Factors in the Germination of *Aira flexuosa*

BY

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I. Introduction.

A considerable amount of attention has been paid to the factors affecting the germination of seeds. Such factors are light supply, the temperature at which the seeds are germinated, and the nature of the substrate or moisture supply. The state of the seed when offered the conditions necessary for germination is also important. The studies to be reported here deal with different combinations of all the factors involved in the germination of *Aira flexuosa*.

A review of all the literature bearing on germination is hardly possible here, but a brief survey of those papers believed to be significant in the present work is attempted.

1. Temperature.

Seeds of different species, and frequently seeds of the same species from different sources, vary considerably in their temperature requirements. Doerfel (1930) records that for certain species germinated at constant temperatures the best results are obtained at 0° C. and 5° C. For most species the optimum temperature is appreciably higher, being in general between 15° C. and 30° C. Edwards (1932) gives a comprehensive account of the maximum, minimum, and optimum germination temperatures of various species, so far as they are known. The optimum temperature for germination may be influenced by other factors. For instance, Harrington (1923*a*) and Munerati (1925) have found for wheat, and Atterberg (1928) for barley, that freshly harvested grain has a lower temperature optimum than grain which has been stored. On the other hand, Gassner (1930) reports for *Poa*, that although at constant temperatures germination is much better in after-ripened than in un-after-ripened seeds, the optimum temperature in both cases is 12° C. Ten species of *Labiatae* and *Cruciferae* have been found to have the same temperature optimum in light as in dark (Bihlmeier, 1929). It is quite possible, however, that this may not apply in all cases.

The seeds of a large number of species show considerably better germination in regular alternating temperatures than they do in

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constant temperature. Thus Harrington (1923*b*) states that redtop (*Agrostis* spp.) parsnip and some Petunia seeds give somewhat better, and celery, orchard grass, Kentucky blue-grass, Bermuda grass, and Johnson grass much better germination with certain alternating temperatures than with constant temperatures. On the other hand, there is no such increase of germination in the case of carrot, parsley, timothy, awnless brome grass, perennial and Italian ryegrass, and meadow fescue. The best results are obtained when the higher of the two temperatures is maintained for only a small part of the day, never more than 8 hours and generally less, and the change to the lower temperature is rapid. It is also shown that different lots of the same kind of seed vary in their temperature sensitiveness. This is probably the effect of differences in degree of after-ripening, or conditions of harvesting and storage. In the case of Johnson grass, Harrington observes that the best germinating lots of seeds are also the least exacting as to temperature conditions.

Gassner (1911) working with *Chloris ciliata* has also found alternating temperatures to be most beneficial when the higher temperature is applied for the shorter and the lower for the longer daily period. Indeed with the reverse arrangement germination of this species is little better than in constant temperatures. When, however, the glume is removed from the grain there is an increase in germination which is not further improved by conditions of intermittent temperature.

Morinaga (1926) has found, like Harrington, that some species show improved germination in alternating temperatures, while others germinate just as well at favourable constant temperatures. He also shows that the alternation most effective for one species is not necessarily the most effective for another. Alternation was found not to be effective if the daily duration of either the higher or the lower temperature was less than 4·5 to 8 hours. It may be noted in passing that other workers have obtained satisfactory results when one of the temperatures has been applied for 4 hours daily. Mechanical injury to the seed-coat or treatment with sulphuric acid produces good germination of *Cynodon dactylon* and *Typha latifolia* at constant temperature, while light and nitrate give increased germination of *Poa compressa* at constant temperature, although the highest percentage germination is only obtained when alternating temperature is applied in conjunction with light and nitrate.

