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J. W. PONT

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PROEFSCHRIFT TER VERKRIJGING VAN DEN GRAAD VAN DOCTOR IN DE WIS- EN NATUURKUNDE AAN DE RIJKS-UNIVERSITEIT TE UTRECHT, OP GEZAG VAN DEN RECTOR MAGNIFICUS DR. H. BOLKESTEIN, HOOGLEERAAR IN DE FACULTEIT DER LETTEREN EN WIJSBEGEERTE, VOLGENS BESLUIT VAN DEN SENAAT DER UNIVERSITEIT TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER WIS- EN NATUURKUNDE TE VERDEDIGEN OP MAANDAG 3 DECEMBER 1934, DES NAMIDDAGS TE VIER UUR

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CONTENTS.

INTRODUCTION.

The object of these investigations was to determine the influence of some seed desinfectants upon the germination of the grains of *Andropogon sorghum*.

The possibility that desinfectants might have a stimulating effect on the germination process made it essential that attention should be paid to the absorption of water and the subsequent swelling of the grains.

This eventual stimulans might, theoretically, affect the rate of swelling and the germ.

In either case an acceleration of the germination process might result.

Since the temperature is an important factor in all physiological processes, the temperature influence on both the swelling and the germination process was investigated.

APPARATUS AND METHOD.

The apparatus consisted of an incubator, fitted with four zinc trays, 45 × 30 × 5 cm, lackered inside. Over each tray three slabs of plate glass, 8 cm wide, could be placed. Each slab could carry five germination beds side by side. The germination beds were circular pieces of filter paper, 8 cm in diameter. The paper was of the same brand as used for the germination tests at the Rijksproefstation voor Zaadcontrôle (Government Seed Testing Station) at Wageningen, and is supplied by the firm SCHUT at Renkum.

Each tray was filled to a certain level with distilled water. Over each glass slab narrow strips of filterpaper were placed, which dipped into the water on either side, one strip to every one germination bed.

The quantity of water in the tray and the width of the strips were adjusted, so as to keep the germination beds soaked with an adequate quantity of water.

The air in the thermostat was saturated with water-vapour before the experiments were started, the seeds consequently did not need to be enclosed altogether in filter paper. This has many advantages, as was pointed out by PRINGSHEIM (1928), whose method resembles mine very closely.

As part of the object was to investigate the influence of desinfectant solutions on the rates of water-absorption and of germination, it was important to choose a certain number of seeds, as standard quantity, which would ensure both comparative reliable mean values and easy manipulation. The standard methods for seed testing, e.g. Rules for Seed Testing (1928), prescribe the use of 4 × 100 seeds for a single sample, or in case of large seeds 4 × 50 each. Such quantities would have involved a cumbersome apparatus. QUANJER and OORTWYN BOTJES (1915) and PRINGSHEIM (1928) used smaller quantities

in their experiments and the latter author pointed out that for comparative analyses small quantities may be used. He did not use larger samples than of 50 seeds each, irrespective of the size of the seeds. A fair degree of accuracy can be obtained with such samples, as is demonstrated in Table I.

The weight increase of the seeds, as a result of steeping, was determined with analytical accuracy by weighing the seeds, before and immediately after steeping, in tared weighing bottles.

The seeds were steeped in water and in three desinfecting solutions, viz. 0.1 per cent $CuSO_4$, 2 per cent $CuSO_4$, 0.25 per cent Uspulun.

The solution of 2 per cent coppersulphate was applied for one hour only, thereafter the seeds were thoroughly washed with distilled water. According to GASSNER and RABIEN (1926) and KOTOWSKY (1926) practically all traces of a salt solution can thus be removed. After washing the seeds were steeped in distilled water.

The other solutions were applied till the seeds were removed to be weighed. Before weighing they were washed, rapidly dried between filter paper and transferred into the stoppered weighing bottles. After reweighing they were arranged on the germination beds. The seeds were always placed with the scutellum on the filter paper, so that uniformity of arrangement excluded variations due to the positions of the seeds.

The vessels used for steeping were small glass dishes with a capacity of about 15 cm^3. A measured quantity of liquid was used in every case which just covered the seeds. This quantity was 8 cm^3. The influence of this arrangement on the process of germination will be discussed later.

The seeds could absorb water during various lengths of time. By these means seeds with varying quantities of water were put to germinate and the influence of presoaking on the germination process could be observed.

The seeds were steeped for 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 30 and 36 hours. In some cases no determination of the quantity of water which was absorbed was made after 18 hours and a duplicate determination after 24 hours was made. Only where a 2 per cent $CuSO_4$ solution was used, no determination could

be made after the first hour. The required washing with water prevented this.

After 2 hours duplicate determinations were made and the mean has been recorded.

In table I are recorded the percentages of weight increase after 24 hours steeping in water at 22.5°, for ten samples. The average germination time, in days, is given in the second column.

TABLE I

Temp. 22.5° Klerksdorp var.

% Water	Av. Germ. Time in days	Seeds germinated
28.61	3.96*	45
29.18	3.68	46
28.33	3.70	43
28.42	3.70	44
29.56	3.77	44
26.85*	3.80	44
28.92	3.67	42
27.45*	3.72	46
28.76	3.70	42
28.61	3.78	46
Av. 28.67 ± 0.26	Av. 3.75 ± 0.025	44

Determinations of mean errors on 10 samples.

The weight increase has been calculated on the basis of the dry weight of the seed.

The average observation appears to be quite accurate, but in a few cases, in particular those marked with asterisks, the variation is much larger than the mean error,

according to the formula $m = \sqrt{\frac{\Sigma v^2}{n(n-1)}}$.

This, however, is a phenomenon frequently met with in investigations on seeds and larger samples nor a larger number of samples would eliminate this. Due allowance will have to be

made for a striking deviation from the mean. In general, it does not influence the accuracy of a number of readings, as the general tendency of a series of observations determines the value to be attached to occasional deviations.

In the following table a number of duplicate determinations of weight increase show that great accuracy can be obtained.

TABLE II

Temp. 34.5° Klerksdorp var.

Time of steeping in hrs.	1	2	4	5	6	12
per cent weight increase	11.01	14.58	20.74	23.21	23.85	28.86
	11.17	14.72	20.53	23.11	24.35	28.88
Averages	11.09	14.65	20.64	23.16	24.10	28.87

Reliability test on duplicate determinations.

The figures in the tables endorse the assumption that samples of 50 seeds each may produce results of a fair degree of accuracy.

The influence of the temperature on the water absorption and on the subsequent germination was studied at 15°, 20°, 25°, 30°, 35°, 40° and 45°. The seeds were soaked and laid out to germinate at the particular temperature.

A germination experiment was taken to be started from the moment of steeping. The number of germinated seeds was determined every 24 hours, those seedlings only being considered which had a developed root system as well as a plumule standing away from the scutellum. This is in agreement with the general rules for seed testing. In some cases the points of the radicles could be observed outside the seeds, but no further development took place afterwards. This was very striking at 45°, where several seeds came to this stage only and no further growth followed; the seeds died, owing to the length of exposure to this high temperature. It would have been erroneous to consider them as germinated. The same phenomenon was observed with seeds which were badly infected with parasitic fungi. The embryos developed to the first stages of germination, where root development can be observed, but before complete development could take place they were killed by the infection.

For all samples the average germination time, as defined by GASSNER, was calculated. This proved to be of great value in determining the effect of the treatment the seeds had undergone.

Sterile conditions were maintained as far as practicable. Before every experiment the incubator was desinfected with dilute alcohol and with formaline. The trays and glass plates were sterilised in the same manner. The filter paper and other apparatus was sterilised in a drying oven at 110° for six hours. On the whole, very little infection from outside could be observed during the course of the investigations. The only fungi which did occur were those inherent to the material itself.

MATERIAL.

Three varieties of *A. sorghum* were used during the experiments, viz. Klerksdorp Kort Rooi, Dwarf Hegari and Dwarf Yellow Milo. The first was obtained from the Faculty of Agriculture of the University of Pretoria, cropped March 1934 at Pretoria. Samples of the other two varieties were kindly furnished by the Division of Plant Exploration and Introduction of the Bureau of Plant Industry, U.S. Department of Agriculture, Washington D.C. They were grown at the Woodward, Oklahoma Field Station of the Division of Cereal Crops and Diseases in 1932.

The South African sample was used in all experiments for the investigation of the temperature influence on the rate of absorption, germination etc. Together with these experiments duplicate tests with the American varieties were made for comparative purposes, so that some interesting data could be collected.

In some additional experiments the American varieties have been used because of the fact that the South African sample became badly infected with grain weevils. The required selecting of undamaged, sound seed required more time than was justified, since the other varieties remained free from this source of trouble.

I want to express my thanks to Prof. A. R. PULLEN, of the University of Pretoria and to Mr. B. Y. MORRISON, of the Bureau of Plant Industry, Washington, for their courtesy and assistance by supplying me with the required material.

THE ABSORPTION OF WATER BY SEEDS.

The absorption of water by seeds is a process which is controlled by a number of factors. Because of the complex nature of the material involved it is possible that other factors than the following influence the swelling process:

a. permeability of the layers enveloping the embryo and endosperm, as well as the permeability of the cellwalls and protoplasm of the seed contents,
b. osmotic forces,
c. swelling force of the colloids,
d. temperature.

It is stated by LEHMANN und AICHELE (1931), that HEINRICH (1913) should have indicated that live seeds absorb water at a faster rate than dead ones. Whereas this might be taken to indicate that vital forces are of influence on the absorption process, it is necessary to point out that this question will be referred to in due course.

SWELLING AND PERMEABILITY.

The permeability of the composite layer of pericarp and testa, — for convenience sake frequently called testa, while the grain for identical reasons will be referred to as seed, — is of principal importance for the absorption process.

This permeability is associated with the anatomical structure and detailed information on the structure of the testa of *A. sorghum* seeds has been given by SWANSON (1926, 1928), to whose articles one is referred.

It was BROWN, who indicated in 1907, that the testa functions as a semipermeable membrane. After having demonstrated its

nature in barley, he was able to do the same for rye, wheat and oats. Soon afterwards semipermeable membranes were observed in a large number of seeds, e.g. rice by VALETON (1907) and NAGAI (1916), maize by NAGAI (1916), *Xanthium* by SHULL (1913), *Cucumis* by VAN DER MAREL (1919), while a list of other seeds with semipermeable membranes was published by RIPPEL (1918). In all these cases the absorption of totally submerged seeds gained attention. It was SCHROEDER (1911), who determined the exact nature of the semipermeable membrane and described the phenomena of selective permeability and localized absorption.

In his initial study on barley, BROWN describes how he observed, that the grains of *Hordeum vulgare*, var. *caerulescens* contain a blue pigment in the aleuron layer. This pigment changes colour and becomes red in acid medium. When such barley grains were steeped in dilute sulphuric acid, they retained their blue colour and cracked or damaged seeds only showed a change of colour.

He noticed that the seeds absorbed water from the dilute acid, thereby increasing its concentration. This simple observation has been repeated many times with several solutions of electrolytes and organic substances and an increase in the concentration of the solution was always noticeable.

BROWN concluded, that the acid did not enter the seeds but water only. This proved even to be the case when a solution of sulphuric acid of 36 per cent was used.

BROWN was able to indicate the position of the semipermeable membrane by steeping the seeds first in a 3 per cent solution of silver nitrate and thereafter in a 5 per cent solution of sodium chloride. A depository layer of silver chloride was formed inside the testa. On exposure to light, after sectioning, it was found that the precipitated silver oxide occurred in the testa itself only and the layer on the inner side of the precipitate was taken to be the semipermeable membrane. BROWN gave it the name of "spermoderm" and did not investigate its morphological nature.

The spermoderm was found to be almost impermeable, but at or near the micropyle some substances could enter the seed. Here permeability was greater.

BROWN observed that a solution of iodine entered the seed at the germinal end and other substances were soon observed to behave in a similar manner.

Of the mineral acids, sulphuric acid was never noticed to enter the seeds. Normal hydrochloric acid entered at high temperatures only (REICHARD, 1909, not seen). Dilute nitric acid, 1 per cent solution, did not enter during the first 24 hours of steeping, but, according to BROWN, appeared to enter with length of time. COLLINS (1918) found no traces of nitric acid inside the seeds, even when a 10 per cent solution of the acid was applied.

Several organic acids were found to enter easily: acetic acid, formic acid, lactic acid, butyric acid, trichloracetic acid, picric acid and (by SCHROEDER) osmic acid.

The only salts that have been observed to pass through the membrane are mercuric chloride and mercuric cyanide (BROWN), traces of mercury from desinfectants like germisan and uspulun (HEUBNER, 1928), traces of copper from copper salts (LUNDEGÅRDH, 1924, 1925), and potassium iodide (BROWN).

