) seeds were also employed in

these experiments and were supplied by the Biology Department of Western Michigan University.

R. kerrioides has a drupe-type seed which consists of a thin exocarp and a hard, stony endocarp similar to that of plums, cherries and peaches. The embryo, with cotyledons, is surrounded by a seed coat referred to by Flemion (31) as the inner seed coat. The inner seed coat has a fleshy, white cuticle which forms a milky paste when macerated in a small amount of water. The drupe is 7 mm long and averages 9.91 gm per hundred; weight will vary depending on the conditions under which the seeds were stored. The radicle and hypocotyl average 2.5 mm in length and the cotyledons average 5 mm in length.

The experimental procedure involved making germination tests on the seeds in six-inch or three-inch petri dishes. Two filter papers were placed in each dish. Fifteen ml of distilled water and/or the extract of macerated seed coats were used in each six-inch dish, and 5 ml of the same were used in each three-inch dish. Because of the slow elongation of R. kerrioides embryos, germination was considered to have taken place when the combined length of the hypocotyl and radicle exceeded 3 mm. Germination counts generally were made at four, six and eight days. Tests for germination were made at room temperature and in darkness.

Seed coat extract was prepared from lots of 100 seeds. The thin black exocarp first was removed, macerated in 15 ml of distilled water, and strained. An endocarp extract

was prepared in a similar manner. The hard endocarp of the seeds was removed by cracking with pliers and splitting the two halves, taking care not to damage the seeds.

Because of the difficulty encountered in separating the inner seed coat from the embryo of dry seeds, the seeds were first soaked in distilled water for 24 hours. A small incision with a razor blade, parallel to and between the cotyledons and opposite the hilum, made possible the separation of the two halves of the inner seed coat, thus freeing the embryo. The inner seed coats were also macerated in 15 ml of distilled water, and the extract was used in germination and elongation tests.

In order to determine the inhibiting nature of the inner seed coat on germination it was necessary to consider several possibilities: the permeability of the inner seed coat to water; the effect of high oxygen pressure; the effect of light; and finally, the effect of various inner seed coat extracts.

EXPERIMENTAL

Permeability of the Inner Seed Coat to Water

Measurements of water uptake were made on R. *kerrioides* seeds in order to determine the permeability of the inner seed coat. The exocarp and endocarp were first removed leaving only the inner seed coat on the embryo. Table 1 shows the results of weighing seeds before and after a 24 hour soaking period. Ground seeds (lot 5) weighed more before soaking and took up less water than any of the other seeds tested. Shrub seeds (lot 4) took up more water than the ground seeds. Two year seeds (lot 2) and refrigerated seeds (lot 3) took up the most water and the one year seeds (lot 1) took up slightly less. This indicates that permeability of the seed coat increases as the period of storage increases. This also was shown by Flemion (34).

Although shrub seeds (lot 4) absorbed water readily, they failed to germinate. Lots 1, 2, and 3 also absorbed water readily but the germination rate was only 4 percent. The ground seeds (lot 5), which were moist when collected, took up less water but germinated well. Since all inner seed coats permitted the passage of water it seems that impermeability of the inner seed coat is not a factor in the germination of R. *kerrioides*.

Table 1. Water uptake in seeds of *R. kerrioides*: lot 1, 1 year dry stored seeds; lot 2, 2 year dry stored seeds; lot 3, 1 year refrigerated seeds; lot 4, shrub seeds; lot 5, ground seeds.

	Dry weight of 100 seeds	Weight after 24 hr in H_2O	Water uptake
Lot 1	2.44 gm	3.70 gm	1.26 gm
Lot 2	2.26	3.60	1.34
Lot 3	2.25	3.58	1.33
Lot 4	2.65	3.88	1.23
Lot 5	3.35	4.10	.75

The Effect of Light on the Germination of R. kerrioides

It was found that while radicles and hypocotyls elongated faster in darkness, light had no discernible effect on germination. The germination rate was only 4 percent for seeds held in darkness and seeds held in light, while that of the controls was 79 percent.

The Effect on Germination of Incising the Inner Seed Coat

A series of experiments was performed to determine whether or not incising the inner seed coat would affect germination. If the inner seed coat were impermeable to air incising the coat should result in a higher rate of germination. Excised shrub seeds (lot 5) were used for the control, but excised seeds of any lot could have been used. Other tests showed that excised seeds of all lots germinated at about 70 percent.

The percent of germination of the control was much greater than the experimentals but cutting the inner seed coat did result in some increased germination (see table 2). Ground seeds (lot 5), however, germinated well with the coats intact and incising the coats increased germination only slightly. Even the cut inner seed coats inhibited germination of these ground seeds. When the coats were completely removed germination was increased by 20 percent. This, however, is not sufficient evidence to show that the inner seed coats of seeds that were on the ground all winter

Table 2. The effect of cutting the inner seed coat upon the germination of R. kerrioides seeds.

