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A STUDY IN CONNECTION WITH THE PROBLEM OF HORMONIZATION OF SEEDS

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CONTENTS

LIST OF ABBREVIATIONS AND COMMERCIAL PREPARATIONS

c.p.a.	—	p-chlorophenoxyacetic acid
2,4-D	—	2,4-dichlorophenoxyacetic acid
2,4-DK	—	potassium salt of 2,4-D
2,4-DNH$_4$	—	NH$_4$ salt of 2,4-D
e.i.	—	3-ethylindole
i.a.a.	—	indole-3-acetic acid
i.a.a.K	—	potassium salt of i.a.a.
i.a.a.Na	—	sodium salt of i.a.a.
i.a.d.	—	indole-3-acetamide
i.b.a.	—	indole-3-butyric acid
i.b.a.K	—	potassium salt of i.b.a.
i.m.m.a.	—	(indole-3-methyl)-malonic acid
i.p.a.	—	indole-3-propionic acid
n.a.a.	—	naphthalene-1-acetic acid
n.a.a.K	—	potassium salt of n.a.a.
n.a.a.Na	—	sodium salt of n.a.a.

n.a.d.	—	naphthalene-1-acetamide
n.o.a.a.	—	2-naphthoxyacetic acid
ph.a.a.	—	phenyl acetic acid
sol.	—	solution
t.i.b.a.	—	2,3,5-triiodobenzoic acid
t.u.	—	thiourea
vit.	—	vitamin

Agrosan	—	a seed disinfectant containing an organic mercury compound
Auxan	—	commercial powder preparation containing a growth substance
Auxilin	—	commercial preparation containing i.b.a. (Pennsylvania Chemical Co)
Belvitan	—	commercial preparation containing i.a.a.+i.b.a. (I.G. Farben Ind., Bayer-Leverkusen)
Ceresan	—	a powder disinfectant containing ethyl mercury phosphate
Euradin	—	commercial preparation containing n.a.a.K
Germisan	—	a seed disinfectant containing an organic mercury compound
Granosan	—	a proprietary mercurial seed dressing manufactured by the Bayer Semesan Co in the U.S.A.
Hormodin	—	commercial preparation containing i.b.a. (Merck Co)
Merck dust	—	commercial powder preparation containing i.b.a. (Merck Co)
Roche 202	—	commercial preparation containing n.a.a. (Hoffmann – La Roche)
Rootone	—	commercial powder preparation containing n.a.a. (American Chem. Paint Co)
Semesan	—	a powder disinfectant containing 30% hydroxymercurichlorophenol
Spergon	—	a seed disinfectant containing tetrachloro-p.quinone

INTRODUCTION

Statement of the problem

The idea of stimulating the germination of seeds by a particular treatment which might improve growth, development and crop as well, evidently dates from long ago. The ancients sought to realize it by the use of manure and urine.

After it became known that simple chemical compounds could act as fertilizers they soon found employment in the treatment of agricultural seeds. About 1760 the increasing knowledge of plant-diseases and their control led to the use of copper salts and mercury salts for seed disinfection.

In a modern sense both processes survive in the form of the so-called seed-dressing: the coating of seed with a combination of nutrients and disinfectants, in order to protect it against attacks by micro-organisms, fungi and insects.

Under the influence of the hormone-theory, conviction gained ground that in plants development is regulated by specific substances from the very beginning. It was found that the endosperm of different kinds of seeds contained one or more hormone-like substances, taken up by the embryo in the initial stages of development, and that subsequent processes were closely interrelated with those occurring during the first stages (149, 171, 178, 182).

So it was obvious to suppose that a lack of germinative power might be due to a shortage of natural auxin, which, in the first place, might arise from prolonged storage, causing inactivation. A possible interference by phytohormones with the processes resulting from the so-called vernalization was also taken into account.

In short, when the time was ripe for it attempts were made to influence the germination of seed and the development of the plant by administration of *synthetic growth substances.*

CHOLODNY, in 1936 (25, 26), was the first to report positive results from seed-treatment with hetero-auxin. From then research in this domain expanded rapidly, until, since 1944, interest waned more and more, as appears from the number of papers on this subject (table I).

TABLE I

Number of papers concerning the treatment of seeds with growth substances during 1936—1951.

Year	Number	Year	Number
1936	3	1944	5
1937	5	1945	3
1938	12	1946	2
1939	9	1947	5
1940	14	1948	6
1941	18	1949	3
1942	20	1950	2
1943	13	1951	1

In the author's opinion this was occasioned by manifold discrepancies in the results. A further explanation is offered in chapter I.

Together with VELDSTRA (115) it may certainly be concluded that in the matter of seed-treatment the application of plant growth substances stands a good chance.

However, before arriving at applications useful in practice, the many factors ruling the processes of germination and development will have to be appreciated.

Therefore the present study was initiated with an analysis of the influence of exogenous factors upon the development of the embryo. The results may tend to obtain some more insight into the correlation-phenomena, playing a part during germination under normal conditions, and their influencing by treatments of the seed with growth substances.

CHAPTER I

SURVEY OF THE LITERATURE

(Treatment of seeds with synthetic growth substances)

Various effects have been pursued in the treatment of seeds with growth substances, mainly in view of practical applications.

In the first place it was tried *to improve the germination* of aged or badly germinating seeds, or *to arrive at larger crops* by influencing subsequent development (7). For, a particular treatment of the seed may raise not only the percentage of *germination* but its *rate* as well (8). An advance like this, as compared with non-treated material, conceivably may subsist down to harvesting. These differences, however, usually vanish during development (52, 64, 121).

In certain circumstances an *acceleration of growth* was observed (23) and, probably owing to this and to an *enlarged root-system* (9, 53), also an *advanced flowering* in comparison with the controls (107, 110, 113).

Breaking the natural dormancy of the seed was reported in a few cases only (14, 100). More recently, the deleterious effects of 2,4-dichlorophenoxyacetic acid and other herbicides have been used to advantage in preventing the germination or in killing the very young seedlings of some weeds. This phase of growth substance effects on germination seems to have much more promise of successful application (122–140, 230).

A phenomenon, stated first of all by GRACE in 1938, looked promising, viz. that the *injury inflicted upon seeds* by fungicides, hot water or other seed disinfectants *might be reduced* or even abolished by means of growth substances (32, 58–62, 116).

Finally it may be mentioned that after treating seeds with growth substances the plants showed *more resistance to diseases* (43, 90, 96).

Unlike other practical applications of growth substances in which the number of methods is but very limited, in seed treatment they widely diverge. Considering the large diversity of objects treated and the varying conditions, it is not to be wondered at that results were often contradictory.

A lengthy discussion of the papers on the subject would involve a great many of details and is intentionally omitted. However, to meet the convenience of those who want to consult the literature and for the purpose of surveying the results obtained so far, a number of data is summed up in table II.

Enumeration of both positive and negative results from the table shows that treatment with growth substances favoured *germination* in 81 cases, whereas an unfavourable effect or none at all was seen in 169 instances. Consequences on further *development* appeared to be positive in 115 cases; there were 104 negative effects.

Summarizing, in the treatment of seeds by means of growth substances negative results are predominant. It certainly will be more conspicuous on closer examination of the data.

Now it may be considered what errors have been made in research and from what sources the numerous, conflicting statements may have sprung.

1. *General experimental technique.* Common shortcomings are: the omission of replications (16, 17, 25), experiments on a scale too small to judge of (24, 25, 107–110) and the lack of control groups, the seeds

of which are sown in the dry state, without any treatment (e.g. 18, 22, 23, 25, 26, 74, 96, 110). Many research-workers have made these mistakes, thus rendering their results anything but reliable. If, owing to some treatment, part of the objects should die off they might procure thus much space in the trial-plot that the remaining plants ultimately would produce a larger yield (42, 49, 51, 55, 105). A wrong interpretation would class such a case among the favourable results obtained with growth substances.

2. *Influences of the object itself.* The effect of growth substance treatment will be highly dependent on the fact, whether or not the plant is able to supply its want of hormones by synthesis. In the former case no result is to be expected, whereas the latter does offer a possibility of overcoming a hormone deficiency (52, 68, 112, 117, 121).

It may be expected, therefore, that certain influences, exerted upon the mother-plant, will continue to take effect via the seed. For example, the seeds harvested after a period of drought, mainly being tiny, will raise a progeny less vigourous than that of larger seeds, formed under better growth conditions.

Though it has scarcely been touched upon in the literature, it may be assumed that some part is played by the nature and by the amount of reserve substances and ergons (i.e. the proportion embryo-size/mass of reserve substance). Reference to this matter is found under 67 and 95. Moreover, there may be influences of *origin* (10), *age* (9, 10, 86, 103), *dormancy*, *after-ripening*, *method of storage*, *differences in the permeability of the seed-coat*, and *inhibitory substances*, being present or otherwise (21, 40).

It would seem that the diversity within a given lot of seed is not properly realized, although several papers have pointed out the interest of *physiological condition* (52), *size* and *weight* of the seed with respect to the resulting crop (200-212). In multispermous fruits differences occur between the component seeds (100).

Many seeds are more or less injured by mechanical threshing. It is conceivable that the effect of a growth substance may be different, as it enters through the seed-coat or by way of a damaged spot. Besides, it is well-known that various, badly germinating seeds may develop well, after the seed-coat has been injured, either mechanically or chemically. This usually means an aid in clearing away a mechanical resistance offered by the seed-coat in germination.

All these points present possibilities of explaining, for instance, the contradictions arising between experiments on a small scale, under well-controlled conditions, and those on a larger scale, made in practice.

The difference in behaviour between varieties and strains with respect to similar treatment was pointed out already by McRostie, Hopkins and Grace (80); it was found again in later experiments (32, 42, 47, 52, 97, 103). Early strains generally would require a concentration of the growth substance lower than that, desirable for late strains (94, 97).

3. *Choice of the growth substance, method employed and manner of*

treatment. As mentioned already at the beginning of the chapter, there are almost as many methods as there are authors; standardization is out of the question.

a. Choice of the growth substance. As a matter of course this choice mostly will determine the ultimate result. It would appear that an injurious side-effect of certain growth substances sometimes may be counteracted by adding thiourea or a combination of it with vitamin C (4, 5, 6, 94, 96). This is contested by others (51), who ascribe it to an effect of pH alone. Some investigators prefer certain salts (8, 86) or a combination of several growth substances (8, 49, 103). A minor contamination of the substance may influence its effect to no small degree (61).

b. Method of administration. Roughly speaking two methods should be distinguished: a *dust-treatment* (growth substance mixed with powdered talcum, charcoal (96) or a seed disinfectant) and the method of *soaking* in a solution, which is subject to two variations: a *superficial spraying* (44, 73, 98) or a *brief submersion* (85).

On this point a good deal of variation is found in the literature, e.g., preliminary germination in water is applied before the growth substance is added (21); the seeds are continuously exposed to the growth substance by allowing them to germinate on a medium containing the active compound (36, 82, 102); after 24 hours' soaking in a solution of growth substance the seeds are rinsed with water (7); after soaking in the solution sowing is done either direct or after the seeds have been rinsed with water and allowed to dry (6).

Too little attention has been paid to the fact that many kinds of seed are apt to meet with 'soaking injury', when soaked in solutions (190–199). That the oxygen content of the solution does affect the growth of oat coleoptiles after the treatment has been made admissible by ALBAUM, KAISER and EICHEL (1).

The leaching of inhibitory substances during wet treatment may be another cause of varying results (21, 40). In addition, it is imaginable that, by soaking in solutions, seeds will lose part of their own growth hormones.

In general the seeds are sown immediately after treatment. Subsequent storage in the dry state would give less favourable results (47, 91).

The usual methods of applying the growth substance in solution, even at a low concentration, in many cases do retard root growth. So a dust treatment is recommended by GRACE and co-workers, as in this way a gradual supply of active material is realized.

c. Duration of treatment and concentration. It must be emphasized that the concentrations, optimal for germination, are quite different from those required in further growth. In normal development of a plant the dosage of natural hormones is a matter of careful regulation owing to correlation. The treatment of seed, however, resembles an 'initial loading therapy' which, obviously, in many cases will not completely fulfil the requirements of the material at the moment.

The *influence of the concentration* is of importance and accordingly has been studied in detail by various authors (50, 117). Even a slight

over-dosage of growth substance may prove harmful; the same applies to traces of alcohol contained in the solvent (27).