BROWN was able to distinguish between the behaviour of an iodide solution which entered readily, as was noticeable by the staining of the seed contents, and the behaviour of a solution of thiosulphate, which decolourised the testa, but not the seed contents.

Several organic substances pass through the membrane, e.g. formaldehyde in solution, alcohols, aether, chloroform, phenolic substances and glycocol (BROWN and TINKER, 1915). It was established soon, that in all cases permeation depends upon the presence of water, a dry membrane being altogether impermeable.

A few tests were made by me to verify the behaviour of mineral acids and picric acid. VAN DER MAREL stated, that while picric acid was found to enter the grains of barley, wheat, maize and *Penicillaria spicata*, it did not enter the grains of sorghum. I have tested the grains of the three varieties of *A. sorghum* at my disposal. They were steeped in a saturated aqueous solution. This did not enter the seeds during the first 6 hours, but after 24 hours it could be observed in all seeds. (Temp. 18°).

The mineral acids nearly all enter the seeds, though the rate of entrance appears to be very slow and unequal for sulphuric, nitric and hydrochloric acid. After 24 hours, at a temperature of 18°, traces of sulphuric and nitric acid only were observed. While it appeared that hydrochloric acid had entered further into the seeds, this observation is obscured by the fact, that the seeds contain a certain amount of soluble chlorides. When due allowance was made, it was found that traces only could have been absorbed.

ATKINS (1909) failed to find a semipermeable seedcoat in beans. He found that the absorption of water by living and dead seeds was identical, until the beginning of germination of the living seeds, at which time the osmotic phenomena became manifested. According to this author, the forces which are concerned in the initial stages of water intake are those of capillarity and imbibition. On germination osmotic forces begin to influence the amount of water taken up by the living seeds. ATKINS, however, did not take into account the open micropyle of the material with which he worked, as was pointed out by SCHROEDER (1911).

This investigator contributed some very important data. He identified the semipermeable membrane with the inner integumentary layer. He observed that seeds, that were steeped in an iodine solution, did not absorb the iodine over their whole surface, but the blue colour could be seen to spread progressively from the germinal end of the grain. This indicated that the solution was absorbed at the germinal end at a rate much higher than that with which it is absorbed over the general surface. To describe this, he introduced the term "selective permeability". This was illustrated by an experiment with halved seeds. These were first soaked in a solution of cobaltous chloride, then dried and thereafter made to absorb water. In both parts the cut surfaces changed colour, but in the germinal parts of the grains an additional change was observed round the embryo.

Absorption of water, therefore, takes place principally at the germinal end of the seed and the water spreads inside the grain along the inner surface.

Penetration of the more central parts takes place very slowly.

The other part of the seed coat is not so much impermeable to water as very little permeable. Further evidence was given by SCHROEDER in 1922.

This proved the existence of semipermeable cellulose membranes. RIPPEL (1918) drew attention to this fact, since semipermeability in plants had been associated always with living matter only.

The observation of SCHROEDER was confirmed by MÖLLER (1921) and by COLLINS (1918).

COLLINS pointed out that the distribution of water inside the grains always takes place from the germinal towards the apical end. This distribution is precisely the path of enzyme desintegration during the germination of the embryo. Water thus prepares the way for the distribution of the enzymes or even may carry the enzymes which are active in dissolving the food reserves.

He noticed, that the impermeability to any solute is not perfect, since with time, small quantities enter the grain at the germinal end. This is the place where selective permeability is located. The cutinised inner integument, on the other hand, remains impermeable to salts for a considerable time. It is because of this property that osmotic cells could be constructed to good effect with these parts of the testas of several seeds: the general testa, e.g. COLLINS (1918), BRAUNER (1928), GUREWITSCH (1929). According to RIPPEL (1918), however, the inner integument of grains should prove to be little suited for such purposes, because it is cutinised and does not readily imbibe water. PFEFFER stated, in 1877 already, that " kein Kork und keine Cuticula absolut impermeabel für Wasser ist". RIPPEL'S opinion did not keep the other investigators back.

GUREWITSCH (1929) determined the permeability of the general wheat testa, by measuring electrically its degree of permeability to various ions. An electric current which passes through a solution of an electrolyte causes the ions to move in the direction of the poles. When the vessel containing the solution is divided in two by a diaphragm constructed with a piece of testa, the ions have to move through this diaphragm and the resistance offered is quantitavily a measure for the ionpermeability of the membrane.

Ions which increased the rate of swelling of colloids did permeate at a faster rate than those which retarded the swelling.

The membrane itself does not swell easily. The effect of certain ions, therefore, could be studied with accuracy.

GUREWITSCH developed the following theory to explain the behaviour of the membrane.

The membrane should consist of a fine network of micels with intermicellary pores. The solvent is taken up between the micels, the ions influence the volume of the intermicellary system. Alcohol causes an irreversible increase in size of the intermicellaries. Substances, like iodine, mercuric chloride, osmic acid and organic dyes, which always pass through the membrane, are marked by their adsorption by the membrane. These substances, therefore, should pass through the micels themselves.

This explanation of GUREWITSCH shows a complete parallelity with the one of SCHÖNFELDER (1930) for the permeability of protoplasm. In both cases the explanation requires a joint application of the ultrafilter and adsorption theories.

Little information is available about the osmotic values of the seed contents. Because of the dry condition of the seeds, they cannot easily be determined. BOUYOUCOS and MCCOOL measured osmotic values of ground seed after mixing the meal with water. They found osmotic pressures for *A. sorghum* of about 7 atmospheres. In how far this value corresponds with the one prevailing under natural conditions escapes analysis.

SHULL (1913) determined the capillary and imbibition forces of *Xanthium* seeds and found these to be very high. Very little water only needed to be imbibed, however, to cause a considerable drop in these forces.

The sole reason for mentioning these factors is to emphasize that the Q_{10} of the osmotic value is only little influenced by a change in temperature. The rate of uptake of water varies chiefly because of the presence of non-osmotic factors regulating the rate of entrance of water.

The water that enters the grain causes swelling. This swelling will be caused principally by the swelling of starch. Both the swel-

ling force and the osmotic force influence the absortion of water. The maximum capacity of the grain to absorb water depends largely upon the elasticity of the testa. The behaviour of the germ, however, is also of great influence.

Own experiments. Seeds, that are steeped in water which just covers them, hardly suffer from oxygen deficiency. The varieties of *A. sorghum*, used in the experiments, germinated at a very fast rate. At 25°, 30°, 35° and 40° the absorption process had practically come to an end after 30 hours steeping. About that time the radicles appeared and absorption was no longer studied on seed, but on germinating embryos joined to their reserves. It was only in case the exchange of oxygen between the seeds and the air was prevented, by using deep steeping vessels filled to utmost capacity, that no germination took place.

It is probably due to the use of deep vessels for steeping, that BROWN (1909, 1912) was able to plot absorption curves over periods of 90 hours.

The absorption of water by seeds may be represented by a graph. BROWN (1909), BROWN and WORLEY (1912) and SHULL (1920) have tried to give an explanation of the curves they found. As the influence of the temperature will be discussed separately, the general information to be obtained from any one curve will be given first.

SHULL (1920) produces a curve for the moisture intake in *Xanthium*, to which he applies the formula:

$$y = a \log (bx + 1) + c,$$

wherein y = total percentage of water already taken up at any given moment, x = time elapsed since the beginning of soaking, a, b, c are constants.

SHULL observed that the constants a, b, c are not the same for every part of the curve, since he could divide his curve into three component parts, each having its own values for the constants. This formula, with different constants, could be applied to all absorption curves which SHULL plotted for *Xanthium* seeds, split peas and maize.

The formula does not tell us anything about the processes which take place inside the seed during absorption, nor does it

give an idea of the exact place where the three components meet at different temperatures. It would be interesting to know if a relation exists between these places and if they express some biological process inside the seed.

I am not able to contribute anything to this side of the problem, since the formula of Shull does not appear to be applicable to my curves. This, however, does not prevent an analysis.

The absorption of water shows an initial rate which is very high. Without doubt this is largely due to imbibition by the testa. The absorption rate thereafter decreases gradually, as saturation increases. The absorption rate is not a logarithmic function of the time.

The process becomes still more complicated, when the influence of the temperature on the swelling process is considered.

From a physico-chemical point of view the temperature influence on swelling has not been investigated. HENNEMANN (1929) has stated that swelling is influenced by the antagonistic influences of cohesion and hydration.

Because the swelling of seeds is such a complicated process it was hoped to simplify the case by observing the swelling of ground-up seeds. The experiments performed to this purpose cannot claim a high degree of accuracy, they merely give qualitative information.

TABLE III

Temp.	Volume in cm^3						% Incr.
0	6.5	6.5	6.5	6.5	6.4	6.4	0
15	6.4	6.6	6.6	6.6	6.6	6.5	3
20	6.5	6.85	6.85	6.85	6.8	6.8	5
30	6.2	6.8	6.8	6.8	6.8	6.8	9
35	6.3	7.0	7.0	7.0	7.0	7.0	11
Time	0	30	60	90	120	300	min.

Volumetric determination of the swelling of meal.

A small quantity of meal was shaken with water in a graduated measuring cylinder of 10 cm^3, divided in tenths of a cm^3. As soon as the material had settled, the volume was read. The cylinders were then placed in incubators, kept at different temperatures and the volumes were read again after certain intervals. The results are tabulated in table III.

The swelling of meal is influenced by the temperature. This swelling takes place at a very fast rate. Equilibrium is reached in about 30 minutes time. This equilibrium proves to depend upon the temperature.

Meal was shaken with water and allowed to settle. The cylinders were placed immediately afterwards in incubators at different temperatures. After one hour they were removed, after the volume had been read, and transferred to a room at 15°. An increase in volume was observed in those cylinders which came from cooler places, while in the others a decrease in volume resulted.

TABLE IV

Volume in cm^3. after

Temp.	0	30	60	150	210	minutes
0	6.55	6.55	6.55	6.55	6.5	H_2O
	7.5	6.85	6.8	6.75	6.7	$NaCl$
	7.5	6.8	6.8	6.7	6.7	$CaCl_2$
20	6.75	6.8	6.8	6.8	6.8	H_2O
	7.1	6.85	6.8	6.75	6.75	$NaCl$
	7.6	6.8	6.65	6.6	6.55	$CaCl_2$
30	6.4	6.6	6.6	6.6	6.6	H_2O
	6.9	6.8	6.75	6.75	6.7	$NaCl$
	7.35	7.0	6.9	6.9	6.85	$CaCl_2$
43	6.4	6.8	6.8	6.8	6.85	H_2O
	6.8	6.7	6.7	6.7	6.7	$NaCl$
	7.3	7.1	7.05	7.0	7.0	$CaCl_2$

The swelling of meal in dilute solutions ($N/1000$) of $NaCl$ and $CaCl_2$.

It had still to be determined in how far the swelling of the meal was due to water only, for dissolved material from the seed might influence the process. About equal weight quantities of meal were shaken with water and with N : 1000 solutions of $NaCl$ and $CaCl_2$. The results are presented in Table IV.

The swelling of meal in water differs from that in dilute solutions of electrolytes. In the latter instance there is a continuous decrease in the volume, while with water only the original volume of the swollen material remains practically constant. The slight decrease in volume, noticeable towards the end of the observations in some cases of Table III, has to be ascribed to the passing into solution of the electrolytes which are present in the meal.

The seed contents absorb different quantities of water at different temperatures. This deduction is of importance for the explanation of the absorption curves of the seeds.

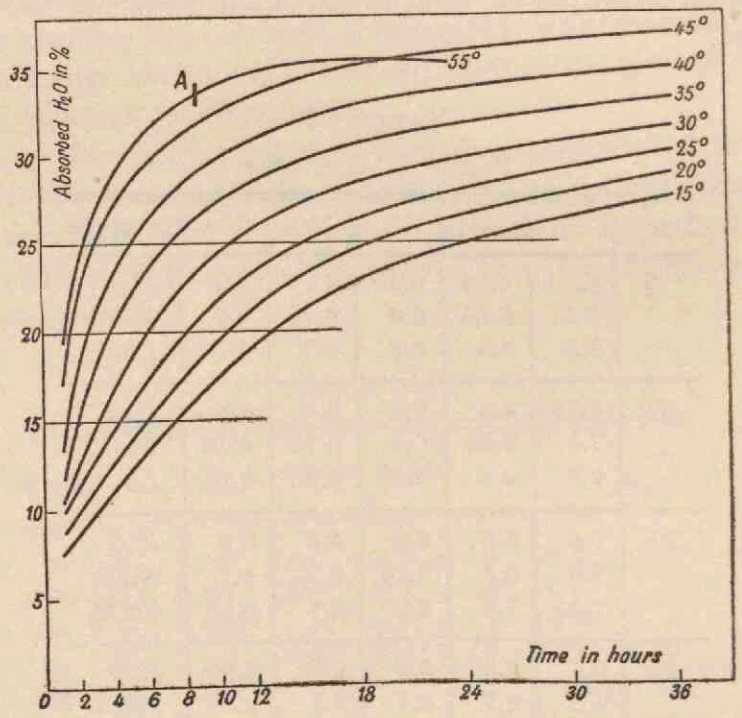

Fig. 1. Absorption curves plotted from the figures for the absorption of water, cf. Tables XII—XVIII.