Days	Control	(lot 1) 1 year dry seed		(lot 2) 2 year dry seed		(lot 3) 1 year refrig.		(lot 4) Shrub seed		(lot 5) Ground seed	
		Incised	Intact	Incised	Intact	Incised	Intact	Incised	Intact	Incised	Intact
4	25%	4%	0%	20%	3%	8%	0%	12%	0%	16%	16%
6	80%	8%	2%	32%	6%	8%	4%	12%	0%	20%	16%
12	88%	12%	2%	40%	12%	16%	4%	48%	0%	48%	40%
14	88%	12%	3%	48%	12%	16%	4%	48%	0%	60%	48%

have an inhibiting effect on germination since some of these seeds may not have fallen from the shrubs until spring and, as a result, would not have been subjected to after-ripening conditions. In view of this it seems probable that the inner seed coat may have no inhibiting effect on seeds that are completely after-ripened. This should be investigated further.

The two year seeds (lot 2) with coats intact showed a higher percentage of germination than the 1 year dry (lot 1) or the 1 year refrigerated seeds (lot 3). Seemingly the inner seed coat and/or embryo undergoes some change during storage that modifies its inhibitory effect. Flemion (34) points out that many changes take place in these seeds during prolonged storage that modifies the germinating characteristics.

None of the shrub seeds (lot 4) germinated unless the inner seed coats were incised. Further experiments revealed that extract prepared from the coats of this lot had very little effect on germination. It seems, then, that mechanical restraint and a non-after-ripened embryo may have been the limiting factors in the germination of these seeds. None of these seeds cracked open as a result of water uptake, while two year seeds (lot 2) cracked open readily following soaking.

These experiments showed that the inner seed coat had an inhibiting effect on germination in all cases and this inhibiting effect was not limited to the lack of water

or to the lack of air.

The Effect of Oxygen on the Germination of R. kerrioides

An attempt was made to determine the oxygen permeability of the inner seed coat. Seeds with either incised or intact inner seed coats were placed in either pure oxygen or in air. Seeds placed in pure oxygen show no greater germination than those placed in air (see table 3). Increased oxygen pressure did not cause a subsequent increase in germination in either seeds with incised coats or in seeds with intact coats. In the light of these experiments it seems that oxygen permeability is not a major factor in the germination of these seeds.

The Effect of Exocarp, Endocarp and Inner Seed Coat Extracts upon Germination of Excised R. kerrioides Seeds

Extracts prepared from the exocarp, endocarp or inner seed coats of R. kerrioides seeds were tested to determine their effects on germination. The exocarp and endocarp were first removed from 100 seeds. After the seeds were soaked in distilled water for 24 hours the inner seed coats were removed. Extracts were prepared by macerating each of the above in 5 ml of distilled water. After maceration the mixture was allowed to settle for two minutes and the supernatant was decanted into a graduated cylinder. An additional 10 ml of distilled water was mixed with the pulp and the supernatant was again decanted. Experimentals were placed

Table 3. The effect of oxygen upon germination of R. kerrioides seeds.

	Shrub seeds			Ground seeds		
Days	Control* in air	Incised seeds in oxygen	Intact seeds in oxygen	Control in air	Incised seeds in oxygen	Intact seeds in oxygen
4	12%	12%	0%	16%	18%	16%
6	12%	16%	0%	20%	25%	20%
12	48%	45%	0%	48%	50%	42%
14	48%	45%	0%	60%	62%	48%

* Controls are incised.

in 6 inch petri dishes, 20 seeds per dish in 15 ml of extract. Controls were placed in distilled water.

The results of the tests, shown in Table 4, indicate that there is no appreciable difference between the rate of germination of controls and of those seeds placed in exocarp or endocarp extracts. However, of the seeds planted in 15 ml of concentrated inner seed coat extract none germinated.

The Effect of Various Inner Seed Coat Extracts of R. kerrioides on Germination

The Effect of Various Concentrations of 1 year Inner Seed Coat Extract

Figure 1 shows the results of tests made to determine the effect of different concentrations of inner seed coat extract on germination of R. kerrioides. It was observed that while fifteen ml of concentrated inner seed coat extract from one year dry stored seeds (lot 1) completely inhibited germination, concentrations of less than 5 ml of extract diluted in 10 ml of water did not suppress germination. When 5 ml of extract was diluted in 10 ml of distilled water and used in tests there was a sharp reduction in germination. Concentrations greater than this resulted in almost no germination and seeds that did germinate in these concentrations died within a few days and emitted an odor of ammonia. Since it seemed possible that

Table 4. The germination of excised seeds of *R. kerrioides* in undiluted extracts of exocarp, endocarp and inner seed coats.*

	Control	Exocarp extract	Endocarp extract	Inner seed coat extract
Germination	75%	65%	75%	0%

* All seed coats and seeds are from lot 1.

