III.

STUDIES ON CHEMICAL METHODS FOR OVERCOMING DELAYED GERMINATION.

Preliminary studies on vapour and gases.
Discussion of results.
Short-period gas and vapour treatments.
Discussion of results.
Solutions of organic and inorganic compounds.
Discussion of results.

Summary .

With ordinary agricultural seeds the problem of delayed germination seldom, if ever, forms a disadvantage in practice and attention has mostly been directed thereto with an object other than that of finding means of hastening the process for purposes of practical application. In contrast to this, however, are the numerous investigations on the chemical stimulation of germination which were generally undertaken for the stimulation of germination for agricultural use though not particularly for the improvement of delayed germination.

It might be mentioned here that practically all the studies reported in this and the following chapter were undertaken long before those on the apparent causes of delayed germination, reported in the previous chapter.

Preliminary studies on vapours and gases.

The following vapours and gases were tested out in order to learn something about the response of dry seeds to such treatment:- Hydrochloric acid, nitric acid, acetic acid,

sulphur / ..

sulphur dioxide, nitrogen peroxide, ammonia, carbon bisulphide, carbon dioxide, oxygen, ether, chloroform and acetylene. An excess of everyone of these substances was used and in all except hydrochloric acid the atmospheres in the various flasks were made humid at the commencement of each test by placing moist filter paper inside.

For every treatment except chloroform and ether, nine kinds of seed (all harvested March 1936) were used and these were placed in small cotton muslin bags in large well-stoppered 3 litre flasks in which they were subjected to the influence of the above-mentioned gases and vapours. The flasks were kept sealed for two months when they were opened and the seeds tested in the usual way at 30°C. The seeds of individual treatments were all kept together in one flask.

Discussion of results.

In the case of chloroform and ether, only two Digitaria ecotypes were employed and for these two treatments as well as for hydrochloric acid, acetic acid, ammonia, nitrogen peroxide and sulphur dioxide the results were negative, these chemicals apparently proving harmful with a two months' treatment.

In these studies were employed :- Digitaria ecotype C.1 (Kuruman) and a selection 24-3 of the ecotype "Inkruip"; Panicum coloratum ecotype C.98; Panicum sp. ecotype "Makarikari"; Panicum minus ecotype F.14; P. maximum ecotype F.6; Chloris gayana ecotype F.15 and Setaria sphacelata ecotype H.10. The latter two were harvested in May 1936, the others all in March, the test being conducted end of July 1936.

In the case of carbon dioxide, the germination over the first eight days' duration of the test showed over 40% stimulation for Panicum coloratum ("Hammanskraal") C.98, 62.5%

for / ..

for Panicum minus v. planifolium F.14, and 16% for Panicum sp. ("Makarikari") C.79, as compared with their respective controls, whereas no or slight stimulation was obtained with the other kinds. F.15 was discarded after a few days owing to fungi but the treatment appeared to have been harmful. Carbon bisulphide gave no or slight stimulation while nitric acid vapours gave for Panicum C.79 a 33% increase and for Digitaria 24-3 a 53% increase (over their controls) within the first eight days of the test, whilst for the others the effect was either harmful or valueless.

With oxygen, Panicum minus F.14, gave a 28% increase, for Panicum C.79 a 41% increase and for Digitaria ("Kuruman") C.1 a 20% increase for the first eight days in comparison with their respective controls. With the rest of the seed (lines) only slight or no increases were registered.

Afterwards carbon dioxide and oxygen were used at room temperature and at 45°C for keeping (storing) seeds in, the gases being renewed after every monthly test. Seven lines, the results for two of these appearing in table 13 , were tested for six months. It will be observed that at 45°C both oxygen and carbon dioxide used alone, were harmful, particularly the former, as the germinative power appeared to be soon lost, although in two of the five grasses a slight increase over the control was registered at the first monthly test. O_2 at room temperature seemed to be about equal to the controls. CO_2 at room temperature did not equal the controls and only in one case showed a gradual increase up to the 6th monthly test. In table 13 the monthly values of two of these grasses are tabulated.

TABLE 13 / .,

TABLE 13: THE MONTHLY GERMINATION OF TWO ECOTYPES TREATED WITH CO_2 AND O_2 AT ROOM TEMPERATURE AND 45°C, FOR SIX MONTHS.