At A. seeds started cracking.

Towards the end of the absorption period the curves run parallel. The general tendency shows, that a continued, very small absorption may be expected. For the curves at 25° and higher, the absorption rate, at least during the last six hours, showed an increase over the expected one. This must be ascribed to the fact that the seeds were actually germinating at the time, as could be observed by the appearance of a number of roottips. The curves have been plotted as they would probably have continued their course, in case this complication had not arisen. The parallelity of the last parts of the curves indicates that the swelling maximum for the various temperatures has been approached. This maximum, however, proves not to be independent from the temperature. This contradicts the statement of COUPIN (1896) that seeds always swell to the same maximum volume.

The first parts of the curves show great differences. The initial intake of water, apparently, is very rapid.

This intake represents the amount of water which is absorbed by the testa. This precedes the swelling of the seed contents and the two processes can hardly be separated from one another, in particular when a number of seeds are used.

The testa itself wil hardly affect the swelling process, as the localised absorption of water by the seed contents is of predominating influence.

The influence of the temperature on the swelling process is very striking. This can be demonstrated best by comparing the velocities of water intake at various temperatures, after certain

TABLE V

Weight percentage of water absorbed

Q_{10}	15%	17%	20%	23%	25%
45/35	—	4.8	2.6	2.5	2.4
40/30	3.8	3.0	2.2	1.8	2.0
35/25	2.3	2.3	2.1	1.9	2.0
30/20	1.7	1.6	1.5	1.8	1.9
25/15	1.4	1.4	1.4	1.6	1.8

Q_{10} ratios at different stages of the swelling process.

equal weight percentages of water have been absorbed. The Q_{10} ratios have been determined for the times that 15, 17, 20, 23 and 25 per cent of water had been absorbed.

The remarkable feature of the swelling process is, that the temperature ratios increase with a rise in temperature, which indicates that the intake of water is inhibited at low temperatures, but that the responsible factor decreases in influence as the temperature rises. These ratios, however, show a steady decrease as the amount of absorbed water increases and this must express that during the first stages of the absorption process something takes place inside the seeds at a diminishing rate which accounts for this phenomenon.

When considering what material is used, it seems possible to find an explanation by advancing the following hypothesis. The temperature ratios give expression to the differences in the rates with which water is absorbed by the colloids. When equal weight percentages of water have been absorbed, more water can be bound at higher temperatures than at lower ones, with constant temperature the quantity of water that can still be bound decreases with time. The swelling of dehydrated colloids takes place at a very high rate when the temperature is high, — the membrane being more permeable, — at a low temperature the permeability limits the absorption. Thus permeability is a limiting factor. It is due to the nature of our material that some of the temperature ratios appear remarkable. The drier the material, the higher these ratios are at high temperatures. The more the seeds approach germination, the more the temperature ratios approach, which are usually considered to be, "normal" values.

The temperature ratio therefore depends upon the degree of saturation of the material.

In all investigations, where temperature ratios Q_{10} have been determined, the material consisted of organs or tissues which were functioning normally, i.e. they were saturated with water. In the present instance, however, little water only is present and that in such a small quantity that the material cannot be taken to be "normally" active. The only physiological process that can be traced in seeds is respiration, and even that takes

place at an extremely low rate. The moment that water enters the seeds, all functions of the protoplasm can start, and the intensity largely depends upon the amount of water present. This saturation again depends upon the temperature, as is shown in the next table.

TABLE VI

Temperatures

Percentage of water	45°	40°	35°	30°	25°	20°	15°
25	2.2	4.9	7.2	10.8	14.7	18.7	24.0
20	1.5	2.5	3.7	5.8	8.1	10.2	12.9
15	—	1.3	1.8	3.0	4.2	5.8	7.4

Time in hours required for the absorption of a given percentage of water.

The fact, that the water absorption depends upon the temperature, should show a parallel in observations made on plasmolysis. A large degree of parallelity does exist between the processes of swelling and plasmolysis, though the direction of the passage of water is opposite in the two. If a principal difference does exist, it must be looked for in the membranes, for in swelling a semipermeable cellulose membrane regulates the process, while in plasmolysis the protoplasm is the layer involved. The influence of the protoplasmic membranes inside the seed may be neglected for the moment. Before comparing my observations with those of other authors, who studied swelling on seed, I shall refer to some observations on plasmolysis.

DELF (1916) published an almost classical study on the subject. She observed that the shrinkage of a tissue followed an almost logarithmic course, but as she probably expected such a relationship between the percentage contraction and the time of contraction, she selected from several duplicate determinations those data which actually did show this. Apart from this, she observed that the rate of plasmolysis was high at high temperatures and low at low temperatures, in particular during the first stages of plasmolysis. Her calculated coefficients of increase are reproduced here:

	Onion leaves	Dandelion scapes
40/30	3.0	2.6
35/25	2.9	3.0
30/20	2.6	3.8
25/15	2.0	3.3

The ratios increase with a rise in temperature. In either experiment the protoplast showed a tendency to contract at high temperatures. This tendency became noticeable already at 35°, and the values for the higher temperatures have been computed from short time observations, during which the protoplasm could be taken not to be injured by the temperature.

De Haan (1933) showed that the increase in permeability with temperature is due to the influence of the temperature on the swelling of the protoplasm. He summarised his observations as follows: "Die Quellung erfolgt bei niederer Temperatur langsamer als bei höherer, folglich wird auch die Permeabilitätszunahme bei niedriger Temperatur langsamer stattfinden. Das Q_{10}, das dabei erhalten wird, ist auch für einen Quellungsprozesz karakteristisch".

While this observation in itself can be corroborated, it is probably the explanation offered by Irwin (1928), which will prove to be most helpful. In her study: On the accumulation of dye in *Nitella*, she describes that the cells of *Nitella* show a remarkable faculty of absorption. The initial absorption rates show temperature ratios which agree with those found in my experiments, viz. 5.9 to 4.6. She considers that the absorption is the result of a chemical combination of the dye and the cell contents. In the theoretical discussion of the experimental data, she comes to the conclusion that this explanation fits the facts.

There is no reason for supposing that water, which is absorbed by seeds does not combine with the colloids; as a matter of fact this would be contradictory to the experimental evidence.

The remarkable temperature ratios may be explained by the swelling of the colloids inside the seed; the rate of swelling of these colloids having been shown to increase with a rise in temperature.

A short survey of previous observations made on swelling seeds may be given at present.

DIMITRIEVICZ (1875) observed already that the swelling rate was increased by a rise in temperature, and he pointed out that this had to be of influence on the velocity of the germination process, since a certain quantity of water has to be present before germination can start.

HORKY (1877) and JUST (1877) made similar observations.

EBERHART (1906) noticed that the germinal parts of grains absorbed more water within a certain period than the apical parts. This phenomenon itself was independent from the temperature. He stated that seeds of wheat and barley swell to the same maximum volume, though the time required to reach this state is dependent on the temperature. His observations were made over 240 hours! We are not informed how it was possible to suppress germination.

BROWN and WORLEY (1912) measured the weight increase of samples of seeds which were steeped in water at 3.8°, 21.1° and 34.6°. The weights were determined after fairly long intervals, altogether five over a period of 90 hours. They were presented as graphs. The temperature ratios are fairly high, as they remarked, viz.:

$$Q\frac{21.1}{3.8} \text{ av.} = 3.40, \quad Q\frac{34.6}{21.1} \text{ av.} = 2.44.$$

They explained the fact, that the velocity with which water is absorbed nearly is an exponential function of the time, with the Hydrone Theory of ARMSTRONG.

DENNY (1917) found a much lower temperature ratio, 1.6 to 1.3, while the Q_{10} decreased with rising temperature.

SHULL (1920, 1924) found a close agreement between his observations and those of BROWN and WORLEY. He considers that the Q_{10} values of 1.55 to 1.83 should indicate that absorption is a physical process.

KONDO (1923) again noticed that the total volume of rice seeds depends upon the temperature and the time, the swelling rate again being dependent upon the temperature.

Other important observations on swelling are those of STILES and JØRGENSEN (1917), while STILES (1924) gave a survey of Permeability.

STILES and JØRGENSEN immersed small discs of storage tissue of potatoes and carrots in water at different temperatures. Changes in weight were recorded and the amount of water absorbed plotted against the time. They noticed an increase in the absorption rate with rising temperatures, up to a critical temperature. Prolonged exposure led to a rapid loss of weight, probably due to protoplasm becoming injured and no longer functioning as a semipermeable membrane. This agrees with the observations of DELF. STILES and JØRGENSEN find the following temperature coefficients:

Temperature range	Temperature coefficient Q_{10}	
in centigrade degrees	Potato Tuber	Carrot Root
10—20	3.0	1.3
15—25	2.75	1.4
20—30	2.7	1.6

The effect of high temperatures made it difficult to obtain reliable information about the absorption under those circumstances. In the graphs which they produce, it is evident that some pronounced change takes place in the material. Considering that the material was saturated to utmost capacity with water, is is not remarkable that it should prove to be very sensitive to heat. The loss in weight was the result of a contraction of the tended cellwalls, which pressed out water.

In seeds, I have not obtained a clear confirmation of this point, though some obvervations show that the same may happen. Seeds that absorbed water at 45° did so very rapidly. Only a few embryos developed, and not further than the initial stages of root growth. Not a single one produced a seedling. A remarkable large number of seeds showed drops of water oozing out, some time after being put out to germinate. The appearance of moisture at first was taken to be a sign of infection by some micro-organism. As none could be observed shortly after their appearance, another explanation had to be looked for, and the most likely one is that of STILES and JØRGENSEN, viz. that the

living seed had died after exposure to heat and that a contraction of the walls caused excessive water to be pressed out.

As seeds which did show such drops never did germinate, the presence of drops was taken to indicate death after swelling.

Another quantity of the seeds became cracked. The latter condition is the result of absorption being carried to such a point that the elastic seed coat could extend no further and had to give way. This also was fatal. I consider it likely, that seeds with lowest viability will succumb due to exposure to high temperatures much sooner than perfectly sound seeds. The first will be killed before the seed contents have absorbed so much water that the testa cracks, the others will die only afterwards.

It was of interest to know what manner of swelling cracked seeds would show. A hundred seeds — Dwarf Yellow Milo — were steeped in water of 55°, and the absorption of water was determined after certain intervals. After about 5 hours, many seeds showed cracked testas. As the weight remained more of less constant from six hours steeping onwards, the seeds were dried back to approximately the original weight in vacuo over concentrated sulphuric acid. Thereafter they were steeped once more in water, now at 30°, and the weight increase was compared with that of another sample of sound seed. These last determinations are reproduced in table VII.

TABLE VII

Temp. 30° Dwarf Yellow Milo

Hours of steeping	per cent weight increase	
	seeds killed at 55°	normal seeds
1.5	27.68	20.56
3	31.74	26.44
4	32.12	31.61
5	34.21	34.04
6	35.38	36.42
9	35.71	38.10

Absorption rates of dead and live seeds.

While the rate of absorption at first is much faster for the cracked seeds than for the normal ones, the total quantity of water absorbed by the first is less. The osmotic forces which are active in the normal seeds have been eliminated in the cracked material. This then may explain the difference in the rate of absorption as noticed by HEINRICH (1913).

We may summarize the factors which are of influence on the swelling of seeds as follows:

1. The permeability of the selective semipermeable membrane. This permeability, as well as that of the protoplasm is increased by a rise in temperature. cf. HENNEMANN and DE HAAN.
2. The swelling force of the colloids. The final amount of water absorbed by the colloids increases with a rise in temperature.
3. The osmotic forces. These are only little increased by higher temperatures.

The influence of salt solutions hardly needs a lengthy discussion after the absorption of water has been analysed. The seed is enclosed by a semipermeable membrane which shows a localised area of greater and selective permeability. By various experiments it has been shown that this part of the testa is fully semipermeable for a certain limited time. This time has been determined to be approximately 24 hours. This is in agreement with observations by other authors on gramineous seeds.

