Massey Agricultural College
(UNIVERSITY OF NEW ZEALAND)

PALMERSTON NORTH, N.Z.

There are a number of obvious errors in this thesis. This is because it was written after the candidate left for U.S.A and no draft copy was submitted for comment.

G. V. Fife

28.4.58

A STUDY OF THE CAUSE OF GERMINATION INJURY FOLLOWING CONTACT PLACEMENT OF DRIED BLOOD FERTILIZER WITH SEEDS

A Thesis

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Nancy L. Baigent

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

The use of organic fertilizers has suffered a decline since the initiation of large scale production of soluble inorganic salts. Nevertheless, a demand for natural organic forms of nitrogen persists, especially in regions of high rainfall and sandy soils, and in connection with the production of crops of high acre value.

In recent years knowledge of the principles and practice of fertilizer application also has expanded rapidly. Localized or contact placement of fertilizer has been found to favour rapid early growth, and to lessen "fixation" of fertilizer nutrients by the soil. Difficulties in the forms of impaired germination and damage to young seedlings have arisen from contact placement of organic fertilizers. Many mechanisms have been put forth to explain the basis of fertilizer toxicity;viz. plasmolysis of root tissues by the high solute concentrations, production of excessive local acidity or alkalinity from fertilizer materials, direct toxic effects on the young plant by free ammonia or cyanide formed by chemical or microbial breakdown of fertilizer materials.

Accordingly, it was decided to evaluate the hypothesis that germination injury to seeds results from the production of injurious quantities of free ammonia during mineralization of dried blood fertilizer placed in contact with the seeds.

Since nitrogen is taken up by the plant chiefly as nitrate, or sometimes as ammonia, complex forms of nitrogen present in organic fertilizers must undergo mineralization in the soil before becoming available to the plant. In this process, micro-organisms convert protein nitrogen into ammonium salts, and thence to nitrites and nitrates. Excessive water, or the absence of oxygen from any other cause, tend to favour an anaerobic microflora, and may result in stopping the nitrification process, and in the reduction of such nitrates as were already formed under more favourable conditions. That ammonification and nitrification are not necessarily correlated was once more confirmed by Fraps and Sterges (1947). Since ammonification was still significant in all extreme cases, the result was an accumulation of ammonia under the influence of such factors as too high and too low pH, and too high and too low temperature.

Many investigations clearly point to a volatilization of ammonia, not directly from organic substances such as dried blood, but from free ammonia after ammonification. (Screenivasan and Subrahmanyan (1935), Willis and Sturgis (1945), Pochon and Tchan (1947), Pochon et al (1947), to name a few such studies.) Accordingly, a review of the relevant literature on injury to germination through the presence of toxic concentrations of free ammonia will now be presented.

Russell and Petherbridge (1913) found that sand cultures containing 10 parts per millon of nitrogen as free ammonia, retarded the germination of turnip seed, whereas with 100 parts per millon of nitrogen no seed germinated. Since the

sand cultures contained 16.7% of moisture, it appeared that 0.006% of nitrogen as free ammonia was injurious and that 0.06% was fatal.

The work of Willis and Piland (1931) supports the idea that free ammonia in high concentrations acts as an inhibitor of seed germination and plant growth. The fertilizer used was a mixture of C.P. diammonium phosphate, potassium nitrate, and potassium chloride. The free ammonia formed by the hydrolysis of the diammonium phosphate was apparently the most toxic component of the fertilizer mixture for the young cotton seedlings. No injury was observed from the use of ammonium as the sulphate, chloride or nitrate. Nor did the alkalinity of the diammonium phosphate appear to contribute to the injurious effect. Monoammonium phosphate produced a lesser degree of injury than did diammonium phosphate.

The hypotheses were investigated that hydroxyl ions or free ammonia were the toxic factors. This was accomplished through the use of calcium carbonate as a treatment supplemental to applications of diammonium phosphate, and through calcium carbonate and calcium sulphate as supplementary treatments to ammonium hydroxide. There was similarity in the degree of injury from ammonium hydroxide and from diammonium phosphate, although this did not prove that the causes were quantitatively alike. However, the effectiveness of calcium sulphate as a corrective of ammonium hydroxide toxicity gave strong support to that conclusion. The failure of calcium carbonate to correct

the injury from the diammonium phosphate was not thought to controvert the fundamental property of calcium in this respect, because of the possibility, which was suggested, that the calcium carbonate might increase the concentration of free ammonia without correspondingly increasing the concentration of calcium available for antagonism.

The slight but probably significant decrease in the extent of injury from ammonium hydroxide when supplemented by calcium carbonate, eliminated the possibility that a reduction in the concentration of hydroxyl ions was the sole cause of the previously noted remedial effect of the calcium salts. This result with calcium carbonate and ammonium hydroxide strongly indicated that the calcium has a direct physiological effect independent of any chemical reaction within the medium. There was the possibility, however, that the greater efficiency of the sulphate was not due to a more intensive antagonism, but to an additive effect of a chemical reaction, probably the formation of the non-toxic ammonium sulphate.

Willis and Piland (1931) concluded that, in practice, fertilization with diammonium phosphate, or other materials productive of free ammonia, might not be injurious on highly absorptive soils, or when the fertilizer was applied long enough in advance of planting to provide for complete absorption. Under other conditions the use of gypsum as a supplement might constitute an effective means of control. Ground limestone would probably not serve the same purpose. They considered that this type of fertilizer injury constitutes a problem only with germinating

and seedling plants, and it is probable that the ultimate effect on the crop would depend on the nature of the rooting systems of the plants fertilized. Taprooted plants would naturally be most subject to damage, but this might be corrected by the later development of the lateral roots.

That injury to germinating seeds by free ammonia occurs was also demonstrated by Barton (1940). She investigated the effects on various organisms of different concentrations of gases applied for several periods of time. Germination of water-soaked seeds of radish exposed for as long as 240 minutes to 1000 parts per million of ammonia gas was not only delayed, but reduced. Extension to 960 minutes killed off all the seeds. In no case did exposure of dry seeds of radish to ammonia gas cause reduction in germination percentage. Rye seeds were more sensitive than those of radish. Exposure of soaked rye seeds to 1000 parts per million of ammonia gas for 240 minutes resulted in 100% kill, while those exposed to 250 parts per million for 960 minutes had a germination capacity of only 48%.

Duisberg and Buehrer (1954) studied the extent of the inhibitory effect on seed germination of applications of ammonia by irrigation or by injection. Ammonia applied by surface irrigation in an amount to give a concentration of 230 parts per million of nitrogen on the dry soil basis, prevented the germination of barley, whereas when applied by injection, a concentration of 270 parts per million of ammonium nitrogen not only permitted germination, but resulted in more vigorous plants than in the controls.

To determine the limit of ammonia concentration at which germination is entirely prevented, pots of soil uniformly injected with ammonia at a series of concentrations were planted to barley. Germination was complete at all ammonia concentrations up to 450 parts per million during the first 10 days after planting. Increase in concentration of ammonium nitrogen tended to delay germination.

Independent germination tests were made to determine whether inhibition is due to the initially high hydroxyl concentration, or to ammonium ion concentration. This was done by surface-irrigating the soil with calcium hydroxide, ammonium chloride, and ammonium sulphate solutions prior to planting. Germination was not inhibited by hydroxyl ions at an initially high concentration as long as the ammonium ion was absent. Ammonium nitrogen at concentrations above 450 parts per million prevented germination completely. At lower concentrations of ammonium ion germination occurred, but the plants were feeble and stunted. The plants growing in the ammonium sulphate-treated soil were, in general, more vigorous than those growing in the ammonium chloride-treated soil, which pointed to a possible toxic effect of the chloride ion.

The literature on the subject of causes of germination injury through contact placement of dried blood with the seed, is limited. Sayre and Clarke (1935) found that many organic fertilizers are injurious to seeds and roots of plants, when first applied to the soil. Their experimental results with peas, beans, and corn in the glasshouse were confirmed by field trials.

They found that the lower rate of solution of organic fertilizers, as compared with inorganic fertilizers, indicated that the cause of injury to roots resided in some factor or factors other than excessive concentration of soluble salts resulting in plasmolysis of root tissue. They considered that injury from organic fertilizers was caused by various soluble organic substances such as amino-acids and soluble peptides. In addition it was found that organic fertilizers greatly stimulated mould growth, with the logical conclusion that they might also stimulate more rapid growth of parasitic organisms that attack roots and seeds in the soil, and thus indirectly cause injury.

The period of injurious concentration was found to vary with the rate of application, the soil moisture content, and the soil texture. In their experiment with dried blood, it required from 9-14 days in the soil before this fertilizer was no longer toxic to roots. The detrimental effects of dried blood were definitely restricted to an area in close proximity to the fertilizer band, as shown by the fact that seeds sown $\frac{1}{2}$" to the side of the fertilizer band germinated satisfactorily.

Sherwin (1923), working with cotton and corn seeds, found that organic fertilizer reduced the germination count by causing the death of the seedling before its appearance at the soil surface. He noted absence of roothairs and considerable root decay. The growth of fungi was also stimulated, especially those fungi which are injurious to root systems.

The mineralization studies and germination injury experiments reviewed in the foregoing section suggest that the most likely source of germination injury through contact with dried blood is the production of free ammonia during the mineralization process. Realizing the practical difficulties of demonstrating directly the role of free ammonia production in a system in which water is present, it was decided to approach an answer by the indirect procedure of showing (1) the production of ammonium-nitrogen in quantities sufficient to constitute a source of germination injury and (2) both qualitative and quantitative similarity in germination injury caused by free ammonia from inorganic sources, and that resulting from application of the dried blood fertilizer.

Chapter II

Experimental Materials and Methods

General methods and selection of materials are described in this section. Special techniques which were developed for particular experiments are described in their approximate context.

Fertilizer

The fertilizer used throughout the experiments was a standard dried blood mix from the fertilizer bins of the Field Husbandry Department. The fertilizer was ground and passed through a 2 mm. sieve, thus enabling a more representative sample to be obtained when small quantities of fertilizer were being weighed. Determination of nitrogen content by the Kjeldahl method showed the dried blood to contain 13.6% nitrogen.

Media For Germination Studies

Three media were used in which to study injurious fertilizer effects on seed germination.

(1) Expanded perlite
(2) Washed river sand
(3) Steam-sterilized soil

The three media were compared with respect to their relative merits in evaluating the injurious effects of organic fertilizers upon germination of seeds.

Three varieties of seeds were used, so as to include a broad spectrum in speed of germination. The varieties were cress (very quickly germinating in 4-5 days), cabbage (germinating in 7-8 days), and carrot (requiring 14 days to complete germination).

The fertilizer was dried blood, applied to the media in 6 inch clay pots, one pot per fertilizer treatment. Treatments could not be replicated because there was a shortage of pots. Fertilizer was applied in contact with the seeds: at 3 levels; namely, 7 g., 14 g, and 25 g. per pot. In another series, fertilizer was broadcast at the rate of 7 g. per pot. Control pots containing only the media were included in the experiment. Where dried blood was applied as a contact fertilizer with the seed, the pots were filled with the appropriate medium to within two inches of the top and the fertilizer was spread evenly over this surface. The pot was watered well until water leached freely from the base. The seeds were then distributed evenly over the surface in contact with the fertilizer, and were covered lightly with a further layer of the medium. Where dried blood was applied as a broadcast application, the fertilizer was worked thoroughly into the top 2-3 inches of medium in the pot.