Name of Grass	No. of monthly test	Percentage Germination: Control	CO_2 at room T	CO_2 at 45°C	O_2 at room T	O_2 at 45°C
Panicum minus var. planifolium B. 7/11	1st	4.0	0.6	5.0	2	0
	2nd	9.7	5.2	8.1	12.7	0
	3rd	11.5	6.1	6.2	10.0	0
	4th	16.2	8.8	3.5	16.3	0
	5th	23.6	8.2	2.1	16.6	0
	6th	26.3	11.8	0	22.2	0
Digitaria sp. "Inkruip" 27-9	1st	0	0	2.2	0	9.8
	2nd	0	0.3	6.3	0.2	0
	3rd	0.4	0.8	0.9	0.7	0
	4th	1.1	0.2	0	0.9	0
	5th	1.1	1.0	0	1.0	0
	6th	1.2	0.5	0	1.6	0

With acetylene, Panicum C.79 and Panicum minus F.14, gave respectively 27% and 14% increases over their controls. In the case of the other seed, the effects were either indifferent or harmful.

The above percentages were secured at even temperatures over an eight-day period. Thereafter all tests were subjected to various combinations of alternating temperatures and it is interesting to record the effects. Amongst those kinds of seeds that received the CO_2 treatment, Panicum C.79 and Setaria H.10, showed very distinct stimulation when room temperature (six hours) plus 30°C (eighteen hours) followed the even temperature treatment at 30°C. Under the same conditions, Panicum C.79 and Panicum minus F.14, with the CS_2 treatment, exhibited marked increases over the even temperature periods, indicating that the lack of germination capacity experienced

after / ..

after two months of this treatment is not all to be interpreted as being due to harmful effects. Perhaps a great deal, if not entirely, is to be ascribed to induced or secondary delay or "dormancy".

With HNO_3 treatment, only Digitaria 24-3 appeared to have exhibited some stimulation on substitution of alternating temperatures. The total germination of 80% is perhaps largely the result of the original treatment. In the case of the O_2 treatment, no marked effect as a result of alternating temperatures is observed. On the other hand, a favourable influence from such treatment was detected in certain of the kinds subjected to acetylene gas. Again Panicum C.79 was distinctly stimulated, but the germinability of Setaria H.10 could also be improved in this way. It is, therefore, not clear whether the lack of germination was due to loss of viability or not. In a few of the cases bacteria and fungi developed abundantly in the seed-beds.

Though other workers have used ammonium salts, the use of ammonia vapour has apparently been rarely used. McCallum (1909), using this gas, obtained favourable results with potato tubers. Barton (1940), employing concentrations of 1000 and 250 p.p.m. for 1, 4, 15, 60 and 960 minutes of continuous flow over moist and dry seeds of radish and rye obtained a toxic effect in the case of moist seeds of both kinds whilst in the case of dry seeds no reduction in percentage was experienced.

CS_2 has often been studied. According to Lehmann and Aichele (1931), Prillieux (1878) obtained harmful effects after three days treatment of wheat. In connection with disinfection studies these authors recorded the harmful effects reported by several authors. Addition to the soil gave stimulation, according to Kooh (1912) - as cited by Lehmann and Aichele etc.. Bokorny (1913) obtained favourable results with it, dissolved

in / ..

in water, and when alcohol was added (1 c.c. per litre of water).

Chloroform and ether have often been employed not only for seeds but also for woody plants, etc., and as ether has been employed by many workers, a complete review would be out of place here. Both dry and moist (frozen or soaked) seeds were employed in past studies. Coupin (1899) and Burgerstein (1906) - cited by Lehmann and Aichele (1931) - demonstrated the importance of moisture content of seeds in this respect. Ether was not only employed as a vapour but also in aequeous solution. That the state of delayed germination is also an important consideration seems clear and the results of Kiessling (1911) - cited by Lehmann and Aichele (1931) - confirm this. He appears to have been the only worker who gave attention to this aspect. Not only the condition of the seed, but also that under which the test is carried out (temperature, etc.) must be important. Howard (1915) - Bulletins 17 and 21 - reviewed most of the earlier work on anaesthetics (plants and seeds) and also reported his own work on the effect of ether on CO_2 production in cuttings under various conditions and on dry, soaked and frozen seeds. Lehmann and Aichele (1931) reviewed much of the work on Gramineae. Both favourable and unfavourable effects were realized .

Chloroform was studied under very similar conditions by a number of investigators and more or less the same remarks apply here as immediately above. Acetylene has been reported as a rootforming substance from the Boyce Thompson Institute.