A non-sterile technique, attuned to practical application, involves the chance of destruction or additional formation of growth substances by the action of bacteria, thus causing uncertainty about the concentration. So positive results, once obtained, probably must not be generalized without comment in order to arrive at a definite instruction for use, the hormone metabolism of the seed playing a part as well.

Growth substances in the form of salts, notably at high concentrations, are alleged to be less poisonous (8). PODEŠVA (96), however, states that particularly at high concentrations potassium salts are more deleterious than free acids.

Reports about the *duration of the treatment* widely differ. If it is a lengthy one, the above-mentioned factors of soaking injury and leaching of inhibitors assert themselves. Here too, it is impossible to generalize, as every type of seed behaves differently.

d. Temperature. As a rule no attention is paid to the temperature of the growth substance solutions, only a few data being recorded. DYKYJ and DYKYJ-SAJFERTOVÁ (50), and PODEŠVA (97) afterwards, found that the penetration of growth substances into the seed is promoted by rise of temperature.

e. Acidity. DYKYJ and DYKYJ-SAJFERTOVÁ not only studied the effect of the temperature but that of the pH as well, principally with a view to applying the seed-treatment in sugar-beet culture (47, 51). CHADWICK and SWARTLEY (24) likewise have drawn attention to this subject.

Low pH and high temperature of a solution may give rise to over-dosage (97). The use of tap water as a solvent is not recommandable either, since it is usually too hard (47, 51).

As the dissociation of acidic growth substances is determined by the pH of their solutions, the growth hormone effect will depend on the pH as well. Some workers, therefore, apply a growth substance together with a buffer (39).

f. The influence of light during treatment. Although there is a possibility of certain seeds being liable to the action of light, no data on this subject are to be found.

4. *Condition during germination.* Different species of seeds certainly must not be regarded from the same point of view, which is already adequately demonstrated in Nature by the wide variation existing in biochemical processes during germination. Thus, HSUEH and LOU (67) have pointed out the completely different effect of the growth substance upon seeds, germinating either under aerobic or under anaerobic conditions.

It is not at all imaginary that some mutual influencing may arise from the excretion of inhibitory substances if, for instance, in laboratory germination tests the seeds are laid down too closely (cf. FRÖSCHEL).

LAFFERTY (73) pointed out that especially the humidity of the medium plays an important part in germination.

5. *Significance of the circumstances during culture.* From various

quarters already attention has been directed to the influence of the *season* (9, 10, 120) and of the *climate* in general (2, 5, 32, 42, 48, 51, 53, 54, 97, 103).

SÖDING and co-workers (103) found differences between *sunny* and *shady* plots (see also 74), whereas it appeared that in experiments like these the *alkalinity of the spraying water* deserves attention too (24).

Of course the *influence of the soil* is a matter of weight. The *temperature of the soil* (47), as well as the *humidity* (2, 5, 46, 66, 73, 92), the *pH* (93) and the *humus content* (93, 120) separately may exert an influence upon the result of a treatment with growth substances. In a cold and moist soil, for example, some authors established a diminishing of the toxic effect of certain treatments (47).

Furthermore, the *condition of the soil* plays a part (2, 5, 10, 31, 34, 42, 47, 48, 51–54, 90, 103, 106, 112, 117), and so do the *manuring* (90, 106, 107, 111, 112) and the *tillage* (42, 48). An abundant occurrence of nutrients in the soil may mask the stimulative effect of growth substance treatment (73, 103, 106, 112). A positive outcome was obtained only on normal supply of nitrogen; neither omission nor excessive administration of nitrogen led to any result (5, 68).

All these factors affecting the treatment with growth substances, several workers have arrived at the conclusion that *results are to be expected only under average growth circumstances* (2, 66, 91, 92, 96, 97, 103).

6. *Consequences in the event of a culture not being adapted to the demands of a treatment with growth substances.* FRIEDRICH (54) has called attention to the fact that the treatment in question may inhibit development, thus causing retarded harvesting, which may involve the risk of the seeds and seedlings becoming attacked by parasites. Preliminary experiments in the hothouse often turn out well, owing to adequate supervision and nurture, while in the field failures are no exception. On account of their retarded development the objects treated will have to be reaped later than the controls and, probably, really will afford positive results, if only the grower does not cling to traditional methods.

Considering the above, it becomes acceptable that various authors advocate the desirability of making small-scale experiments beforehand (103, 117). Apart from the difficulties attended with it, this method does not afford the solution of the main problem, namely, *the explanation of the numerous contradictions in the matter of seed-treatment with growth substances.*

In the present case a prosecution of the investigations will profit greatly by strictly standardizing the circumstances of the experimental technique. This will require carefully selected material, the properties of which are known as fully as possible.

When sufficient knowledge has been gathered about the hormone metabolism of the seed and about the mechanism of germination (after administration of growth substances or otherwise), the connection between these processes and the many variable, external factors will have to be elucidated systematically. Only then one may expect this complicated matter to yield important results in practice.

TABLE II

Data on the treatment of seeds with synthetic growth substances

Species	Treatment [1])	Results [2])		References
		1. in germination	2. in development and yield	
CONIFERAE				
Chamaecyparis lawsoniana	i.b.a.	+		120
Ginkgo biloba *)	i.a.a. or n.a.d. mixed with talc powder	—		24, 109
Juniperus procumbens *)	i.a.a. or n.a.d. mixed with talc powder	—		24, 109
Larix decidua	Roche 202	+?	+?	76
„ *leptolepis*	Roche 202	+?	+?	76
Picea excelsa	Roche 202	—	—	76
Pinus lambertiana	i.b.a.	+		120
„ *maritima* *)	sol. of i.a.a.	+		35
„ *monticola*	i.b.a.	—		120
„ *silvestris*	Roche 202	—	+	76
Pseudotsuga douglasii	i.b.a.	—		120
MONOCOTYLEDONES				
Agrostis tenuis *)	talc powders containing n.a.a. or n.a.a. mixed with t.u.; Auxan, Hormodin, Rootone	—		43
Allium cepa	Germisan powder with n.a.a.		+?	5
„ „ *)	Merck and Rootone dust; sol. of n.a.a.K	—		13
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a. + vit. C + t.u.		+	97
„ *libani* *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
„ *porrum*	sol. of n.a.a.K + vit. C + t.u.		+?	5
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
Amaryllis belladonna *)	Rootone powder	+		69
Asparagus officinalis *)	Belvitan sol.	+		9
Avena sativa	sol. of i.a.a.		+	23
„ „	sol. of i.a.a.		+	25, 26
„ „ *)	sol. of e.i., i.a.a., i.m.m.a., i.p.a.	—	—	36, 37
„ „	sol. of i.a.a.	—	—	52
„ „	damage caused by disinfectants reduced by addition of i.a.a., n.a.a.	+	+	59
„ „ *)	sol. of i.a.a.		—	68
„ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—	—	71

[1]) List of abbreviations and commercial preparations on p. 2.

[2]) Results have been divided into effects upon *germination* and upon *development and yield*. A positive result, in the opinion of the author cited, is marked by a +; no effect, or a negative one, by a —, while +? indicates that the investigator himself does not attach much importance to the positive results. ? signifies lack of further indications.

*) Of this plant the scientific name was mentioned in the paper cited. The other plant names have been translated from the native names which includes the possibility that a wrong interpretation is given.

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Avena sativa	sol. of i.a.a.	+	+	74
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc powders and sol. containing i.a.a., i.b.a., n.a.a., n.a.d., ph.a.a.		—	106
„ „	Agrosan and talc powder with n.a.a.		—	112
„ „ *)	sol. of i.a.a.		+	113
„ „ var. *Fulghum* *)	sol. of i.a.a.		+	1
„ sp. *)	Merck and Rootone dust	—	—	13
Callicore Brunsvigia hybr. *)	Rootone powder	+		69
„ *rosea* = *Amaryllis belladonna* *)				
Cattleya warneri *)	i.b.a., n.a.a.	+	+	82
Festuca pratensis *)	Merck and Rootone dust	—	—	13
Gladiolus sp. *)	Rootone powder	—		69
Haemanthus katherinae *)	Rootone powder	+		69
Hordeum vulgare	powders with i.a.a. or n.a.a.	—	—	11
„ „	sol. of c.p.a., i.a.a., n.a.a., n.o.a.a., t.i.b.a.	—		15
„ „	sol. of i.a.a.		—	25
„ „	talc powders containing i.a.a., i.b.a., n.a.d.	—	—	31
„ „	mercurial disinfectant powder with i.a.a. or n.a.a.		+	56
„ „	talc powder with i.a.a.	—	+?	66
„ „	sol. of 2,4-D	+		67
„ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—	—	71
„ „	powders and sol. with i.a.a., n.a.a.	—	—	73
„ „	sol. of i.a.a.		+	96
„ „	Agrosan and talc powder with n.a.a.		—	112
Hordeum sp. *)	Mn, Cu and Fe salts of n.a.a.	—	?	86
Leucojum sp.	sol. of n.a.a., n.a.d., Roche 202	—		117
Lilium auratum *)	sol. of different growth substances	—		14
„ *regale* *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
Lolium multiflorum	talc powders containing i.b.a., n.a.a. or n.a.a. + t.u.; Rootone and Hormodin A	—	+	29
„ *perenne* *)	Merck and Rootone dust	—	—	13
Moraea sp. *)	Rootone powder	—		69
Oryza sativa	sol. of i.a.a., n.a.a.	+	+	33
„ „	sol. of i.a.a.	—	—	52
„ „	sol. of 2,4-D	+		67
„ „ *)	sol. of i.a.a.	—	—	78
„ „	sol. of i.a.a.		+	84
„ „	sol. of i.a.a.	—		102
„ „ *)	sol. of i.a.a., i.b.a., n.a.a.		+	110
Panicum miliaceum	sol. of i.a.a.		—	23

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Panicum miliaceum	sol. of i.a.a.		—	25
„ „	sol. of i.a.a.	—		46
Poa pratensis *)	Merck and Rootone dust	—	—	13
„ „	talc powders containing i.b.a., n.a.a. or n.a.a. + t.u.; Rootone and Hormodin A	—	—	29
„ „ *)	talc powders containing n.a.a. or n.a.a. mixed with t.u.; Auxan, Hormodin, Rootone	—		43
Secale cereale	Germisan powder with n.a.a.		+?	5
„ „ *)	Merck and Rootone dust	—	—	13
„ „	sol. of i.a.a.		—	25
„ „	talc powders containing i.a.a., n.a.d.	—	—	31
„ „	sol. of i.a.a.		—	98
„ „ *)	sol. of n.a.a., n.a.d., Roche 202	+	—	117
Sorghum sp.	talc powder with 2,4-D		+	27
„ *sudanense*	sol. of i.a.a.		+	91
Sprekelia sp. *)	Rootone powder	+	—	69
Streptanthera sp. *)	Rootone powder	—		69
Triticum aestivum *)	talc powders containing i.a.a., i.b.a., n.a.a. Rootone. Combinations with Semesan	—	—	119
„ „ *)	sol. of n.a.a., n.a.d., Roche 202	+	—	117
„ *spelta*	sol. of n.a.a., n.a.d., Roche 202		—	117
„ *vulgare* (= *T. aestivum*)	sol. of i.a.a.K, i.b.a.K, n.a.a.K		+?	9
„ „ *)	sol. of i.a.a., ph.a.a. and mixtures	—	+	18
„ „	sol. of i.a.a.		—	23
„ „	sol. of i.a.a.		—	25
„ „	talc powder with 2,4-D		+	27
„ „	talc powders containing i.a.a., i.b.a., n.a.d.; Ceresan + growth substance		+	31
„ „ *)	sol. of Rootone		+	34
„ „	sol. of i.a.a.	—		46
„ „	sol. of i.a.a., n.a.a.	—		50
„ „	sol. of i.a.a.	+	+	74
„ „ *)	sol. of i.a.a.	—	—	78
„ „	Ceresan powder with i.a.a., n.a.a. and mixtures		+	80
„ „	sol. of i.a.a.	—		87
„ „	sol. of i.a.a.		+	90, 91, 92, 93, 95
„ „	sol. of i.a.a., n.a.a., n.a.a. + vit. B_1 and C	+	+	96
„ „	Agrosan, Granosan and talc-powder containing i.a.a., n.a.a.		—	112
„ „	fungicidal dust containing i.a.a., n.a.a.	+		116
„ „	sol. of n.a.a., n.a.d., Roche 202	+	—	117
„ „ *)	talc powders and sol. containing n.a.a. to reduce damage caused by hot water disinfection	—	—	**)