Results: The effects of fertilizer applications in different media, on seed germination of cress, cabbage and carrot are presented in Table I.

With increasing rates of application of dried blood in contact with the seed, there was a corresponding decrease in percentage germination in all media. Though the treatments were not replicated for each variety, the same trend was observed with the three

TABLE I

Influence of Medium On Germination Injury By Contact Placement of Dried Blood Fertilizer.

Seed Variety	Medium	Percentage Germination				
		No Fertilizer	Contact 7g. dr.b.	Contact 14g. dr.b.	Contact 25g. dr.b.	Broadcast 7g. dr.b.
Cress	Perlite	92	33	10	2	78
	Sand	90	49	25	6	91
	Soil	92	75	49	30	89
Cabbage	Perlite	96	8	9	2	67
	Sand	90	39	26	16	83
	Soil	96	81	66	35	98
Carrot	Perlite	65	0	0	0	0
	Sand	63	0	0	0	0
	Soil	75	20	0	0	24

different varieties, in greater or lesser degree. The depressing effect on seed germination of increasing rates of application of dried blood was apparently less severe in the cases of quickly-germinating seeds such as cress and cabage, than in the case of carrot which required a full 14 days to complete germination.

Dried blood in combination with perlite appeared to have the most damaging effect on germination of cress and cabbage. Dried blood, applied as a contact fertilizer in soil had the least depressing effect on germination of cress and cabbage, while dried blood in sand was intermediate in effect. Broadcast applications of dried blood did not appear to have any effect on germination of cress and cabbage.

For carrot seed, since only the control pots showed any germination of seeds planted in perlite and sand, no distinction can be made between these two media, under fertilizer treatments. Approximately 20% of the carrot seed germinated when dried blood was applied at the lowest rate in contact with the seed in soil, or as a broadcast treatment in soil. Higher rates of application completely depressed germination in this trial.

The effect of the dried blood was most marked in perlite, not only in depression of emergence of seedlings, but also in the general thrift of the seedlings immediately after emergence. Affected seedlings were stunted and deformed in many cases. The young radicles were foreshortened, brown, swollen at the tips, and roothairs were absent. The dried blood fertilizer placed in contact with the seed formed a band, or zone, of injury. Once the young radicle had succeeded in penetrating and

developing beyond this zone, the seedling started to grow strongly.

The glasshouse, being in the last stages of construction, was not being run as a propagating unit. The lack of shading on the glasshouse and the fact that it could not be maintained at high humidity, resulted in wide fluctuations in temperature, and made maintenance of water content within the pots difficult. In view of these facts the next experiment was set in the propagating pits of the Horticulture glasshouse. These pits were thermostatically controlled to 75°F (24°C) with associated high humidity.

During these experiments with different media containing fertilizer, difficulties were experienced in wetting the material to which the fertilizer was added, probably due to the nature and fine particle size of the layer of dried blood.

Perlite retained water extremely well and no watering was necessary after the initial wetting. Sand and soil tended to dry out at the surface. Watering was arbitary and was carried out when necessary. All the pots were watered at the same time, including those containing perlite.

From this trial it would appear that soil exerts a slight "buffering" action on the injurious effects of dried blood. This may have been due to the adsorptive properties of the soil in removing from the soil solution substances which may otherwise have been toxic to germination.

Sand was selected as the medium for further experimental

work since it appeared to be intermediate in effect between soil and perlite, and exhibited no great absorptive properties.

Seeds

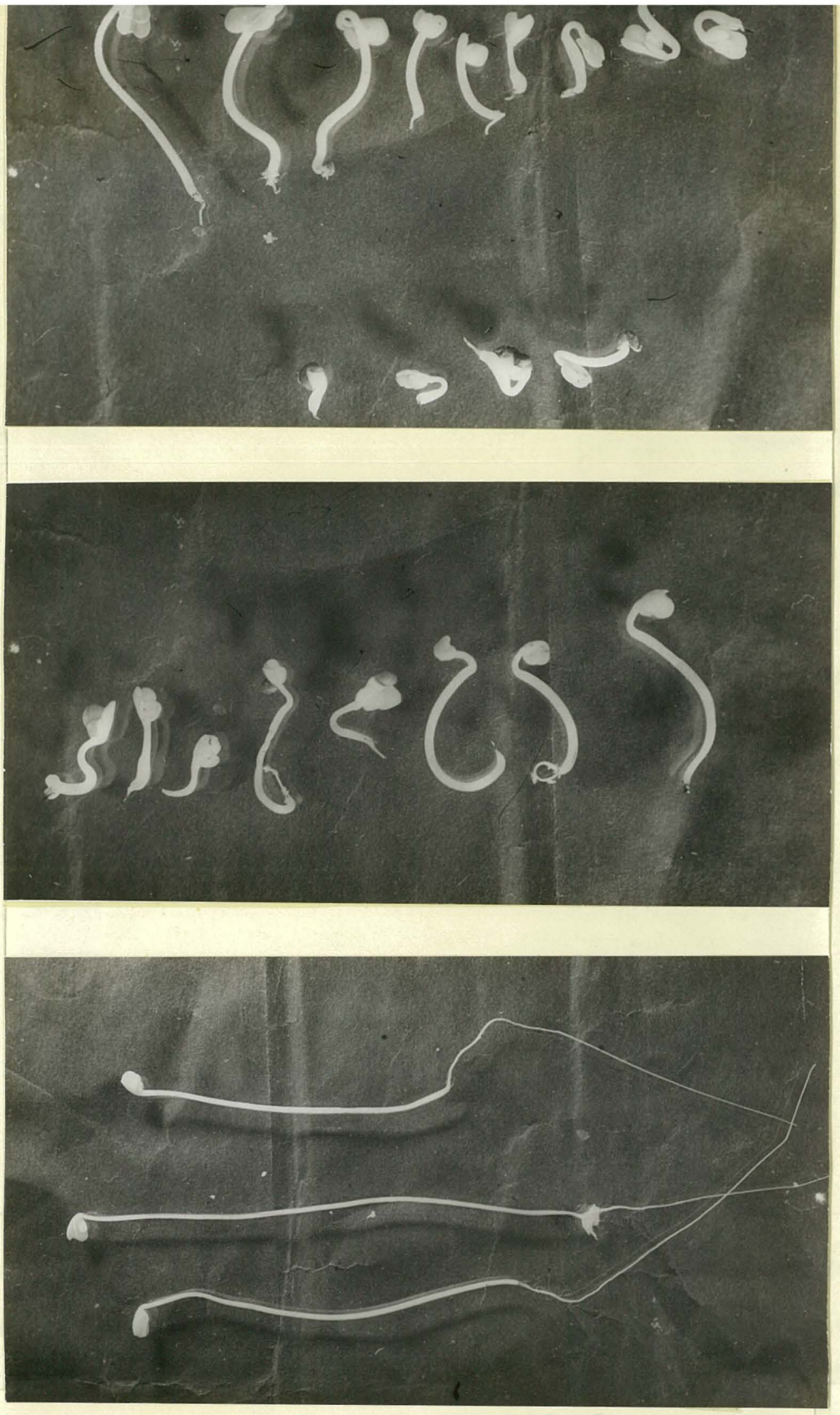
Since percentage germination figured prominently in all experiments, thorough familiarity with the germination process of the seeds was considered essential.

Seed Varieties

The word "variety" is used in the horticultural sense throughout this study. Seed varieties examined were:

Cress (*Lepidium sativum*)
Cabbage (*Brassica oleracea*)
Turnip (*Brassica rapa*)
Lettuce (*Lactuca sativa*)
Radish (*Raphanus sativus*)
Leek (*Allium porrum*)
Tomato (*Lycopersicum esculentum*)
Parsley (*Petroselinum crispum*)
Parsnip (*Pastinaca sativa*)

Five hundred seeds of each variety were placed on moistened filter papers in petri dishes, one hundred per dish, the seedlings being counted daily and removed from the petri dishes at the first green leaf stage. As incubator space was not available, the seeds were germinated on an open bench, at room temperatures ranging from 13° - $21^{\circ}C$. (55° - $70^{\circ}F$)



A. Deformed and Normal Germination of Cabbage Seedlings.

(1) Deformed seedlings, germinated in contact with 0.00375N ammonium hydroxide solution.

(2) Deformed seedlings, germinated in contact with 2g. dried blood fertilizer in sand.

(3) Normal seedlings germinated in contact with distilled

Germination Counts.

Germination may be defined as the emergence and development from the seed embryo of those essential structures which are indicative of its ability to produce a normal plant under favourable conditions. The observation was made in fertilizer injury experiments, that the radicle emerged from many seeds, but this was as far as germination and subsequent growth proceeded. Stunting of the seedling and ultimate decay frequently resulted. It was found necessary in making germination counts, to set some arbitary point in the development of the young seedling, at which germination was regarded as being satisfactorily concluded. This point was taken as the opening of the first green leaves, or cotylendonary leaves. At this stage, assuming no interference from other factors, the seedling could be expected to emerge from the soil and grow under natural conditions.

The seeds were individually examined for percentage germination, ease of handling of seeds in large numbers, speed and evenness of germination, and the percentage of healthy and deformed seedlings.

In recording results, seedlings were classed into three groups:-

(1) Normal ----- denoting seedlings which might have been expected to continue growth to normal plants.

(2) Deformed --- denoting seedlings where the plumule was present, but where the radicle was foreshortened, swollen, and without roothairs.

TABLE II

Speed Of Germination In Sand Of 10 Seed Varieties, Recorded As Percentage Germination

Seed Variety	Speed Of Germination																				
	4 days	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Cress	73	17	5	1	1	1															
Turnip	8	44	23	5	3	2															
Radish	8	41	25	21	1	1															
Cabbage		80	15	2	0	2															
Lettuce			88	5	0	2															
Leek				12	28	17	14	5	4	2	1	0	1								
Tomato					8	9	13	20	7	10	4	7	5	5	2	3					
Carrot						10	27	20	15	3	1	1	1								
Parsley												7	8	6	9	5	6	5	5	3	1
Parsnip												18	13	8	10	8	5	2	3	0	1

Stunting and subsequent death of seedlings usually resulted.

(3) Abnormal - - denoting diseased seedlings which had decayed, seedlings where germination had gone only so far as the emergence of the radicle with subsequent decay of seed and radicle, and hard seeds which had not germinated or were no longer viable.

Results:-

Speed of Germination:-

The results of seed examination are presented in Table II.

Seedlings were counted and removed from the petri dishes as soon as the first leaves appeared. Results presented in this Table are the average germination percentages of 5 replicates of 100 seeds for each variety.

Some seed varieties e.g. cress, lettuce, cabbage, turnip, radish, proved to be very quick in germinating, 7 days being the total germination period as recommended by the International Seed Testing Association. Germination was characterized by the majority of the seeds reaching the first green leaf stage at the same time. The initial germination count was therefore very high, and later counts were very low. This group was therefore designated, "quick-germinating."