Giglioli (1895), after 16 years storage of lucerne seed in an alcoholic solution of SO_2, still found 0.15% germination. It was also found most harmful by Barton (1940) who studied its effects on dry seeds and moist seeds at 250 and 1000 p.p.m. for periods varying from 1 min. to 16 hours. Apparently only these workers have employed this gas in such

studies / ..

studies. Work on NO_2 has not come to the writer's notice.

For the marked disagreement between the two sets of experiments with CO_2 and O_2 the only explanation that suggests itself is the question of moisture or humidity of the atmospheres in the two series.

The above results with CO_2 and O_2 treatments do not appear to be very consistent and it is perhaps surprising that stimulation was obtained with both gases, though not with the same ecotypes. That these gases play an important role in delayed germination seems certain, if only under certain conditions, and it appears not unlikely that with further investigation it should be possible to employ them for the overcoming of delayed germination. That these gases, alone and in mixtures, play and can play a very decisive role in the events associated with the disappearance of delayed germination, has been established by various workers though pre-treatment of dry seeds has hardly ever been reported on.

Giglioli (1893), using O_2 and CO_2 (dry) realized respectively 0.68% with lucerne seed after 16 years and 0% with lucerne, wheat and vetch after 17 years storage. Kiessling (1911) found that with oats and barley, increased O_2 is deleterious to fully after-ripened, but favourable to non-after-ripened seeds. Crocker (1906) amd Shull (1914), working with Xanthium seeds, found that by increasing O_2 supply an immediate increase in absorption and germination resulted as was later (1935) found by Thornton. Atwood (1914) obtained similar results with Avena fatua. Becker (1912) - cited by Shull (1914)- studying Dimorphotheca pluvialis found better germination in O_2 than in air (especially the ray seeds). Exposure for 30 hours to O_2 (though not 15 hours) favoured further germination in atmosphere. Increased O_2 pressure favoured the germination of Calendula eriocarpa seeds, whilst two Atriplex spp. were

injuriously / ..

injuriously affected. Kidd (1916) considered that "the widely occuring phenomenon of delayed germination in the case of the moist resting seeds are related to an inhibitory partial pressure of CO_2 in the tissues of the embryo". Working with potato tubers he, 1919, found an O_2 and CO_2 concentration, above 5.1% and 20% respectively, harmful, the deleterious action of the former increasing in the presence of CO_2. Harrington (1917) showed that CO_2 (in a wide range of concentrations) induced germination of non-afterripened Johnson grass seeds. Gardner (1921) promoted the germination (in darkness) of Rumex Crispus and carrot seeds by increased O_2 pressure. Harrington (1923), working with cereals, obtained similar results but non-afterripened seeds of Johnson's grass failed to respond to an increase of O_2 (Harrington and Crocker 1923). With Typhia latifolia seeds, Morinaga (1926) found poor germination in air but reduction of this concentration of O_2 by addition of H or N to the extent of 40-80%, gave approximately 90% germination. A 94% germination was secured with a 99% H mixture. When seedcoats were broken, the germination took place at O_2 pressure of 1-90% of that of the atmosphere. Cynodon dactylon seeds also germinate better with reduced O_2 partial pressures (dilution with H or N) but the effects were not as marked. Kondo and Okamura (1930) found hulled rice stored in CO_2 retained germination capacity perfectly for 4 years. Braun (1931) found that 5-8% CO_2 was most favourable for the hastening of the sprouting of dormant potato tubers.

Thornton (1933) reported on the influence of CO_2 on the O_2 uptake and acidity of tubers, bulbs etc. He, 1933, further recorded the effects of CO_2 and O_2 and mixtures of these on the sprouting of potato tubers. In 1935, after studying the O_2 and CO_2 requirements and development of dormancy in the upper and lower seeds of Xanthium, he also found that the

needs / ..

needs for germination were very different for the two types of seed (intact) and their naked embryos at different temperatures. Like Shull (1914), he drew attention to the normal procedure in germination with O_2: "Germination of the intact seeds in O_2 takes place with the growth of the cotyledons before the growth of the radicle rather than by growth of the radicle followed by an enlargement of the cotyledons as is the normal procedure." He, 1936, showed that lettuce seeds ordinarily requiring 20°-26°C to germinate (in darkness) would give good germination in the presence of CO_2 even at 35°C. Higher CO_2 concentration (in conjunction with O_2) was needed for germination at higher than at lower temperatures. In 1939 the same author reported on the relationship of O_2 to CO_2 in breaking the dormancy of potato tubers, CO_2 in presence of 20% O_2 being found very effective. After further investigations (1939), he claimed that too much O_2 prevented sprouting and that freshly harvested potatoes would sprout in 7 days if held in 5 to 10% of O_2 under moist conditions; in 9 days with 2% O_2 under dry conditions. Under natural conditions this takes place because of the reduced permeability of the periderm to O_2, subsequent to the suberization of its tissues.