In a recent paper on certain species of *Poa*, Gassner (1930) has found that a single temperature fluctuation, either from warm to cold or cold to warm, gives better germination than constant temperature, provided that the length of exposure to the first temperature is neither too short nor too long, the actual optimum exposure depending on the temperature. He attributes the harmful effect of too long an exposure in the first temperature to a "Hemmungsvorgang": "Die Versuchsergebnisse lassen sich dahin deuten, dass jeder längere Aufenthalt bei einer konstanten Temperatur zu einer Selbsthemmung des Keimungsvorganges führt; diese Hemmungsvorgänge sint offenbar temperaturspezifisch, da sie sich durch Anwendung einer abweichenden Temperatur unterbrechen lassen." Here again it has been found that the best results with alternating temperature are obtained if the higher temperature acts for the shorter time. But this, although generally true, is not always the case. Gassner (1915*a*) reports the reverse to be true for *Oenothera biennis*, and Doerfel (1930) observes that for *Hyoscyamus* germination is better if the higher temperature acts for the longer time. It is also necessary for this species that the temperature interval should be sufficiently large. The small variation 25°–30° gives lower germination than constant temperature.

Several suggestions have been put forward as to why germination should be better in alternating than in constant temperature. Liebenberg (1884) has suggested that at any given temperature the available reserve materials are largely used up in respiration, but if the temperature is suddenly lowered the surplus immediately on hand can be used for growth. This is either an incorrect or an incomplete explanation in view of the fact that a single alternation from cold to warm has been found to give better germination than constant temperature. Harrington (1923*b*) suggests almost the converse of this, namely, that at low temperatures there is an accumulation and metabolism of oxygen in a form which becomes immediately available for growth at the onset of the higher temperature. This is also invalidated as a general explanation, since a single temperature change from warm to cold causes increased germination. Vanha (1899) points out that differences in temperature between different parts of the seed, the germinating bed, and the air immediately surrounding the seeds, following a rapid temperature change would be liable to cause different gas densities which might set up gas movements leading to the removal of carbon dioxide and renewal of oxygen, conditions favourable for respiration, and presumably for germination.

When Gassner (1911) found that removal of the glumes of *Chloris ciliata* brought about good germination at constant temperatures, and, moreover, that the glumeless grains were no longer susceptible to intermittent temperature, he concluded that intermittent temperature acted more or less by annulling the glume function and allowing free ingress of oxygen. " Für *Chloris ciliata* halte ich den Nachweis für erbracht, dass die keimungsfördernde Wirkung der intermittierenden Temperaturen nicht, wei bisher meistens angenommen, in einer Reizwirkung, sondern in einer Verbesserung der Sauerstoffverhältnisse bei gleichzeitig möglicher Amvendung hoher Keimungstemperaturen besteht. Die Wirksamkeit der intermittierenden Behandlung zeigt sich an die Existenz einer sauerstoffzutritterschwerenden Schicht gebunden, als welche bei den Scheinfrüchten von *Chloris ciliata* die Spelzen wirksam sind."

In his more recent work on *Poa*, Gassner (1930) finds that grains from which the glumes have been removed are still susceptible to alternation of temperatures, so that in this case at least increase of oxygen cannot be the mode of action of the temperature change. In view of the fact already mentioned, that too long an exposure to one temperature, before alternation to another, proves harmful, he considers that regulated intermittent temperatures give increased germination not because they are directly beneficial, but because they allow evasion or overtaking of temperature-specific hindrance effects: "Wenn regelmässig intermittierende Temperaturen die Keimung von *Poa*-Samen im Gegensatz zur Einwirkung dauernd konstanter Temperaturen ermöglichen, also nicht eigentlich 'Auslosen,' so liegt das daran, dass durch die Kombination einer tiefen und einer hohen Temperatur im richtigen Zeitverhältnis (bei 12°: 28° ca. 7 Zeiteinheiten 12° auf 1 Zeitenheit 28°) die Ausbildung der Temperaturspezifischen Hemmungsvorgänge vermieden oder überholt wird."

Doerfel considers that intermittent temperatures probably operate on certain physiological processes, the connection of which with certain temperature points would explain the marked superiority of some temperatures and temperature combinations.