**) Unpublished results of experiments by the author in collaboration with Ir W. Kakebeeke.

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Triticum sp. *)	sol. of i.a.a., i.a.a.K, i.b.a., n.a.a., n.a.a.K; Merck and Rootone dust	—	—	13
„ „	mercurial disinfectant powder with i.a.a. or n.a.a.		+	56
„ „	damage caused by disinfection reduced by addition of i.a.a., n.a.a., ph.a.a.		+	58
„ „	damage caused by disinfectants reduced by addition of i.a.a., n.a.a.	+	+	59
„ „	formaldehyde containing i.a.a. or n.a.a.	+		60
„ „	formaldehyde containing n.a.a.	+	+	61
„ „	talc dust with i.a.a., n.a.a.K and combinations with KNO_3 and ethyl-mercuric bromide	—	—	62
„ „ *)	Mn, Cu and Fe salts of n.a.a.	+		86
„ „ *)	sol. of i.a.a.		+	113
Tritonia sp. *)	Rootone powder	—		69
Zea mays	Germisan powder with n.a.a.		+?	5
„ „	sol. of i.a.a.		+?	9
„ „ *)	sol. of i.a.a.		+	22
„ „	Rootone dust and mixture with Ceresan	—	+	30
„ „	sol. of i.a.a.	—		46
„ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—	—	71
„ „ *)	sol. of i.a.a.	—	—	78
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc powders with n.a.a.		—	106
DICOTYLEDONES				
Achillea ptarmica *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Alyssum saxatile *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Anemone pulsatilla rubra *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Anthemis tinctoria kelwayi *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Antirrhinum majus *)	Merck and Rootone dust; sol. of n.a.a.K	—	—	13
„ „	Rootone powder	+	+	69
Apium graveolens	sol. of n.a.a.K + vit. C + t.u.		+?	5
„ „	sol. of i.a.a.		+	91
Aquilegia "Crimson Star" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Arachis hypogaea *)	Merck and Rootone dust; sol. of n.a.a.K	—		14
„ „	lanolin with i.a.a.	+		100

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Arachis hypogaea	sol. of i.a.a.		+	101
Artemisia vulgaris *)	Belvitan sol.	+		9
Asclepias tuberosa *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Atropa belladonna *)	Belvitan sol.	—	+	9
Aubrietia deltoidea "*Monarch Mixture*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Beta vulgaris *)	Merck dust; sol. of n.a.a.K	—	—	13
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
„ „ var. *altissima*	sol. of n.a.a.K		+	2
„ „ „ „	sol. of Euradin		+	3
„ „ „ „	sol. of n.a.a.K	?	?	4
„ „ „ „	Germisan powder with n.a.a.		+	5
„ „ „ „	sol. of i.a.a.		+	7, 8
„ „ „ „	sol. of i.a.a.K, i.b.a.K, n.a.a.K		+	9
„ „ „ „	sol. of n.a.a.K		+	10
„ „ „ „	sol. of n.a.a.K		—	20
„ „ „ „	sol. of i.a.a., n.a.a., combination with adenine, vit. B_1 and nicotinic acid		+	21
„ „ „ „	sol. of i.a.a. + adenine + vit. B_1 + nicotinic acid		+?	38
„ „ „ „	sol. of i.a.a., n.a.a., Euradin	—	—	39
„ „ „ „	sol. of n.a.a., Euradin, mixture of adenine + vit. B_1 + nicotinic acid + i.a.a.		+	40
„ „ „ „	different commercial preparations	—	—	41
„ „ „ „	sol. of n.a.a.		—	42
„ „ „ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a. and commercial preparations		—	44
„ „ „ „	sol. of n.a.a.	—	—	47
„ „ „ „	sol. of i.a.a., n.a.a.	—	+?	48
„ „ „ „	talc powders with i.a.a., n.a.a. and mixtures; sol. of i.a.a., n.a.a., i.a.a. + n.a.a., and combinations with t.u.; Euradin	—	—	49
„ „ „ „	sol. of i.a.a., n.a.a.		+?	51
„ „ „ „	sol. of n.a.a.		—	55
„ „ „ „	sol. of i.a.a., n.a.a.		—	63
„ „ „ „	sol. of Euradin		+	81
„ „ „ „	sol. of n.a.a.K		+	85
„ „ „ „	Hormodin powder; sol. of Hormodin A		—	89
„ „ „ „	sol. of i.a.a., n.a.a.	+	+	90
„ „ „ „	sol. of i.a.a.		+	91
„ „ „ „	talc powders and sol. with i.a.a., n.a.a.		+	97

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Beta vulgaris var. *altissima*	sol. of i.a.a., i.b.a., n.a.a., ph.a.a., Euradin, Belvitan, Roche 202		—	105
„ „ „ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
„ „ „ „	talc powders and sol. containing i.a.a., i.b.a., n.a.a., n.a.d. Rootone		—	108
„ „ „ „	Agrosan and talc powder containing n.a.a.		—	112
„ „ „ „	sol. of i.a.a., n.a.a.		+	121
Boltonia asteroides *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Brassica campestris subsp. *chinensis* *)	sol. of i.a.a.	—	—	78
Brassica oleracea	sol. of n.a.a.K + vit. C + t.u.		+	5
„ „ *)	sol. of i.a.a.K	+		7
„ „ *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		8
„ „	Belvitan sol.	+		9
„ „	sol. of i.a.a.	—		46
„ „	sol. of i.a.a.		+	90, 91, 96, 97
„ „ *)	sol. of i.a.a., n.a.a.K		+	94
„ „ var. *acephala*	Belvitan sol.	+		9
„ „ „ *capitata*	sol. of i.a.a.		+	90, 96
„ „ „ „ *)	sol. of i.a.a., n.a.a.K		+	94
„ „ „ *capitata* f. *alba*	sol. of i.a.a.		+	91
Brassica oleracea var. *gongylodes*	sol. of n.a.a.K + vit. C + t.u.		+?	5
„ „ „ „	sol. of i.a.a.	—		46
„ „ „ „	sol. of i.a.a.		+	90, 91, 96
„ „ „ „	sol. of i.a.a., n.a.a.K		+	94
„ *rapa* *)	Merck dust; sol. of n.a.a.K	—	—	13
„ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
Calendula officinalis	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
Cannabis sativa	sol. of i.a.a.		—	23
„ „	sol. of i.a.a.		+	27
Capsicum frutescens	sol. of i.a.a.	?	?	96
Carya illinoensis *)	sol. of i.b.a., n.a.a.	—		19
Chaenomeles japonica *)	sol. of n.a.a., n.a.d., Roche 202	—		117
Cheiranthus linifolius *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24
Chrysanthemum coccineum "*James Kelway*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+	+	109
Cichorium endivia *)	Belvitan sol.	+	+	9
„ „ *)	sol. of i.a.a.	—		46
„ *intybus*	Germisan powder with n.a.a.		+?	5
„ „ *)	Belvitan sol.	+	+	9
„ „	sol. of i.a.a.		+	91
Clematis tangutica *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Coreopsis lanceolata "*Double Sunburst*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Cornus florida *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
„ *stolonifera* *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
Cotoneaster divaricata *)	talc powder with i.a.a. or n.a.d.	—		24, 109
„ *foveolata* *)	talc powder with i.a.a. or n.a.d.	—		24, 109
Cucumis sativus	Germisan powder with n.a.a.		+	5
„ „ *)	Belvitan sol.	+		9
„ „ *)	sol. of i.a.a.	—		70
„ „	sol. of i.a.a.		+	96, 97
„ „	sol. of i.a.a., n.a.a.		+	115
Cucurbita pepo *)	sol. of i.a.a.	—		104
„ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
Cynara cardunculus *)	Belvitan sol.	+	+	9
Daphne mezereum *)	Belvitan sol.	+	+	9
Datura stramonium *)	sol. of i.a.a.K	—		7
„ „ *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		8
Daucus carota	sol. of n.a.a.K + vit. C + t.u.	?	?	4
„ „	Germisan powder with n.a.a.; sol. of n.a.a.K + vit. C + t.u.		+	5
„ „ *)	Belvitan sol.	+		9
„ „	sol. of i.a.a.	—		74
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a., n.a.a.	—	+	96
„ „ *)	sol. of i.a.a.K + vit. B_1, vit. C, oestrone, caffeine and nicotinic acid		+	103
„ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
„ „	sol. of n.a.a., n.a.d., Roche 202		+	117
„ „ var. *sativa* *)	Merck dust; sol. of n.a.a.K	—	—	13
Delphinium ajacis *)	sol. of i.a.a. or i.a.a.K	+		7, 8
„ „ *)	Merck and Rootone dust; sol. of n.a.a.K	—	—	13
„ "*Martin's Stock*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Dianthus alpinus allwoodii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—	+	24, 109
Digitalis lanata *)	sol. of n.a.a.	±		115
„ *purpurea gloxiniflora* "*Rose*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Diospyros virginiana *)	talc powder with i.a.a. or n.a.d.	—		24, 109
Erigeron coulteri *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Erysimum linifolium *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Fagopyrum esculentum	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc powders with n.a.a.; sol. of i.b.a., n.a.a.		—	106
Geum chiloense "*Orange Queen*"*)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Glycine = *Soja*				
Gossypium sp.	dust containing i.b.a., n.a.a.K and combinations with Spergon and Ceresan	—	—	75
„ „	sol. of i.a.a.		+	101
Helianthemum polifolium *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
„ *roseum* *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
„ *vulgare* *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
Helianthus annuus *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		8
„ „ *)	sol. of i.a.a.	—		104
Heliopsis pitcheriana *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Heuchera lithophila *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Ilex opaca *)	talc powder with i.a.a. or n.a.d.	—		24, 109
Impatiens sp.	sol. of n.a.a., n.a.d., Roche 202	—		117
Incarvillea delavayi *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Inula helenium *)	Belvitan sol.	+	+	9
Lactuca sativa	Germisan powder with n.a.a.; sol. of n.a.a.K + vit. C + t.u.		+?	5
„ „ *)	Merck and Rootone dust; sol. of i.a.a.K, n.a.a.K	—		13
„ „	sol. of i.a.a.		+	90, 91, 96, 97
Lavendula spica *)	Belvitan sol.	+		9
Lens sp. *)	Mn, Cu and Fe salts of n.a.a.	—	?	86
Lepidium sativum *)	sol. of e.i., i.a.a., i.m.m.a., i.p.a.	—	—	36, 37
„ „	sol. of i.a.a., i.p.a.	—	+	79
Liatris pycnostachya *)	talc powders containing i.a.a., or n.a.d. mixed with t.u.	—		24, 109
Linum usitatissimum	sol. of i.a.a.		—	23
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a.	—		102
Lobelia cardinalis *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Lupinus luteus *)	sol. of n.a.a., Roche 202	+	+	117
„ *polyphyllus* *)	sol. of n.a.a.		+	117
„ „ "*Blue King*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Lupinus polyphyllus "Rose Queen" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		24, 109
Lychnis arkwrightii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		24, 109
Lycopersicon = *Solanum*				
Lythrum salicaria roseum superbum *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		109
Maclura pomifera *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
Matricaria inodora "Dwarf Golden Ball" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		24, 109
Matthiola incana *)	Merck and Rootone dust;			
	sol. of n.a.a.K	—	—	13
Medicago sativa	sol. of i.a.a.K		+	9
Melissa officinalis *)	Belvitan sol.	+	+	9
Mespilus germanica *) . . .	sol. of n.a.a., n.a.d., Roche 202	—		117
Nicotiana tabacum	Germisan powder with n.a.a.;			
	sol. of n.a.a.K + vit. C + t.u.		+?	6
„ „	i.a.a., 2,4-D		—	65
Ocimum basilicum *)	Belvitan sol.	+	+	9
Oenothera fruticosa youngii *) .	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		24, 109
Orlaya grandiflora *)	sol. of i.a.a.	+		8
Paeonia suffruticosa *)	sol. of i.a.a.K, n.a.a.K . . .	—		13, 14
Papaver nudicaule "Stanford's Giant" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	+		24, 109
„ *somniferum*	Germisan powder with n.a.a.		+	5
„ „ *)	sol. of i.a.a.		+	91
Pentstemon gloxinoides "Sensation" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	+		24, 109
Perilla ocymoides	sol. of i.a.a.		—	23
„ „	sol. of i.a.a.		+	27
Petroselinum sativum *) . . .	sol. of i.a.a.K	+?		7
„ „ *) . . .	Belvitan sol.	+	+	9
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
Phacelia tanacetifolia *) . . .	talc dust and sol. with i.a.a.		+	96
Phaseolus angularis *)	sol. of i.a.a.		—	118
„ *lunatus*	Rootone dust	—		30
„ *mungo* var. *radiatus* *)	sol. of i.a.a.	+	—	78
„ *vulgaris*	sol. of i.a.a.	+		74
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d. . .		—	83
„ „ *)	sol. of i.a.a.	—		104
„ sp.	Rootone dust; mixture with Ceresan	—	—	30
Phaseolus sp.	dry seed dressings (mercurial and cuprous oxide) containing n.a.a., mixed naphthylidene acetic acids and i.b.a. . . .	—	—	32
Physostegia virginiana grandiflora *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	+		109