Potzoff (1936) found that CO_2 or O_2 atmospheres did not influence after-ripening of tobacco seeds, Zimmerman and Hitchcock (194) obtained favourable results by treating Althea cuttings with high concentrations of CO_2.

Though several authors have employed HCl, HNO_3 and acetic acid solutions for promotion of germination with varying success, exposure to vapours has not been recorded and few have done work on their effect on delayed germination. Harrington (1917) found no forcing action with HCl and acetic acid solutions on Johnson's grass seeds. Gardner (1921) promoted the germination of tobacco seeds and Rumex crispus in darkness by

means / ..

means of HCl solutions. Ray and Stewart (1937) subjected non-after-ripened Paspalum seed to 37% HCl, obtaining slight improvement in germination. Barton (1939) employed it in high concentration as a scarifying agent for the seed-coats of certain grasses.

Short-period gas and vapour treatments.

As the abovementioned results appeared to indicate the possibilities of this type of treatment, a number of gases and vapours were tested out over short periods. The following were employed : Thioacetic acid, thioglycollic acid, ethyl thiocyanate, ethyl iodide, ethylene dichloride, carbon tetrachloride, chloroform, carbon bisulphide, ether, formalin, ammonia, glacial acetic acid, toluol, nitric acid, nitrogen peroxide, carbon monoxide. The seeds of seln.24-3 from the "Inkruip" Digitaria ecotype, with an extended delayed germination, was used in all these tests unless otherwise stated, because it was considered that if good stimulation could not be secured with this, it was hardly worth while testing out seed of other ecotypes, although it was realized that the response might vary with the ecotype. Germination tests were conducted in the usual way at ca. 30°C. The seed was harvested in March 1936 and practically all the tests were conducted during that year. In all these tests a standard type dropping bottle was used to measure the quantity of the chemical and no water was used unless stated.

Thioacetic acid at the rate of six drops per two litre flask (air) gave 6.7%, 5.3%, 47.8% and 0% germination after one hour, two hours, 10 hours and 24 hours respectively. Employing only one period (eight hours) but varying concentrations, viz. 1, 2 and 4 drops p. 2 L., the germinations were 4.6%, 7.4% and 3.4% respectively. With the control at approximately / ..

proximately 6%, the maximum stimulation was over 40%.

Thioglycollic acid with the same ecotype gave 29% germination, using 2 drops per 2 L. flask for 1 hour; with 1 drop the germination was 27.9% and 17.5% respectively for six and 19 hours; with 4 drops, 34%, 25.6% and 8.8%, for 2, 11 and 24 hours respectively; and with 5 drops, 30.6%, 15.1% and 10.6% for 2, 3 and 4 hours respectively. The control tested about 1[illegible].

Ethyl thioyanate at a concentration of 0.75 c.c. per 2 L. flask, yielded after 6 hours with Digitaria seln. 24-3, Panicum coloratum C.91 and Panicum maximum F.7, 37.2%, 5.5% and 0% germination respectively; after 24 hours, 29.1%, 14.6% and 0% respectively; after 3 and 5 days no germination and after 11 days, 7.8%, 6% and 0% respectively, the controls being all approximately 2%. The response is somewhat unusual.

Ethyl iodide at a concentration of 4-5 drops per 2 L. flask exhibited 19.5%, 26.6%, 32.1%, 17.9% and 0% germination over 1 hour, 3 hours, 6 hours, 22½ hours, 11 days, 14 days and 21 days respectively; for a 17 hour period, 4.8%, 13.5% and 17.6% germination were realized with 12 and 5 drops respectively. Using 5 drops over periods of 3, 6, 12, 24 and 48 hours, the percentage germination was respectively 12.4%, 14.0%, 27.8% 13.7% and 16.2%. Over a period of 3 hours, 10 drops gave 13.0% germination and with 1 drop over periods of 7½ hours, 24 hours, 2 days and 5 days the percentages were 4.1, 4.7, 8.3 and 1.0 respectively. The control was approximately 14% germination.