2. Light.

As regards light, seeds fall into three classes: (1) light-aided, (2) light-indifferent, (3) light-hindered. Depending on other factors different samples of the same species may belong to different groups or at least vary in their degree of light-sensitiveness. Lehmann (1912) has reported that different samples of *Epilobium roseum* exhibit different degrees of light-requirement, hence it is necessary to work with a pure line to obtain reliable results. In the case of *Verbascum thapsiforme*, seeds gathered from the under part of the inflorescence gave only 10 per cent. less germination in the dark than in the light in ten days, while if the seeds were gathered from the upper part of the inflorescence germination was 40 per cent. less in the dark. Maier (1932) notes that harvesting time affects the light-sensitiveness of *Poa nemoralis*. The most decisive factor in light-sensitiveness is perhaps degree of after-ripening. Jönsson (1893) has shown that seeds of *Poa pratensis* in the un-after-ripened condition germinate up to 88 per cent. in the dark and not at all in the light, while after twelve months they germinate equally well in either. Gassner (1911) finds that the un-after-ripened grain of *Chloris ciliata* requires light, while the after-ripened does not. The same writer later reports (1915) that after-ripening brings about progressive alteration in the seeds, so that they are at first aided by light, next indifferent to it, and finally hindered by it as regards their germination. Germination temperature comes into the question inasmuch as with low temperatures the inhibiting action of light appears earlier in after-ripening than it does at higher temperatures, so that with seeds at a medium stage of after-ripening light is hindering at low temperatures and stimulating at high temperatures.

Busse (1926) using a number of varieties of tobacco, which are mainly light germinators, finds that germination itself is not affected by darkness, but only the time required for it to take place. Wieser (1927) finds that the seed of *Lythrum salicaria*, which can germinate up to 50 per cent. in the dark, show decreased ability to germinate in the dark with age. Kinzel (1917) believes that light and dark play an important part in the germination of seeds in nature, and that different reactions in this respect are to be regarded as a response to environmental conditions. The seeds of dune plants are subject in nature to deep burial, and they generally germinate better in dark than in light. On the other hand, the seeds of plants inhabiting firm ground are usually hindered by dark. It is quite possible that this view may be correct, but until more data is available it cannot be generally applied.

Maier (1932) has found that *Poa* species, particularly *Poa nemoralis*, are sensitive to light and give better germination under its influence. This light-sensitivity increases after the seeds are put to germinate, and, after reaching a maximum, falls away again. The point of highest light-sensitiveness depends on temperature. Parallel with this there is a variation in sensitiveness to temperature fluctuation. Maier considers that the term " Lichtempfindlichkeit " (Light-sensitiveness) should be replaced in certain cases by the term " Lichtbedürfnis " (Light-requirement), and that the statement that light-sensitiveness decreases with after-ripening should be amended in this respect. In other words, it is light-requirement which decreases in the course of after-ripening.

As a general rule it has been found that temperature conditions have a profoundly modifying effect on the action of light. Lehmann (1912) observes that within the temperature limits that allow of germination, with the light-germinating seeds examined (*Epilobium roseum*, *E. hirsutum*, *E. palustre*, *Veronica longifolia*), the higher temperatures encourage germination in the dark, while with the dark-germinating seeds (*Phlox Drummondii*, *Nemophila insignis*, *Whitlavia grandiflora*) the lower temperatures promote germination in the light. Fluctuating temperature has an even more marked influence on the light action. Gassner (1915*a*, 1915*b*) demonstrates this in the case of *Ranunculus sceleratus*. This species shows negligible germination in both dark and light in both high and low constant temperatures. With suitable temperature alternation, however, it germinates in either light or dark. In constant dark the best results are obtained with a large temperature interval (14° C.) and the higher temperature applied for the shorter time. No data is given for germination in constant light, but when the seeds are exposed to daylight for part of the day the germination percentages are higher and the necessary temperature interval smaller. As no experiment was carried out using fluctuating temperature and constant daylight, it is not known whether light or alternation of light and dark causes the increase in germination in this instance. Fassbender (1925) states that intermittent illumination of the seeds of *Epilobium hirsutum* has a greater effect than constant light, while Lakshmana Rao (1925) using *Lythrum salicaria* finds intermittent light to have the same effect as the sum of the light intervals. Gassner (1915*b*) next turns his attention to the question of whether light fails to act as an aid to germination at constant temperatures because it is ineffectual at constant temperatures, or because, though the seeds are altered in some way by the light, they fail to germinate because of the need for temperature fluctuation. Since he finds that seeds illuminated at constant temperature and then transferred to darkness and fluctuating temperature give a higher percentage germination than seeds germinated in darkness and fluctuating temperature without previous exposure to light, he concludes that light does exercise some effect on the seeds at constant temperature, an effect which remains latent until the seeds are presented to suitable temperature conditions.