Leek, carrot, and tomato seeds were placed in a second group of "medium-fast-germinating" seeds, requiring 7-16 days to complete the process to the first green leaf stage. Germination counts were moderately low, but were consistent

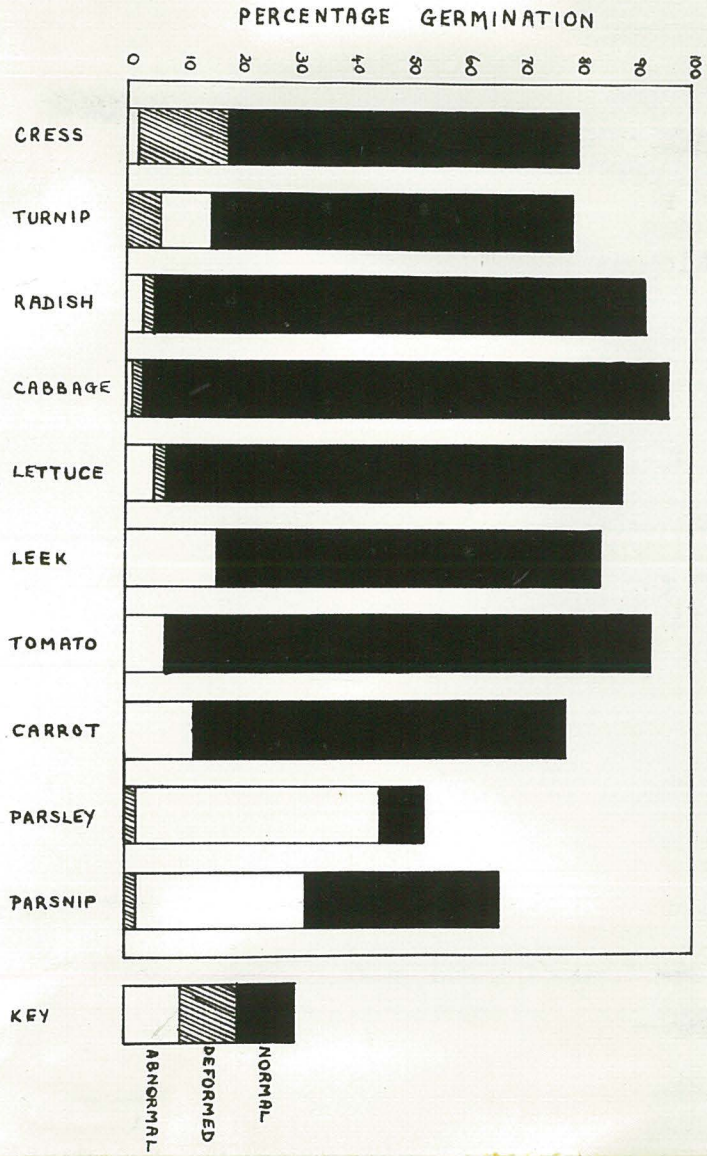


FIG. 1. Analysis of Germination into 3 Classes: Normal, Deformed, and Abnormal.

over this period.

The remaining seeds, parsnip and parsley, were classed as "slow-germinating" varieties, since not only did 14 days elapse before initial germination counts could be made, but the germination process was prolonged with very low daily counts.

Analysis of Seed Germination:-

An analysis of seed germination is presented in Fig. 1. Percentage germination was at, or above, the 80% level for most varieties. Total germination for parsnip and parsley was low, and a high percentage of the seed fell into the "Abnormal" class, apparently from lack of viability. Cress showed a high percentage of deformed seedlings.

As a result of the germination trials several varieties of seeds were discarded as being unsuited for the experiments to follow.

Tomato seeds, being extracted from pulp in the seed production process, tended to be stuck together in groups of 2 or 3 seeds. A percentage of the seed was also broken and chipped. Both these factors made seed counting slow and tedious. Though germination percentages were high, the germination process was very prolonged.

Parsley seed was discarded as unsuitable for further experiments as a result of the very long period required for germination to proceed, the ultimate low germination capacity, and the aggregation of the seeds into groups of twos and threes.

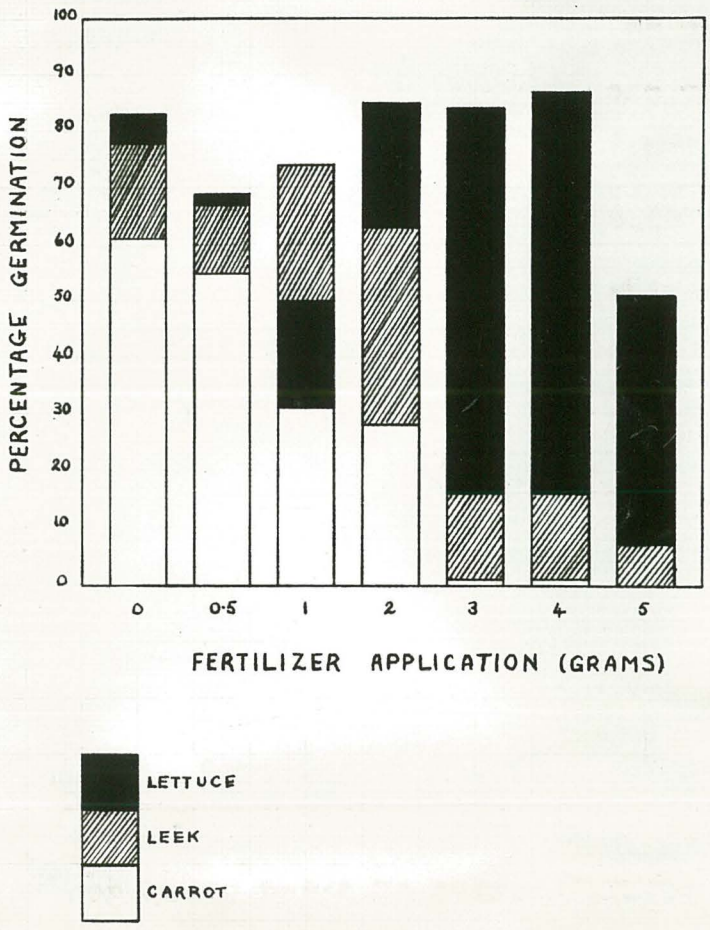
Parsnip seed was likewise discarded because of the long period required for germination, the low germination percentage in relation to other varieties, and the presence of fungal contaminants which could have been carried on the seed, or could have been favoured by nutrient substances from germinating seeds. Fungal contaminants were not present in the petri dishes in which parsley seeds were germinated, although the conditions and period required for germination of seeds were similar. The stimulation of moulds by organic fertilizers has been cited by some workers as a possible indirect cause of germination loss. Therefore, the possibility of fungal contamination of seed could not be permitted.

From the remaining varieties, lettuce, cabbage, radish, leek, and carrot, were selected for further experiments. These varieties all showed high percentage germination, a range in speed of germination, and the seeds were small but easily counted and handled. It was realized that difficulties in maintaining an even moisture content in pot experiments in the glasshouse would be experienced with seed varieties requiring periods greater than 14 days to complete germination. The choice of these varieties therefore gave 3 "quick-germinating" varieties in lettuce, cabbage, and radish, one relatively quickly-germinating variety in leek, and one variety requiring a 14 day germination period in carrot.

Effect of Contact Placement of Dried Blood Fertilizer on Percentage Germination.

Lettuce, leek, and carrot were selected for further trials, under glasshouse conditions. The speed of germination was the important factor in selecting these varieties. The range

FIG. 2. Glasshouse Trial: 3 seed varieties sown in contact with increasing rates of application of dried blood.



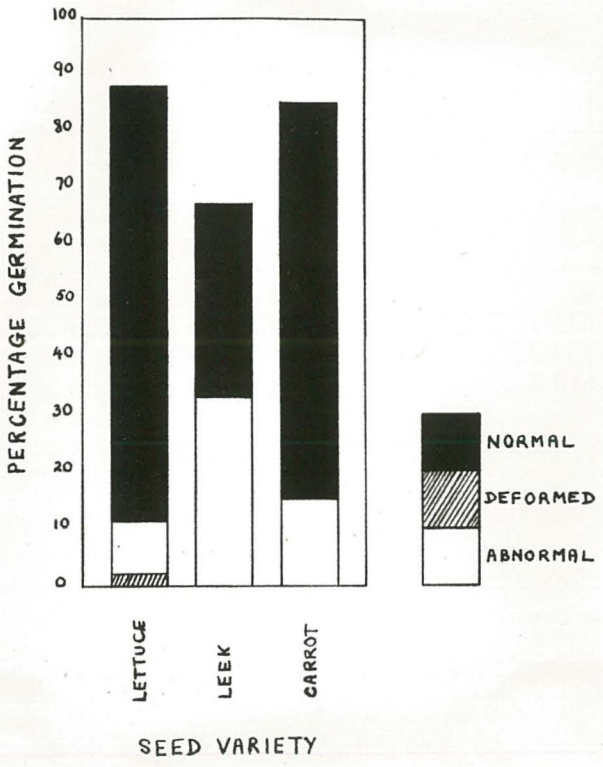
covered by germination period in this way, was from 5-7 days with lettuce seed, from 6-12 days with leek seed, and from 9-14 days with carrot seed. Though the nature of the seed varieties differed, there was in some part, a check on time effects of the organic fertilizer injury to germination of seeds.

Therefore, lettuce, leek and carrot seeds were germinated in sand, in contact with dried blood fertilizer. Fertilizer applications were at the rates of 0, 1, 2, 3, 4, and 5g. of dried blood. For each seed variety there were two 5 inch plastic pots containing 100 seeds per fertilizer treatment. The pots were filled with clean, washed river sand, the fertilizer sprinkled over the surface, the seeds sown and lightly covered with 1/8 inch of sand. The pots were watered until water leached freely from the base. On draining, the pots were covered with sheet glass, placed on the open bench in the glasshouse, and the seeds left to germinate. Germination counts were made daily.

Results:-

The results of the experiment, presented in Fig. 2, show the percentage germination of the 3 seed varieties under increasing rates of application of dried blood fertilizer. Increased rates of application of dried blood generally resulted in decreased total germination of seeds. Lettuce seed, except in one instance, showed the highest total germination at all rates of application, followed by leek and carrot seed, in that order. At rates of application of dried blood greater than 2g. per pot, the total germination of carrot seed was negligible.

FIG. 3. Analysis of germination of 3 seed varieties under incubator conditions.



Lettuce seed, a very "quick-germinating" variety, would appear to escape the maximum effect of decomposition products of the dried blood. Leek and carrot seeds with longer germination periods, were more subject to fertilizer effects. Though one seed variety may be more or less susceptible than another seed variety to organic decomposition products, it is likely that speed of germination is one of the most important factors in degree of injury to germination.

Germination Under Incubator Conditions:-

Since the original germination trials were carried out under fluctuating room temperatures, further samples of carrot, lettuce and leek seeds were tested for germination percentages in an incubator at 24°C. (75°F). The seeds were germinated in petri dishes, 100 seeds per dish, in the same manner as previously described. Five hundred seeds were examined for each variety.