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Physostegia virginiana rosea *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24
Phytolacca decandra *)	sol. of i.a.a., i.b.a., n.a.a.	—	—	13
Pisum sativum *)	Merck and Rootone dust;			
	sol. of n.a.a.K	—		13
„ „ *)	sol. of i.a.a., ph.a.a.	—	+	16
„ „ *)	sol. of i.a.a. and ph.a.a. in combination with manganese sulphate and uranyl nitrate	—	+	17
„ „	sol. of i.a.a.		—	23
„ „	sol. of 2,4-DNH$_4$	?	?	28
„ „	Rootone dust	—	+	30
„ „	dry seed dressings (mercurial and cuprous oxide) containing n.a.a., mixed naphthylidene acetic acids and i.b.a	+	+	32
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc dust containing i.a.a.		+	97
„ „ *)	sol. of i.a.a.	—		104
„ sp. *)	Mn, Cu and Fe salts of n.a.a.	—	?	86
Platycodon grandiflorum mariesii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Polemonium richardsonii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Potentilla nepalensis *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Prunus persica	sol. of i.b.a.	—		99
Pyrethrum = *Chrysanthemum*				
Pyrus malus var. *niedwetzkyana* *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
„ sp. *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
Raphanus sativus *)	sol. of i.a.a.K	+	+	7
„ „ *)	Merck and Rootone powder;			
	sol. of n.a.a.K	—	+	13
„ „	sol. of i.a.a., i.b.a., n.a.a.	—	—	53
„ „	sol of i.a.a.	+		74
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	sol. of i.a.a.K, n.a.a.K		+	88
„ „	sol. of n.a.a.K and combinations with vit. C, t.u. and i.b.a.K		+	94, 96
„ „	talc powders and sol. containing i.a.a., i.b.a., n.a.a., n.a.d., n.o.a.a., ph.a.a.		—	106
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
Rhododendron obtusum kaempferi *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Ricinus communis *)	sol. of i.a.a.	—		104
Rudbeckia hirta hybr. "*Autumn Forest*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Satureja hortensis *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
Saxifraga cordifolia *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Silene saxifraga *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Sinapis alba (=*Brassica alba*)	sol. of i.a.a.		+	23
„ „ *)	sol. of e.i., i.a.a., i.m.m.a., i.p.a.	—	—	36, 37
„ „ *)	sol. of i.a.a., i.b.a., n.a.a.		+	110
„ „ *)	sol. of i.a.a.Na, n.a.a.Na		—	111
Soja max (=*Glycine max.* = „ *soja*) *)	sol. of i.a.a., i.b.a., n.a.a., and mixtures	+		7, 8
„ „ *)	Merck and Rootone dust	—		13
„ „	sol. of i.a.a.	—		46
„ „	talc powder with i.a.a.; sol. of i.a.a.	—	+	56
„ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—	—	71 71
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
„ „ *)	sol. of i.a.a.		—	118
„ „ *)	talc powders containing i.a.a., i.b.a., n.a.a.; Rootone, combinations with Semesan	—	—	119
Solanum melongena *)	Belvitan sol.	+	+	9
„ „ var. *esculentum* *)	Merck and Rootone powder; sol. of n.a.a.K	—		13
„ *lycopersicum*	sol. of n.a.a.K + vit. C + t.u.	?	?	4
„ „ *)	Merck and Rootone dust; sol. of i.a.a., i.a.a.K, i.b.a., n.a.a., n.a.a.K	—	—	13
„ „	sol. of i.a.a., i.b.a., n.a.a.	—	+	53
„ „	sol. of i.a.a.	—	—	64
„ „	sol. of i.a.a.	+		74
„ „	sol. of i a.a.		+	91
„ „	talc powders with i.a.a., i.b.a., n.a.a., ph.a.a.	+	+	107
„ „ *)	sol. of i.a.a., i.b.a., n.a.a.		+	110
„ „ *)	sol. of i.a.a.		+	113
„ „	sol. of i.a.a., n.a.a.		+	115
„ „ *)	talc powder with n.a.a.; sol. of i.a.a.	—	—	***)
„ *tuberosum*	sol. of i.a.a., i.b.a., n.a.a.		—	53
Spinacia oleracea	Germisan powder with n.a.a.		+	5
„ „ *)	Belvitan sol.	+		9
„ „	sol. of i.a.a.		+	27
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115

***) Unpublished results of experiments by the author.

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Tagetes erecta *)	Merck and Rootone powder; sol. of n.a.a.K	—		13
„ *patula* *)	Merck and Rootone powder; sol. of n.a.a.K	—		13
Thalictrum aquilegifolium *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Thymus vulgaris *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
„ „ *)	Belvitan sol.	+	+	9
Trifolium hybridum	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		8
Trollius europaeus *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Tropaeolum majus *)	Merck and Rootone dust; sol. of n.a.a.K	—	—	13
Ulmus americana *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	+		14
Valeriana officinalis	sol. of i.a.a.	—		46
Valerianella olitoria *)	Belvitan sol.	+	+	9
Veronica longifolia hendersonii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Viburnum lantana *)	talc powder with i.a.a. or n.a.d.	—		24, 109
„ sp. *)	sol. of different growth substances	—		14
Vicia faba *)	sol. of i.a.a.		—	68
„ „ *)	Mn, Cu and Fe salts of n.a.a.	—	?	86
„ *sativa*	sol. of i.a.a.		—	23
Viola arkwright rubra *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24
„ *cornuta chantreyland* *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
„ „ *"Rose Queen"* *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Zinnia sp. *)	Merck and Rootone dust; sol. of n.a.a.K	—	—	13

CHAPTER II

GENERAL METHODS

§ 1. MATERIAL

The common pea was used, Nr 756, "Groene Lente" from the firm of Messrs. Turkenburg, of Bodegraven, Holland. Usually seeds were chosen from the latest crop available.

§ 2. STERILIZATION AND PRE-SOAKING

As mere traces of some ergons may influence the development of the embryo, it is obvious that the present investigation can be made *in vitro* only, under strictly aseptic conditions. Many micro-organisms produce substances which have been proved growth factors for higher plants, thus their presence would confuse the results.

The peas were sterilized externally by immersion in alcohol (96 %) for 10 minutes, in $HgCl_2$ (0.1 %) for 15 minutes and by washing four times with sterile aq. dest. Then the seeds were pre-soaked in sterile water or in sterile solutions of the compounds under investigation (growth substances, inhibitors, etc., of various concentrations) by keeping them in sterilized Petri dishes at about 24° C for some 18 hours (i.e. overnight). After carefully washing the seeds under sterile conditions the embryo*) was separated from the cotyledons. The instruments were sterilized by dipping in alcohol (96 %) and subsequent flaming before each operation. In a number of series, parts of the cotyledons were left attached to it. Only those seeds were used, the coat of which was not ruptured after soaking.

Attempts at isolation of the embryo from seeds, not soaked beforehand, were unsuccessful. The embryo in dry seed being very brittle, a slight injury during the manipulations is hardly preventable. Uninjured embryos possibly might be isolated from the dry, hard seeds by means of a special, though time-devouring technique (243) which, however, never would provide the material required for sufficiently large experiments.

All the operations were carried out in a transfer room previously sterilized by U.V. lamps.

§ 3. Media and culture

Culture, under sterile conditions, took place in the dark at 24° C. Culture tubes were used, containing 10 ml of a standard nutrient medium according to Bonner and Addicott (232). It is composed as follows:

$Ca(NO_3)_2.4H_2O$	236 mg
$MgSO_4.7H_2O$	36 „
KNO_3	81 „
KCl	65 „
KH_2PO_4	20 „
$Fe_2(SO_4)_3$	2 „
sucrose	40 g
double-distilled water, up to	1000 ml
agar	±4 g

$Ca(NO_3)_2.4H_2O$ was prepared from $CaCO_3$ (A.R.) and nitric acid (halogen-free); crystallized from water.

KH_2PO_4 was prepared from K_2CO_3 (pure grade, from tartar) and H_3PO_4 (pure grade); recrystallized till meeting buffer grade specifications.

The sucrose employed was commercial sugar, whereas commercial agar, before use, was thoroughly leached with distilled water for a week.

The synthetic growth substances, di-n.amyl-acetic acid, rac. (trans)-tetrahydrothiophene-α, α'-dicarboxylic acid and the cis isomer were prepared in the Research Laboratory of the N.V. Amsterdamsche

*) "embryo" in the text stands for "embryo devoid of cotyledons".

Chininefabriek, Amsterdam. All the other chemicals were of the purest grade available.

Sterilization, as a rule, was effected by autoclaving at 110° C for 15 minutes. If thermolabile substances had to be added to the nutrient medium, 5 ml portions of a solution containing twice the amount of sugar, salts and agar were sterilized in the tubes. Before cooling, the substance under investigation was added, viz. 5 ml of a solution sterilized by filtration through a sintered glass funnel (17 G 5, SCHOTT, Jena).

§ 4. ESTIMATION OF RESULTS

Biological materials always show variability to a large extent. In order to obtain results reliable statistically, groups were made up of 20 specimens at the least.

After culture for ten days or three weeks, dependent on the kind of the experiment, abnormal plants were discarded and results were recorded photographically. Lengths of shoot, hypocotyl and root were measured and, the numeration of lateral roots being performed, fresh and dry weight determinations were carried out.

The standard error was used, if possible, as an estimate of the dispersion of the means and it was calculated according to the equation

$$E = \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}.$$

CHAPTER III

PRELIMINARY EXPERIMENTS

§ 1. IMPORTANCE OF THE SIZE OF SEED AND EMBRYO

Any given lot of peas will consist of seeds of rather varying size. It is imaginable that the seeds of such an assortment may develop unequally, owing to a different content of ergons and of reserve substances, and on account of disparate size of the embryos. In the literature this theme is mentioned several times (F, 200–212), though any possible bearing upon the *in vitro* culture of embryos or of roots has been ignored.

Hence, in the present investigation the material was divided into five lots by means of thin, wooden sieves, fit with holes 5.5, 6.0, 6.5 and 7.0 mm wide. So, lots resulted of the following diameters: under 5.5, 5.5–6.0, 6.0–6.5, 6.5–7.0 and over 7.0 mm.

After a night's pre-soaking it is distinctly visible that the larger peas have swollen most and have been the first to rupture the seed-coat and to show the root-tip, whereas the smaller peas have swollen least and have not ruptured.

During pre-soaking of peas of a size, differences prove to appear since not all of them will swell to the same degree. Prior to the excision of embryos, therefore, the material must be sized once more. As already stated, seeds were chosen the coat of which was not yet ruptured.

TABLE III

Ratio of embryo to mass of reserve substances after pre-soaking. Average of 100 specimens. Weights in mg.

Diameter of the seed	Weight of embryo		Weight of cotyledon				Ratio: embryo / reserve substances	
			fresh		dry			
	fresh	dry	left *)	right *)	left *)	right *)	fresh	dry
< 5 mm	4.8	1.2	97.8	99.5	39.4	40.0	1 : 41	1 : 68
6.0-6.5 mm	6.1	1.7	157.0	153.5	69.5	68.5	1 : 51	1 : 82
> 7 mm	7.7	2.2	248.7	248.0	114.3	114.6	1 : 65	1 : 102

*) see fig. 1.

It appears that, in peas of different diameter, both the size of the embryo and the ratio embryo/reserve substances widely diverge (see table III) and that these large differences lead to unequal development (table IV).