Fluctuating temperature, however, does not always enable light-sensitive seeds to germinate in the dark. Hutchings (1932) reports that the seeds of *Mimulus ringens* do not germinate in the dark either with high or low constant temperatures or with alternation of temperature. In diffuse light, high constant temperatures cause an increase in germination, but there is no data on the effect of fluctuating temperature in diffuse light.

Light-sensitive seeds appear to be particularly sensitive also to the acidity of the substrate. Ottenwälder (1914) has established that eight species which otherwise require light for germination will germinate in the dark at suitable temperatures if provided with an acid substrate, while two other species closely related to several of these eight, but differing from them in that they are not light-sensitive, fail to receive any benefit from similar concentrations of acid. Gassner (1915*b*) has followed this up with a fairly wide survey of the effects of chemical substances, both germination-aiding and otherwise, in relation to light. Knops' solution at the most favourable temperature combinations enables *Ranunculus sceleratus* to germinate as well in dark as in light. Of the other chemical substances it may be said in general that while weak solutions may or may not aid germination according to their chemical nature, strong solutions have a deleterious effect.

Both Kuhn (1916) and Lehmann (1919) have found that light-hindered seeds are affected by the substrate. The former has shown that suitable concentrations of hydrochloric, sulphuric, or nitric acid enable the seeds of *Phacelia tenacetifolia* to germinate in light. The actions of dark and acid are, however, not additive, since the same concentration of acid which produces germination in light does not give increased but rather poorer germination in dark. Lehmann reports that the light-hindered seeds of *Veronica Tournefortii* are favourably affected by nitrate.

Nelson (1927) using several species of *Poa* has observed that although potassium nitrate and other salts were stimulating in culture they had a depressant effect when the seeds were germinated in soil. Maier (1932) also reports nitrates and to some extent hydrochloric acid as aiding the germination of *Poa*.

Light-requirement can sometimes be altered by the removal of glumes or testas. *Chloris ciliata* with glumes requires light, but is indifferent to it after their removal (Gassner, 1911), while the removal of the testa of the light-hindered *Phacelia tenacetifolia* in the same way dispenses with the necessity for dark (Böhmer, 1928). In both cases the effect is attributed to the increased supply of oxygen reaching the seed. This is not necessarily the case. The two light-hindered species, *Phacelia tenacetifolia* and *Nigella sativa*, can germinate in light if the oxygen content of the medium is increased, but the removal of the seed-coat of *Nigella sativa* does not enable it to germinate in light. Axentjev (1929) considers that seed-coats may hinder germination by restricting the supply of oxygen to the seed. It is at least evident that light does not always act through the medium of oxygen intake, since Wieser (1927) has found that *Lythrum salicaria* can germinate in light in the complete absence of oxygen.

A few workers have carried out investigations in connection with the amount of light necessary to promote the germination of light-requiring seeds. In the case of *Lythrum salicaria* Lehmann (1918) finds that using 730 c.p. there is a perceptible effect with illumination of 1 minute at 20° C., or $\frac{1}{10}$ second at 30° C., and Wieser (1927) using 200 c.p. gets a comparable effect in 1 minute, while the maximum effect is usually reached with about 12 hours illumination. Hutchings (1932) finds that much longer exposure to light is necessary in the case of *Mimulus ringens*. Using continuous illumination with light of different intensities, Ottenwälder (1914) has shown for a sample of *Epilobium* that the minimum effective light-strength is 3 to 0·5 c.p. at 20° C., while at 25° C. $\frac{1}{400}$ c.p. does not reach the lower limit. As might be expected, the light-requirements of different species, and even different samples of the same species, are by no means uniform. Lakshmana Rao (1925) finds that the percentage germination of *Lythrum salicaria* at 31° C. and 35° C. is directly proportional to the amount of light received, within a certain range. Above this range the position is reversed. Kommerell (1927) proves for the same species that the effect of light on germination is directly proportional to the wave length.