Figure 1. The effect of the inner seed coat extract from 1 year dry stored seeds (lot 1) on the germination of 1 year dry stored seeds.

a - 15 ml of distilled water, control

b - 2 ml of extract diluted in 13 ml of distilled water

c - 4 ml of extract diluted in 11 ml of distilled water

d - 5 ml of extract diluted in 10 ml of distilled water

e - 8 ml of extract diluted in 7 ml of distilled water

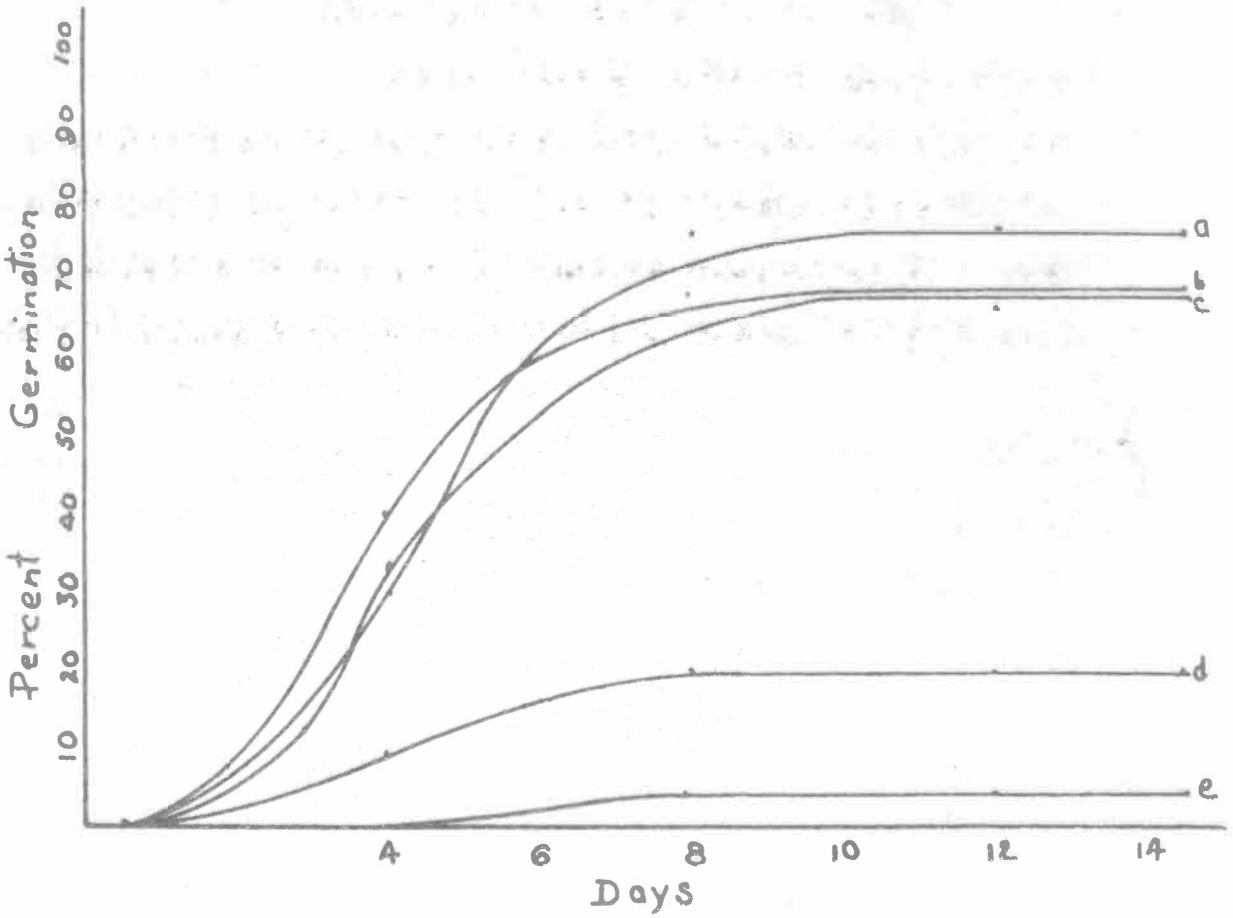
Percent Germination
10
20
30
40
50
60
70
80
90
100
4
6
8
10
12
14
Days
a
b
c
d
e

Figure 1.

bacteria or enzymatic action might be responsible for inhibiting germination of seeds in inner seed coat extract, tests were made using autoclaved extract and sterile dishes. Although the results of the tests varied somewhat, there was no apparent difference between the germinating characteristics of seeds placed in the autoclaved extract or in the non-autoclaved extract. Figure 2 shows that a 5 - 10 solution (5 ml of inner seed coat extract diluted in 10 ml of distilled water) of autoclaved extract caused a sharp reduction in germination and greater concentrations resulted in complete inhibition of germination. Similar results were obtained with non-autoclaved extract and with extracts that had been centrifuged.