Results:-

An analysis of the germination trials is presented in Fig. 3. Results are given as average germination percentages. Save for a general speeding up of the whole germination process for all the varieties, results merely confirmed the earlier work. Average germination percentages were slightly lower for lettuce and leek, and slightly higher for carrot. All varieties were judged suitable for further experimental work.

Chemical Analysis

Sampling:-

Sampling was carried out at all times immediately before analysis for ammonia and nitrate content.

Ammonia and Nitrate Determinations:-

Ammonia was determined by an adaptation of Olsen's method (Piper, 1942). The sample of approximately 80 g. sand (fertilizer-treated) was placed in a beaker, to which was added 50 ml. of 2N potassium chloride solution acidified with 0.1N hydrochloric acid pH 2. After stirring intermittently for 30 minutes, the suspension was filtered and then leached with 150 ml. potassium chloride solution.

For the determination of ammonia, the filtrate, together with 200 ml. distilled water and 3-4 g. of magnesium oxide, was heated in a distillation apparatus. The distillate was collected in standard sulphuric acid. The excess of acid was titrated with standard sodium hydroxide using a bromocresol green-methyl red indicator. The amount of ammoniacal nitrogen is recorded as milligrams of nitrogen per core.

For the determination of nitrate, the volume of the liquid in the distilling flask was restored to the original level with distilled water, and approximately 2g. of powered Devarda's alloy were added. The distillation was recommenced into a further quantity of standard sulphuric acid. The excess of acid was again titrated with standard sodium hydroxide, using a bromocresol green-methyl red indicator. The amount

of nitrate present is expressed as milligrams nitrogen per core.

Blank determinations were carried out using all the reagents employed in making the sand extracts.

Indicator:

The indicator used during all ammonia and nitrate determinations was a bromocresol green-methyl red mixture. 0.1% aqueous solutions of bromocresol green and methyl red were mixed in the ratio of 5:1 respectively.

Methyl red by itself was found to give an unsatisfactory end point. With the bromocresol green-methyl red mixture there was a definite blue-grey pink colouration following an alkaline-neutral-acid change.

pH Determinations:

pH determinations were made using a Cambridge portable pH meter fitted with Beckman glass and saturated calomel electrodes.

CHAPTER III

THE EFFECT OF ADDED CARBOHYDRATE ON THE SUSCEPTIBILITY OF SEEDS TO INJURY BY DRIED BLOOD

Since dried blood contains more than sufficient nitrogen to satisfy the demands of the proteolytic microflora, substantial amounts of both ammonia and nitrate will inevitably appear in a system in which dried blood is the major material undergoing decomposition.

If the extent of germination injury is determined by the concentration of either, or both, of these mineralized forms of nitrogen, it should be possible to reduce this effect progressively, by the addition of increasing levels of carbohydrate to a fixed amount of dried blood. Addition of finely-divided starch or cellulose should effectively raise the C:N ratio, and, because of the relatively slow degradation of these polysaccharides, it was expected that their effects would be continued over a lengthy period. The pot experiment described hereafter was designed to determine whether the incorporation of starch with dried blood resulted in an increased percentage germination of leek seed over that obtained in the presence of dried blood alone. The similar effect of cellulose is described in another connection in Chapter VI. below.

Method

The experiment was set up in 5 inch plastic pots, using sand as the medium to carry fertilizer and carbohydrate

treatments. Carbohydrate was added in the form of powered starch, commercial "Robin" brand.

Leek seed was used; one hundred seeds being sown per pot. Leek had certain advantages for this test: the percentage of germination was high in the standard trials on moist filter paper, the seeds were easily counted and handled, and the germination of individual seeds extended over 14 days. In the choice of seed variety for this study, the speed of germination is regarded by the writer as being a very important factor. By using leek seed, the effect of fertilizer and carbohydrate treatments could be evaluated over the full 14 day germination period. Germination was considered complete when the slender, looped cotyledon turned green.

Treatments

The treatments comprised:-

(1) Control pots containing only sand with the seeds.

(2) Sand, 3g. dried blood in contact with the seeds.

(3) Sand, 3g. dried blood and Ig. starch, in contact with the seeds.

(4) Sand, 3g. dried blood and 3g. starch, in contact with the seeds.

(5) Sand, 3g. dried blood and 9g. starch, in contact with the seeds.

(6) Sand, 3g. dried blood and 21g. starch, in contact with the seeds.

(7) Sand, 21g. starch in contact with the seeds.

(8) Sand, 9g. starch in contact with the seeds.

(9) Sand, 3g. starch in contact with the seeds.

Each of the treatments 1-7 was replicated 15 times, while treatments 8 and 9 were replicated 5 times only. In this way germination counts were replicated 5 times for each treatment and there remained 10 pots per treatment which were sampled for chemical analysis. Treatments 8 and 9 were not sampled, since any effects of starch treatments on germination would be magnified, presumably, at the higher rate of application of starch, 21g. of starch per pot.

The pots were set up in the following manner which modified according to the treatment applied. Brick crocks allowed for adequate drainage at the base of the pot. The pot was filled to the rim with sand. The powered starch and dried blood mixture which had been previously ground and mixed with a mortar and pestle, was sprinkled evenly over the sand, and mixed into the top inch. The pot was watered until water drained freely from the base. Leek seeds were sprinkled over the level surface and covered with a thin layer of sand. The pots were set in a heated pit of the Horticulture propagation glasshouse.

The pots were not directly watered again throughout the experiment. With the high humidity at which the pit was maintained, syringing of the sand surface with a mist spray was all that was necessary. The pit was thermostatically controlled at 75°F. (24°C.). However, glasshouse routine required the opening of the pit 3 times daily for syringing and ventilation of material being propagated by the Horticulture Department. This resulted in unavoidable temperature fluctuations, despite the temperature

barrier provided by the glasshouse. Maximum and minimum thermometers placed within the pit showed fluctuations in one 24 hour period of 65°F.-94°F. (18°C.-34°C.)

Sampling:

Sampling was carried out immediately before analysis for ammonia and nitrate content. Pots to be sampled were brought into the laboratory. The sampler consisted of a cork borer, 1.25 in. in diameter, with a rubber plunger to remove the core of material after the sampler had been pressed into the medium. A mark made on the sampler gave a core, 1.5 in. in length, altogether providing a moist weight of approximately 80g. The sampler was washed thoroughly with distilled water between samplings.

Exact weights of samples were not deemed necessary, since treatments were in all cases applied only to the top inch of sand. Equal cross-sections of treated material were taken in the sampler. The moisture content in the pots was never high enough to permit the leaching of nutrients, or of injurious factors, from the surface inch of medium. Samples were not taken immediately around the circumference of the pot where fertilizer distribution may not have been even. Duplicate analyses were made on each pot. A fresh pot per treatment was sampled for each new series of analyses.

Chemical Analysis For Ammonia and Nitrate.

As the sample was taken it was placed in a beaker

TABLE III

Periodic Ammonium Nitrogen Analyses Of Sand Containing Starch and Dried Blood

Treatments		Ammonium Nitrogen (in mg. per core.)							
		Incubation Period in Days							
Dr. Blood (in g.)	Starch (in g.)	2	3	5	7	9	11	13	17
3		1.38	1.85	3.48	2.88	3.42	2.97	2.49	2.94
3	1	0.44	0.19	0.88	2.01	2.47	2.12	3.07	3.61
3	3	0.44	0.41	0.46	0.86	1.76	1.64	2.01	3.01
3	9	0.39	0.49	0.82	0.75	1.74	1.52	1.04	1.24
3	21	0.25	0.36	0.21	0.55	0.40	0.59	0.70	0.75
	21	0.27	0.12	0.21	0.08	0.03	0.14	0.03	0.14
No Treatment		0.24	0.33	0.38	0.16	0.01	0.14	0.09	0.12

and covered with 50 ml. of 2N potassium chloride solution acidified to pH 2. After 30 minutes with occasional stirring, the suspension was filtered and the residue leached with an additional 150 ml. potassium chloride solution. Analysis for ammonia content was then carried out, followed by a determination of nitrate content. Both methods have been described under Materials and Methods.

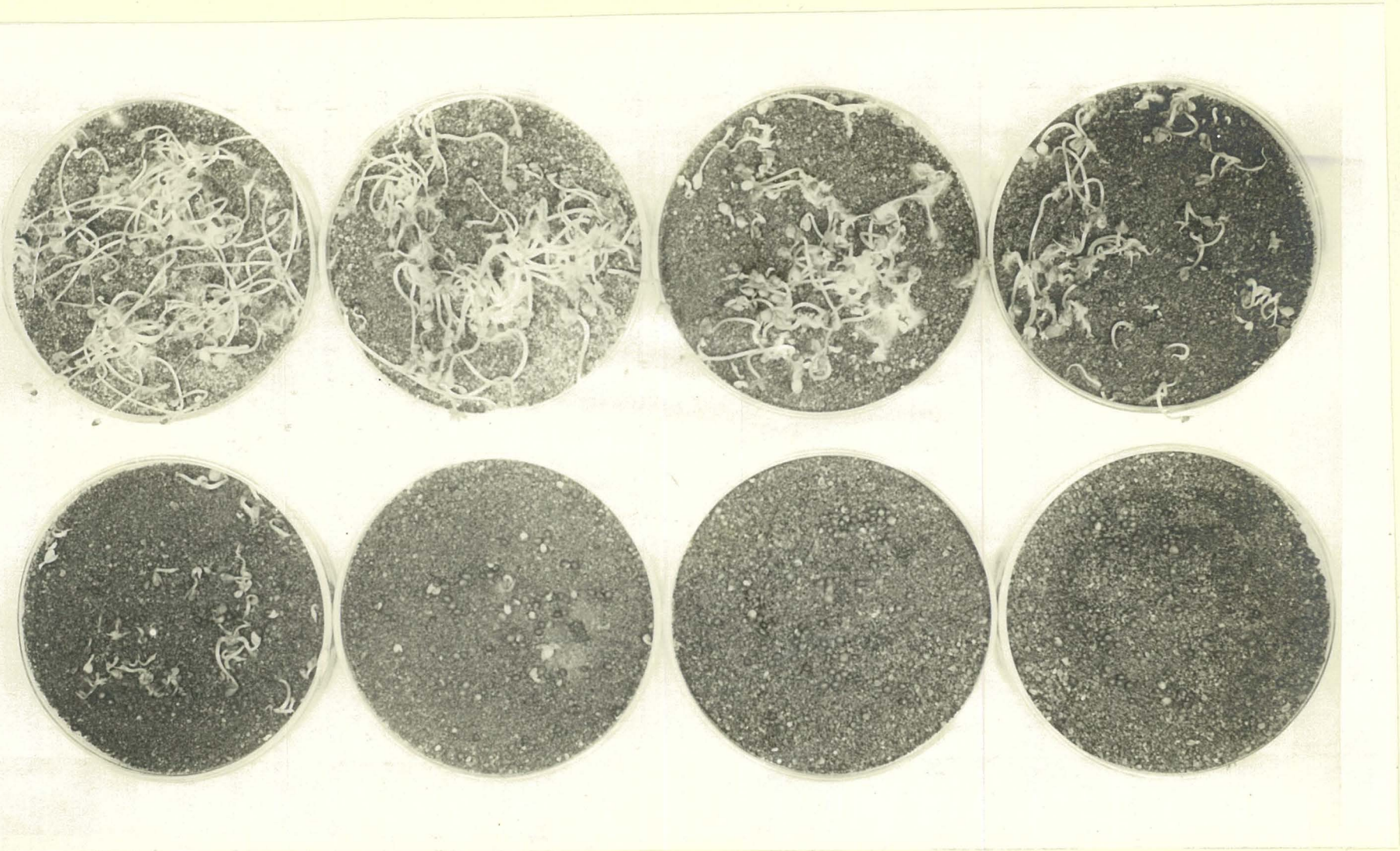
pH Determinations

Samples from treated pots were taken for pH determinations at the same time as samples were taken for chemical analysis. Using the sampler, approximately 50g. moist weight were taken and washed into a beaker with 50 ml. distilled water. pH determinations were made immediately.