There are a number of more or less opposed theories as to the mode of action of light in promoting germination. Lehmann (1912) and Lakshmana Rao (1925) consider that in the case of *Lythrum salicaria* and *Epilobium hirsutum* light acts as a stimulus. This is argued on the basis of certain similarities which appear to exist between the light action on these seeds and the behaviour of light acting as a stimulus on other plant organs. In another paper (1913) written in conjunction with Ottenwälder, Lehmann declares that light has a catalytic effect, and offers the three hypotheses (1) that it hastens and increases enzyme action ; (2) that it activates zymogen present in the resting seed ; (3) that it itself acts as a catalyser in the presence of certain substances. Ottenwälder (1914) finds that light can be replaced by acid, and considers that both have a catalytic action. Gassner (1915) is opposed to the idea of light as a stimulus on account of the fact that light-requiring seeds, given a sufficient exposure to light in temperate conditions unsuited to germination and then subjected to drying, subsequently germinate, given suitable temperature, in the dark. He considers that an inhibiting factor operates during germination, whose action is hindered by light. Finally Kommerell believes light to have a photochemical action.

3. After-Ripening.

The seeds of some species will not germinate immediately after they are ripened and shed from the plant. This may be due to " hardness," mechanical resistance of the seed-coat, reduction of oxygen by the seed-coat, morphological immaturity of the embryo, or it may be due to the need for a period of after-ripening. The changes which take place during this process are as yet imperfectly understood, but alterations in acidity, enzymes, and food-reserves have been observed. After-ripening seems to occur most rapidly at comparatively low temperatures (0°–5° C.). This is reported by Pack (1921) for *Juniperus*, by Davis (1927) for *Cornus florida* and *Sambucus canadensis*, by Joseph (1929) for *Betula*, and by Flemion (1933) for *Rhodotypos kerrioides*. As Joseph points out, this would enable seeds to after-ripen on the ground during winter.

As regards changes in acidity, Eckerson (1913) has found that the dormancy of the *Crataegus* embryo is due to its dormant hypocotyl, and that this organ, at first slightly alkaline or neutral, becomes distinctly acid with after-ripening. Rose (1919) has shown that a slight increase in acidity accompanies the after-ripening of *Tilia* seeds. In both, increased acidity is accompanied by increased water-holding power, of the hypocotyl in the former, and the embryo in the latter. Jones shows that in the sugar maple the embryo is always basic, although the hydrogen ion concentration may increase in the embryo as it after-ripens, and that there is no rise in the water-holding capacity of the seeds. Increased water-holding power is probably connected with increased acidity, owing to the hydrophilous colloids having a greater water-holding capacity in an acid medium.

As regards enzyme activity, Eckerson (1913), Rose (1919), Jones (1920), and Davis (1927) all find a rise in catalase activity as after-ripening progresses, while Crocker and Harrington (1918) report an increase in the activity of both catalase and oxidase.

The chemical changes which accompany after-ripening in general seem to involve an increase in carbohydrate and a decrease in stored protein. Jones reports that after-ripening in sugar-maple is accompanied by an increase in the amount of free reducing sugars, and the same is found by Okada (1930) for *Euryale ferox*. Pack finds a decrease in the amount of stored fat and protein, with increase in sugar content, and first appearance of starch in the case of *Juniperus*. He also finds an enormous increase in the degree of dispersion of stored fat, translocation of food in the form of fats or fatty acids from the endosperm to the embryo, increase in soluble proteins and marked hydrolysis of stored proteins, a sevenfold increase in amino acid content, and complete disappearance of histidine. Davis observes in the case of *Cornus florida* an increase in starch, sugar, and amino acids, with little or no change in fats, acidity, or phosphatides.