With ethylene dichloride, used at the rate of 1, 2 and 5 drops per 2 L.flask, Digitaria 24-3 germinated 2.4%, 1% and 2.6% respectively and with 10 drops over periods of 1 hour, 3 hours, 10 hours, 24 hours, 9 days and 15 days, resulted in 5.4%, 2.3%, 2.4%, 4.1%, 4.6%, and 23% germination respectively, the control being approximately 6%.

Employing carbon tetrachloride, at 10-11 drops per

2 L. / ..

2 L. flasks gave 19.5% and 13.9% respectively, the control being about 14%. Formalin, 40%, used at a concentration of 10 drops for 1 hour and 4 hours, resulted in 11.8% and 25.3% respectively, while 5 drops for 4 hours yielded 23.5% and 2 drops for periods of 4 hours , 25½hours and 2 days resulted in 10.7%, 21.0% and 18.5% respectively, the control being about 14%.

Ammonia, tested out in the concentrations of 1, 2 and 4 drops per one L. flask for 1 hour periods showed 32.4, 35.0 and 19.6% germination respectively, whereas 1 drop (per 2 L.flask) at periods of ½, 1 and 1½ hours showed respectively 9.4%, 25.3% and 9.0%, with the control at about 14%. Carbon tetrachloride used together with ammonia gave lower values than for the separate compounds. On the other hand, glacial acetic acid tried out with concentrations of 1, 2 and 4 drops for 1 hour periods, yielded only 10.3%, 9.0% and 3.5% germination respectively, the control being about 14%. Toluol gave even lower values.

Nitric acid which had previously, with two months' treatment, given very promising results with seed of Digitaria seln. 24-3, was again used at shorter intervals and controlled concentrations. In subdued light 10 drops per 2 L. flask gave after 1¾ hours slightly better germination in a dry atmosphere than in a humid atmosphere; after 3 days the moist atmosphere still gave a germination but not the dry atmosphere; after 10 days, however, the seeds in the moist atmosphere had also lost their germinating power. Using (in dark) 2, 5 and 10 drops (the acid spread out in the flask) as well as 5 c.c.acid, per 2 L. flasks for three days, best germination was obtained with 2 drops, 1 drop yielding no improvement in germination and the other concentrations no germination. In the dark set, the 2 drops for 2, 5 and 26½ hours yielded 36.6%, 13.5% and 27.4% respectively with contral at 11%. Using 2 drops per 2 L. flask for 1½ hours, 4 days, 10 days, 25 days and 70 days in darkness,

../ ..

the percentage germination obtained was respectively 4.6, 33.0, 35.0, 0.0 and 0.0, with control at 9.8%. Panicum minus var. planifolium, F.14, with concentrations of 3 drops per 3000 c.c. jars, with and without $CaCl_2$ germinated as follows :-

	with $CaCl_2$	without $CaCl_2$	with $CaCl_2$	without $CaCl_2$	with $CaCl_2$	without $CaCl_2$	
for:	2 hrs	2 hrs	5 hrs	5 hrs	10 hrs	10 hrs	Control
	%	%	%	%	%	%	%
	76.4	63.1	82.4	58.2	2.6	40.6	28.7

The importance of the humidity factor is clearly indicated, the effect of humidity being reversed for the 10 hour period as compared with the 2 and 5 hour periods. Employing the same strain and the same concentrations for 2 hours, (a) acid alone, (b) acid with $CaCl_2$; (c) acid with pyrogallic acid; (d) acid with pyrogallic acid plus $CaCl_2$, gave 42.7%, 64.6%, 33.6% and 36.7% germination respectively, indicating the importance of the presence of oxygen for a favourable reaction. The action of nitric acid fumes is no doubt a complex one and the substances formed are naturally influenced by temperature, illumination, acid concentration, humidity, amount of seed, the period of treatment and volume of air.

Nitrogen peroxide being set free by nitric acid when it fumes, was also tested out as it was thought that the stimulative properties of nitric acid fumes might be due to this gas. Using P. minus F.14 and employing the reaction excess copper plus nitric acid, the following concentrations were studied for 2 hours in 3000 c.c. jars:- (a) No $CaCl_2$ plus 3 drops acid; (b) $CaCl_2$ plus do.; (c) No $CaCl_2$ plus 10 drops acid; (d) $CaCl_2$ plus do. The percentage germination was respectively 71.8, 44.3, 85.4 and 75.8 proving the value of a humid atmosphere for low concentrations of the treatment which seems contrary to the nitric acid treatment alone. That the presence or absence of $CaCl_2$ has little or no value with 10 drops acid for 2 hours,

was / ..

was also established for 5 other strains with 10 drops acid and no $CaCl_2$ for 2 hours. Other strains varied in their response from fair to nothing. For a period of 1 hour this treatment appeared less effective. Lead nitrate, as a source of the gas, was also tried. No doubt the 2 months' treatment mentioned above was too long to be effective.