When a grain is steeped in a salt solution, the water from the solution is absorbed and the rate depends on the osmotic concentration of the steeping solution.

In the experiments very dilute concentrations have been used, viz. 0.1 per cent $CuSO_4$, 0.25 per cent Uspulun and 2 per cent $CuSO_4$. The latter solution was applied for one hour only and cannot have had any noticeable influence upon the absorption rate after the seeds had been thoroughly washed. The molar concentration of Uspulun cannot be given, since the exact formula is unknown. Thus the 0.1 % solution of coppersulphate only remains. The molar concentration is $N/125$, and this cannot have influenced the absorption rate to any large extent.

In no case can one expect an acceleration of the water absorption to result from the application of desinfectant solutions.

With low concentrations, the depressing effect which they could have must be negligible. This is fully supported by the experimental data supplied in the tables XII—XVIII, and may be illustrated by the graph, fig. 2, which presents the absorption of water at 30° from water and desinfectant solutions.

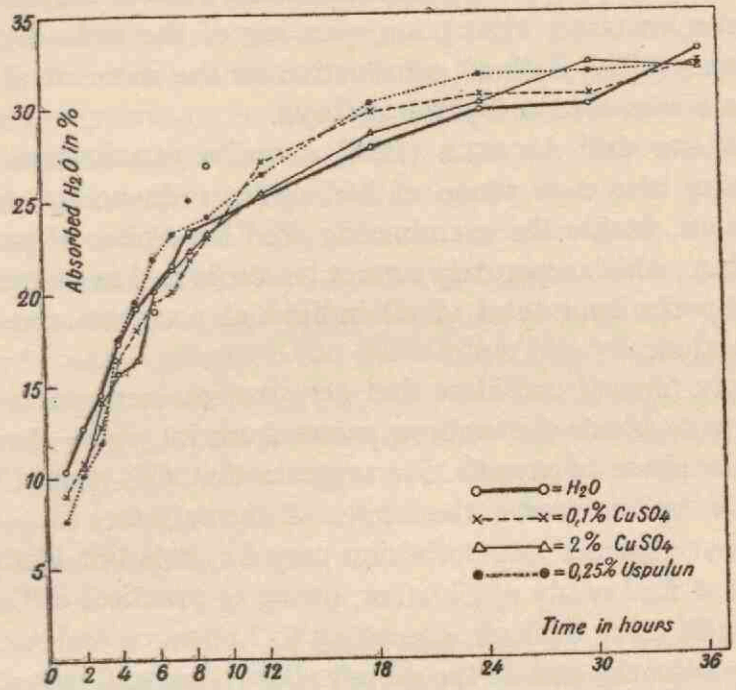

Fig. 2. Absorption curves at 30°, from Table XV.

GERMINATION AND THE INFLUENCE OF THE TEMPERATURE ON GERMINATION.

The term germination does not lend itself to a clear definition. KISSER (1932) has given a contribution in which he tries to clear this point. He gives a survey of the definitions of previous authors and this is shortly reproduced here.

NOBBE (1876) considered the swelling of seeds as a mechanical process, preceding the development of the embryo. As a result of swelling the cellwalls were stretched but no change in cell numbers nor growth of the cell walls was caused by it. The chemical changes in the food reserves of the seed facilitated sub-

sequent growth of the embryo. Germination commenced at the moment, that the radicle, which had increased in size by cell division, pierced the testa.

DETMER (1880) identified the absorption of water by the seeds with the beginning of germination.

KLEBS (1885) regarded germination as a number of consecutive processes, as water absorption, piercing of the testa etc.

SCHMID (1902) defined germination as the moment at which the testa was broken by the embryo.

LEHMANN and AICHELE (1931) describe germination as the beginning of a new phase of life, under influence of external conditions. Inside the germinating seed a number of processes take place, which separately cannot be considered as germination, but only the sum total of all individual processes constitutes germination.

KISSER himself considers that germination commences at the moment at which the embryo passes from its phase of relative rest to a phase of growth. He suggests that this should be determined by measuring the length of the radicle.

However correct this definition may be, it is but likely that it will not find ready application, owing to practical difficulties.

It is still more difficult, according to LEHMANN and AICHELE, to determine the end of the germination process. Gradually the embryo develops into a seedling and the seedling into a plant. Clear limits do not exist. They cite the "Technische Vorschriften für die Prüfung von Saatgut, 1928", which like the "Rules for Seed Testing, 1928", consider those seeds as germinated which show a normal sprout and a root with roothairs, but principally those which may reasonably be expected to continue their development under favourable conditions. General experience is required as a guide.

The official rulings, to a certain extent, have simplified the general position and while the definition remains rather vague it is more readily applicable than the one of KISSER for the beginning of the germination process.

A seed was considered as germinated when either the radicle had developed normally or adventitious roots had developed, so that the embryo possessed organs for absorption of water and

other substances which could function if the seedlings were developing in soil. The plumule had to develop so far that it did stand away from the scutellum; this includes that it did react to its position and showed geotropic response. The length of the plumule was not considered of importance, neither the fact that the first blade could still be enclosed by the coleoptile.

When counting the number of seeds that had germinated, I followed, as far as possible, the Rules for Seed Testing. Because the average germination time had to be determined, the germinated seeds were counted and removed every 24 hours.

In the tables presenting the results of the germination experiments No. XII—XVIII, the first double column deals with untreated seeds, steeped in water only. Each table gives the results obtained at one temperature. As the influence of desinfectants was studied at the same time, the various columns facilitate comparison.

For every sample the average germination time has been calculated. These figures have been used to calculate the means for the samples soaking from 1 to 6 hours, from 1 to 12 hours and from 12 to 36 hours. They are presented in the following table.

TABLE VIII

Temp.	Aver. 1st. 6 hrs.	Aver. 1st. 12 hrs.	Aver. last 24 hrs.
15	5.55	5.57	5.62
20	3.03	3.07	3.48
25	2.33	2.32	2.65
30	1.70	1.77	2.15
35	1.70	1.74	2.40
40	1.98	2.03	2.83

Average germination times in relation to length of period of presoaking and temperature.

While there is hardly any difference between the values in the first two columns, a striking difference is noticeable between them and the values in the last column. It is only at 15°, where germination proceeds very slowly, that the difference between the values is small. On the whole, the longer period of soaking

has a retarding influence on the rate of germination. When comparing this with the total number of seeds germinated in each case, it is clear that the duration of soaking does not influence the final count, except at 40°, where a decrease in the germination percentage demonstrated the injurious effect of long soaking.

It was likely that the oxygen supply caused this phenomenon.

This was put to a test. 100 seeds were soaked in the usual way, while a second sample of 100 seeds was placed in a flask with little water on a shaking machine. 24 hours later the seeds were put out to germinate and the average germination time was calculated. The test sample germinated in 3.05 days, the control in 3.25 days. The temperature varied, since the shaking machine could not be placed in an incubator at constant temperature. The difference was not due to a greater absorption of water, though the shaken seeds absorbed water at a slightly faster rate than the controls.

TABLE IX

Room temp. ± 20° Klerksdorp var.

Time in hours	per cent water absorbed	
	shaken	control
2	11.72	10.19
4	15.92	14.41
6	18.73	16.68
12	25.31	23.33
24	29.18	29.10
36	32.64 [1])	30.61

Percentage water absorption under influence of shaking.

The amount of oxygen available to the seeds is increased by shaking, the shaking itself is of no influence. This could be proved by another experiment. One set of samples were soaked in water, about 25 cm^3 to 100 seeds, with an oxygen atmosphere above the water, a second group in stoppered vessels, filled to utmost

1) seeds were germinating.

capacity with water and a third one in stoppered vessels filled with boiled water. The samples in boiled water were placed on the shaking machine. After 24 hours the percentages of water absorbed were 30.08, 28.85 and 29.37 respectively, while the average germination times in the same order were 2.80, 2.97 and 3.00 days.

The presoaking of seeds can have a depressing influence on the rate of germination. Whenever the layer of water over the soaking seeds prevents or obstructs the gas exchange of the seeds, the germination rate is retarded and the average germination time is lengthened. The method as employed in my experiments gave nearly optimum conditions for development.

Observations of this kind were made by JUST. HABERLANDT (1877) had stated that presoaking had no influence on the rate of germination. JUST (1877) remarked that HABERLANDT had not stated the height of the water column in his experiments. JUST found this to be an important item, since the presence of water might influence the gas exchange. He observed that seeds which had been covered by a water layer of ½—1 cm in depth germinated more readily than seeds which had been covered by 4—6 cm water.

The effect of presoaking depends altogether upon the height of the water column above the seeds.

EBERHART (1906) observed that presoaking barley at 10° during 100 hours had about the same effect as 48 hours presoaking at 20°. If the seeds were soaked for longer times, germination was inhibited to a larger or lesser extent.

GEIGER (1928) noted that soaking seeds show inhibited gas exchange, as gradually all oxygen is used; anaerobic conditions may therefore develop.

PEI SUNG TANG (1931) passed air through the water in which seeds were soaking. Up to a certain rate of air flow, a relation existed between this rate and the germination percentage. A further increase in the air supply did not cause a corresponding increase in germination.

The influence of the temperature on the rate of germination is very pronounced. The influence on the absorption of water has been discussed already and the effect of the absorption on

the living organism is left for discussion. From the experimental data we may deduce that a relatively small quantity of water suffices to start the germination process. How small that quantity actually is, could not be determined by my experiments, for the seeds could absorb water from the filter paper after having been soaked.

The average germination time clearly shows the effect of the temperature. This has been presented in a graph (Klerksdorp var.), fig. 3. The average of the germination times for the samples soaking from 1—6 hours has been plotted together with the averages for the samples soaking from 12—36 hours. The differences become more and more pronounced as the optimum temperature is approached. This must be ascribed to the inhibiting effect of presoaking. When the optimum has been passed the injurious effect of the temperature causes a lengthening of the average germination time.

The averages for 24 hours soaking are about the means of the values shown in the graph. The varieties Dwarf Yellow Milo and Dwarf Hegari have been soaked for 24 hours at all temperatures. The values for the absorption of water, average germination time and final count have been listed with those of Klerksdorp, which underwent the same treatment.

TABLE X

Temp.	Absorption in % H_2O			Av. Germ. Time			Final Count out of 50		
	1	2	3	1	2	3	1	2	3
15	22.30	34.92	30.39	5.6	5.8	6.1	42	48	29
20	27.26	38.85	34.04	3.4	3.0	3.2	43	46	39
25	28.76	43.23	38.26	2.3	**2.0**	2.3	48	49	47
30	29.68	43.31	40.56	**2.1**	**2.0**	**2.0**	47	43	47
35	31.52	44.04	41.55	**2.1**	**2.0**	2.3	45	37	33
40	33.62	43.06	44.57	2.4	2.7	2.3	41	12	31
45	37.32	43.26	46.14	—	—	—	—	—	—

1 Klerksdorp Kort Rooi. 2 Dwarf Hegari. 3 Dwarf Yellow Milo.

Absorption, Av. Germination time and Final count of the three varieties after 24 hours soaking at different temperatures.

The average germination times of the three varieties show different optima.

For Klerksdorp Kort Rooi, the optimum temperature lies between 30° and 35°. The final counts do not show any difference in the germination percentages. From the graph, fig. 3, however, it is evident that the optimum temperature is influenced by the

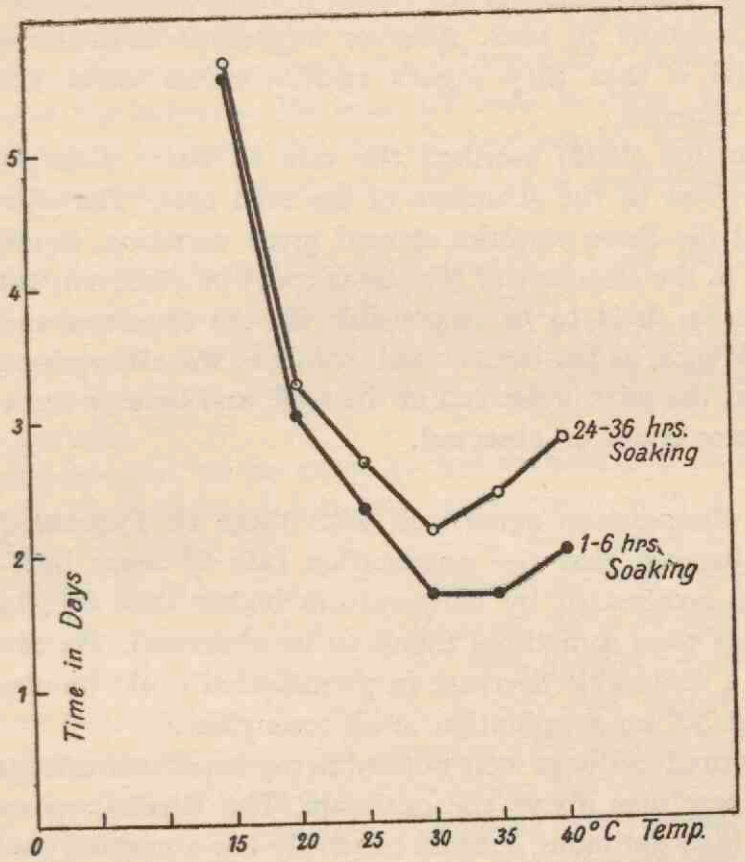

Fig. 3. Influence of temperature and length of presoaking on the germination time.

presoaking period. When this period is short, there is no distinction to be made between the influences of the two temperatures.