§ 2. Effect of age and storage conditions

It is obvious to assume that the age of the seeds may be of consequence to the process of germination and to further development. In 1952 an experiment was made with peas of one size, harvested in 1945, 1947, 1949 and 1951, respectively. Only minor differences in development were seen.

Nevertheless, errors if any from this source were eliminated by always using seeds from the latest crop available. Cool and dry storage was maintained as much as possible.

§ 3. Influence of pre-soaking

A short period of soaking was deemed indispensable for the excision of embryos in undamaged condition. The method, for all that, is liable to objections. For, it is not only thinkable, but also probable that already during pre-soaking certain components from the cotyledons will be absorbed by the embryo (see 238), in which case results are not unequivocal.

So, it seemed worth while studying the factors involved in pre-soaking as well as their importance. Soaking was always performed in the dark.

a. Effect of the temperature

If, during pre-soaking, any substances are transferred from the cotyledons to the embryo, one might try to check this process, e.g. by lowering the temperature. On the other hand, there is some danger of certain enzymatic processes being favoured by low temperature (vernalization!), so that subsequent development would be influenced again.

Temperatures of 4°, 25°, 35° and 45° C were studied and so were various soaking-periods. The numerical data show that further development of the embryo is either not affected by the temperature

TABLE IV

Influence of the size of the seed on the ultimate development of the dissected embryo. Results after three weeks. Average of 35 specimens.

Diameter of the seed	Length of the embryo	Average weight of the two cotyledons after pre-soaking period		Sprout			Root + hypocotyl			Average number of lateral roots
		fresh	dry	length	fresh weight	dry weight	length	fresh weight	dry weight	
mm	mm	mg	mg	mm	mg	mg	mm	mg	mg	
< 5.5	4.0	215	87	61 ± 1.39	45	3	54 ± 0.98	26	3	8
5.5 - 6.0	4.5	257	106	66 ± 1.33	49	3	59 ± 1.34	29	3	9
6.0 - 6.5	5.0	318	135	73 ± 1.41	54	3	64 ± 1.69	33	4	9
6.5 - 7.0	5.0	375	138	75 ± 1.62	55	3	64 ± 1.04	32	4	10
> 7.0	5.5	478	209	78 ± 1.79	60	4	67 ± 1.34	36	4	10

during pre-soaking, or to a small degree only. Both the former and the latter temperature caused an increase of abnormal individuals. A lengthy treatment at 25° C was also fatal.

b. Duration of pre-soaking

For the above reasons pre-soaking must be stopped in good time. BONNER and BONNER (see 238) mention a period of six hours at most. At 25° C, it will require at least a five hours' soaking before enough seeds have swollen satisfactorily. The process takes even more time at 4° C; besides, the peas not only remain wrinkled a good long while, but also become tough and keep retaining a hard centre. As a rule, the embryo cannot then be properly excised.

In experiments, carried out at the same temperature, the duration of pre-soaking scarcely affected the development of the young plant. So, if any substances are transferred from the cotyledons to the embryo, it will take place already in the first stage of swelling. In *rye*, according to DE ROPP, the transport of growth-stimulating substances would take place within the first two hours (238).

c. Water-supply

When soaked in water, the seeds exude various substances. Peas not only give off inhibitory compounds: a red pigment was isolated from "pea-diffusate" by VELDSTRA.*)

It is quite possible, therefore, that the substances exuded will influence the embryo inside the seed if their concentration becomes too high or if pre-soaking is protracted. In Nature, after all, such substances are adsorbed by the soil, or decomposed by the action of micro-organisms.

Peas of one size, in lots of fifty, were pre-soaked in 10, 20 or 40 ml of water, under sterile conditions at 24° C. After this it appeared that in the first dish the peas had absorbed the whole of the water; in the next, enough water was left, whereas in the third dish the greater part of the peas had remained submerged, which involves some danger of "soaking injury" (190–199).

On the whole, the development of the embryos showed no large differences. So, if not too many peas are put together in one dish and if so much water is added that the peas are submerged only half, no substances given off by the seeds will influence the future development of the embryo.

§ 4. POSITION OF THE EMBRYO ON THE NUTRIENT MEDIUM

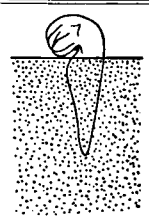
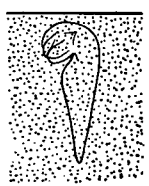
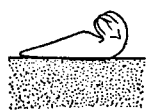
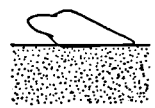
If an excised embryo is transferred into a culture-tube (containing a nutrient medium) by simply dropping, its position will wholly depend on chance. It is conceivable that the initial position may influence future development; therefore, it was tried to demonstrate this by a number of tests.

Starting from peas of 6.0–6.5 mm diameter the embryos were placed upon and into the nutrient medium in five, diverse positions: upright, its plumule rising above the medium; upright, completely immersed;

*) Unpublished.

TABLE V

Influence of the position of the embryo on the nutrient medium (at the beginning of the culture) upon ultimate development. Standard medium with 4% sucrose and 0.4% agar. Results after 21 days. Average of ± 30 specimens.

Position of the embryo at the beginning of the culture						
Sprout	average length . . . . . (mm)	78 ± 1.6	76 ± 1.6	70 ± 2.0	69 ± 1.5	64 ± 2.3
	„ fresh weight. . . . . (mg)	57	53	50	51	48
	„ dry weight . . . . . (mg)	3	3	3	3	3
Root plus hypocotyl	average length . . . . . (mm)	63 ± 1.4	62 ± 1.7	59 ± 1.4	62 ± 1.3	56 ± 0.9
	„ fresh weight. . . . . (mg)	34	35	26	27	26
	„ dry weight . . . . . (mg)	3	3	3	3	3
Average number of lateral roots		10.6	9.9	10.0	10.8	9.8
Percentage of plants with the root growing above the medium		0	0	10	38	20

lying on its back (i.e. ,the side facing the seed-coat in the whole pea, see fig. 1); with its back aloft, and, finally, lying on its side.

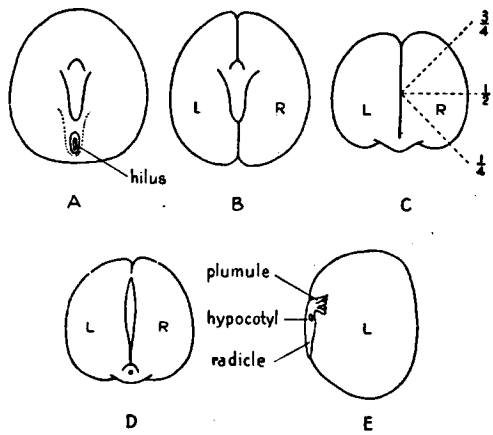


Fig. 1. Seed of pea. A, as seen from attached edge (back of the embryo). B, same as A, seed-coat removed. L = left cotyledon; R = right cotyledon. C, as seen from above, seed-coat removed. D, cross-section through plumule, seed-coat removed. E, seed-coat and right cotyledon removed.

From the results, summarized in table V, it may be concluded that development is influenced indeed by the position of the embryo. Especially in quantitative experiments, therefore, attention should be paid to this point.

§ 5. Effects of injury

Various workers have stressed the point that an embryo is very sensitive to certain manipulations. In order to check these statements, embryos, freshly excised, were either pricked with a needle or grasped with fairly hot tweezers before placing them into the culture-tubes. In comparing their development with that of embryos handled with utmost care, scarcely any trace of injurious after-effects was found in the former.

If, then, the embryos are isolated cautiously and if the instruments are allowed to cool after flaming no ill effects are to be feared.

§ 6. Influence of the medium; concentration of the agar

No doubt the composition of the medium will affect development. In this investigation the medium according to Bonner and Addicott (232) has been used, though, in another connection, it could be shown that its composition is certainly not optimal for the culture of pea-embryos. Besides, it is to be expected that the demands of the plant will vary during development.

Attempts at making embryos develop on a liquid medium have failed of effect. Finally, an agar medium was preferred for culture and its optimal concentration was ascertained.

As appears from fig. 2, the standard medium containing 4 % sucrose yields the best results both in sprout and in root development if about 0.3 % agar is present. A concentration of 0.2 % is generally too low; "drowning" of the embryo gives rise to abnormal development.

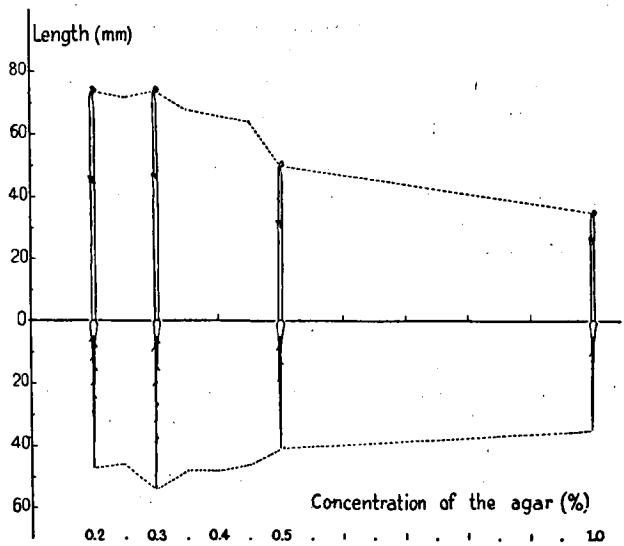


Fig. 2. Influence of different agar concentrations in the standard nutrient medium. Results after three weeks' culture in the dark at about 24° C.

§ 7. Aeration

It is obvious that during *in vitro* culture the gas-volume may play a part. Even a small variation of the diameter of the tubes leads to different levels of the medium, thus the space available to the aerial parts of the plant is different too. Trouble will be caused not only by mechanical influences (growth against the cotton plug and, therefore, immersion of the basal parts of the plant), but also by mutual variations of the composition of the gas-milieu.

In order to eliminate possible errors in advance, only tubes of one size were used (150 mm in length and 17–18 mm wide).

Another point requiring attention is the firmness of the cotton plug. It may, after all, affect the interchange with the air. Comparisons were made between plugs of the usual, medium texture and both very loose and very tight plugs. The results obtained with the various kinds were very much alike, so, from that time normal plugs were used, made up always by the same person.

Summary, and survey of methods

1. Peas, usually seeds of the latest crop available, were assorted by sifting shortly before use (cf. note on p. 61).

2. As a rule, peas of one size were employed. They were externally sterilized by immersion both in 96 % alcohol for 10 minutes and in 0.1 % $HgCl_2$ for 15 minutes, and wahsed four times with sterilized, distilled water.
3. To facilitate excision of the embryo, the peas were pre-soaked overnight in water or in certain solutions, at about 24° C, in the dark.
4. Seeds, of which the seed-coats had remained intact, were calibrated again before the excision of the embryos. After removal of the seed-coat, the cotyledon-stalks were cut close by the embryo, in order to obtain similar objects.
5. These embryos, after being placed upright into culture tubes, were adjusted right in the centre of the nutrient medium by means of a transfer loop, the plumule rising just above the surface.
6. A medium containing 0.3–0.4 per cent agar ensured optimal development, unless substances were present affecting the gelation properties.
7. In the proceedings mentioned under 4 and 5, the influence of the time-factor was limited as much as possible. If, for instance, a number of treatments was tested on 24 objects each, the successive manipulations were finished with 8 objects only of each group; this sequence was repeated until all of the material had been handled.
8. Culture took place in the dark, at about 24° C.
9. After ten days or three weeks, dependent on the kind of experiment, the results were recorded.

CHAPTER IV

EARLY DEVELOPMENT AND CORRELATION PHENOMENA; INFLUENCE OF VARIOUS SUBSTANCES

§ 1. INFLUENCE OF THE COTYLEDONS

The cotyledon, as a depot of reserve substances and ergons, certainly will have a large influence upon normal development. If seeds are treated with growth substances, it may be imagined that their action on the embryo will not only be direct, but also indirect, via the cotyledons. For, the latter strongly swell when soaked in water or in growth substance solutions. Considering the volume of the cotyledons, being often much larger than that of the embryo (see table III, p. 24) it is quite possible that the after-effect of a growth substance treatment becomes very pronounced, if exerted via the cotyledons.