Figure 2 shows that seeds placed in a 2 - 13 solution had a higher percentage of germination than did the control. Other experiments, not shown here, indicated that seeds might possibly be stimulated by weak extract concentrations, whether the extract had been autoclaved or not. Additional work in this area would be of interest.

An interesting similarity was noted in excised seeds growing in inner seed coat extract and seeds growing with the inner seed coats incised. If seeds with incised coats were held in darkness in distilled water, the portion of the cotyledons that extended from the slit in the seed coat turned yellow, indicating the presence of protochlorophyll, but the portions covered by the seed coat remained white. Elongation of this protruding portion of the cotyledon was

Figure 2. The effect of autoclaved inner seed coat extract from 1 year dry stored seeds (lot 1) on the germination of 1 year seeds of B. kerrioides.

a - 15 ml of distilled water, control

b - 2 ml of extract diluted in 13 ml of distilled water

c - 5 ml of extract diluted in 10 ml of distilled water

d - 8 ml of extract diluted in 7 ml of distilled water

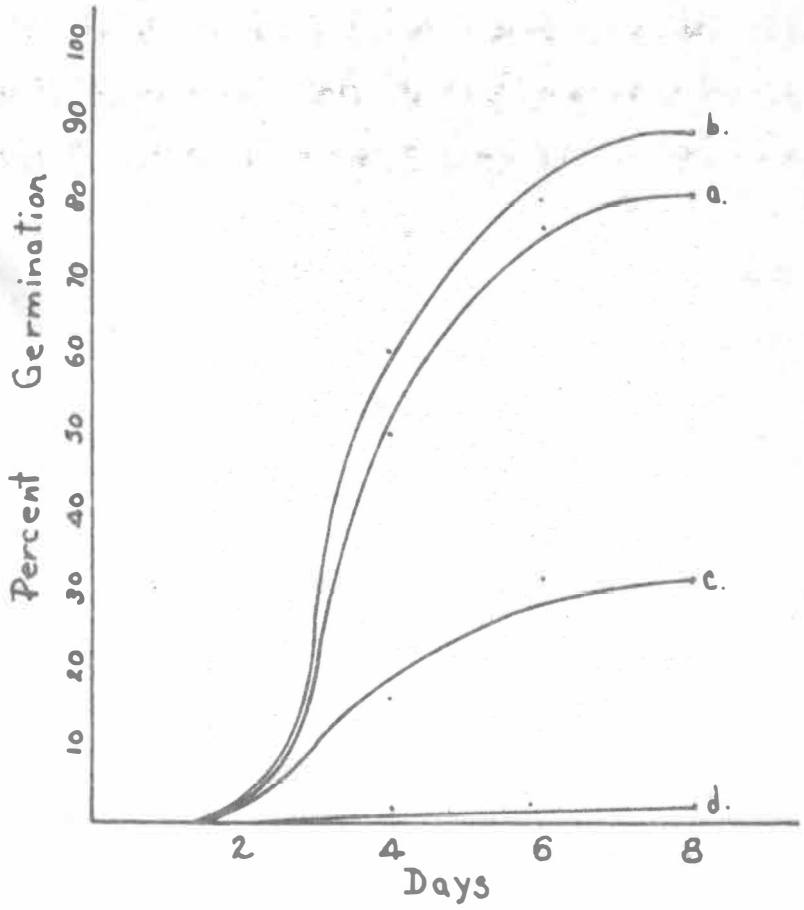
Percent Germination
10
20
30
40
50
60
70
80
90
100
2
4
6
8
Days
b.
a.
c.
d.

Figure 2.

more rapid than the portion which was covered by the seed coat. In many cases the part covered by the coat failed to make any growth. The cotyledons of excised seeds growing in inner seed coat extract also remained white and made little if any growth if they were in contact with the soaked filter paper even if the extract concentration was weak. The cotyledons that extended into the air, on the other hand, turned yellow and made rapid growth. When seeds were placed on filter paper in distilled water the cotyledons in contact with the paper tended to become more yellow than the cotyledons that extended into the air. This is the opposite result that occurred in inner seed coat extract of any of the non-after-ripened seeds tested.