For Dwarf Hegari the shortest germination time equals two days. The final counts at the temperatures 25°, 30° and 35° show such pronounced differences, that the optimum temperature appears to be nearer 25° than 30°. Strictly this applies only to

the experimental conditions. Dwarf Hegari proved to be the fastest in germination of the three varieties at medium temperatures. The radicle-tips became visible: at 15° after 48 hours, 20°, 24 hrs., 25°, 12 hrs., 30°, 9 hrs., 35°, 5 hrs., 40°, 12 hrs. The variety appears to be very sensitive to heat.

For Dwarf Yellow Milo the optimum is found at 30°.

Of the three varieties the South African one seems to be the most resistant to heat. Another important character of this material is that they absorb relatively less water than the other varieties.

SWANSON (1926) ascribed the rate of water absorption by *A. sorghum* to the structure of the seed coat. The absorption rate of the three varieties showed great variation. Small differences in the structure of the testas could be observed, but these cannot be held to be responsible for the observed variation. All evidence, as has been stated, points to the absorption taking place at the micropylar end of the seed, and here no remarkable difference could be observed.

My observations agree well with those of TJEBBES (1912), who observed that the germination rate of sugar beet seeds became accelerated by temperatures higher than 30°, but the seedlings were sometimes found to be abnormal. He saw that at 40° a noticeable decrease in germination could be observed, while at 50° no germination at all took place.

Abnormal seedlings were noticed in my experiments frequently at temperatures above the optimum. The Klerksdorp variety, with a high optimum, showed relatively few abnormal seedlings, while the two American varieties germinated badly at high temperatures. The abnormal feature was that the radicle did not grow more or less straight, but developed spiral-wise, forming either a longitudinal or a ring-spiral. Because this was observed more frequently in Klerksdorp var. in desinfected seeds, it was at first taken to be a result of desinfection. Experiments showed that this phenomenon could result as well after short time exposure to high temperatures and seeds which had been soaked at 55°, for one hour, showed large numbers of abnormal roots. This has to be regarded as a general symptom

of injury to the root or radicle, irrespective of the cause of the injury. Generally the radicles recovered after a few days at lower temperatures, as was shown by their further growing straight out.

Somewhat similar observations were made by FRIESEN, and PRINGSHEIM (1928). FRIESEN observed that the radicles of seeds which had been exposed to heat or had been treated with chemicals showed less geotropic response than normal radicles. He considers this to be the result of a reduction of number of starch grains in the calyptra. He does not want to draw the parallel between heat influence and that of chemicals too close, because the alteration in the behaviour of the root is caused by different agents.

PRINGSHEIM observed the spiral growth of roots, but ascribed this to the influence of desinfectants only.

My own observations have shown that the reaction of the root is independent of the cause of the reaction and is merely symptomatic.

Reference has to be made to the experiments of WASSINK (1934), which induced my own experiments with a shaking-machine. WASSINK was able to grow *Phycomyces* in a liquid medium by shaking an inoculated culture solution. He suggested that the development of the spores normally is retarded because they sink in the solution and suffer from oxygen deficiency. The rate of gas exchange by diffusion is very slow and the oxygen supply in the immediate neighbourhood of the spores is exhausted after some time. By shaking the solution, the spores are brought in continuous contact with a medium with normal oxygen content, so that diffusion of oxygen from the air needs to take place through a thin layer of water only and this hardly influences the diffusion rate.

Seeds also depend upon the oxygen supply for germination. The parallel with the material of WASSINK is obvious. In this case, however, the conditions which suppress germination could not all be eliminated. This may be due to the fact that the seeds on the germination beds are in still more favourable conditions, as they are in immediate contact with the air, at most separated from it by a thin film of water.

THE INFLUENCE OF DESINFECTION ON THE GERMINATION PROCESS.

As stated in the introduction, the influence which desinfectants may have on the seed and on the germination process has drawn much attention during the last 20 years.

Some investigators tried to demonstrate that seeds could be treated in such a manner that, as a result, the growth of the embryo became accelerated and germination took place, in a shorter time than normally, while because of stimulation of the embryo the seedlings might show more vigorous growth than untreated ones.

Others again considered the stimulation of seeds as not proved, or otherwise not sufficiently controllable to warrant advocating its general application in practical agriculture.

Many opinions have been aired and data have been collected to support the one or the other theory.

The principle of stimulation itself has ruled botanical physiology for quite a long time. The controversy was started by the publication of RAULIN (1869), who indicated that some elements, up to then considered of no value to plant nutrition, were essential for plant growth. The list of these elements, started by RAULIN with zinc and silicon, has become much extended since and a large number of elements are now accepted as essential which formerly were considered as perfectly useless. RAULIN, already, pointed out that the concentration of the elements in the culture solution was of importance and that some substances in every concentration had to be considered as poisons, e.g. silver and mercury. It was RICHARDS (1897), who interpreted the observations of RAULIN by suggesting that elements like zinc, cobalt, nickel, manganese and others, were not so much of essential value for nutrition itself as they might be stimulants for metabolism. This remark was made in the days that PFEFFER and CZAPEK had built up a physiological terminology in which the term stimulus had a principal value and was applied and abused in several instances. Phototropism and geotropism were processes, wherein a stimulus was perceived by the plant. A minimum stimulus was required to start

off any reaction. The chemical stimulation of plants was reduced to a phenomenon comparable with others in human and animal physiology. A substance could be poisonous in large quantities and would, sometimes, show an enhancing influence on some process in lesser concentration. Later on this was defined by PRINGSHEIM (1914), who distinguished between chemical stimulants and nutritive substances by applying the following test: When a substance causes an increase of one third in the dry weight of a plant when applied in double the normal quantity the substance is of nutritive value; when the increase is no more than one seventh the substance is a stimulant. That this distinction is merely arbitrary needs no comment.

This line of thought may have been fruitful to a certain degree, it has never explained what did happen. Seeds of different plants and, in case of cereals, of many varieties have been subjected to numerous treatments, so that an extensive literature has been produced on the subject. The conclusions and deductions of the investigators sometimes escape analysis, because of the scantity of information supplied. Many have been published before a standard method for analysis and observations on seeds was demanded and described by GASSNER (cf. page 40).

The term stimulation is justly vague. PFEFFER (1897—1904) uses it extensively, likewise CZAPEK (1922). BENECKE—JOST (1923) state that the term stimulation will have to be dropped most probably in seedwork, as the observed phenomena could be reduced to catalytic functions. According to these authors, chemical stimulants have no nutritive value. From this one might deduct that they consider it essential that no stimulation experiments should be performed with nutritive salts. In KOSTYTSCHEW-WENT (1931) a similar opinion is stated. The principal experiments which would support this statement are those of STEPHAN and LANTZ. STEPHAN (1929) observed a more rapid germination after stimulation and a greater catalase activity. By what method the germination rates are determined and compared is not commented. The catalase activity needs not to be a result of the previous treatment, but may be a parallel phenomenon with a more

rapid germination in some samples. LANTZ (1927) noticed that 0.25 per cent Uspulun has very little influence on the amount of water absorbed within a given period. No direct relation between desinfection and catalase production can be found in his article.

Seed stimulation with chemicals began to receive a great deal of attention since the publications of POPOFF and his school.

The basic principle for his investigations was the assumption, that, in the course of its life, every organism looses water and the loss of water causes ageing. Thus ageing is linked to a process of dehydration. The rate of vital processes depends upon the degree of hydration of the protoplasm. When the latter is increased a greater activity results and such an increase can be induced by applying certain, well-chosen stimulants.

This point of view, in itself a rather fascinating theory, has started research workers looking for the adequate chemical stimulants. At first they were found to be rather numerous, but increasing accuracy in experimental methods and thorough interpretation of the results have gradually reduced the number to a dwindling few. Some nitrates are still considered as stimulants, though the objection of BENECKE-JOST should apply to them, while others again look for stimulating substances principally among the poisonous seed-desinfectants.

As SENF (1925) stated the case of the adherents of the stimulation-idea, a treatment of the seed with chemicals may result in:

1. making available a small quantity of food for the developing embryo,
2. increasing the permeability of the testa,
3. destroying the spores of injurious organisms, attached to the testa,
4. increasing the viability of the embryo.

These points may be analysed as follows:

1. The practice of using nutritious substances should be abolished for this purpose, since the object is to study stimulation of the embryo and not the best method of supplying the young seedling with food.

2. The observations on the existence of semipermeable membranes provide the answer. The testa is not easily made more permeable and if so, at great risk to the embryo only. Greater permeability may be caused by the application of alcohol and other substances which cause an irreversible change in the membrane. These substances are all very poisonous to the embryo. The use of known seed-desinfectants does not increase the permeability and never causes stimulation, much sooner retardation of the germination process.
3. The destruction of spores of fungi can only ameliorate the conditions for the seedling after development and thus may influence the germination percentage, as well as the rate of germination. This will be demonstrated by the results from my experiments.
4. This point has been formulated in a very vague manner and is included in the other ones.

BREDEMANN (1924, 1926), in his plot- and field-experiments, failed to find a stimulating effect for salts of magnesium, manganese and potassium, when applied in concentrations of 3 to 4 per cent during from 4 to 24 hours.

LUNDEGÅRDH (1924) considered that *Cu* was absorbed from solutions of copper sulphate. The absorption should take place periodically and in a serial experiment the periodical stimulations should correspond with the absorption of minute quantities of copper. His method, however, leaves doubt about the value of the observations, for the small variations in the percentage of coppersulphate in the steeping solution may come within the limits of accuracy of the method.

KOTOWSKY (1927) drew attention to the fact that the theory of POPOFF opposes the theory of semipermeability. He observed that the testa of cereals prevented the absorption of potassium nitrate and other salts from solution and washing the seeds for 1 minute resulted already in very heavy losses in the amount of salts absorbed in the testa. The behaviour of the testa in the presence of stimulants should be known, before any further attempt should be made to apply stimulation on a larger scale, in order to escape failures.

NIETHAMMER (1927, 1928) is very cautious in expressing an

opinion. While for some time associated with POPOFF's periodical, she has apparently become convinced by GASSNER and considers that since the desinfectant, in this case Uspulun, has a killing effect on the spores of several micro-organisms, it is very likely that its stimulating effect should be regarded as a result of its desinfectant properties.

KISSER (1933), in a largely theoretical contribution, considers stimulation not proved and the possibility of stimulation not likely.

The present author is in no way advocating the use of any particular desinfectant and it is partly because of the historical value of coppersulphate that its effect has been chosen as an object for closer investigation and comparison with that of one of the products of modern industry, Uspulun.

The influence of coppersulphate has attracted the attention of numerous investigators.

VOGT (1926) records the use of this salt in the year 1761, and consequently it is impossible to give a complete survey of the development of its application. Many statements have been made which discourage the continuation of the application of this fungicide in seed practice. One of the most recent is that of HEALD (1932):

"The injury from coppersulphate treatment has generally been measured in terms of the reduction in the percentage of viable seeds, which may frequently show a drop from 90 to 100 per cent germination of untreated wheat to 36 to 60 per cent germination when given the standard bluestone treatment (1 pound to 5 gallons for 5 to 10 minutes)". This is practically a 2 per cent solution.

"It has been shown that the toxic action of the copper also causes a pronounced retardation of growth when the treated seed is planted in the field and that many seedlings which do grow make an abnormal development, with curved, deformed plumule and poor root growth".

The question arises, whether the influence of coppersulphate really is as injurious as stated here. For it has been in use so long, that one wonders why it has remained so at all, if its action is as dangerous as stated by HEALD; it would be no compliment

to the ability of observation and deduction of former generations.