II. Material and Methods.

Except where otherwise stated, the "seed" of *Aira flexuosa* used in these studies was gathered on the island of Bute, Scotland, during August and September 1931, and allowed to dry in light until the middle of October, being thereafter stored in seed-packets until used. Owing to the presence of many empty glumes the sample was rough cleaned by means of a small blower, and only "seed" possessed of a caryopsis, as surely as could be ascertained, selected for test. The germination tests were carried out in glass-topped Copenhagen tanks on blotting-paper pads as described by Nelson (1927).

In the case of seeds germinated in the dark, the glass domes over the pads were covered with lightproof paper; where fluctuating temperatures was one of the conditions the pads were transferred between one tank at 20° C. and one at 30° C., being left for eight hours at 30° C., and 16 hours at 20° C. The solution of potassium nitrate and acids were supplied to the pads by dropping the ends of the wicks into small bottles of the solution standing in the foot of the tanks.

The first two germination tests were carried out, one from 9th till 27th February 1932, one from 10th till 30th November 1932. Each test comprised thirty-two pads, each carrying 50 seeds. Eight different combinations of factors were investigated, four pads being subjected to each set of conditions. All the combinations of the following pairs of factors were employed, (*a*) light *v.* dark, (*b*) constant *v.* fluctuating temperature, (*c*) tap water *v.* a 0·2 per cent. aqueous solution of potassium nitrate. A third test, similar to these except for the omission of the potassium nitrate factor, was made from 8th till 29th May

1933. The effects of 0·1 M solutions of KNO_3, HNO_3, H_2SO_4, and HCl were investigated in December 1932, the test running from 2nd till 17th December, and of 0·01 M and 0·001 M solutions of HNO_3, HCl, and KNO_3 in March 1933 (14th till 24th). Further investigation of constant temperature was made from 14th May till 26th June 1933.

Unless stated to the contrary the figures given in the tables of results are percentages calculated from the average of four pads of fifty seeds each—two hundred seeds in all.

III. Experimental Results.

A. The Effect of After-ripening.

TABLE I.

Germination Conditions.	Per cent. Germ. Feb. 1932.	Per cent. Germ. Nov. 1932.	Per cent. Germ. May 1933.
Light, water fluct. temp.	17·5	60·0	59·5
,, ,, const. temp.	58·0	67·0	72·0
Dark, ,, fluct. temp.	9·5	51·0	61·5
,, ,, const. temp.	30·5	75·0	64·5
Light, KNO_3, fluct. temp.	13·5	57·0	—
,, ,, const. temp.	45·0	68·0	—
Dark, ,, fluct. temp.	8·0	51·0	—
,, ,, const. temp.	31·0	66·5	—
Average	26·6	61·9	64·4

As shown in Table I., germination for any given set of conditions was much better in November than in February 1932, the increase varying with different sets of conditions. On the other hand, the germination in May 1933 does not differ greatly from the germination in November 1932, but shows only a slight increase or decrease.

The conclusion to be drawn is that seed harvested in August–September 1931 was not fully after-ripened in February 1932. The process of after-ripening had probably been completed by November 1932, since in the following May there was either little increase or slight decrease in germination under similar conditions. The discussion of the relative effects of after-ripening on germination under different conditions will be deferred until after consideration of the effect of these conditions themselves on germination.

B. The Effect of Temperature.

1. *Alternating Temperature.*

Table I. also shows that *Aira flexuosa* germinates considerably better at a constant temperature of 20° C. than it does in an alternation of 20° C. for 16 hours, followed by 30° C. for 8 hours. This is the case whether the other conditions be light or dark, water or KNO_3. It is to be noted that the depressing effect of the fluctuation in temperature is much less marked in the November and May germinations than in the February tests. This will be discussed in a later paragraph.

2. *Constant Temperature.*

TABLE II.