The young seedlings from non-chilled R. kerrioides seeds did not appear to respond phototropically or geotropically. Cotyledons often tended to grow downward against the filter paper while the roots extended into the air. When the seedlings were turned so the cotyledons extended upward and the roots downward they often reversed themselves in a few days. As the roots elongated they became convoluted and showed no apparent geotropic response. The plumule always showed a positive phototropic response, however, regardless of the position of the cotyledons. It is not known if seedlings from non-chilled seeds exhibit the same growth habits when planted in soil.

The Effect of 2 Year Extract

Extract prepared from the inner seed coats of 2 year seeds (lot 2) caused less inhibition of germination (see Table 5) than the extract from 1 year seeds (lot 1). In similar concentrations of 1 year extract no germination took place. Seeds placed in 15 ml of undiluted extract of either 2 year (lot 2) or 1 year (lot 1) inner seed coats failed to germinate. The cotyledons of these seeds in contact with the filter paper failed to enlarge, and they did not turn yellow in darkness as did cotyledons that were not in contact with the soaked paper.

The Effect of Extract from Refrigerated Seeds

The extract from the inner seed coats of seeds that were held at a temperature of 0 to 5 degrees centigrade for a 4 month period inhibited germination less than extract from 1 or 2 year seeds (lots 1 and 2). Table 6 shows that both 1 year dry stored seeds and refrigerated seeds will germinate in the undiluted extract of refrigerated seeds. When these seeds are placed in a 5 - 10 concentration of this extract no inhibition was noted. Similar concentrations of 1 year seed extract (see Table 5) greatly inhibited germination. Although the extract from refrigerated seeds had little effect on germination, Table 2 indicates these seeds were not after-ripened since only a small percent germinated unless the inner seed coats were

Table 5. The effect of extracts from inner seed coats of one and two year dry stored seeds on germination of excised seeds of R. kerrioides.

		2 year inner seed coat extract		1 year inner seed coat extract	
Seeds	Control	8 - 7 sol*	undiluted	8 - 7 sol	undiluted
lot 1	80%	62%	0%	0%	0%
lot 2	52%	48%	0%	0%	0%

* 8 ml of extract diluted in 7 ml of distilled water

Table 6. The effect of inner seed coat extract from one year R. kerrioides seeds that had been refrigerated dry for four months on the germination of R. kerrioides seeds.

	Control 15 ml distilled water	15 ml inner seed coat extract from lot 1 seeds	5-10 sol from 1 year lot 1 seeds	5-10 sol from refrig. lot 3 seeds	15 ml extract from 1 year refrig. lot 3 seeds
Germination of lot 1 seeds	76%	0%	22%	68%	40%
Germination of lot 3 seeds	72%	0%	32%	64%	36%

removed. Flemion (34) shows that R. kerricides must be stored in a cold and moist situation before after-ripening occurs.

Although holding R. kerricides seeds in the cold modified the inner seed coat, cold did not have the same effect on extract that was refrigerated after it was obtained from non-chilled seeds; such extracts greatly inhibit germination. In addition, this inhibition was demonstrated in Avena (oats) and Cucumis (cucumber) (see Figure 3).

The Effect of Extracts on Shrub Seeds and Ground Seeds

The drupe-type seeds of R. kerricides are anchored securely to the receptacle and some seeds remain on the shrubs all winter. Others will drop after leaf fall, however, and will be found on the ground the following spring. Those seeds that remain on the ground during the winter were found to be after-ripened in that they germinated with the inner seed coats intact and subsequent development of normal seedlings followed (see Table 2). As previously reported, seeds were collected from the shrubs as well as from the ground and used in tests to determine the effect of the inner seed coat extracts on germination.

Three inch petri dishes were used to determine the effects of extracts from ground and shrub inner seed coats. Extracts were prepared as previously explained. Preliminary tests showed that weak concentrations of extracts from shrub and ground seeds had no observable effect on germination.

Figure 3. The effect of undiluted inner seed coat extract on the germination of oat seeds.