Carbon monoxide used alone in 1, 2, 5, 20 and 50% mixtures with air to germinate Digitaria seln. 24-3 in, resulted in only the 2% mixture giving stimulation. With 200 uncleaned seeds the germination was 8.5% and of 20 cleaned seeds 15 germinated. The rest of the mixtures yielded no germination.

Discussion of results.

On the whole the results are somewhat disappointing, though it is perhaps remarkable to find that of the 18 compounds used for the treatment of dry seeds, only four, toluol, acetaldehyde, ethylene and HCN showed no improvement in germination over the control. Experience has, however, shown that the kind of seed used is one of the most difficult to stimulate and seed of other ecotypes would perhaps have given much higher values. Thioacetic acid, carbon tetrachloride and HNO_3 (NO_2) gave the best results.

Thioacetic and thioglycollic acids were reported as effective in breaking the dormancy of potato tubers (Miller 1930). Ethyl thiocyanate has not (?) been employed by other workers. Ethyl iodide was found effective for forcing of plants by Stuart (1909) and Denny and Stanton (1926). Ethylene dichloride vapours gave favourable results in the forcing of early sprouting of potato tubers and the shortening of the rest period of woody plants at the Boyce Thompson Institute (Denny 1926a, 1926b ; Denny and Stanton 1926). Carbon tetrachloride (vapours) has apparently been rarely used. Stuart (1909) and McCallum (1909)

used / ..

used it for plant forcing, it being reported effective by the latter. Müller (1928) usedit with barley and considered that a more intensive germination was indicated. The influence of formalin solutions on germination has been studied by many workers, both favourable and unfavourable results being recorded. Hurd (1921) demonstrated the effect of injury to seed-coats and of subsequent drying. Atwood (1922) showed the importance of after-treatment, whereas Molz and Müller (1925/6) - cited by Lehmann and Aichele (1931) - found that temperature of germination was important. Zeuschner (1926) claimed that coat thickness variarions in various wheats were not responsible for the different responses. The use of carbon monoxide has apparently only been reported by Giglioli (1895) who recorded 84% germination for lucerne after 16 years' dry treatment and from the Boyce Thompson Institute (Zimmerman et al, 1933) where it was found to induce root initiation.

Solutions of organic and in-organic compounds.

Solutions of the following compounds were used for seed treatment as a means of overcoming delayed germination, the tests being conducted in 1936 and 1937: Thioglycollic acid, thiourea, potassium thiocyanate, semi-carbazide hydrochloride, sodium thiosulphate, alpha naphthalene acetic acid and beta indolyl acetic acid. Also, nitric acid, phosphoric acid, boracic acid, ammonium dithiocarbonate, hydrogen peroxide, succinic acid, sodium hydroxide, sulphuretted hydrogen, acetaldehyde, and potassium permanganate were tested out, but so far have shown little or no promise. The same methods of testing as above, were employed.

When seed of Digitaria seln. 24-3 was steeped in a 2% thioglycollic acid solution for 1, 3, 10, 24 and 49 hours,

the / ..

the germination obtained was respectively 9.3%, 17.6%, 33.7%, 10.6% and 2.1%; with a 0.5% solution and 1, 3 and 6 hours, the germinations obtained were 5.6%, 8.0% and 5.6% respectively, whereas with a 0.1% solution and 1, 3, 9½ and 50 hours, the percentages were 6.9, 4.9, 9 and 8,8 respectively. When the latter concentration was used as a medium, the germination was 15.8%. The control was approximately 6%.

Thiourea, when used as in the last-mentioned, at a concentration of 1%, then after 1¼ hours Digitaria seln.24-3, Panicum coloratum C.91 and P. maximum F.7 gave respectively 6.4%, 0% and 31.4%; after 4 hours, 7, 0 and 30.8% respectively; at 6 hours, 4.3%, 0% and 25.8% respectively; whilst after 12 hours the values were respectively 8%, 0% and 14.0%. All three controls were about 2%.