(*Figures derived from 2 pads of 50 seeds each.*)

Germination Conditions.	Per cent. Germ. 30° C. 12 days.	Per cent. Germ. 20° C. 21 days.	Total per cent. Germ. 33 days.
Light . .	15	49	64
Dark . .	7	57	64

In this investigation the seeds were kept at a constant temperature of 30° C. until there was no further germination on 3 consecutive days. The pads were then transferred to a constant temperature of 20° C. until no further germination occurred.

From the figures given in Table II. it appears that a constant temperature of 30° C. is unfavourable for the germination of *Aira flexuosa*, but not sufficiently high to cause injury to the seeds, since subsequent exposure to 20° C. caused the germination to rise to over 60 per cent.

At first sight it might appear that the decreased germination of *Aira flexuosa* in a temperature alternation of 20° C.–30° C. as compared with germination at a constant temperature of 20° C. is attributable to the fact that 30° C. is unfavourable for germination. But it must be remembered that it is only the un-after-ripened seed which shows a really low germination in fluctuating temperature, and that with after-ripening the difference to a large extent disappears. The results shown in Table II. are derived from after-ripened seeds, and if the temperature of 30° C. employed in the temperature alternation were the main cause of the decrease in germination under fluctuating temperature, then one would expect the decrease to remain fairly marked even when after-ripening is complete, and this is not the case.

C. *The Effect of Light.*

The figures for February 1932 in Table I. show that *Aira flexuosa* in the un-after-ripened condition germinates better in light than in dark. As with temperature, the difference is not nearly so marked when after-ripening has taken place. Indeed in two cases slightly better germination occurred in the dark.

D. *The Effect of Potassium Nitrate and Acids.*

TABLE III.

Germination Conditions.	Feb. 1932.		Nov. 1932.	
	H_2O	0·2 per cent. KNO_3	H_2O	0·2 per cent. KNO_3
Light, const. temp. .	58·0	45·0	67·0	68·0
,, fluct. ,, .	17·5	13·5	60·0	57·0
Dark, const. temp. .	30·5	31·0	75·0	66·5
,, fluct. ,, .	9·5	8·0	51·0	51·0

Apparently a 0·2 per cent. solution of potassium nitrate has no effect on the germination of *Aira flexuosa*. Table III. shows slight variations from 1 per cent. better germination in water, with a mean difference in percentage germination of 3·5 in favour of water. This cannot be regarded as significant.

TABLE IV.

Germination Conditions.	Per cent. Germination.
Tap water	44·5
0·05 M KNO_3	47·0
0·1 M KNO_3	15·0
0·1 M HNO_3	0·
0·1 M H_2SO_4	0·
0·1 M HCl	4·0

TABLE V.*

Germination Conditions.	Per cent. Germination.
Tap water	40·0
0·01 M KNO_3	29·0
0·001 M KNO_3	35·0
0·01 M HNO_3	22·0
0·001 M HNO_3	43·0
0·01 M HCl	26·0
0·001 M HCl	19·0

The question of the effect of substrate on the germination of *Aira flexuosa* was further investigated, using solutions of potassium nitrate, nitric acid, sulphuric acid and hydrochloric acid of various concentrations. Commercial seed of unknown history was employed in these experiments, which were carried out at 20° C. in light.

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* Figures derived from 2 pads of 50 seeds each.

It appears that 0·1 M H_2SO_4 and 0·1, 0·01, 0·001 M HCl retard germination. Nitric acid and nitrate on the other hand are harmful only in the stronger concentrations, and in weaker concentrations have no appreciable effect on germination, neither retarding nor stimulating.

E. The Relationship of After-ripening to the other Factors affecting Germination.

TABLE VI.