a - 5 ml distilled water, control

b - Undiluted extract of shrub seeds

c - Undiluted extract of refrigerated seeds

d - Undiluted extract of ground seeds

e - Undiluted 2 year seed extract

f - Undiluted refrigerated extract

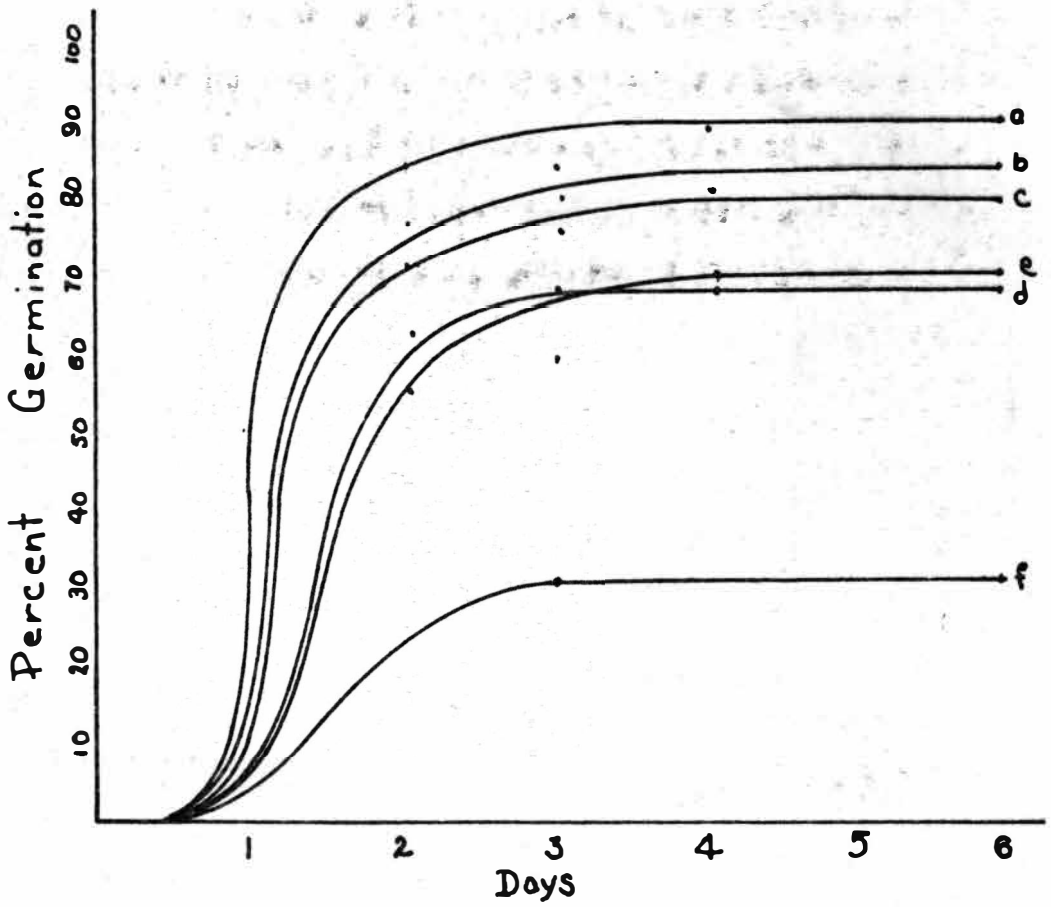
Percent Germination
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
Days
a
b
c
e
d
f

Figure 3.

These tests were made with undiluted extracts. Results are shown in Table 7. Excised shrub seeds (in fact all excised seeds) germinated almost equally well in either distilled water or the undiluted extract from the inner seed coats of ground seeds. Elongation of the seedlings, however, was greater in the distilled water.

Table 7 shows that excised ground seeds did best in distilled water. Germination here was 90 percent. Excised ground seeds in undiluted extract from ground seed coats showed 76 percent germination. No germination of ground seeds took place in the extracts from lots 1 and 2.

The Effect of Various Inner Seed Coat Extracts on the Germination and Elongation of Oats

The tests to determine the effect of the inner seed coat extract on the germination and elongation of oats were made with undiluted extracts since preliminary tests showed weaker concentrations had little effect. The oat seeds were placed in 3 inch petri dishes on 2 sheets of filter paper soaked with 5 ml of various inner seed coat extracts. The results of the tests show that the germination of oats was inhibited by inner seed coat extracts. Test seedlings elongated at a much slower rate than control seedlings, but showed no differences in appearances other than length. The total elongation of controls was 2186 mm in 6 days while that of experimentals was only 147 mm. None of the test seedlings died and there was no browning

Table 7. The effect of undiluted extracts from inner seed coats of various lots of seeds on the germination of excised shrub and ground seeds of R. kerrioides.

	Control	Shrub extract	Ground extract	lot 1 extract	lot 2 extract
Germination lot 4 (shrub) seeds	68%	44%	60%	0%	0%
Germination lot 5 (ground) seeds	90%	68%	76%	0%	0%

of the root tips (see Figure 4).

Figure 3 shows that extract refrigerated at 0 to 5 degrees centigrade for 1 month produced the greatest inhibition of germination. Oat seeds that failed to germinate in the 6 test extracts employed in this experiment were taken from the petri dishes, washed and replanted in distilled water. No germination resulted in any case.

The extract from shrub seeds (lot 4) and ground seeds (lot 5) had less inhibitory effect than any of the other extracts tested. Refrigerated extract inhibited germination and elongation most.