This may also account for the contradictory results obtained in testing several kinds of seeds. BLOCISZEWSKI (231) already showed that in various seeds large differences do exist in the proportion size of embryo/volume of reserve substances:

Zea mais	1 : 4.73
Trifolium	1 : 5.66
Raphanus	1 : 5.76
Avena	1 : 15.75
Secale	1 : 17.25
Pisum	1 : 32.40
Lupinus	1 : 49.88

As to the pea, this statement was put to the test with seeds of various size (see p. 24).

The above discrepancies, however, need not only arise from a different proportion of embryo-volume to cotyledon-volume, perhaps *the nature of the reserve-substances in the cotyledons too may be determinative for the action of the growth substance.*

Prior to the treatment of this problem, an analysis was made of the correlation-phenomena appearing in early development and of their eventual influencing by constituents of the nutrient medium.

a. The early stages of development in the presence of various amounts of cotyledon-tissue

Information about the role of the cotyledons may be obtained, e.g., by determining the growth of both sprout and root in embryos, connected with different amounts of reserve substance tissue.

Partial amputation of the cotyledons of this object proved feasible, without functional disturbances appearing; that is to say, barring possible effects of reducing the amount of reserve substance tissue. For, the cotyledon of the pea, on microscopical examination, proves fairly homogeneous; inside the epidermis parenchyma is found, filled up with starch. Close by the attachment with the embryo some vascular tissue is present. In amputating, this part was always left intact, by cutting the cotyledon parallel to the embryo (see fig. 1, C).

For this purpose an experiment was made with peas, 6.0–6.5 mm in diameter, which, after sterilizing, were pre-soaked in distilled water. Five groups were arranged, consisting of: embryos without cotyledons, embryos with part of a cotyledon (a quarter, or a half) and embryos with either one or two complete ones. Whether a single cotyledon or part of it was left attached to the embryo, it was always on the right side (see fig. 1, C).

Cultivation was carried out in the dark, at about 24° C. The lengths of both the sprout and the root (including the hypocotyl) were measured regularly with a celluloid rule, while from time to time some objects were set apart for fresh and dry weight determinations.

This led to the following observations:

1. Development of plain embryos proceeds regularly. The more cotyledon-tissue is left, the larger the divergence between objects, otherwise treated similarly. Despite of the uniformity of the peas, their cotyledons are often mutually different. A different content of nutrients and growth factors probably will be the cause of variations in development.
2. Development starts with vigorous root-growth; not until the root has attained some length does sprout-growth begin.

3. Growth, of both the root and the sprout, is the more vigorous, the more cotyledon-tissue is left to the embryo. The mere presence of a small piece takes an enormous effect.

Therefore, in studying the influence upon isolated embryos, of substances added to the nutrient medium, one must cut the cotyledon-stalks close to the embryo, lest errors should appear in quantitative tests.

4. If cotyledon-tissue is present and the sprout grows against the cotton plug, so that both the hypocotyl and the basal part of the sprout are pressed down into the nutrient medium, the agar at once starts deliquescing, until a clear solution results. A preliminary communication (239) treats of the subject.

5. The more cotyledon-tissue is present, the less the boundary line between the hypocotyl and the root is distinguishable. The young plant, grown from an embryo without reserve food, shows a dark root, clearly discernible from the hypocotyl.

6. An embryo without cotyledons produces hardly any lateral roots (at best some tiny points, after three weeks), whereas the object, remained intact, possesses a large number of well-developed, lateral roots. Here too the amount of cotyledon-tissue is related to the average number of lateral roots. Most of the latter are usually located on the side where the cotyledon was left, though it is often difficult to judge of, owing to torsion of the main root.

Several of the correlation-phenomena outlined have been observed in seedlings by other authors, like DOSTÁL (213), KOŘÍNEK (215), MATON (216) and PLCH (217). It seemed desirable, however, to analyse these relations under the circumstances of the present experiments, in view of making comparisons when, afterwards, growth substances would come into play.

b. Influence of the cotyledons after amputations at different stages of development

The above results were obtained after the embryos, attached with cotyledon-tissue, had been cultivated for about eight days. In view of the subsequent tests with growth substances, it was deemed essential to determine the lapse of time, during which the cotyledon-tissue has to remain connected with the embryo, in order that, after amputation, a distinct growth-stimulating influence may be exercised.

Beside a group of twenty plain embryos, one hundred objects with both of the cotyledons were put into tubes and cultivated as usual. Every twenty-four hours 20 objects were deprived of their cotyledons, one group of 20 specimens being left intact for the sake of comparison. After nine days the results were recorded (table VI).

Just as in normal development, the growth of the root was stimulated before that of the sprout became vigorous. Evidently, the early growth processes were sufficiently stimulated if the connection of the cotyledons with the embryo was maintained for 24 hours.

The promotion of sprout development by the cotyledons went on as long as these organs were present (in this experiment for nine days).

TABLE VI

After-effect of cotyledons, amputated at various stages. Average of about 20 specimens. Results after nine days' cultivation on standard medium.

		Cotyledons amputated					
		at once after pre-soaking	after 24 hours	after 2 × 24 hours	after 3 × 24 hours	after 4 × 24 hours	not at all
Sprout	average length . . . . . (mm)	33 ± 1.16	37 ± 1.83	55 ± 3.10	73 ± 2.62	95 ± 4.32	149 ± 5.33
	„ fresh weight . . . . . (mg)	42	51	99	167	245	462
	„ dry weight . . . . . (mg)	2	3	5	9	13	30
Root with hypocotyl	average length . . . . . (mm)	37 ± 1.33	50 ± 2.47	68 ± 2.52	96 ± 1.81	97 ± 2.93	90 ± 3.33
	„ fresh weight . . . . . (mg)	25	39	57	78	99	98
	„ dry weight . . . . . (mg)	3	4	5	7	10	12
Average number of lateral roots		0.4	1.1	2.3	7.0	16.5	15.1

Root growth, however, came to a temporary stand-still after 3 × 24 hours already.

Furthermore, the formation and the growth of lateral roots were clearly influenced by the cotyledons. The average number of these roots was doubled every 24 hours, until the maximum (about 16) was reached after four days. The growth-promoting action of the cotyledons was dependent on a normal connection with the embryo: if amputated cotyledons or parts of them were placed into the medium, around the embryo, scarcely any influence upon root growth was observed.

c. Regeneration-phenomena after amputation of the plumule, in the presence of various amounts of cotyledon-tissue

An interesting correlation between the development of the sprout and that of the lateral roots reveals itself in the regeneration of the sprout, after the plumule has been amputated simultaneously with part of the cotyledon-tissue, viz. on one side of the embryo.

The matter was investigated further. Peas, 6.0–6.5 mm in diameter, were pre-soaked in water. Immediately after this, the left cotyledon was cut off in whole, whereas the right one was either curtailed (to one quarter, or one half, resp.) or kept intact. Beside these groups, with normal embryos, similar ones were arranged from which the plumule was removed as well.

After four days' culture in the dark, lateral roots began to appear, while after six days part of the objects, deprived of their sprouts, started developing a new one from the axillary bud of the failing cotyledon.

When the test (table VII) was finished after eight days, the largest number of lateral roots was found with the objects which had developed a new sprout, especially if parts of a cotyledon were present.

As to the development of lateral roots, the objects with intact sprouts differed the more from the amputated ones, the less cotyledon-tissue was present. No explanation can be offered for the present.

So, there is a distinct correlation between the growth of the regenerated sprout and the development of lateral roots.

A better understanding of the mutual influencing of sprout and root will not be gained until these organs have been cultivated, both separately and together, on the same nutrient medium, by the technique of organ-culture (240). STEPHENSON (218) showed for *lettuce* seedlings that, in a standard solution lacking certain growth-factors, root growth was promoted indeed if the sprout was present in the medium. The administration of nicotinic acid took a similar effect. On the other hand, the main root exerted a favourable influence on the formation of adventitious roots to the sprout. This stimulating action was also observed if nicotinic acid or aneurin were added to the medium in which the sprout was cultivated.

TABLE VII

Correlation between regeneration of sprouts and development of lateral roots, in the presence of cotyledon-tissue on one side of the embryo. Average of about 20 specimens per group. Results after 8 days' cultivation on standard medium.

Cotyledon-rest		1			½			¼		
Plumule amputated		—	+	+	—	+	+	—	+	+
Regeneration of sprout		—	—	+	—	—	+	—	—	+
Sprout	average length (mm)	121 ± 4.51	—	27±3.50	105 ± 4.26	—	36 ± 2.73	88±4.79	—	27±2.60
	„ fresh weight (mg)	308	—	60	208	—	74	144	—	47
	„ dry weight (mg)	21	—	5	12	—	5	8	—	3
Root with hypocotyl	average length (mm)	100 ± 1.22	40±3.72	93±3.32	91 ± 4.08	64±7.04	102 ± 2.02	66±4.98	59±2.08	97±2.48
	„ fresh weight (mg)	76	57	119	59	83	120	41	70	98
	„ dry weight (mg)	9	6	13	7	10	13	5	8	10
Average number of lateral roots		14.7	0.9	13.6	10.7	4.9	13.6	1.5	2.9	10.7

d. Effect of amputating the top of the sprout (terminal bud) at various stages of development

Under natural circumstances too, it often happens that injury is inflicted upon the plumule, or—at a somewhat further stage—upon the terminal bud, of the seedling. As a rule, regeneration occurs by the development of either an axillary bud of a cotyledon or a lateral bud of the sprout. Now, a study was made of the correlation-phenomena arising on intentional removal of the plumule, or, at a further stage, of the terminal bud.

From peas, 6.0–6.5 mm in diameter, the embryo was isolated and transferred on to the standard medium, containing 0.4 per cent agar. At the very beginning, twenty objects were deprived of their plumule. After 4, 6, 14 and 17 days, resp., the terminal buds were removed from twenty specimens. A number of control groups was left intact (in this respect, for the rest, they lacked the two cotyledons from the start of the experiment).

It appeared that an axillary bud of the cotyledon developed into a new sprout, if the plumule was removed immediately at the beginning. Its growth was somewhat retarded in comparison with a normal sprout; the root, however, was more vigorous. Moreover, the root was distinctly whiter than in the controls; lateral roots were longer and the root-cap had developed more clearly.

If the terminal bud was amputated after 4 or 6 days, part of the sprout was still present, whereas another one developed from one of the axillary buds of the cotyledon. Here too, root growth surpassed that of the controls; the lateral roots had increased both in number and in length and the root-cap had developed well.

If, finally, the terminal bud was removed much later (after 14 or 17 days), a lateral bud usually developed because, meanwhile, the sprout had finished already the first internode. The growth of the main root was not stimulated any longer by this operation.

The results are summarized in table VIII.

§ 2. Early development of the embryo under the influence of various substances

It may be imagined that the correlation between the development of the various parts of the seedling can be interfered with, not only by amputating organs or parts of them, but also by adding one or more substances to the nutrient medium. Previous to studying the subject proper—the influence of growth substances—it seemed advisable to investigate the constituents of the nutrient medium as to their effect at different concentrations. The effects of adding several other organic compounds to the medium and those, following variations of pH were studied as well.

a. The inorganic ingredients of the medium

By varying the concentrations of all the inorganic ingredients (see p. 22) optimal growth was found at the following concentrations:

TABLE VIII

Effect of amputating the top of the sprout upon development of both sprout and root, and upon the number of lateral roots. Average of about 20 specimens. Results after 22 days' culture on standard medium.

		Plumule or terminal bud amputated					
		not at all	at once	after 4 days	after 6 days	after 14 days	after 17 days
Sprout	average length . . . (mm)	72 ± 1.69	61 ± 2.47	61 ± 3.23 *)	57 ± 3.09 *)	57 ± 2.49 *)	60 ± 2.06 *)
	„ fresh weight . (mg)	45	43	50	43	31	34
	„ dry weight . (mg)	3	2	3	3	2	2
Root with hypocotyl	average length . . . (mm)	48 ± 1.18	73 ± 2.83	64 ± 3.07	61 ± 2.72	50 ± 1.47	51 ± 1.69
	„ fresh weight . (mg)	28	44	37	35	27	27
	„ dry weight . (mg)	3	5	5	4	4	3
Average number of lateral roots . . .		3.4	9.9	9.8	11.9	6.1	6.2

*) In these groups the lengths of both the old and the regenerated sprout were put together. The same applies to fresh and dry weights, respectively.

calcium nitrate 3×10^{-3} mol. (708 mg/l), *potassium nitrate* 3×10^{-3} mol. (303 mg/l) and *potassium dihydrogen phosphate* 4×10^{-3} mol. (544 mg/l).