Germination Conditions.	Per cent. Germ. Feb. 1932.	Per cent. Germ. Nov. 1932.	Incr. in per cent. Germ.
Light, water, fluct. temp. .	17·5	60·0	42·5
,, ,, const. temp. .	50·0	67·0	9·0
Dark, water, fluct. temp. .	9·5	51·0	41·5
,, ,, const. temp. .	30·5	75·0	44·5
Light, KNO_3, fluct. temp. .	13·5	57·0	43·5
,, ,, const. temp. .	45·0	68·0	23·0
Dark, ,, fluct. temp. .	8·0	51·0	43·0
,, ,, const. temp. .	31·0	66·5	35·5
Average . . .	26·6	61·9	35·3

In the consideration of the various factors affecting germination it has been noted in passing that the percentage germinations under different conditions in November show much smaller differences than do the percentage germinations under different conditions in February of the same year. In other words, the increase in percentage germination with after-ripening varies with the conditions of germination. The extent of this variation is seen in the third column of figures in Table VI.

For further consideration of the relationship of after-ripening to the other factors, it has been necessary to gather together all the data concerning each factor in turn by taking the average of all the germination percentages of the seed exposed to each factor. The figures in the following tables have been derived in this way, *i.e.* the figure given for constant temperature is an average of the percentages for Light, water, constant temp.; Dark, water, constant temp.; Light, KNO_3, constant temp.; and Dark, KNO_3, constant temp.

If the factors are considered in pairs, *i.e.* light and dark, fluctuating and constant temperature, where one of a pair has a depressing effect on the germination of un-after-ripened seed the increase in percentage germination with after-ripening is much greater for seeds germinated in the presence of that factor than it is for seeds germinated in the presence of the other member of the factor pair.

Table VII.

Factor.	Per cent. Germ. Feb.	Per cent. Germ. Nov.	Increase.
Const. temp.	41·1	69·1	28·0
Fluct. temp.	12·1	54·7	42·6
Light	33·5	63·0	29·5
Dark	19·75	60·85	41·1
Water	28·9	63·2	34·4
Nitrate	24·4	60·6	36·2

As is shown in Table VII., the increase in germination due to after-ripening is 42·6 per cent. in the case of fluctuating temperature, and only 28 per cent. in the case of constant temperature, although constant temperature is the more favourable condition for germination. In the same way there is an average increase of 41·1 per cent. in dark and 29·5 per cent. in light. Moreover, the greater the influence of a factor on the germination of un-after-ripened seed, the less is the increase on after-ripening in germination of seeds under the influence of this factor, relative to the increase in germination of seeds under the influence of the other member of the factor pair.

Table VIII.

Factors.	Per cent. Germ. of Un-after-ripened Seed.	Difference.	Incr. in per cent. Germ. with After-ripening.	Difference.
Const. temp.	41·1	29·0	28·0	14·6
Fluct. temp.	12·1		42·6	
Light.	33·5	13·75	29·5	11·6
Dark.	19·75		41·1	
Water	28·9	4·5	34·4	1·8
Nitrate	24·4		36·2	

In the February tests (*i.e.* un-after-ripened seed) constant temperature gave an average of 29 per cent. better germination than fluctuating temperature, light an average of 13·75 per cent. better germination than dark, and water an average of 4·5 per cent. better germination than nitrate. If the differences in increase in germination on after-ripening are examined (Table VIII. last column), it is found that they occur in the same order.

As after-ripening proceeds the factors which influence germination of the un-after-ripened seed become less important, so that germination of after-ripened seed is much nearer constancy than is the germination of un-after-ripened seed.

IV. Summary and Conclusions.

I. After-ripening is an important consideration in the germination of *Aira flexuosa*. The process was found to be incomplete 5 months after harvesting and complete 14 or 15 months after harvesting.

II. *Aira flexuosa* in the un-after-ripened condition germinates better at a constant temperature of 20° C. than in an alternation of 20° C. for 16 hours with 30° C. for 8 hours. A constant temperature of 30° C. is unfavourable for germination.

III. The un-after-ripened seed germinates better in light than in dark. On the completion of after-ripening this difference largely disappears. There is no indication, however, that the seeds originally light-requiring become dark-requiring with after-ripening.

IV. Germination is not aided by weak solutions of potassium nitrate, nitric acid, sulphuric acid, hydrochloric acid, and is depressed by stronger solutions.

V. In the after-ripened condition the seeds of *Aira flexuosa* are less sensitive to external factors than in the un-after-ripened condition.

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