Figure 4. The effect of undiluted inner seed coat extract on the elongation of roots of oat seedlings.

a - Control

b - Shrub extract (lot 4)

c - Ground extract (lot 5)

d - Refrigerated seed extract

e - 2 year extract (lot 1)

f - 1 year extract (lot 2)

g - Refrigerated extract

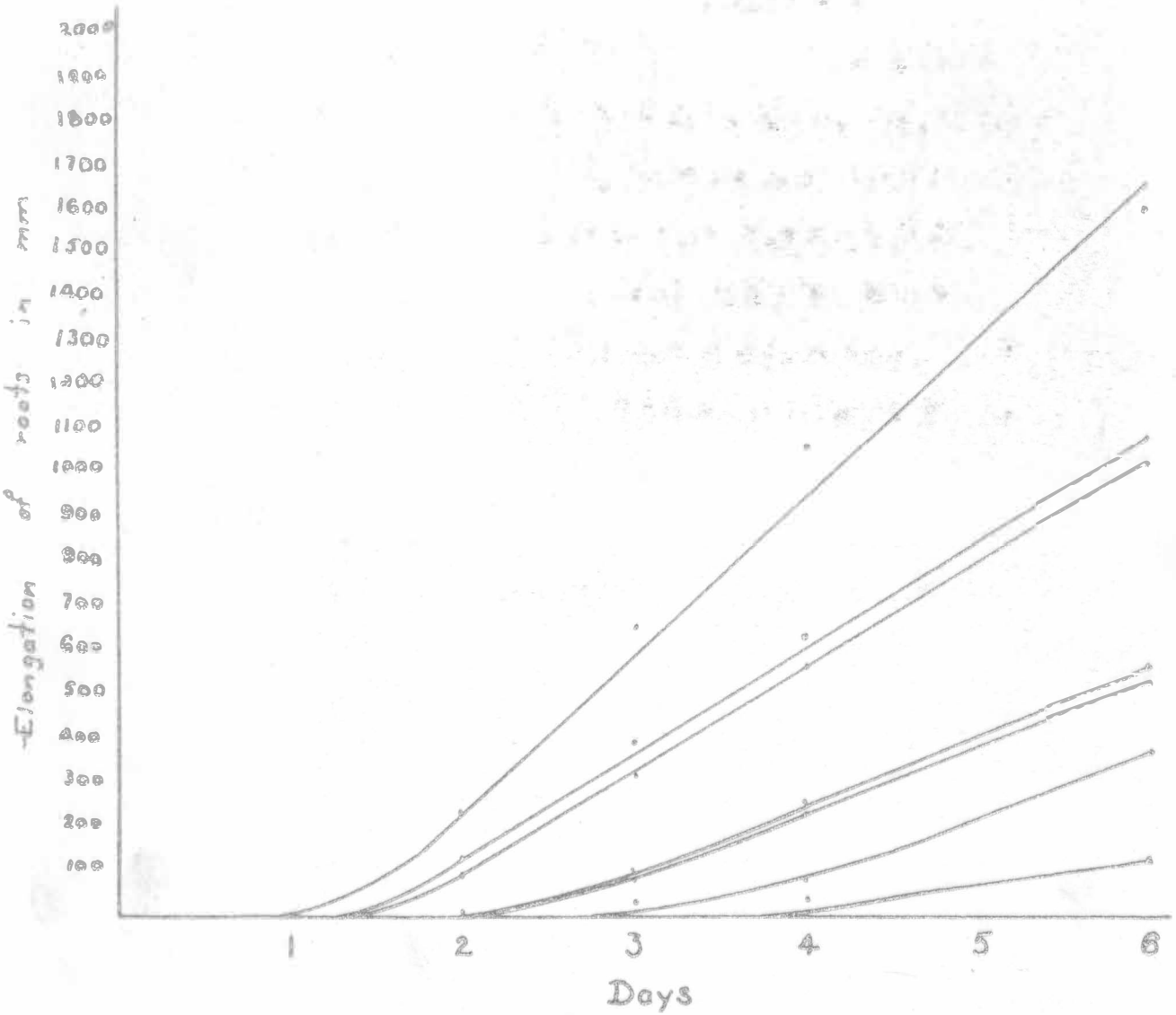
Elongation of roots in mm
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
1
2
3
4
5
6
Days

Figure 4.

DISCUSSION

Although work on germination is voluminous, little work has been done on the germination of Rhodotypos kerrioides seeds. Flemion (31,32,33,34) made several studies on the after-ripening and dormancy of R. kerrioides but no other work on the subject has been published. This study is an attempt to determine the effect of the inner seed coat on the germination of R. kerrioides, a problem that has not been previously investigated.

The dormancy of R. kerrioides can be broken by a 3 month wet-cold treatment. That the seed coat has something to do with this dormancy is evidenced by the fact that when the seed coat is removed the seeds will germinate, although they develop into "physiological dwarfs" (31).