Results

Results of nitrogen determinations are recorded in Table 3. The units appear as milligrams N per core, since water content in the pot was not regulated nor measured at any stage. The figures given are the average values of duplicate analyses for each treatment, corrected by blank determinations of nitrogen on the reagents used. Eight sets of analyses were made. Times of sampling are recorded in the table.

Dried blood treatment by itself gave the highest ammonia values. These values rose to a peak which was sustained over several days, and which fell only slightly by the time the experiment was terminated after 18 days. Ammonia values for



B. Germination of cabbage seed in contact with increasing concentrations of dried blood in sand.

Levels of Application of Dried Blood.

l. to r.	0g.,	0.5g.,	1g.,	1.5g.
	2g.,	2.5g.,	3g.,	3.5g.

sand alone, and for the treatment incorporating 21g. starch, were so low that they could be eliminated as a source of germination injury.

With increasing amounts of starch applied with the fertilizer in the sand medium, ammonia values were initially very low and comparable to those values obtained using sand only. In all cases, however, as the experiment progressed there was an increasing quantity of ammonia determined in the analyses. With the lowest application of starch, Ig. per pot, this buildup of ammonia was apparent at the third sampling. Treatments comprising 3g. starch and 3g. dried blood, showed the rise in ammonia concentration at the fourth sampling. With 9g. starch plus 3g. dried blood, it appeared in the fourth and fifth samplings, and with 21g. starch in association with dried blood, the rise was not apparent until the seventh and final sets of samplings.

Germination counts are not recorded since germination of the leek seed was suppressed or did not take place, except in one or two isolated cases. An extremely difficult physical condition for seed germination, in the form of very hard encrustation of the surface sand in the pots, may have been the cause of germination failure. Even at the lowest rate of application of starch this surface crusting was evident. Normal germination occurred in the control pots. No seedlings appeared in the treatments with 3g. dried blood. This was to be expected however, as germination counts were very low at this rate of application

TABLE IV

Periodic pH Values Of Sand Containing Starch And Dried Blood.

Treatments		pH Values							
		Incubation Period In Days							
Dr. Blood (in g.)	Starch (in g.)	2	3	5	7	9	11	13	17
3		7.46	7.80	8.03	7.92	7.47	8.20	7.35	7.26
3	1	7.60	6.66	7.39	7.87	7.84	8.19	7.59	7.67
3	3	8.05	7.36	7.25	8.03	8.00	8.00	7.87	8.43
3	9	8.52	7.64	8.25	8.04	8.22	7.96	7.94	8.05
3	21	8.60	8.26	8.53	8.61	8.39	8.22	7.88	8.64
	21	8.76	8.44	8.05	8.60	8.59	8.56	8.56	8.57
No Treatment		7.19	7.19	7.24	7.21	7.17	7.15	6.92	7.07

of dried blood in a pilot trial run prior to the experiment.

pH measurements made at the times of sampling are presented in Table IV. The high pH values obtained from treatments including starch, indicated that alkalinity in the medium might have been a factor influencing germination in this experiment. Accordingly, the pH of a slurry of the "Robin" brand starch in water was determined, giving a value of pH 9.06. Since this starch was finely powered for laundry use, it was possible that some additive was present which would raise the pH to the mentioned level. Analar starch gave a pH value of 7.55 when tested in the same way.

All treatments in which starch was applied together with dried blood showed an initial fall into pH, followed by a rise to highly alkaline values.

Nitrate determinations were made, but only negligible amounts were detected over the 18 days of the experiment.

Following the failure of germination of the leek seed, this experiment developed into an indirect study of the rate of mineralization of dried blood in sand. As such, it confirms the results of other workers such as Owen and Winsor (1950). The mineralization of dried blood is accompanied by the production of appreciable amounts of ammonia and a corresponding rise of alkalinity in the medium. The initial fall in pH observed could have been caused by the release of organic acids in the decomposing dried blood.

Following the difficulties with crusting of the medium, high pH values for starch, and fluctuations in moisture content and the temperature of the pit, it was decided to experiment further along these lines, but under more controlled conditions in the laboratory.

CHAPTER IV.

SEED GERMINATION PERCENTAGES, PH LEVELS, AND AMMONIA AND NITRATE CONCENTRATIONS IN SAND-DRIED BLOOD MIXTURES.

The aim of this experiment was comparable with that of the starch method just described, but rather than raising the C:N ratio in the medium, rates of application of dried blood in contact with the seeds were progressively increased. The experiment was carried out in petri dishes in an incubator maintained at 24°C. Sand was used as the medium, 80g. dry weight being contained in each dish.

Treatments

The treatments comprised:-

(1) Control, containing sand only.
(2) 0.25g. dried blood per 80g. sand.
(3) 0.5g. dried blood per 80g. sand.
(4) I.0g. dried blood per 80g. sand.
(5) I.5g. dried blood per 80g. sand.
(6) 2.0g. dried blood per 80g. sand.
(7) 2.5g. dried blood per 80g. sand.
(8) 3.0g. dried blood per 80g. sand.

All treatments were replicated 12 times.

Setting Up The Petri Dish

Where fertilizer was to be used, the 80g. dry sand together

with the appropriate rate of fertilizer application were ground and mixed with a mortar and pestle. Three filter papers, placed at the bottom of each petri dish, were moistened with 20ml. distilled water. The fertilizer-sand mixture was evenly distributed over the surface. The water spread upwards by capillarity through the sand in the petri dish. The amount of water added was just sufficient to give a moist substrate for seed germination. Fifty cabbage seeds were spread evenly over the surface and covered with some fertilizer-sand mixture which had been previously withheld. Incubation followed.

Germination counts were made every two days, and were totalled at the end of the 7 days germination period.

Relation of Seed Sowings to Incubation of Fertilizer

All 12 replicates of each treatment and the control dishes containing sand only, were placed in the incubator at the same time. However, sowing of seeds was done in relays. As the seven days forming the germination period for one set of seeds passed, the seedlings and dead seeds were removed from the surface, and a fresh number of seeds were sown. Seeds were sown 1, 3, 5, and 8 days after initial incubation of the petri dishes. Results are recorded as final germination counts. Thus, for the first seed sowing, the figure given is the average percentage germination for 6 dishes of 50 seeds each, and the figures for the second, third and fourth sowings are the averages of four dishes of 50 seeds each.

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Table V.
Incubation period before sowing seeds (days)
Should read I, 3, 5, 8, days instead of I, 2, 3, 4, days.

TABLE V.

Effects On Germination Of Concentration Of Dried Blood In Sand, And Duration of Incubation Prior To Sowing of Cabbage Seed

Treatment Dr. Blood	Percentage Germination			
	Incubation Period Before Sowing Seeds (Days)			
	1	3	5	8
Control	96	96	96	96
0.25g.	92	94	80	74
0.5g.	92	94	86	68
1.0g.	94	74	0	0
1.5g.	94	76	0	0
2.0g.	94	50	0	0
2.5g.	88	34	0	-
3.0g.	90	26	0	-

pH Measurements

pH measurements were made on the contents of the petri dishes before ammonia and nitrate determinations were made. The contents of each dish were washed into a beaker with 50ml. distilled water. After thorough stirring, a pH measurement was obtained.

Chemical Analysis

Immediately following the pH measurement of each sample 50ml. acidified potassium chloride were added to the beaker. The contents were then allowed to stand with occasional stirring for 30 minutes. Filtering of the suspension and subsequent leaching of the residue with the addition of 150ml. acidified potassium chloride, then proceeded as before. Determinations of ammonia and nitrate were made 3, 5, 7, 8, 12, 15, and 21 days after starting the experiment.

RESULTS

Germination results

Germination results are recorded in Table V. as final germination percentages. The control plates showed an average of 96% germination throughout the germination trial which ended at the sixteenth day of incubation. For all treatments the first sowing of seed showed high percentage germination. However, with increasing length of incubation period, and increasing rates of application of dried blood, germination percentages fell rapidly. After the fifth day of incubation, germination was completely inhibited for all rates of application greater than Ig. Seed counts were not made on treatments containing 2.5g. and 3g. dried blood

TABLE VI

Ammonium Nitrogen Analyses of Dried Blood-Treated Sand Cultures

Treatment Dried Blood (in g.)	Ammonium Nitrogen (in mg. per ml.) Incubation Period in Days						
	3	5	7	8	12	15	19
Control	0.014	0.028	0.010	0.020	0.034	0.045	0.088
0.25	0.046	0.108	0.407	0.456	0.740	0.603	0.649
0.5	0.046	0.130	0.647	0.389	0.617	0.622	0.818
1.0	0.091	0.192	1.018	1.415	2.019	1.705	1.999
1.5	0.098	0.225	1.200	1.514	2.352	1.960	2.132
2.0	0.168	0.608	1.964	2.558	3.273	3.136	3.430
2.5	0.175	0.664	2.489	2.758	-	-	-
3.0	0.253	0.753	2.695	3.572	-	-	-

per dish after the sixth day.

Chemical Analyses

Results of analyses for ammonia are presented in Table VI as milligrams nitrogen per ml. of soil solution.

Low values of ammonia were obtained from extracts of control plates containing sand only, but these values were negligible compared with the values obtained from treated plates. For all treatments there was a rapid increase in ammonium-nitrogen content as the length of incubation increased. The production of ammonium-nitrogen would, from these results, appear to be directly proportional to the rate of application of dried blood. The pungent odour of ammonia which had volatilised from the petri dishes, was apparent in the incubator after three days of incubation.

Nitrate determinations were made following ammonia determinations, but until the last few days of the experiment, amounts analysed were negligible and are not recorded here.

pH Results

These results are presented in Table VII. For plates containing sand alone, the pH remained constant. With the lower rates of application of dried blood (0.25 and 0.5g. per dish) there was a rapid rise in pH value in the petri dishes, followed by a levelling off at relatively high values. With the remaining treatments (1 - 3g. per dish) there was an initial drop in pH followed by a gradual rise. The fall and rise of pH value appeared to be proportional to the rate of application of fertilizer.