Potassium thiocyanate as a 1% solution and with one hour soaking gave with lines seln.24-3, C.91 and F.7 respectively, 11.3%, 1.2% and 27.9% ; with 2 hours, respectively 9.8%, 0% and 27.2%; with 4 hours, 4.6%, 0% and 31.3% respectively ; with 6 hours, 6.7%, 0% and 14% respectively and with 12 hours, respectively 6.1%, 0% and 3.45%. A 2% solution showed after 12 hours respectively the following : 21.7%, 0% and 30%; after 18 hours, 21.7%, 0% and 26.4%; after 24 hours, 24.5%, 0% and 15.4% ; and after 41 hours, 18.3%, 2.1% and 29.2%. As a medium of ca. 0.09% concentration seln.24-3 gave 36.6% germination and as a medium of 0.009% the germination was 12.8% for the same time and kind. All three the controls exhibited about 2% germination.

Semi-carbazide hydrochloride with Digitaria seln. 24-3, giving as control about 6%, yielded respectively 8.1%, 6.1% and 3.5% germination in a 0.1% solution for 1, 3 and 9½ hours; in a 0.2% solution for 1, 3¼. 10, 24 and 50 hours the percentages were respectively 12.0, 7.4, 12.5, 14.2 and 10.5,

whereas / ..

whereas with a 0.5% solution for 1, 3, 6 and 50 hours, 7.9%, 10.4%. 5.3% and 4.6% respectively were realized. A 5% solution gave 9.1%, 7.0%, 4.8% and 2.5% with ½ hour, 1 hour, 2 hours and 19 hours treatment respectively, whilst a 0.1% solution used as a medium, gave 8.3% germination.

Sodium thiosulphate was tried out on line seln.24-3 in several concentrations, each for 2 periods, but with no success.

Alpha naphthalene acetic acid and beta indolyl acetic acid were studied in more detail, as indicated in the table below. With the Panicum F.14 it proved fairly beneficial giving increases of over 35%, whilst the Digitaria 15-7 was deleteriously affected.

T A B L E 14 / ..

TABLE 14 : THE PERCENTAGE GERMINATION OF DIGITARIA SELN: 15-7 AND PANICUM ECOTYPE F14, WHEN TREATED WITH ALPHA NAPHTHALENE ACETIC ACID AND BETA INDOLYL ACETIC ACID

Treatment	1hr. Soaking F14 %	1hr. Soaking 15-7 %	2hrs. Soaking F14 %	2hrs. Soaking 15-7 %	6hrs. Soaking F14 %	6hrs. Soaking 15-7 %	As a Medium F14 %	Control F14 %	As a Medium 15-7 %	Control 15-7 %
Alpha naphth.acetic acid. 0.01 gm : 100 cc. water	57.7	2.4	57.2	2.6	68.4	2.7	29.0	ca.30	8.3	ca.17
Beta indol. acetic acid. 0.01 gm : 100 cc. water	57.6	6.0	34.5	5.4	62.8	5.1	46.6	do	14.4	do
Alpha naphth. acetic acid 0.05 gm : 100 cc. water.	37.6	2.8	-	-	47.2	3.0	-	do	-	do
Beta indol. acetic acid. 0.05 gm : 100 cc. water.	37.1	3.3	-	-	41.6	4.3	-	do	-	do

Treatment	18hrs. Soaking	18hrs. Soaking	12hrs. Soaking	12hrs. Soaking						
Alpha naphth.acetic acid. 0.005gm : 100cc. water.	-	-	37.2	11.7	62.6	6.3	22.8	do	8.0	do
Alpha naphth.acetic acid. 0.0005gm : 100cc.water.	63.4	22.3	55.4	7.8	-	-	46.4	do	16.4	do

63.4
57.7
37.6

22.3
2.4
2.0

Solutions of alpha naphthalene acetic acid of 1 pt. per 20,000; 1;100,000; 1;1,000,000; 1:10 million and 1:100 million, used as media with lines selns 24-3 and 15-7 showed no trend of response in either direction and when Panicum ecotype F.14, was soaked in all of the latter solutions for 12 hours, no definite trends were obtained either. In the case of F. 14 the control gave the same percentage germination as the different media but, if anything, a slightly better value in the soaking trial. Digitaria line 24-3 also showed no definite response to this chemical, used as media (with quartz sand) in the above concentrations. There seemed to be no improvement in germination when, after soaking, the seeds were first dried in the sun or room and thereafter stored for 16 days or as long as 5 months.