In the middle of the last century, KÜHN advocated the use of coppersulphate. Some years afterwards, in 1872, NOBBE wanted to determine the influence of a coppersulphate treatment on the germination of seeds which had been threshed by hand and by machinery. Machine-threshed seed were found to contain sometimes up to 20 per cent of seeds which were damaged, with cracked testas. These seeds, under normal conditions and when not vitally injured, germinated faster as they could absorb water more rapidly than sound seeds. They were, however, much more sensitive to the desinfectant. NOBBE used a solution of 0.1 per cent and steeped for 24 hours. Hand-threshed seed germinated faster, after steeping, than machine-threshed. The differences between the two groups became less pronounced in about three weeks time. Wheat was relatively more sensitive than rye, barley and timothy.

KÜHN (1873) replied by stating that NOBBE should have applied a 0.5 per cent solution for 12 hours only, since then no injury to wheat was noticeable. In this way the effect of coppersulphate became the object for exact investigations and all kinds of concentrations have been applied as well as different treatments after the application thereof.

NOBBE noticed that the treatment had impaired the quality of the seedlings. Some were rootless, others had injured roots. This, however, was observed in both the machine- and hand-threshed material and had to be ascribed to the influence of the coppersalt on the germ. As NOBBE stated: "Die vom gebeiztem Korn erzeugten Pflänzchen sind nämlich durchweg entweder gänzlich wurzellos, oder doch mit einem sehr geschwächten Wurzelsystem versehen".

TJEBBES (1912) observed that a 2 per cent solution did not cause any injury to the seeds of sugar beets, provided the seeds were spread out to dry before sowing, immediately after steeping. When dry, they could be sown without any danger. Sowing in moist condition led to heavy losses.

QUANJER and OORTWYN BOTJES (1915) used coppersulphate in a very concentrated solution, which was applied as a spray. The argument in favour of this treatment was that the seeds do

not swell in such a solution and no salt can enter the micropyle. The germs on the testa are killed effectively. In all their experiments, the treated seeds show a slightly retarded germination as compared with the controls. The method itself, from their accounts, leaves little to be desired. They noticed that a more dilute solution of coppersulphate, a 2 per cent solution, caused injury. They present here and there slightly flattered results by including rootless seedlings in their germination counts.

This point will always remain a subject for discussion, for seeds which have produced normal and vigorous plumules may reasonably be expected to continue growth. The present international rules have been laid down fairly recently (1928). My own experiments show that rootless seedlings were not retarded in their complete development by more than about 24 hours.

During the following years a great effort was made to increase the size of crops to the utmost. The prevailing conditions favoured the publications of POPOFF and his school. According to his theories, one should be able not only to increase the quantity but also the quality of crops, by applying small amounts of inorganic substances. The outlook was decidly promising, till exact investigations began to shake the foundations of the stimulation hypothesis.

It was GASSNER (1923, and following years), who exploded the theory. In a series of publications he gave a reliable basis to seed work. Germination tests had to be made on 4 × 100 seeds. The average germination time had to be calculated for every sample, to enable comparison, together with the germination percentage. The quotient of the percentage and the germination time gave a figure which he called the "Wertungszahl" — germination value. By comparing these values the results of different treatments could be determined.

All field tests should be preceded by laboratory tests under controlled conditions. The effect of any treatment could thus be studied accurately, interfering environmental factors had to be eliminated.

In a laboratory experiment it was necessary to wash the seeds after desinfection to prevent injury and this washing was found to be quite effective.

In this way he prepared the way for the determination of the dosis toxica and dosis curativa, the concentrations of the desinfectant which caused injury to the seed and which prevented infection by fungi.

GASSNER worked exclusively with one variety of wheat at one temperature and studied the action of formaldehyde and of organic mercury compounds. He noticed, that while washing removed practically all desinfectant from the testa, small quantities, traces, could stay behind. As a result root injury could occur, the other parts of the germ were not injured. This is indirect evidence in favour of localized absorption. The explanation offered was that some of the desinfectant became adsorbed by the testa. A similar observation had been made already by HURD (1921), who observed this for coppersulphate. It is but likely that the desinfectant will enter more or less deeply into the micropyle during steeping and that subsequent washing will remove only the most readily accessible deposit.

GASSNER observed that the influence of the desinfectant depends upon the temperature. While this statement refers more in particular to the mercury compounds and formaldehyde, the same could be observed for coppersulphate. At high temperatures swelling and development of the embryo take place at a higher rate and the radicle will pierce the testa much sooner than at low temperatures, consequently root-injury may become more frequent. GASSNER considered that low temperatures should favour desinfection, but other observations made it impossible to lay down a hard and fast rule. A simple relation between length of time, temperature and concentration in a desinfecting process could not be found.

VOGT observed that the action of Uspulun, "weizenfusariol" — a mixture of mercuric chloride and coppersulphate —, and coppersulphate was not influenced by a change in temperature.

PLAUT (1925) and NAGEL (1925) both noted that the toxic effect of desinfectants increased with a rise in temperature and the dosis curativa had to be reduced for both Uspulun and coppersulphate. No stimulation was observed by PLAUT in sugar beets, either in seedling growth or in sugar content of the beets after applying various desinfectants and magnesiumchloride.

BECKER (1926) used coppersulphate on wheat and applied concentrations of 0.05, 0.1, 0.25 and 0.5 per cent. After 36 hours(!) he counted the number of germinated seeds and found a larger percentage germinated after the 0.1 per cent treatment than in all others, while 0.5 per cent retarded germination. After 3 days the difference had disappeared, the injurious effect of the concentrated solution remained visible. The time of steeping was 4 hours, at 18°, germination taking place at 10°.

With Uspulun concentrations of 0.05 and 0.1 per cent stimulated slightly, while those of 0.25 and 0.5 per cent retarded germination. After 48 hours the effect of the highest concentration was still noticeable, that of the others was about the same.

Little injury could be observed on the whole.

Rice was found to be stimulated by 0.02 to 0.03 per cent coppersulphate after 24 hours steeping, but the seedlings were injured. During later stages of development this effect wore off altogether. Similar observations were made with Uspulun.

NIETHAMMER advanced the opinion that the stimulating effect of Uspulun might be due to sterilisation rather than to stimulation.

Some attention must be paid to the statements of SENF (1925). His observations on the effect of coppersulphate require careful analysis. He treats his material, amongst others, with the following concentrations of coppersulphate:

0.25 %	for	30	min.	germination percentage	85.3
0.25 %	,,	60	,,	,,	90.8
0.5 %	,,	30	,, (normal)	,,	84.5
0.5 %	,,	60	,,	,,	85.5
water	,,	30	,,	,,	87.5
water	,,	60	,,	,,	88
untreated			,,	,,	87.5

Material: wheat, Rimpaus Dickkopfweizen. From these figures he concludes that the seeds have been injured by the treatment. The average germination time was found to vary from 3.1 to 3.4 days.

The results with Uspulun have been treated in a similar offhanded manner. The material for attention is wheat, Kirsche Dickkopf. With the normal treatment, 0.25 per cent Uspulun

for 1 hour, he finds a germination percentage of 97 per cent, time 3.4 days. Other related figures are:

1	% for	15	min.	99 %,	time	3.5	days
0.5	% „	30	„	98 %,	„	3.3	„
0.25	% „	120	„	97 %,	„	3.3	„
0.25	% „	30	„	96 %,	„	3.5	„
0.1*)	% „	120	„	98 %,	„	3.1	„
0.1*)	% „	60	„	98 %,	„	3.2	„
water	„	120	„	95 %,	„	3.5	„
water	„	60	„	96 %,	,	3.2	„
water	„	30	„	95 %,	„	3.7	„
untreated				95 %,	„	3.5	„

The marked treatments should have stimulated. The variation, in the average germination time should make one rather cautious not to rely too much on the deductions made. The material shows marked variations and the mean error, apparently, has not been considered.

The fact that conclusions as to the influence of a certain treatment are based on the means of the results obtained with a number of different varieties, which each show a different behaviour towards the desinfectant, expose his deductions to serious objections. It is of little importance that he finds that coppersulphate should be unsuitable for desinfection.

Own Experiments: Material: Dwarf Yellow Milo.

To determine the influence of time, temperature and concentration of a desinfectant solution, two concentrations of coppersulphate were used, viz.: 0.1 and 1.2 per cent. The seeds were steeped for 1, 6 and 12 hours at 0°, 30° and 45°, and laid out to germinate at 30°. A control test was made wherein water took the place of the coppersulphate solution.

The samples consisted of 100 seeds each.

If any simple relation did exist, 12 hours steeping in 0.1 per cent should have an equal effect as 1 hour steeping in 1.2 per cent. The influence of the temperature should become evident from the results. The results have been tabulated in Table XI.

TABLE XI

Sample No.	Temp.	Time hrs.	Treatment	Germinated after hrs.					Germ. %	A.G.T.	Germ. value
				24	48	72	96	120			
			$CuSO_4$								
1	0	1	0.1%	49	42	1	—	2	92	1.59	116
2		6	,,	5	75	4	3	—	84	2.13	79
3		12	,,	—	81	10	2	—	91	2.20	83
4	30	1	,,	56	35	8	—	—	99	1.52	130
5		6	,,	40	36	8	—	—	84	1.62	104
6		12	,,	—	89	3	—	—	92	2.03	91
7	45	1	,,	56	31	4	—	—	91	1.43	127
8		6	,,	4	36	6	—	—	46	2.04	45
9		12	,,	—	19	12	2	1	31	2.81	22
			$CuSO_4$								
10	0	1	1.2%	21	14	56	—	—	91	2.38	76
11		6	,,	—	4	84	—	—	88	2.95	60
12		12	,,	—	3	76	—	—	79	2.97	53
13	30	1	,,	12	9	69	—	—	90	2.63	68
14		6	,,	—	7	83	2	1	90	3.07	59
15		12	,,	—	8	59	5	—	67	3.18	42
16	45	1	,,	15	10	67	—	—	92	2.57	72
17		6	,,	—	1	21	—	4	26	3.27	16
18		12	,,	—	—	4	3	2	9	3.78	5
19	0	1	water	53	34	2	—	—	89	1.43	124
20		6	,,	—	84	4	—	—	88	2.05	86
21		12	,,	—	84	2	—	—	86	2.02	85
22	30	1	,,	59	32	—	—	—	91	1.35	135
23		6	,,	38	52	1	—	—	91	1.59	114
24		12	,,	11	83	1	—	—	96	1.92	100
25	45	1	,,	63	25	—	—	—	88	1.28	138
26		6	,,	1	21	5	—	—	27	2.15	25
27		12	,,	—	3	2	—	—	5	2.40	4

Sample No. 24, steeped in water for 12 hours, at 30°, was taken as the one with which the others might be compared.

Effect of time, temperature and concentration of $CuSO_4$, upon further development at 30°.

Analysis of the results:

a. The control samples.

The highest germination values are obtained where the seeds are steeped in water for one hour only, as long as the temperature is high. Little difference exists between the values of 30° and 45°. The low temperature retards the rate of development and this effect has not yet worn off after about a day and a half. Longer exposures to 45° cause a rapid drop in the germination percentage and everything points to the material being vitally injured. At 30° the longer steeping causes lengthening of the germination time, which phenomenon is less pronounced at 0°, though quite prominent.

b. The treated samples.

The temperature influence is here of the same nature as in the controls. In the dilute coppersulphate series the different top values agree closely with those of the controls. This agrees well with the observations made on the rate of water absorption and germination with the Klerksdorp variety. A slight retarding influence of dilute coppersulphate is evident. The more concentrated solution has a pronounced inhibiting influence on the average germination times and consequently on the germination values.

A remarkable effect is that of the dilute salt solution on the seeds exposed to high temperatures. When comparing the figures of samples 8 and 9 with those of 26 and 27, it is clear that the first values show a great advance over the second ones. Considering all sets of samples, it appears as if the coppersulphate in this case has assisted in the development of the seedlings. The only explanation which I could find is, that the seedcoats of the seeds, during the swelling process, cracked and that some small quantity of the salt itself has come into contact with the embryo and has counterbalanced the effect of the temperature. To a certain extent this might be considered as stimulation, though it is preferred not to use that term, since the term has generally been applied for an increase of the germination percentage at normal temperatures. At and below the optimum temperature there is no stimulation.

The relative toxic effect of coppersulphate is not increased by a rise in temperature.

The general development of the experiment was indicated already after 12 hours. At that time the following samples were germinating, showing the tips of the radicles: 1, 4, 5, 6, 7, 10, 13, 16, 19, 22, 23, 24 and 25.

This is an ideal illustration of the effect of the optimum temperature and of the inhibiting action of the concentrated salt solution, when applied for 6 and 12 hours (14, 15).