TABLE VII

pH Values Of Dried Blood-Treated Sand Cultures

Treatments	pH Measurements					
Dried Blood	Incubation Period In Days					
(in g.)	5	7	8	12	15	19
Control	7.29	7.27	7.26	7.30	7.19	7.21
0.25	7.43	7.82	8.11	8.46	8.26	8.17
0.5	7.39	7.88	8.09	8.33	8.41	8.26
1.0	7.46	7.45	7.71	8.45	8.79	8.99
1.5	7.53	7.45	8.04	8.57	8.84	8.94
2.0	7.71	7.40	7.65	8.79	8.59	8.89
2.5	7.72	7.47	7.66	-	-	-
3.0	7.76	7.42	8.01	-	-	-

From the very high ammonium-nitrogen values obtained, the high pH values associated with them, and from the corresponding injury to germination as recorded by successive seed sowings, it appeared that there were several factors or combinations of factors, which might be responsible for germination failure. These were the presence of the ammonium ion, the presence of high concentrations of hydroxyl ions, or the presence of toxic concentrations of free ammonia. The following experiments were designed to test these hypotheses.

CHAPTER V.

INVESTIGATION OF THE EFFECTS OF AMMONIUM ION, HYDROXYL ION AND FREE AMMONIA CONCENTRATIONS ON GERMINATION.

In the preceding section it has been shown that germination injury became increasingly marked as mineralized nitrogen accumulated. This injury could arise from any one, or combinations of the following factors.

(1) a specific injurious effect of the ammonium ion.

(2) a specific injurious effect of the hydroxyl ion.

(3) a specific injurious effect of free ammonia.

The experiments reported in this section were designed to enable a decision to be reached on this matter.

Special Techniques For Dilution Work

Following the incubator experiment, it was decided to attempt to produce injury to germination by supplying either the ammonium ion, the hydroxyl ion, or free ammonia, from inorganic sources and then to reproduce this effect through the agency of the dried blood fertilizer.

Difficulties were soon experienced in handling seeds in contact with solutions. In pilot trials, 50 cabbage seeds were germinated on filter papers which were moistened with 10ml. of ammonium hydroxide over a range of concentrations. The lids of the petri dishes were greased with vaseline to prevent the escape of any volatilised ammonia, but the method was not satis-

factory. Very little ammonium hydroxide solution could be added to the petri dishes without waterlogging the seeds.

Attention was directed towards germinating the seeds in contact with a larger supply of standard solution, so that pH measurements also could be made. Straight-sided glass bottles, 5.5 in. in height and 2.5 in. in diameter, were obtained. The bottles were provided with screw-top, bakelite caps. Two hundred ml. of the solution under test were taken in each bottle. A volume of approximately 100ml. air remained for the germinating seeds. This proved to be sufficient for the purpose.

In the first trials, 5.5cm. filter papers were floated on the surface of the solutions with the aid of small cork rings. The seeds placed on the filter paper surface, while free of waterlogging conditions, were still in contact with a large volume of standard solution. However, the ammonium hydroxide solutions were discoloured by the cork rings and it was thought advisable to adopt some other means of floating the seeds.

A trial was made with paraffin wax floats. The wax was cast in a circular mould, 1.5 in. in diameter, to form a block 0.25 in. in depth. As with the cork rings, the seeds were germinated on a filter paper supported on this wax float. This was a more successful method but germination percentages were still much below those obtained in the original germination tests with the seed varieties. The filter paper, despite the wax float, tended to become waterlogged.

To overcome this problem, it was decided to attach stands to the floats. In this way seeds could be germinated on a platform

slightly above the surface of the test solutions. A crucible was inverted, and the paraffin wax float attached to this by means of a column consisting of an inch of household candle. The entire stand was approximately 3 in. high. This stand had to be revised later, since the candle proved to soften and become plastic in dilute ammonia solutions.

The final form of the stand was satisfactory. Both the platform and the stem of the stands were formed from pure paraffin wax. All results given hereafter are germination counts of seeds grown on pure paraffin wax stands.

The procedure in filling the bottles was as follows. All bottles, lids, and wax stands were washed thoroughly and rinsed in distilled water. The stands were placed in the bottles and the appropriate solution was added until the liquid was at a level slightly below that of the paraffin wax platform. Fifty or one hundred seeds, depending on the variety, were then germinated on a filter paper laid across the platform. The filter paper was kept moist by contact with the solution below. The lids on the bottles were screwed down for the period required for completion of germination of the seed variety under incubation at 24°C. (75°F.). The bottles were then opened and the seeds examined individually.

TABLE VIII

Effect Of Ammonium And Other Ions On Germination Of Cabbage Seed

Normality of Solutions	Percentage Germination					
	$(NH_4)_2CO_3$	K_2CO_3	NH_4Cl	KCl	NaCl	$(NH_4)_2SO_4$
0.3 - 0.5	0	0	0	0	0	0
0.2	0	0	0	25	8	0
0.1	0	0	33	74	46	81
Solutions Corrected For O.P.						
0.2			0			0
0.1			38			33
0.05			57			52

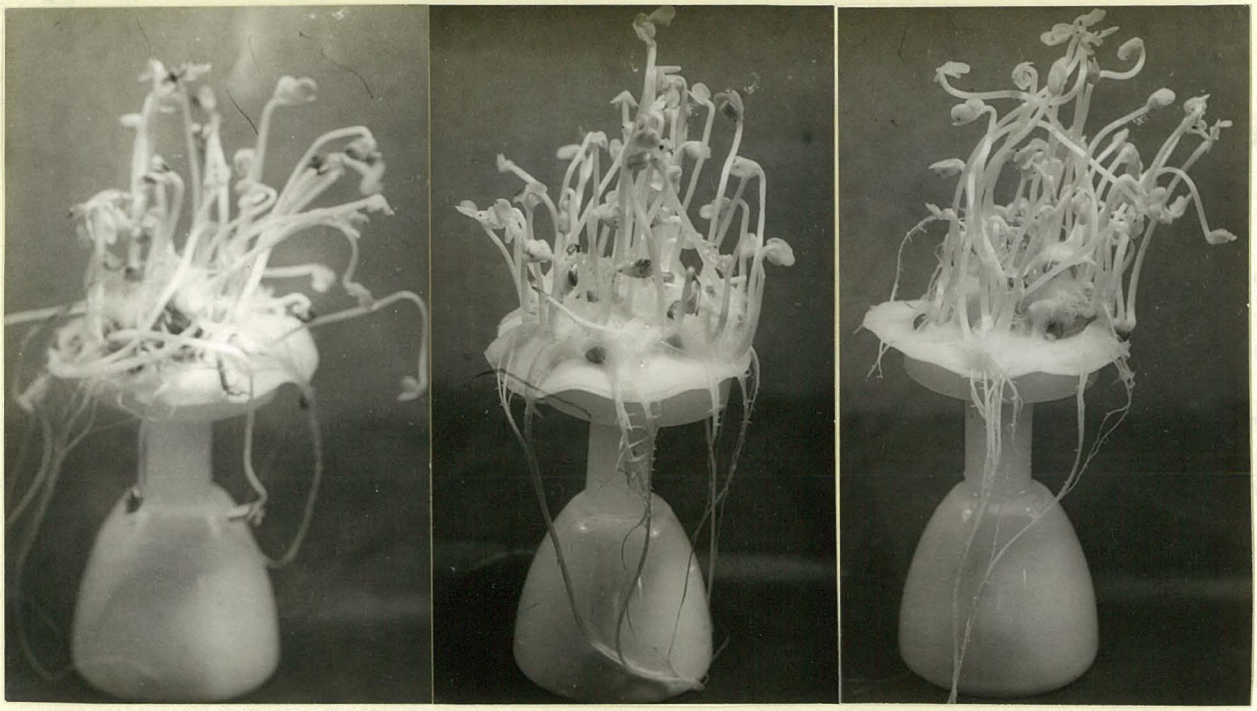
Effect of Ammonium Ion Concentration on Germination

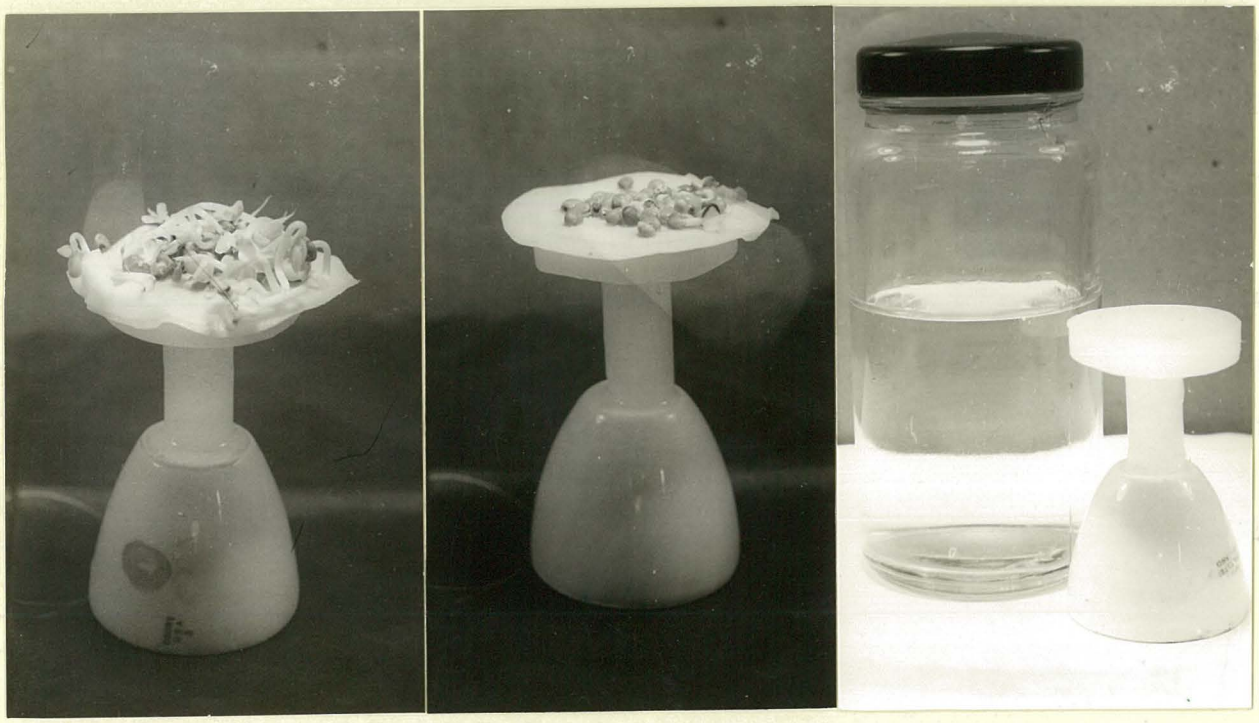
It has been well established by many workers that fertilizer injury to young seedlings can occur through plasmolysis of cell tissues by high electrolyte concentrations in the soil solution. In order to test the hypothesis that the ammonium ion has a specific injurious effect on germination over and above the plasmolytic effect, a number of solutions were prepared from salts composed of varying combinations of similar anions and cations: ammonium carbonate, ammonium chloride, ammonium sulphate, potassium carbonate, potassium chloride and sodium chloride. From each of these six salts, 0.5N, 0.4N, 0.3N, 0.2N, and 0.IN solutions were prepared. Distilled water was used as the control.

The treatments were replicated 4 times. Cabbage seed was used as the seed variety. The experiment was carried out in glass bottles as described under Special Techniques. The bottles were incubated at 24C. (75F.) for 7 days.