No optimum could be ascertained for either *magnesium sulphate* up to 3×10^{-3} mol. (740 mg/l) or *potassium chloride* up to 6×10^{-3} mol. (448 mg/l).

However, the concentration of *ferric sulphate* proved important, a maximum number of lateral roots being produced at 10^{-4} mol. The same applied to *ferric chloride.*

b. Various sugars

If sugar was absent in the medium, development was but scanty; some elongation of the embryo occurred. The addition of sugar caused growth; however, the optimum concentrations for sprout development and for root growth generally did not coincide. The formation of a root-cap, as well as the maximum number of lateral roots, were dependent on particular concentrations.

Under the circumstances of the present investigation the optimum concentrations for sprout growth, root growth and development of lateral roots, resp., were the following:

dextrose : 3×10^{-1} mol. (54 g/l); 4×10^{-1} mol. (72 g/l); and 2×10^{-1} mol. (36 g/l);
laevulose : 2×10^{-1} mol. (36 g/l); 4 (to 5) $\times 10^{-1}$ mol. (72–90 g/l); and 4×10^{-1} mol. (72 g/l);
sucrose : 2×10^{-1} mol. (68 g/l); 3×10^{-1} mol. (103 g/l); and 4×10^{-1} mol. (137 g/l);
maltose : 1 (to 2) $\times 10^{-1}$ mol. (36–72 g/l); 10^{-1} mol. (36 g/l); and 10^{-1} mol. (36 g/l).

A comparison of these sugars clearly demonstrated their importance in development. It is a matter of specific physiological action, rather than a realization of certain osmotic conditions. This was confirmed once more by testing the effect of combinations of a sugar with *d-mannitol*, which, in itself, is inactive biologically.

So far, the action of *sucrose* seemed to show the largest resemblance to the natural development under the influence of the cotyledons. In all likelihood, in the intact plant as well, sucrose will play an important part in these processes, considering its abundant occurrence in the cotyledons (234).

c. Amino acids

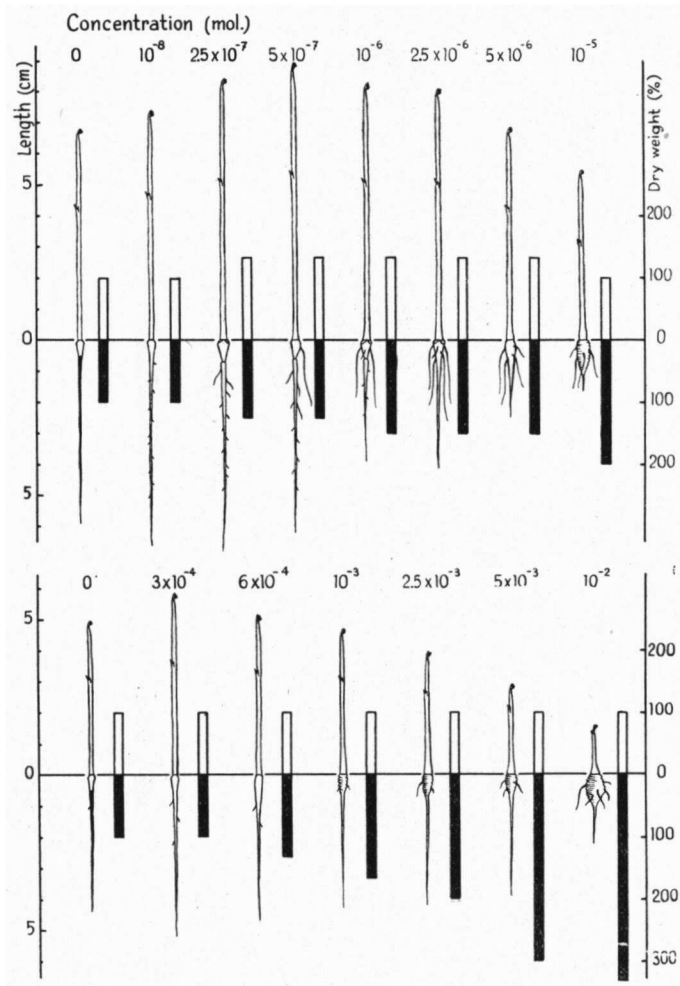
Among the amino acids tested, little or no influence was exerted by *glycine*, *l*(+)*alanine* and *l*(−)*asparagine*. *l*(—)*Aspartic acid* caused some stimulation of growth in sprouts and in roots, at 5×10^{-6} and 5×10^{-4} mol., respectively. From 10^{-3} mol., inhibition revealed itself, which, apart from that, was also observed with glycine at the same concentration.

l(+)*Glutamic acid* scarcely affected growth, though, at 10^{-7} mol. there was a slight stimulation of the development of lateral roots. Sprout growth was promoted by both *dl-methionine* (10^{-4} mol.) and

l(—)*histidine* (5×10^{-5} mol.). A combination of these two acids acted yet more favourable.

d. Growth substances

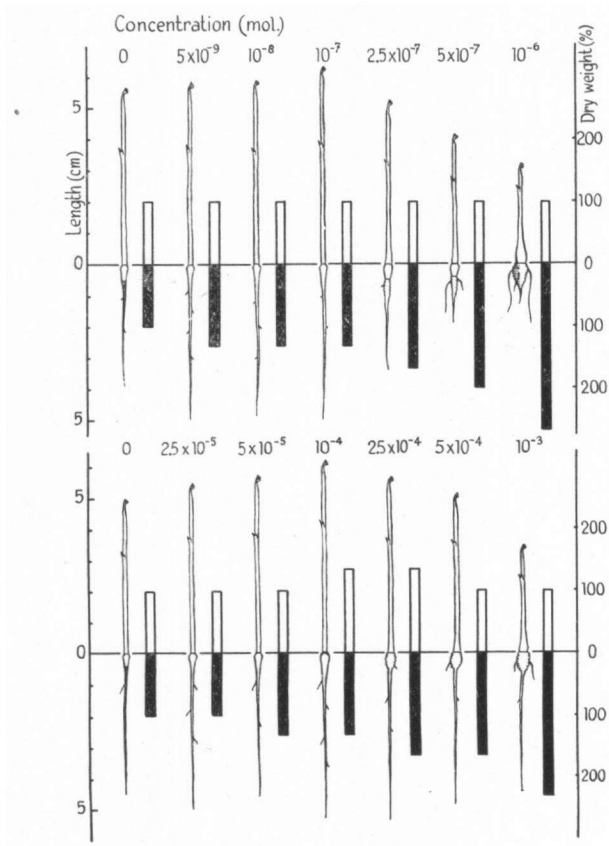
The main object of this investigation is studying the influence of growth substances upon the embryo, after its isolation from the seed, as an attempt at elucidation of the many discrepancies in the results described in the literature.



Figs. 3 and 4. Influence of different concentrations of the potassium-salt of i.a.a. administered via the nutrient medium (above, fig. 3) or during the pre-soaking period (below, fig. 4). The plants pictured show the *development* after three weeks, in proportion to the control-group. Vertical columns represent *dry weights* of both the sprout (above the zero-level) and the root, together with the hypocotyl (below the zero-level). Values in percentage of controls (= 100%). Average of ± 20 specimens.

For this purpose, the potassium salts of *indole-3-acetic acid*, of *naphthalene-1-acetic acid* and of *2,4-dichlorophenoxyacetic acid* were added to the standard nutrient medium, in such a way that concentrations largely diverged.

The phenomena observed proved very specific, and quite different from those produced by other substances. In general, the growth of both sprout and root was stimulated at very low concentrations (i.a.a.K: 10^{-8} mol.; n.a.a.K: 2.5×10^{-8} to 10^{-7} mol.; 2,4-DK:



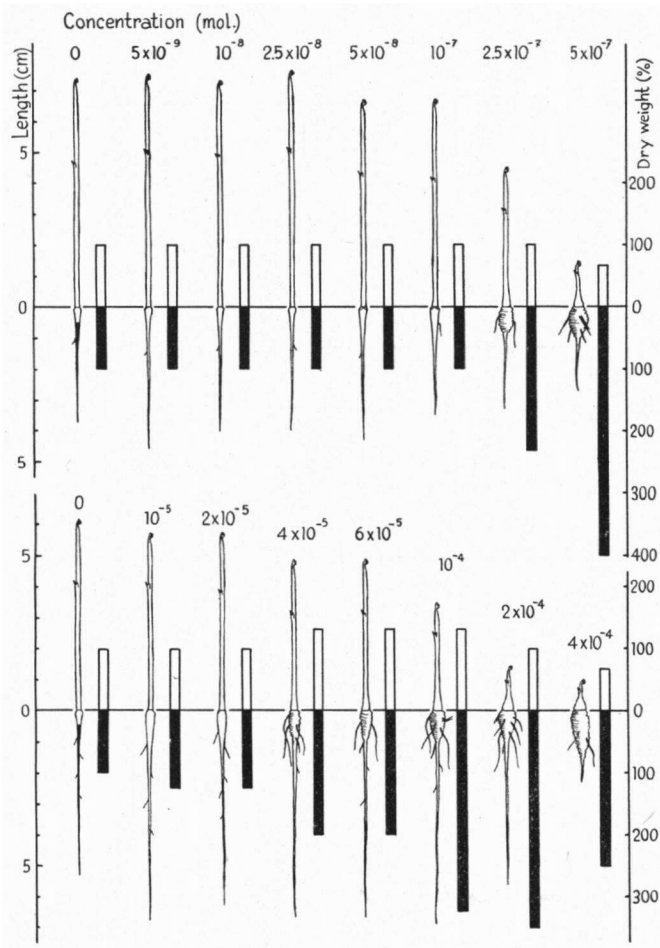
Figs. 5 and 6. Influence of different concentrations of the potassium-salt of n.a.a. administered via the nutrient medium (above, fig. 5) or during pre-soaking period (below, fig. 6). For explanation cf. figs. 3 and 4.

5×10^{-9} to 2.5×10^{-7} mol.). At higher concentrations (from 2.5×10^{-7} mol.) inhibition of growth occurs, accompanied by a swelling of the hypocotyl and by an increase of the amount of lateral roots.

In contradistinction to WHITE's view (247, p. 188) it was noticed that, though the main root was reduced to a short axis, the number of

lateral roots showed an absolute increase by the action of growth substances.

Much importance was attached to the fact that, on increasing concentrations of the growth substance (up to a certain maximum) both the fresh and the dry weights (especially those of the root) kept on rising, in spite of the inhibition of development on the whole. The



Figs. 7 and 8. Influence of different concentrations of the potassium-salt of 2,4-D administered via the nutrient medium (above, fig. 7) or during pre-soaking period (below, fig. 8). For explanation cf. figs. 3 and 4.

boundary line between the hypocotyl and the root finally disappeared, while in that case fasciation of roots frequently occurred. Details are pictured in figures 3, 5 and 7.

It would be worth while tracing the cause of this gain in weight in the presence of a growth substance. Investigations on full-grown

plants, carried out by various authors, revealed an influence upon both the potassium balance and the carbohydrate metabolism.

e. Other organic compounds

Reductone, in general, did not stimulate growth, though at 10^{-3} mol. the longitudinal growth of the lateral roots was promoted, as compared with the controls. From 5×10^{-3} mol. it acted as an inhibitor.

If *d-mannitol* or *inulin* were substituted for sucrose, development failed. The former caused inhibition, if added to a medium with an adequate source of carbon.

Coumarin slightly stimulated sprout growth at 10^{-5} mol. Inhibition was seen from 10^{-4} mol., whereas in roots this occurred already from 2×10^{-5} mol. Up from 5×10^{-5} mol., both the hypocotyl and the root became warty and yellowish.

The potassium salt of *di-n.amyl-acetic acid* inhibited the growth of the sprout (from 5×10^{-6} mol.) and of the root (at 5×10^{-7} mol.). *Adenine* was without any influence.

Adenosine triphosphate (ATP) effected growth stimulation at 7.5×10^{-4} mol., the number of lateral roots being on the increase.

Glutathione somewhat promoted development of lateral roots at 10^{-4} and 2.5×10^{-4} mol.

The addition of (+)*biotin* to the standard medium hardly took any effect, in contrast with the observations by KÖGL and HAAGEN-SMIT (236); more lateral roots and some growth-stimulation were not seen below 5×10^{-5} mol.