The effect of the treatment on the development of parasitic fungi was carefully noted. Dwarf Yellow Milo, at least the sample at my disposal, was infected with *Helminthosporium*.

The controls showed no infections on seed which had germinated within 24 hours. It is possible that some of these seeds were infected, but the development of the embryo left no time to observe this. After 48 hours the following observations could be made:

sample	19,	on	germinated	seeds	23	infections,	on	rest	of	18,	8 inf.
,,	20	,,	,,	,,	38	,,	,,	,,	,,	16, 13	,,
,,	21	,,	,,	,,	48	,,	,,	,,	,,	16, 8	,,
,,	22	,,	,,	,,	14	,,	,,	,,	,,	9, 5	,,
,,	23	,,	,,	,,	10	,,	,,	,,	,,	10, 4	,,
,,	24	,,	,,	,,	13	,,	,,	,,	,,	6, 2	,,

samples 25, 26 and 27 no infections at all.

The exposure to 45° for one hour or longer reduces the number of infected seed to practically nil. It is in agreement with the general observations on desinfection that the percentage infection decreases when the temperature is raised above the optimum of the parasite. The hot water treatment of cereals requires a 10 minutes steeping in water of 51° to 53°, and a longer period at a lower temperature appears to be equally effective. This could be observed on Klerksdorp as well, where the seeds that had been soaked longer than 12 hours at temperatures close to and above the optimum showed no infection at all.

The following observations were made on the seeds treated with 0.1 per cent coppersulphate:

sample 1. normal in every respect, 2 seeds infected.

sample 2. and 3, roottips, in particular the tip of the coleorhiza, slightly brown, roots developing normally, though slower than in controls.

„ 4. 5, 6, 7, roottips slighly brown, roots developing slower than controls, root-hair development delayed.

„ 8 and 9, germinating slowly, no signs of injury.

After 72 hours more infection appeared on 1, 2 and 4, the others remained perfectly free from infection. The radicles were generally thinner than in the controls and in some the development of root-hairs took place rather late. It was impossible to determine in how far this is an important feature, it did occur in some of the controls as well.

The treatment with 1.2 per cent coppersulphate resulted in complete desinfection. After 48 hours many seeds had developed normal plumules, but as no radicles were present or any other roots, the seeds were not considered to be germinated. It appeared as if the coleorhiza could not be pierced by the radicles and in some cases the radicles themselves were injured. The colour of the affected parts was deep brown to black.

After 48 hours the following notes were made:

sample 10, plumules normal in 53 seeds, but roots undeveloped.
„ 11 „ „ „ 79 „ „ „ „ „
„ 12 „ „ „ 63 „ „ „ „ „
„ 13 „ „ „ 69 „ „ „ „ „
„ 14 „ „ „ 60 „ „ „ „ „
„ 15 „ „ „ 61 „ „ „ „ „
„ 16 „ „ „ 61 „ „ „ „ „
„ 17 „ „ „ 14 „ „ „ „ „
sample 18 showed no remarkable features.

Table XI shows that within 24 hours these seeds had all produced roots. In some the radicle had finally pierced the coleorhiza and was visible as a white tip, in others adventitious roots had developed.

No relation between time, temperature and concentration could be observed.

A much better comparison between the effects of coppersulphate and Uspulun could be made on the Klerksdorp seeds, as reported in the tables XII to XVII. They are shown in the germination values.

TABLE XII

Temp. 15°

Time of soaking in Hrs.	H_2O		0.1% $CuSO_4$		2% $CuSO_4$		0.25% Uspulun		I	II	III	IV
	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.				
1	7.80	5.5	8.28	6.0	—	—	7.59	6.2	40	34	—	36
2	9.14	5.5	8.84	6.3	7.34	6.6	9.10	6.4	43	37	36	37
3	10.58	5.6	11.09	6.3	9.93	6.7	10.86	6.4	32	41	36	36
4	11.64	5.5	11.78	6.6	10.42	6.7	11.19	6.8	40	41	35	37
5	11.68	5.6	11.39	6.7	11.58	6.7	12.49	6.3	43	41	39	38
6	12.99	5.6	12.18	6.6	12.92	6.7	13.52	6.3	43	44	39	41
7	14.48	5.6	14.56	6.5	14.16	6.9	14.21	6.3	39	39	44	41
8	15.56	5.5	14.66	6.5	14.04	6.9	15.32	6.6	42	40	40	43
9	16.51	5.8	15.55	6.6	14.18	6.8	15.44	6.6	31	43	39	39
12	18.17	5.5	17.24	6.6	16.57	6.7	21.09	6.5	40	44	41	43
24	22.14	5.6	22.31	6.6	23.30	6.7	24.14	6.5	42	36	39	37
24	22.45	5.6	22.16	6.6	23.30	6.6	24.05	6.4	42	36	35	39
30	25.67	5.6	25.06	6.6	23.76	7.0	25.30	6.3	39	41	34	42
36	27.10	5.7	26.42	6.8	26.17	6.7	25.82	7.0	39	39	34	38
Av. 1st. 6 hrs.		5.55		6.42		6.68		6.40				
Av. 1st. 12 hrs.		5.57		6.47		6.74		6.44				
Av. last 24 hrs.		5.62		6.65		6.75		6.55				

Absorption of water, average germination time and final count as influenced by desinfectants at 15°.

TABLE XIII

Temp. 20°

Time of soaking in hrs.	H_2O		0.1% $CuSO_4$		2% $CuSO_4$		0.25% Uspulun		I	II	III	IV
	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.				
1	8.46	3.0	7.97	3.9	—	—	7.33	3.3	40	39	—	45
2	11.42	2.9	10.77	3.4	10.34	3.8	10.01	3.3	44	44	42	45
3	13.54	3.1	11.53	3.7	12.52	3.8	12.54	3.6	45	45	44	40
4	14.03	2.9	13.67	3.7	13.83	3.6	14.00	3.5	43	45	41	47
5	16.16	3.0	14.25	3.7	15.29	3.8	14.68	3.4	43	45	42	45
6	17.25	3.3	16.65	3.8	15.25	3.5	16.63	3.5	42	41	46	46
7	17.94	3.1	18.16	3.7	17.27	3.5	17.20	3.5	45	41	42	47
8	19.01	3.1	18.03	3.7	17.28	3.6	17.91	3.5	44	40	47	42
9	20.51	3.1	20.08	3.7	19.51	3.7	18.86	3.8	41	43	41	47
12	21.57	3.2	21.35	3.9	20.38	3.7	22.19	3.8	44	40	45	41
18	24.35	3.3	27.46	4.2	24.79	3.8	24.95	3.9	44	40	40	43
24	27.26	3.4	28.06	4.2	26.01	3.7	28.13	3.9	43	40	43	43
30	28.88	3.4	29.53	4.0	28.70	4.0	28.11	4.0	45	42	42	46
36	30.53	3.8	29.88	4.2	28.89	4.1	31.02	4.6	41	43	42	36
Av. 1st 6 hrs.		3.03		3.70		3.74		3.43				
Av. 1st 12 hrs.		3.07		3.72		3.69		3.52				
Av. last 24 hrs.		3.48		4.15		3.90		4.10				

Absorption of water, average germination time and final count as influenced by desinfectants at 20°.

TABLE XIV

Temp. 25°

Time of soaking in hrs.	H_2O		0.1% $CuSO_4$		2% $CuSO_4$		0.25% Uspulun		I	II	III	IV
	% H_2O	A.G.T.	% H_2O	A.G.T.	H_2O %	A.G.T.	% H_2O	A.G.T.				
1	8.69	2.4	6.83	3.2	—	—	7.43	2.9	45	45	—	44
2	10.18	2.3	8.74	2.7	8.60	3.2	10.23	2.7	47	49	48	46
3	13.64	2.3	11.14	2.7	11.26	2.8	13.04	2.9	46	45	47	45
4	14.45	2.3	13.71	2.7	13.72	2.8	16.43	2.2	48	48	47	41
5	17.70	2.4	14.97	2.8	14.95	2.6	17.67	2.4	47	46	45	46
6	17.66	2.3	16.00	2.8	16.00	2.7	18.04	2.1	48	48	42	49
7	18.35	2.3	18.37	2.8	18.58	2.6	20.82	2.3	46	47	44	45
8	18.96	2.3	18.60	2.8	18.32	2.7	20.62	2.4	49	46	44	44
9	20.90	2.3	19.62	2.7	20.16	2.7	22.31	2.3	48	48	46	47
12	21.82	2.3	22.15	2.6	22.51	2.5	25.41	2.2	49	48	44	42
18	27.28	2.3	27.27	2.6	27.78	2.6	28.00	2.3	46	44	48	46
24	28.76	2.3	28.88	2.9	30.02	2.6	29.87	2.6	48	47	45	44
30	28.56	3.0	30.97	2.9	31.60	2.7	30.23	3.0	47	46	46	47
36	31.27	3.0	31.52	3.0	—	3.1	32.43	3.1	46	46	46	46
Av. 1st 6 hrs.		2.33		2.82		2.82		2.53				
Av. 1st 12 hrs.		2.32		2.78		2.73		2.44				
Av. last 24 hrs.		2.65		2.85		2.75		2.75				

Absorption of water, average germination time and final count as influenced by desinfectants at 25°.

TABLE XV

Temp. 30°

Time of soaking in hrs.	H_2O		0.1% $CuSO_4$		2% $CuSO_4$		0.25% Uspulun		I	II	III	IV
	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.				
1	10.38	1.7	8.97	2.1	—	—	7.55	2.1	43	45	—	47
2	12.72	1.7	10.82	2.0	10.57	2.0	10.09	2.1	48	48	47	47
3	14.47	1.7	12.80	1.8	14.08	1.9	11.93	2.0	48	45	48	42
4	17.34	1.8	16.36	1.8	15.68	1.9	17.48	2.0	49	47	46	49
5	19.24	1.6	18.04	1.8	16.34	1.9	19.52	2.0	46	46	48	47
6	19.00	1.7	19.46	1.8	20.93	1.9	21.87	2.0	46	46	48	45
7	21.52	1.8	20.07	2.0	21.27	1.8	23.20	2.0	47	48	44	47
8	23.27	1.9	21.84	2.0	22.29	2.1	25.03	2.0	40	48	46	43
9	23.53	1.9	22.98	2.0	23.14	2.0	24.12	1.9	47	48	44	48
12	26.90	1.9	27.07	2.0	25.31	2.1	26.38	2.0	48	46	48	47
18	27.66	2.0	29.68	2.1	28.54	2.1	30.10	2.0	46	48	47	42
24	29.68	2.1	30.50	2.1	30.06	2.1	31.64	2.0	47	48	49	45
30	29.91	2.1	30.51	2.0	32.26	2.0	31.74	2.2	45	46	46	46
36	32.75	2.4	32.16	2.2	31.86	2.2	32.09	2.4	48	48	45	45
Av. 1st 6 hrs.		1.70		1.88		1.92		2.03				
Av. 1st 12 hrs.		1.77		1.93		1.96		2.01				
Av. last 24 hrs.		2.15		2.10		2.10		2.15				

Absorption of water, average germination time and final count as influenced by desinfectants at 30°.

Table XVI

Temp. 35°

Time of soaking in hrs.	H_2O		0.1% $CuSO_4$		2% $CuSO_4$		0.25% Uspulun		I	II	III	IV
	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.				
1	11.82	1.7	9.51	2.3	—	—	7.85	2.0	46	46	—	46
2	15.33	1.8	13.17	1.8	11.75	2.5	16.22	1.8	45	46	45	45
3	17.82	1.7	15.42	2.4	14.58	2.4	16.48	2.1	50	46	47	46
4	21.67	1.8	18.55	2.2	18.74	2.3	20.76	1.8	43	48	47	44
5	22.75	1.6	20.36	2.0	18.77	2.2	20.23	2.0	50	47	50	47
6	25.29	1.6	23.86	2.1	21.60	2.2	22.94	1.8	43	48	47	50
7	25.08	1.8	23.05	2.0	22.39	1.9	24.54	2.0	45	46	44	46
8	25.74	1.7	24.75	1.9	25.60	2.0	26.22	2.0	46	46	40	40
9	25.73	1.9	26.60	1.9	26.11	1.9	25.29	2.0	48	46	44	42
12	29.37	1.8	32.39	1.9	28.35	2.0	28.81	2.0	46	47	39	39
18	30.66	2.0	30.64	2.2	30.20	2.1	30.37	2.2	42	41	43	38
24	31.52	2.1	31.50	2.1	32.11	2.2	31.71	2.4	45	45	43	41
30	32.18	2.5	31.82	2.3	32.56	2.5	30.01	2.9	43	41	44	28
36	33.48	3.0	33.26	2.9	34.35	2.8	31.97	3.0	41	46	39	22
Av. 1st 6 hrs.		1.70		2.13		2.32		1.92				
Av. 1st 12 hrs.		1.74		2.05		2.16		1.95				
Av. last 24 hrs.		2.40		2.38		2.40		2.63				

Absorption of water, average germination time and final count as influenced by germination at 35°.