Results

The results of this experiment are presented in Table VIII which records average percentage germination for the 4 replicates. The following points emerge. Germination was completely inhibited by the three most concentrated solutions of salts employed (0.5N, 0.4N, and 0.3N). With 0.2N solutions, all three ammonium salts completely inhibited germination of cabbage seeds; however, both potassium and sodium chlorides





C. Germination of cabbage seeds on Paraffin wax stands over Ammonium Hydroxide Solutions.

l. to r. Distilled water, 0.00120N, 0.00250N
0.00375N, 0.00500N, Paraffin wax stand.

permitted some germination but at levels substantially lower than the control in distilled water. The reason for the lower percentage germination in sodium chloride solution as compared with potassium chloride is not clear, although it suggests that the sodium ion has some specific repressive effect. The same effect was apparent with the O.IN solutions.

Germination percentages with O.IN solutions of potassium chloride and ammonium sulphate were high. Ammonium chloride (O.IN) also permitted germination at low level. Germination was completely inhibited by both ammonium and potassium carbonates.

The solutions of the salts at the O.IN and O.2N levels were checked for pH values immediately prior to, and immediately following the experiment. These pH values are recorded in Table IX.

If hydrogen ion concentration limits germination, a possible explanation for the complete inhibition of germination by all concentrations of potassium carbonate emerges from the high pH values recorded in the table for solutions of potassium carbonate.

In the case of ammonium carbonate, germination was inhibited at all concentrations. Three factors could have been responsible, singly, or in combination: plasmolytic effects, pH effects, or free ammonia effects. Inhibition of germination solely as a result of a specific injurious effect on germination of the ammonium ion, can be eliminated on the results of this

TABLE IX

pH Values Of Ammonium And Potassium Salt Solutions

	pH Values	
Salt	0.1 N Soln.	0.2 N Soln.
Ammonium chloride	6.15	5.94
Ammonium carbonate	8.77	8.70
Ammonium sulphate	6.02	6.04
Potassium chloride	6.38	6.32
Potassium carbonate	10.94	10.86

experiment, since germination occurred with equimolar solutions of ammonium chloride and ammonium sulphate.

It will be observed that two sets of results are given for the lower concentrations of ammonium chloride and ammonium sulphate. In the first set of results, the ammonium chloride solutions apparently had a much greater repressing effect on germination than the ammonium sulphate. These results were difficult to interpret and were finally solved by preparing further solutions of the two salts, this time of equivalent osmotic concentrations. The fact that one molecule of ammonium sulphate produced three ions to the two ions produced by ammonium chloride, had been overlooked. Thus, a molar solution of ammonium sulphate produced an osmotic pressure 1.5 times as great as a molar solution of ammonium chloride. Further, a 0.IN solution of ammonium sulphate produced an osmotic pressure 0.75 times as great as a 0.IN solution of ammonium chloride. Accordingly, adjustments were made in the weights of the salts required per litre of solution, to obtain comparable osmotic values.

Fresh solutions were prepared at 0.2N, 0.IN, and 0.05N concentrations. These results are presented at the bottom of TableVIII.Comparable germination percentages were obtained for ammonium chloride and ammonium sulphate solutions in this experiment.

Effect of Hydroxyl Ion Concentration on Germination

The hypothesis that the hydroxyl ion might be the factor repressing germination was investigated through the use of calcium hydroxide, ammonium carbonate, and potassium carbonate solutions over a wide range of pH values.

Potassium carbonate and ammonium carbonate solutions were used over a range from pH 10.8 to pH 6.3. The lower values of potassium carbonate were obtained by depressing the initially high pH value with 0.1N hydrochloric acid. Smaller changes in pH were obtained by dilution with distilled water. The pH of the ammonium carbonate solution was initially 8.48. The pH range was extended above this point by addition of drops of ammonia solution, and the lower values were obtained by progressive addition of hydrochloric acid. The calcium hydroxide solution used gave a pH value of 11.86. Hydrochloric acid was added to obtain two lower pH values as a check on the effect of the calcium ion. The concentrations of all the solutions used were not great enough to have plasmolytic effects on the cell tissues of the young seedlings.

Since the carbonate ion is common to both ammonium and potassium carbonate, potassium carbonate was used as a check on the effect of the ammonium ion, or of free ammonia formed by the ammonium carbonate. Calcium hydroxide was used as a check on the effect of hydroxyl ion concentration, since ammonium hydroxide was used to raise the pH of the ammonium carbonate

TABLE X

Effect Of Increasing Hydroxyl Ion Concentration
On Germination Of Cabbage Seed

pH of Solutions	Percentage Germination		
	$Ca(OH)_2$	K_2CO_3	$(NH_4)_2CO_3$
11.86	83		
10.75			
10.58		78	
10.48	90	81	
10.38		86	0
10.18		83	
10.05		82	
9.92			0
9.85		87	0
9.71		92	
9.48			0
9.36			
9.22			0
9.01			0
8.90			0
8.85			0
8.75			0
8.69		100	
8.65			0
8.48			0
8.36			0
8.30			0
8.27			0
8.10			0
7.91			
7.75			80
7.66			85
7.25			92
7.14			
7.02		94	
6.95			
6.80		100	
6.55		92	
6.38		86	
5.25	65		

solution. The control for the experiment was distilled water.

The experiment was carried out in glass bottles, using paraffin wax stands as previously described. The pH of the solution under test was established in each bottle immediately before sowing 100 cabbage seeds per bottle. The lid of each bottle was then screwed down and the bottle was incubated for 7 days. The pH was determined after germination counts had been made, at the end of the germination period. The pH values given in Table X, are the average values of these two sets of measurements. Little variation was observed.

Results

The results of this experiment are given in Table X. as percentage germination, corrected for the control. Three sets of experiments carried out under the same conditions contributed to these results.

With ammonium carbonate solutions, germination was completely inhibited at all pH levels above pH 8.I. Germination percentage was high below pH 7.75.

Germination was very high over the entire range of potassium carbonate solutions from pH 6.38 to pH I0.58, although there were signs in the slightly stunted growth of seedlings at the highly alkaline level that the limit of tolerance was being approached.

Germination percentage at pH II.86 in calcium hydroxide solution was slightly lower than that of the controls in distilled water, but the vigour of these seedlings was comparable with

that of the controls. At the low pH of 5.25, germination percentage was still considerable, but the thrift of the seedlings was much poorer.

Since with potassium carbonate and calcium hydroxide solutions germination was excellent over very high pH levels, these results must again point to an injurious effect on germination of either the ammonium ion, or of free ammonia. The following experiments were designed therefore, to test the effect of a range of concentrations of ammonium hydroxide on seed germination.

Effect of a Range of Dilutions of Ammonium Hydroxide on Germination

The effect of a range of ammonium hydroxide concentrations on germination of several varieties of seeds was determined in the experiments described in this section.

Cabbage seed was used in pilot trials designed to find the limit of tolerance of this seed variety to increasing concentrations of ammonium hydroxide. Ammonium concentrations below 0.004N were tolerated, but with low percentage germination. Above this concentration germination was completely inhibited.

As a check on possible differences in susceptibility of seed varieties to germination injury in the presence of ammonium hydroxide, 12 other seed varieties were tested over a range of concentrations. These varieties were, lettuce, radish, leek, turnip, boracale, parsley, mustard, beet, marigold, cress, celery and spinach.

A series of dilutions were prepared from a standard 0.IN ammonium hydroxide solution. A check was made on the normality of the standard solution whenever dilutions were made. Seeds were germinated on wax stands in glass bottles as previously described. Incubation at 24C. (75F.) followed. The bottles were not opened between sowing of the seeds and the germination counts, to avoid loss of ammonia by volatilization. After the 7 day germination period has elapsed, the bottles were opened and germination counts were made.

TABLE XI

Effect Of Increasing Concentrations Of Ammonium Hydroxide On Germination Of 13 Varieties Of Seeds

Seed Variety	Percentage Germination			
	0.00125N NH_4OH	0.00250N NH_4OH	0.00375N NH_4OH	0.00500N NH_4OH
Radish	100	99	92	8
Leek	91	86	91	16
Beet	84	47	21	5
Cabbage	90	76	11	0
Cress	100	99	5	0
Mustard	100	86	5	0
Marigold	61	17	4	0
Boracale	90	51	0	0
Turnip	81	37	0	0
Lettuce	82	10	0	0
Celery	0	0	0	0
Spinach	0	0	0	0
Parsley	0	0	0	0

Originally the entire range of dilutions tested for cabbage seed extended from a 0.015N solution to a 0.00045 N solution, with 16 intermediate dilutions. These were not replicated because of the limited incubator space available. When the particular range of tolerance of ammonium hydroxide for each seed variety was established, replicates were made in that range, and just bordering it. Control germination counts were made with seeds germinated over distilled water.

Results

Results are presented in Table XI. With seed varieties requiring very long periods for germination (spinach, parsley, celery) no germination occurred, emphasizing the importance of the speed of germination, in germination injury of this kind. The remaining seed varieties appeared to fall naturally into 3 groups, according to susceptibility of seedlings to ammonium hydroxide.

With boracale, turnip, and lettuce, tolerance was very low. Germination was completely inhibited by a 0.00375N solution. With cabbage, cress and marigold, low germination percentages were recorded in the presence of 0.00375N solutions. Germination was completely inhibited with 0.005N ammonium hydroxide solution. Radish, leek and beet formed a third group in which germination occurred at a low level in contact with a 0.005N ammonium hydroxide solution.

One seed variety from each of the three mentioned groups was selected for further detailed study. Radish, cabbage and

TABLE XII

Effect Of Increasing Concentrations Of Ammonium Hydroxide On Germination Of Radish, Cabbage And Lettuce Seeds

Normality of NH_4OH Solns.	Percentage Germination		
	Radish	Cabbage	Lettuce
0.00750	0		
0.00675	0	0	0
0.00500	6	0	0
0.00400			
0.00375	90	15	
0.00350			
0.00300			
0.00250	98	73	9
0.00200			
0.00150			83
0.00135			
0.00125	100	84	
0.00100			96
0.00050			
0.00045			

lettuce seeds were selected, since they all germinate within 8 days, thus enabling a quick turnover of replications.

Dilutions of ammonium hydroxide were made ranging from 0.0075N to 0.001N, with 11 intermediate values. Replicates were made around the critical level of tolerance for each variety.

Results

Results of this experiment are presented in Table XII in which the figures recorded are the average values of ten replicates for each treatment. Germination percentages have been corrected by the control results.

These results clearly establish an order of susceptibility of the three seed varieties to increasing concentrations of free ammonia or of the ammonium ion. The critical range for radish was established as falling between 0.005N-0.00375N solutions; for cabbage, 0.00375N-0.0025N solutions; and for lettuce, 0.0025N-0.0015N solutions. This range in susceptibility of different seed varieties to ammonium hydroxide solutions suggested the following experiment.

CHAPTER VI.