Tests showed that the seed coat was not impermeable. Measurements of water uptake revealed that dormant seeds absorb water at a fairly rapid rate, but permeability may increase with age as shown by Flemion (34). Two year seeds took up water slightly faster than 1 year seeds.

Numerous workers have shown that the inability of oxygen to diffuse through the seed coat often limits germination. That this could be the case with R. kerrioides is suggested by the increased rate of germination when inner seed coats are completely removed. However, merely

cutting the seed coats causes no significant increase in rate of germination and it appears, therefore, that oxygen permeability is not a limiting factor in the germination of these seeds. Increased oxygen pressure causes no increase in rate of germination.

Extract prepared from macerated inner seed coats inhibit the germination of R. kerrioides, but if the seeds are subjected to cold storage before the coats are removed the inhibiting effect is much less. Cold treatment of the seeds seemed to be more effective in reducing the inhibiting effect of the inner seed coat extract if the seeds were stored under conditions of moisture.

Experimentation with germinating seeds suggested a relationship between chlorophyll formation and inner seed coat extract. When the excised seeds of R. kerrioides germinated in weak concentrations of non-chilled extract, the cotyledons in contact with the filter paper did not turn yellow in darkness as did the cotyledons that extended above the solution. When the excised seeds were placed in distilled water the cotyledons in contact with the filter paper turned deeper yellow than the cotyledons that extended into the air. Some seedlings in either medium developed white spots on one or both cotyledons that failed to enlarge as the cotyledons grew. Only the yellow portions of the cotyledons turned green in light indicating that the yellow pigment was prochlorophyll. Further investigation would be of interest.

The young seedlings from non-chilled R. kerrioides seeds did not appear to respond phototropically or geotropically. Roots became convoluted and often extended into the air while the cotyledons grew downward.

Since tests indicated that permeability or mechanical restraint did not appear to be limiting factors in the germination of R. kerrioides, the inner seed coats were macerated in an attempt to determine whether or not an inhibiting compound was present. When excised R. kerrioides seeds were planted in concentrated inner seed coat extract of one year non-chilled seeds, no germination took place. Excised seeds did germinate in weak concentrations, but the rate of germination and elongation was less than the control. The extract from seeds that were refrigerated at about 0 degrees centigrade for a 4 month period had much less inhibiting effect on germination. The extract from seeds that after-ripened on the ground had little, if any, effect on germination and elongation of R. kerrioides, Avena, and Cucumis. All of the seeds of this lot may not have been after-ripened, however, since some seeds probably fell from the shrubs shortly before the collection was made. Since the extract from ground seeds had so little effect on germination, it seems questionable whether the extract from 100 percent after-ripened seeds would have any effect on germination. This possibility needs to be investigated further.

When inner seed coat extract from non-chilled seeds

is refrigerated at 0 degrees centigrade for 1 month the inhibitory effect on germination increases. During cold storage the appearance of the extract changes from milky to clear. There appears to be no precipitate. The disappearance of this milky substance might be associated with the increased inhibiting effect of the extract.

It seems highly probable, in view of the above work, that a compound, or compounds, is present in non-chilled inner seed coats that inhibits germination. During cold treatment this compound may be degraded. That this action is reversible is evidenced by the fact that the undiluted extract of seeds gathered from the bushes in spring inhibits germination slightly, but when seeds gathered from bushes are dry stored for several months inhibition of undiluted extract is great.

SUMMARY

1. Impermeability of the inner seed coat to water or air did not appear to be a limiting factor in the germination of R. kerrioides.
2. Exposure of the inner seed coat to light did not cause increased germination in seeds of R. kerrioides.
3. Cutting the inner seed coat resulted in some increase in germination.
4. High oxygen pressure did not cause an increased rate of germination in either incised or intact R. kerrioides seeds.
5. Extracts prepared from the inner seed coats of non-chilled seeds of R. kerrioides caused inhibition of germination in seeds of R. kerrioides, Avena and Cucumis.
6. Extracts prepared from the inner seed coats of 2 year non-chilled seeds of R. kerrioides caused less inhibition of germination than did the extract from 1 year non-chilled seeds.
7. Refrigerating inner seed coat extract after it was obtained from non-chilled seeds resulted in increased inhibition.
8. Extracts prepared from the inner seed coats of R. kerrioides seeds that were refrigerated had little

effect on germination.

9. Extracts from the inner seed coats of seeds of R. kerricides that remained on the shrubs all winter caused little inhibition of germination, but if these seeds were stored for several months extracts of the inner seed coat caused great inhibition.
10. Extracts prepared from the inner seed coats of R. kerricides seeds that remained on the ground all winter had almost no effect on germination.

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