TABLE XVII

Temp. 40°

Time of soaking in hrs.	H_2O		0.1% $CuSO_4$		2% $CuSO_4$		0.25% Uspulun		I	II	III	IV
	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.	% H_2O	A.G.T.				
1	13.04	1.9	12.26	1.7	—	—	10.47	2.1	45	46	—	47
2	17.06	1.9	16.58	1.9	15.45	2.3	15.80	2.3	47	44	45	46
3	20.92	2.2	19.69	2.0	19.54	2.3	20.56	2.0	46	44	45	45
4	24.21	1.9	22.95	2.0	21.43	2.1	24.11	2.0	47	40	44	41
5	25.67	1.9	25.37	1.9	24.13	2.0	25.40	2.2	47	44	44	47
6	25.77	2.1	27.04	2.1	24.45	2.0	26.30	2.0	45	45	46	49
7	27.04	2.0	27.43	2.0	27.03	2.0	27.93	2.1	43	46	46	42
8	27.26	2.1	26.01	2.3	27.79	2.1	31.06	2.2	47	43	42	41
9	29.63	2.1	28.99	2.1	29.00	2.1	29.88	2.2	43	45	40	40
12	30.92	2.2	30.34	2.2	30.02	2.2	31.65	2.5	45	42	40	39
24	33.60	2.4	34.45	2.6	33.45	2.5	33.58	2.8	43	43	28	27
24	33.63	2.4	33.69	2.4	33.42	2.4	34.01	2.8	38	43	40	13
30	34.06	2.9	34.49	3.0	34.06	3.2	35.42	3.5	40	42	37	8
36	35.02	3.2	34.13	3.2	30.77	3.1	35.42	3.3	38	40	35	7
Av. 1st 6 hrs.		1.98		1.93		2.14		2.10				
Av. 1st 12 hrs.		2.03		2.02		2.12		2.16				
Av. last 24 hrs.		2.83		2.90		2.92		3.20				

Absorption of water, average germination time and final count as influenced by desinfectants at 40°.

TABLE XVIII

Temp. 45°

Hrs.	H_2O	0.1% $CuSO_4$	2% $CuSO_4$	0.25% Uspulun
2	21.90	20.05	18.14	14.13
4	27.75	25.73	26.01	23.60
6	29.61	28.91	27.89	28.14
8	31.26	30.89	30.53	30.11
10	32.73	32.73	31.09	32.08
24	36.29	37.06	36.14	37.32
30	36.23	36.79	37.08	37.66
36	37.05	36.42	36.62	38.16

Absorption of water from different solutions at 45°.
No germination took place.

Before considering these data, however, we have to know the mean error of the material. The figures of Table I give a mean germination value of 100 ± 1.1, the mean error does scarcely exceed one per cent. The germination values are calculated and tabulated for the main experiments, in the same manner as the average germination times of table VIII have been calculated, and are presented in Table XIX.

The values have been calculated to the basis of 1 to 6 hours steeping in water at 35°.

The values obtained at 15° have not been included in the table, since the desinfected seeds develop at a much slower rate than the controls and do not require any further discussion.

Considering the mean error of the material nothing points to a favourable stimulating effect. Furthermore these figures show that the effect of coppersulphate about equals that of Uspulun, so that there is little preference to be made for the one or other desinfectant. Both have a decidedly depressing influence on the germination process.

Very little distinction can be made between the values of the controls at 30° and 35°, except that prolonged steeping at

TABLE XIX

Temp.	water	0.1% $CuSO_4$	2% $CuSO_4$	0.25% Uspulun		
20	52	43	42	48	1— 6 hrs.	soaking
	52	42	43	47	1—12 hrs.	,,
	46	37	39	46	12—36 hrs.	,,
25	74	61	60	66	1— 6 hrs.	,,
	75	62	61	68	1—12 hrs.	,,
	65	59	62	62	12—36 hrs.	,,
30	101	90	91	84	1— 6 hrs.	,,
	96	89	87	85	1—12 hrs.	,,
	80	83	83	76	12—36 hrs.	,,
35	100	81	76	88	1— 6 hrs.	,,
	98	84	76	84	1—12 hrs.	,,
	66	67	65	45	12—36 hrs.	,,
40	86	84	77	80	1— 6 hrs.	,,
	83	80	76	74	1—12 hrs.	,,
	52	53	44	16	12—36 hrs.	,,

the higher temperature depresses the germination value. This is a regular occurrence, but most striking at high temperatures. The values for 1 to 6-, and for 1 to 12 hours soaking are practically identical. While Table XI showed that a one hour's period of soaking may be considered more advantageous, the desinfection of the seed is frequently not completed within such a short period. The notes made during the course of the experiments support this.

At 15° the controls showed, after 3 days, 16 per cent of the total number of seeds to be infected with *Helminthosporium*, and one seed infected with *Fusarium*. Those treated with 0.1 per cent coppersulphate showed only 1 per cent infected. A slight increase in the number of infected seeds was observed during the following days, in the first case by 1 per cent, in the second ½ per cent. One infected seed only was found in the series treated with 2 per cent coppersulphate, in the Uspulun series nearly 8 per cent were infected, and these seeds had all been treated less than 6 hours.

At 20° the infections were counted after 48 hours. At that time only 6 per cent were infected in the controls, but this

number increased to about 8 per cent during the next 24 hours. Of the seeds treated with 0.1 per cent coppersulphate only 1 per cent became infected, all when treated less than 6 hours; 2 per cent coppersulphate caused complete sterilisation; Uspulun again required about 6 hours before desinfection was completed.

At 25° the conditions for the development of the seed became much better, while those for the infection had gone backwards. The required times to bring about desinfection are reduced and no fungi were noticeable on seeds which had been desinfected longer than 4 hours.

At 30° infection of the controls was visible here and there after 24 hours. In the one sample, steeped in water for 1 hour only, infection was rather severe, in all others the seedlings outgrew the fungus.

On some treated seeds *Helminthosporium* developed, but not until another 24 hours later. The total percentage of infected seed, in all series together, did not exceed ½ per cent, and again the 2 per cent coppersulphate was free from infection.

At 35° hardly any fungi did develop. The desinfected seeds showed none at all. Some of the seeds of the american varieties were killed by heat as was concluded from the appearance of droplets on the surface, in particular the seeds of the variety Dwarf Hegari appeared to be injured. Some of the seedlings of this variety showed rootlets with injured tips. This injury resembled that caused by 0.1 per cent coppersulphate and by Uspulun, the tips of the radicles were black. Adventitious roots appeared very soon and a complete seedling emerged well within 24 hours later. In the desinfected seeds several showed pronounced signs of root-injury, though here as well the injury was of passing nature.

At 40° the two american varieties were perfectly free from infection, but nearly killed by heat. All the seeds that did not develop showed droplets. Klerksdorp had practically no infections at all, the influence of the temperature was noticed by the curling of the roots, in particular of those seeds which had been treated with Uspulun. 2 per cent coppersulphate and the more dilute concentration also caused root-injury and root-curling but not to the same extent.

From these data no deduction can be arrived at which would support the opinion that seed desinfection causes stimulation. In all cases the desinfected seeds show a retarded development, the degree of retardation being about the same for the three different treatments.

The toxic action of coppersulphate does not increase with a rise in temperature in my experiments, Uspulun, however, has a pronounced toxic effect when the seeds are steeped for periods longer than 12 hours. This effect might perhaps be ascribed to decomposition of the mercury-compound at high temperatures, as a result of which inorganic mercury salts pass into solution and injure the germs. Coppersulphate is not influenced by a change in temperature and an increase of the toxic effect of coppersulphate could be associated only with a direct influence of this salt on the radicles of the germinating embryos.

Observations have shown that generally those seeds which are either dead or show a low viability are infected with *Helminthosporium* and succumb to the infection. In desinfected samples the percentage of seeds which might have been considered vitally injured by the treatment corresponds with that of the seriously infected seeds of the controls. The amount of damage, as a result of desinfection, closely corresponds with the percentage of seeds with low viability.

Injury can hardly be prevented when a desinfectant solution is applied. If such a solution has any killing effect on the spores of micro-organisms, it must be a potential danger to the embryos. If desinfection leads to selection of the material, this should not be considered a disadvantage. In agricultural practice this will be preferable to an infected crop.

While the application of mercury compounds will have to be preceded in tropical and sub-tropical countries by a determination of the dosis toxica and of the dosis curativa of these substances, the simpler inorganic salts do not require these tests to the same degree.

The results of the experiments described here show, that the practicability of the application of coppersulphate should obtain further attention.

SUMMARY.

1. Three varieties of *Andropogon sorghum Brot.* have been used for the investigations: Klerksdorp Kort Rooi, from South Africa, and Dwarf Hegari and Dwarf Yellow Milo, from the United States. Of these the first one was employed in the major number of experiments.
2. The swelling of seeds at different temperatures has been observed. The swelling maximum was found to be dependent on the temperature. Ground-up seeds, meal, showed a similar phenomenon.
3. The principal factors controlling the swelling process have been analysed: the rate of permeability of the testa is a limiting factor in this process, while the degree of permeability is influenced by the temperature.
4. The temperature ratios of the swelling process have been indicated to be determined by the degree of saturation of the material.
5. The germination rate has been observed to be inhibited by a period of presoaking of more than 12 hours, shorter periods of presoaking causing no principal differences.
6. The optimum temperatures for germination have been found to differ in the three varieties.
7. Abnormal root development in the seedlings has been observed and interpreted as a symptom of a slight degree of root injury; the causal factors in these experiments were the influence of heat and of desinfecting solutions.
8. The application of desinfecting solutions, coppersulphate: 0.1 per cent and 2 per cent and Uspulun: 0.25 per cent, did not increase the germination value of the samples which were tested; no stimulation could be noticed.
9. The temperature has no influence on the degree of toxicity of a 0.1 per cent coppersulphate solution; the dosis toxica of Uspulun decreases at higher temperatures.

10. A remarkable effect of 0.1 per cent coppersulphate at 45° has been observed (page), for which a tentative explanation is offered.

The study of the behaviour of ground-up seeds in the presence of solutions of electrolytes offers scope for further investigations.

Swelling and germination are linked processes. To determine the moment at which germination commences, it is suggested that this may be effected by exposing the seeds, after different periods of presoaking, for a short time, to a high temperature of about 55°; seeds which are germinating will then be killed, those which have not yet started growth will survive.

I want to express my sincere appreciation of the hospitality extended to me at the Botanical Institute of the University to Prof. Dr. F. A. F. C. WENT and Prof. Dr. V. J. KONINGSBERGER, as successive Directors of the Institute.

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STELLINGEN.

I

Het temperatuurquotient, Q_{10}, is, bij processen van wateropname en afgifte, afhankelijk van de graad van verzadiging met water. Bij alle proeven over Q_{10}'s dient men zich hiervan rekenschap te geven.

II

Het is onmogelijk in de voedingsphysiologie der planten te onderscheiden tusschen voedingsstof en prikkelstof.

III

Er is geen reden om aan te nemen dat kopersulfaat in het bijzonder beschadiging van zaden tijdens het beitsen veroorzaakt.

IV

De proeven van WALDRON laten niet toe te besluiten dat een toename van het 1000 korrel gewicht alleen aan Helminthosporium-infectie wordt toegeschreven.

J. Agr. Res. 48, 1934.

V

De proefopstelling van SUMNER, om het bestaan van beschuttende kleuren te bewijzen, is foutief.

Proc. N. A. S. 20, 1934.

J. W. PONT

VI

De proeven van SIERP maken waarschijnlijk dat de beweging van huidmondjes aan een photochemisch proces is toe te schrijven.

Flora, N. F. 28, 1933.

VII

De onderzoekingen van JAMES wijzen er op dat het geenszins zeker is dat de sapstroom bij worteldruk door de houtvaten plaats vindt.

New Phyt. 32, 1933.

VIII

Het bestaan van „mitogenetische" stralen is nog niet voldoende bewezen.

IX

De samenstelling van de Flora capensis en die van de Zuid Afrikaansche flora in het algemeen wijzen op een migratie in zuidelijke richting onder invloed van klimaatsverandering.

X

De omgrenzing van de families Liliaceae en Amaryllidaceae van HUTCHINSON verdient aanbeveling.