Discussion of results.

Thioglycollic acid proved to be no more stimulative in the solution form than in the vapour state. The optimum concentration appeared to be about 2%. Thiourea, though it stimulated one of the panicums, was apparently harmful to the Digitaria. This chemical has proved most useful for hastening the sprouting of potatoe tubers (Denny, 1926) and for breaking their dormancy (Miller, 1933). Also, Deuber (1931) found it to have a beneficial effect on maple seeds. Potassium thiocyanate has proved to be somewhat similar to the last-mentioned. Though Digitaria selection 24-3 could not be stimulated by means of soaking, a solution thereof, used as a medium for germinating seeds in, gave an increase of over 30% in germination. For the sprouting of potato tubers, Denny (1926) found it excellent. Semi-carbazide hydrochloride has not (?) been used by other workers; it proved to be of no value under the conditions of the above test as was the case with sodium thio-

sulphate / ..

sulphate . The latter was, however, found excellent for the sprouting of potato tubers (Denny 1926, 1935).

The two growth substances alpha naphthalene acetic acid and beta indolyl acetic acid have been fairly extensively used for rootformation on cuttings, for which they have been found very effective. In this connection the investigations of the workers at the Boyce Thompson Institute perhaps deserve special mention. Few have studied the effects on seeds. Amlong and Nauendorf (1937) found that strong heteroauxin solutions gave better germination of old seeds. Lustig and Wachtel (1938) reported that heteroauxin had no effect on the germination of cress seeds. Barton (1940a), treating non-dormant seeds with vapours, liquids or dusts, found these of little or no value. With dormant seeds of apple, etc., no beneficial and even some harmful effects were shown. The present tests have throughout given harmful or indifferent results with the Digitaria, whilst the Panicum, under certain conditions, was found to be well stimulated.

Summary.

1. Preliminary studies on the effects of vapours and gases on dry seeds were undertaken to ascertain whether this method would offer any possibilities of overcoming delayed germination.

2. Chloroform, ether, hydrochloric acid, acetic acid, ammonia, nitrogen peroxide, sulphur dioxide, carbon bisulphide, acetylene, nitric acid, oxygen and carbon dioxide were tested out, an excess of each substance being employed. Nine lines of seed were subjected to treatment for 2 months. The first 7 substances enumerated produced negative results, which, in some instances, may have been due to the period of treatment having been too long.

3. / ..

3. CO_2 produced as much as 62.5% stimulation with P. minus F.14, whilst 2 other lines were also distinctly benefited, little or no increase being registered with the other seed. O_2 gave distinct increases in 3 of the lines, the maximum being 41% for Panicum C.79.

4. CS_2 proved of little or no value under these conditions, whilst with acetylene an increase of 27% over the control was realized with Panicum C.79 , the other lines being harmfully or indifferently affected. With HNO_3 , 2 kinds showed appreciable benefit, amounting to 53% increase for Digitaria seln.24-3, within the first 8 days of test, which was also the period used in the above comparisons.

5. O_2 and CO_2, used at room temperature and at 45°C, for storing seeds in, were found to be of no value over a period of 6 months, and proved to be harmful at 45°C, after about the first month. At room temperature these gases equalled or were below the controls.

6. Short-period treatments, using mainly Digitaria seln.24-3, were tested out with gases or vapours of thioacetic acid, thioglycollic acid, ethul thiocyanate, ethul iodide, ethylene dichloride, carbon tetrachloride, chloroform, carbon bisulphide, ether, formaline, ammonia, acetic acid, toluol, nitric acid, nitrogen peroxide, carbon monoxide, acetaldegyde, ethylene and hydrocyanic acid gas.

7. Though improvement in germination was realized with all except 4 of these chemicals, the results appear to be of little practical value. Thioacetic acid, CCl_4 and HNO_3 were the best, registering maximum increases of 43%, 43% and 54% respectively over the controls.

8. Solutions of thioglycollic acid, thiourea, potassium thiocyanate, semi-carbazide hydrochloride, sodium thiosulphate,

alphanaphthalene / ..

alphanaphthalene acetic acid, beta indolyl acetic acid, acetaldehyde and others, were used for overcoming delayed germination.

9. Seeds were either soaked in solutions of these chemicals or, in some instances, the solutions were used as media. The results were less favourable than with the gases and vapours, the best increases being realized with the two growth substances, though one of the two lines of seed employed was unfavourably affected.