EFFECT OF DRIED BLOOD ON GERMINATION OF RADISH, LETTUCE, AND CABBAGE SEEDS IN SOIL CULTURES

The results of the preceding experiments, together with the observed similarity in form of injury to seedlings following germination in contact with both ammonium hydroxide and applications of dried blood, indicated that germination injury was caused by toxic concentrations of free ammonia in the medium. The high values for ammonium nitrogen obtained from chemical analyses of the sand cultures described in Chapters III and IV supported this view.

It was a problem to differentiate between the ammonium ion and free ammonia, since ammonia is readily soluble, resulting in an equilibrium being set up thus:-

H_2O NH_3 NH_4OH NH_4 OH

The range in susceptibility of different seed varieties to injury from increasing concentrations of ammonium hydroxide, suggested an indirect means of solving this problem. In ammonia dilution experiments, radish exhibited the greatest degree of tolerance, cabbage was intermediate in reaction, and lettuce was extremely intolerant. An experiment was set up in which soil was used as the medium for increasing rates of a application of dried blood. Radish, cabbage and lettuce seeds were tested for percentage germination in contact with the fertilizer. It

was reasoned that if results showed that injury to germination of the three seed varieties fell in the same order as with ammonia dilutions, this would further point to the similarity of the cause of injury in both cases.

As an extension to this experiment, two soils were taken. One soil was an acid soil of pH 4.2. The other soil was alkaline, of pH 7.3. Both soils were of the same soil type, having been taken from a block of fertilizer plots maintained by the Field Husbandry Department. Theoretically, if ammonia were released from decomposing dried blood with a corresponding rise in pH in the environment, less injury should have been evident in the acid soil treatments than with the alkaline soil.

The experiment also included a small retrial of the effect of addition of excess carbohydrate to the medium. Powdered methyl cellulose was used.

The soils obtained from the fertilizer plots were air-dried and passed through a 2mm. sieve. Twenty gram soil samples were taken per petri dish. Where fertilizer treatments were applied, the soil and fertilizer were ground and mixed in a mortar. For both acid and alkaline soils, three rates of application of dried blood were used, Ig., 2g., and 3g., per petri dish. The three seed varieties were sown with all treatments, and with the soils alone as control treatments. Treatments were replicated three times. In the trial with cellulose as an additive, increasing amounts of cellulose, Ig., 2g., and

TABLE XIII

Effect Of Dried Blood Fertilizer On Germination Of Radish, Lettuce And Cabbage Seeds In Soil Cultures

Seed Variety	Percentage Germination					
	Acid Soil			Alkaline Soil		
	1g. D. Bld.	2g. D. Bld.	3g. D. Bld.	1g. D. Bld.	2g. D. Bld.	3g. D. Bld.
Radish	96	98	94	98	96	96
Cabbage	96	88	86	95	87	90
Lettuce	89	0	0	90	0	0

3g., were sown with a fixed amount of dried blood (3g.) in 20g. of soil. Both acid and alkaline soils were used in this trial.

Each dish was set up in a manner similar to that already described with the sand cultures in Chapter IV. Briefly, 10 ml. of distilled water was used to moisten 20g. of soil in each petri dish. The water spread upwards through the dish by capillarity. One hundred seeds of the particular variety were scattered over the soil-fertilizer mixture. The petri dishes were incubated at 24°C. (75°F.) for the 7 day germination period. Germination counts were then made.

Results

Results of this experiment are presented in Table XIII. The figures represent the average germination percentages for 3 replicates of each treatment, as corrected for the control. Percentage germination of both radish and cabbage varieties was very high, and little difference could be detected in the results. With lettuce, germination was high in the treatments of Ig. dried blood per 20g. of soil. However, although the seeds reached the first green leaf stage within the first 4 days of the experiment, development ceased at this stage and the seedlings subsequently rotted. At the 2g. and 3g. levels of dried blood application, germination was completely inhibited in both soils. With radish and cabbage seeds, although germination percentages were high at 3g. application of dried blood, the

same lack of further development of the seedlings was apparent, and the seedlings ultimately decayed.

After the seedlings had been counted, all seed debris was removed from the soil surface, and 100 fresh seeds were sown in each petri dish. The dishes were then incubated for an additional week. Germination was completely inhibited in all cases.

Although this experiment does not distinguish between radish and cabbage seeds in their susceptibility to increasing concentrations of dried blood, it does point out the intolerance of lettuce seeds in this respect. From the complete inhibition of germination of all three seed varieties when sown during the second week of incubation of dried blood-soil mixtures, the experiment also demonstrates clearly once again, the way in which rapidly germinating seeds may escape injury, by emerging before the full effect of the fertilizer develops.

The results obtained with both acid and alkaline soils were similar for all three seed varieties. As demonstrated in the section of Chapter II describing choice of media, soil as a medium tends to alleviate the injurious effects of dried blood on seed germination. This is a possible explanation for the results of the present experiment. The results could be interpreted also as significant evidence of the importance of the presence of free ammonia alone, as the injurious factor to germination, rather than the association of free ammonia with a high pH in the medium.

Where cellulose was used as an additive, no toxic effect on germination was observed, but differences were not apparent between treatments involving different amounts of cellulose. Since cabbage seed was used as the seed variety in this trial, it is likely that the germination period was too short for germination to be influenced to any great extent by the fertilizer and cellulose treatments.

CHAPTER VII.

DISCUSSION

A considerable part of this study has been concerned with the production from inorganic salts of free ammonia, and the evaluation of its role in inhibiting germination of seeds and injuring young seedlings. This work was necessarily carried out on a non-horticultural basis in the laboratory. As stated previously, practical difficulties prevent the direct demonstration of germination injury by free ammonia in a moist soil system, although it is reasonably certain that free ammonia does exist there in equilibrium with ammonium hydroxide.

Workers such as Barton (1940) and Duisberg and Buehrer (1954) have shown beyond doubt that free ammonia from inorganic sources inhibits germination of certain varieties of seeds. This inhibitory effect has been demonstrated for additional varieties of seeds in the present study. Furthermore, as would be expected from the diverse natures of the seed coats, seeds of different plants were shown to differ in susceptibility to increasing concentrations of ammonium hydroxide. For each seed variety there was demonstrated a critical level of ammonium hydroxide concentration, above which point the percentage germination dropped rapidly. In general, it was observed that at a particular concentration of ammonium hydroxide, seeds which germinated and developed rapidly in the seedling stage appeared to escape the full force of the injury. Where the seeds were

not exposed to high concentrations of ammonium-nitrogen until they had reached the young seedling stage, the plants were stunted and eventually decayed, unless removed from the high concentrations of ammonia. Injury was most serious when it occurred early in the germination process. Qualitative and quantitative similarity in the germination injury resulting from treatments with dried blood and with ammonia-yielding inorganic materials, was found in germination experiments wherein critical concentrations of organic and inorganic fertilizer were determined for selected seed varieties.

The type of injury to germination resulting from organic fertilizers noted by Sayre and Clark (1935) and by Sherwin (1923) was confirmed by the writer's experience, both with experiments using dried blood and with dilution experiments where the seeds were germinated in contact with ammonium hydroxide. Typical symptoms were the twisting, browning, stunting and swelling of the young radicles, and the lack of root hairs.

Sayre and Clark (1935) compared injury from inorganic and organic fertilizers on germination of peas, beans, and corn; their experiments were concerned mainly with the duration of injurious concentrations of fertilizer in the region round the seeds. They did not investigate the cause of injury, but suggested that it was induced by various organic substances such as amino-acids and soluble peptides. However, further support for the hypothesis that free ammonia is the factor injurious to germination, comes from an observation of Owen et al (1950), that most amino-acids from organic nitrogenous materials are readily

deaminated in the soils in which they worked, yielding large quantities of ammonia.

Interest was centered in the present study, mainly on the ammonification stage of the mineralization process. It has been shown that increased levels of application of dried blood fertilizer resulted in increased production of ammonium-nitrogen as determined by direct analysis. This ammonia production was accompanied by a rapid and profound rise in pH of the medium. Owen et al (1950) reported a very slow mineralization of dried blood in their experiments, but despite this, they considered that dried blood exerted its maximum injurious effect on germination in the first 14-16 days after mixing with soil. Sayre and Clark (1935) reported 9-14 days as the period of maximum injurious effect of dried blood applications.

In the experiments described in this study, the full injurious effect of the fertilizer was evident 5-7 days after initial "incubation" of the medium. There was a pungent odour of ammonia given off by the cultures, germination percentages fell rapidly, and the periodic ammonium-nitrogen analyses showed a very rapid increase over this period. That the highly injurious factor was maintained for a period of at least 20 days was demonstrated by continued inhibition of germination of fresh samples of seeds.

In the series of experiments in which sand was incubated with dried blood, or with dried blood plus carbohydrate, it was found that considerable quantities of ammonia or ammonia-producing substances formed intermediate in the mineralization process were not immediately converted to nitrate. Under the unfavourable

conditions of temperature, moisture, and aeration, these high concentrations of ammonia may persist for some time. Thus, germinating seedling may be exposed for the lengthened period of time to high concentrations of ammonia in the soil solution, ceasing only as ammonia is lost by volatilization, or by biological or chemical soil processes.

The use of soil cultures was found to be complicated by the presence of organic matter, and possibly by the level of colloidal materials, which would provide sites for adsorption of the ammonium ion from the soil solution. It was observed that where soil was used as the medium for applying fertilizer treatments, a "buffering" effect against the injury resulted. It is possible that this was due to removal from the soil solution of substances or ions injurious to germination, by adsorption or by replacement of other ions in the soil complex.

That the injurious effect is certainly very specific was demonstrated by the series of dilution experiments with ammonium hydroxide and other solutions. Seeds germinated very satisfactorily at very high pH values and when the ammonium ion was present. However, in the presence of any free ammonia, marked germination injury or complete inhibition of germination was evident.

All the evidence in this study points towards free ammonia production as the source of germination injury. The only indication of the production of any other injurious factor was the observed initial fall in pH in the germination medium. This may be explained by the production of organic acids early in the mineralization process.

In the experiment using increased concentrations of dried blood in alkaline and acid soil cultures, it was expected that the injurious effect would be intensified in the alkaline soil culture. No such observation was made, and this can be explained only be the facts that the "alkaline" soil was not markedly alkaline, and that the speed of germination of the cabbage seeds used was sufficient to permit escape from the full effect of the fertilizer.

SUMMARY

1. Dried blood fertilizer during mineralization gave rise to free ammonia as an intermediate product, in sufficient quantity to cause severe injury to seedlings at the lower levels of application, and complete inhibition of germination ~~at higher the higher concentrations and complete inhibition of germination~~ at higher concentrations.
2. Typical symptoms of injury were the swelling, browning, stunting, and twisting of the young radicle, and lack of root hairs.
3. The medium for germination was shown to have a considerable influence on the injurious effect of the fertilizer.
4. There was an initial fall in pH followed by a rapid and profound rise in pH, after incubation of dried blood in sand and soil cultures.
5. In dilution experiments over a wide range of concentrations, ammonium and hydroxyl ions had negligible specific injurious effect. Where free ammonia was present above a certain critical level, marked inhibition of germination was observed.
6. Seed varieties were shown to differ in susceptibility to increasing concentrations of ammonium hydroxide.
7. Speed of germination of the seed variety was shown to be one of the most important factors in escape of the full injurious effect of the dried blood fertilizer during germination.

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