According to ŘETOVSKY and HORÁK (246), thiophane-2,5-dicarboxylic acid would equal biotin in stimulating the growth of yeast. Aqueous solutions, as low as 10^{-12} mol., would generate adventitious roots in epicotyls of *Vicia faba*, growth-promotion of isolated plant embryos being reported as well.

This certainly justified its testing on the pea. Both *rac.* (*trans*)-*tetrahydrothiophene-α,α'-dicarboxylic acid* and its *cis-isomer* (10^{-4} mol.) promoted the growth of either sprout and root; lateral roots increased.

Vitamin B_1 favoured sprout growth (3×10^{-7} to 3×10^{-6} mol.) and root growth (1.5×10^{-6} mol.); lateral roots tended to increasing (3×10^{-5} mol.). *Vitamin* B_2 was practically inactive.

Nicotinic acid, scarcely affecting the growth of sprouts and roots, was not quite inactive towards lateral roots (5×10^{-5} mol.).

l-Pantothenic acid proved a weak stimulant for root growth only; from 10^{-5} mol. inhibition took place.

Thus, neither of these substances caused the characteristic phenomena, to be observed after adding a growth substance to the nutrient medium.

f. Effect of pH

The connection between pH and the development of the embryo was studied by adding phosphate buffers (acc. to SØRENSEN) to the nutrient medium.

When investigating the effect of pH on growth and, possibly, on

certain correlation-phenomena, it should be borne in mind that one never can detect the influence of pH in itself. There are always possibilities of additional influences by certain combinations of salts, or salt concentrations, or by changes in the viscosity of the agar.

From pH 7.2 upwards, decomposition of sucrose occurred on sterilizing. A distinct optimum was found at pH 5.1, for growth (of both the sprout and the root) as well as for the formation of lateral roots. Below pH 5.1 development was satisfactory, but growth declined. From pH 5.8 upwards, development was inhibited more and more.

SUMMARY

It was supposed that on treating seeds with a growth substance, its influence upon the embryo was exerted not only directly, but also indirectly, via the cotyledons. Therefore it was studied first, what influence might be exercised on early development by the two cotyledons and by parts of them.

Correlation-phenomena occurring normally were examined, as well as those occurring after amputation of cotyledons and those, observed on regeneration, after the terminal bud had been amputated.

The addition of a growth substance to the nutrient medium produced a very specific effect on the development of the embryo. By varying the concentrations of all the ingredients of the medium and by testing the effect of several organic compounds, it was ascertained that their action can be distinguished clearly from that of the growth substances.

Finally the effect of pH on the development of the embryo was studied.

CHAPTER V

ACTION OF GROWTH SUBSTANCES ON THE EMBRYO, DIRECT AS WELL AS INDIRECT (VIA THE COTYLEDONS)

§ 1. THE ACTION OF GROWTH SUBSTANCES IN SEED-TREATMENT

Some authors have moved certain points that should be considered in seed-treatment, the more so as they might be related to varying results (compare Chapter I). Beside these points, however, there seem to be a few other ones, deserving more attention than they have received before. The following items are worth while considering:

1. Both size and origin (viz., location in a multispermous fruit) and ripeness may differ in a given lot of seed.
2. The selective action of the seed-coat may differ as well, in dependence on the foregoing. As a semi-permeable membrane it will make difference when substances are passing through, towards the embryo. Thus, the way in which the growth substance is administered —e.g. as such, or as a salt—may play some part.
3. Too little allowance is made for the fact that the growth substance acts not only direct on the embryo, but also by some indirect way. For, after being absorbed at the swelling of the cotyledons, it may keep

exerting an influence during the development of the seedling. In that case the cotyledon would act like a reservoir, gradually giving off its contents.

Ad. 1. The connection between size and subsequent development of the seed was already outlined in Chapter III, § 1. It will be shown below that this point may be important in growth substance treatment too.

Ad. 2. In the object chosen, semi-permeability of the seed-coat probably will play little part, which was already demonstrated by VAN DER MAREL (241)—in contrast to the seeds of, e.g., Cucurbitaceae. For the matter of growth substances, however, nothing has been proved so far.

The influencing of water uptake during the earlier phases of germination (viz., swelling) by growth substance treatment was tentatively studied (see Chapter VI).

Ad. 3. If such an indirect influence should prove a general phenomenon in seed-treatment, different results might be expected if the same growth substance (even of the same concentration) would be applied to seeds, varying in the nature of the reserve substances (e.g. carbohydrates, proteins, or lipids) present in their cotyledons. In that case, small variations in growth substance concentrations, together with differences both in the size of the seeds and in the proportion of embryo-size to volume of reserve substances, might lead to variability of results.

The extent of the above-said, indirect influence was approximated in three ways:

by comparing the development of embryos from seeds pre-soaked in water (on a medium containing growth substance) with those from seeds pre-soaked in growth substance solutions (cultivated on a standard medium, i.e., without growth substances): § 2;

by studying the development of embryos with different quantities of reserve food, isolated from seeds, pre-soaked either in water or in growth substance solutions: § 3;

by estimating the development of embryos from seeds, pre-soaked either in water or in growth substance solutions, on a medium without growth substances, after the cotyledons had been amputated at various stages: § 4.

§ 2. COMPARISON OF THE DEVELOPMENT OF EMBRYOS FROM SEEDS, PRE-SOAKED IN WATER OR IN GROWTH SUBSTANCE SOLUTIONS, ON NUTRIENT MEDIA WITH OR WITHOUT GROWTH SUBSTANCES, RESPECTIVELY

The influence of the three growth substances, tested in the present investigation (i.a.a., n.a.a. and 2,4-D), was mentioned before (p. 39–42); it proved most characteristic (figs. 3, 5, 7).

The action of growth substances, administered during pre-soaking only, was studied under absolutely sterile conditions by adding 20 ml of their solutions (of various concentrations) to peas placed in Petri dishes. After soaking for some 16 hours, in the usual way at 24° C

in the dark, the seeds were carefully rinsed three times with sterile, distilled water. Any interference by residual growth substance thus being avoided, the isolated embryos were transferred into the culture tubes.

a. Pre-soaking in potassium indole-3-acetate

The experiment was terminated after three weeks (fig. 4). Treatment with a 3×10^{-4} mol. solution had stimulated the longitudinal growth of both sprout and root. The result after this short period of cultivation comes up to expectations of seed-treatment.

At higher concentrations growth was inhibited, though fresh and dry weights of the root kept increasing. From 6×10^{-4} mol. onwards the influence of the growth substance clearly manifested itself by an increasing number of specimens, the hypocotyl of which got swollen. The number of lateral roots augmented, and attained its maximum at 10^{-2} mol. i.a.a.K.

The boundary line between the white hypocotyl and the dark root, clearly visible at first, became less distinct from 10^{-3} mol. onwards because the hypocotyl got brown as well. Besides, swelling occurred at the basal part of the sprout.

Generally speaking, the above phenomena are in keeping with the observations made during the culture of embryos on nutrient media, containing various amounts of i.a.a.K (see p. 39 and fig. 3). However, the concentration required for a similar effect is many times higher if the growth substance is applied during pre-soaking; quite obvious, because the administration during a sixteen hours' soaking period is quite different from that during a three weeks' culture.

b. Pre-soaking in potassium naphthalene-1-acetate

The results of an experiment with n.a.a.K are summarized in fig. 6. In general, they resemble those obtained after pre-soaking in i.a.a.K. An increase of longitudinal growth of sprout and root was seen only at higher concentrations (from 2.5×10^{-5} to 10^{-4} mol.) Subsequently both organs showed shrinking, whereas fresh and dry weights of the root, including the hypocotyl, kept rising.

The hypocotyl, from 10^{-4} mol. onwards, started swelling and it became more and more callous at increasing concentrations. The basal part of the sprout became swollen too, from 5×10^{-4} mol. onwards. The average number of lateral roots attained its maximum at 10^{-4} mol.

A comparison of the results with those obtained with nutrient media containing n.a.a.K (p. 40, fig. 5) shows their similarity.

c. Pre-soaking in potassium 2,4-dichlorophenoxyacetate

Stimulation of sprout growth did not appear at the concentrations tested (fig. 8). Fresh and dry weights, however, were raised; the optimum being attained at 4×10^{-5} to 10^{-4} mol.

Root growth was distinctly favoured between 10^{-5} and 10^{-4} mol. Optimum conditions for fresh and dry weights were found at 2×10^{-4}

mol. Again it was chiefly the hypocotyl that became swollen at the lowest dose applied; from 4×10^{-5} mol. onwards it turned brown, so that the boundary line with the root proper got lost.

Pre-soaking in this growth substance raised the number of lateral roots and, from 4×10^{-5} mol. onwards, the larger part of them was found implanted on the swollen hypocotyl. The lateral roots had grown longer than those of specimens, pre-soaked in plain water; now and then fasciation of roots was observed.

Just as with i.a.a.K and n.a.a.K, the effect of 2,4-DK, applied during pre-soaking, is generally comparable to that, produced via the nutrient medium (comp. the data in figs. 7 and 8).

So, it may be concluded that the effect of a treatment with these three growth substances during pre-soaking is fairly comparable to the influence exerted on the isolated embryo, via the nutrient medium. In the former case, however, a similar effect is only obtained at much higher concentrations.

Most striking is the influence of the growth substances upon root development. Low concentrations stimulate longitudinal growth, higher doses cause inhibition, whereas fresh and dry weights keep increasing.

It should be noted that the inhibitory influence upon root growth is less radical if the growth substances are applied during pre-soaking.

TABLE IX

Comparison of optimum concentrations of growth substances, applied to peas, 6.0 — 6.5 mm in diameter

	i.a.a.K	n.a.a.K	2,4-DK
Action on the embryo, via nutrient medium:			
promotion of sprout growth	10^{-8} mol.	10^{-7} mol.	5×10^{-9} mol.
promotion of root growth	10^{-8} „	2.5×10^{-8} „	5×10^{-9} „
incipient inhibition of sprout growth	10^{-7} „	2.5×10^{-7} „	5×10^{-8} „
incipient inhibition of root growth	2.5×10^{-7} „	2.5×10^{-7} „	10^{-7} „
swelling of hypocotyl	5×10^{-7} „	2.5×10^{-7} „	2.5×10^{-7} „
distinct inhibition, all over	5×10^{-7} „	2.5×10^{-7} „	2.5×10^{-7} „
maximum number of lateral roots	10^{-7} „	6×10^{-6} „	5×10^{-7} „
Action after soaking the seed, via cotyledons:			
promotion of sprout growth	$\pm 3 \times 10^{-4}$ mol.	$\pm 10^{-4}$ mol.	?
promotion of root growth	$\pm 3 \times 10^{-4}$ „	$\pm 10^{-4}$ „	$\pm 10^{-4}$ mol.
incipient inhibition of sprout growth	$\pm 10^{-3}$ „	$\pm 9 \times 10^{-4}$ „	$\pm 10^{-5}$ „
incipient inhibition of root growth	$\pm 10^{-3}$ „	$\pm 9 \times 10^{-4}$ „	$\pm 3 \times 10^{-4}$ „
swelling of hypocotyl	$\pm 10^{-3}$ „	$\pm 10^{-4}$ „	$\pm 2 \times 10^{-5}$ „
distinct inhibition, all over	$\pm 10^{-3}$ „	$\pm 9 \times 10^{-4}$ „	$\pm 10^{-4}$ „
maximum number of lateral roots	$\pm 10^{-3}$ „	$\pm 10^{-4}$ „	$\pm 10^{-4}$ „

It is distinctly visible (figs. 3 and 4, 5 and 6, 7 and 8) that swelling is restricted to the original junctures of the cotyledons.

Table IX summarizes the optimum concentrations of the growth substances for certain, discernible phenomena, appearing after administering in both ways.

The figures show that, on the whole, 2,4-DK is the most active agent; the optimum concentrations of n.a.a.K are higher and those of i.a.a.K even a little more.

For each effect recorded, one can calculate the proportion of the concentrations required in direct and in indirect application of each of these substances. Now, if attention is paid to a very specific effect (e.g., the swelling of the hypocotyls), considerable differences prove to exist (table X).

TABLE X

Approximate factors, obtained by dividing the growth substance concentrations required in action via the cotyledons (during pre-soaking) by those required in direct action (via the nutrient medium), for a similar final result.

	i.a.a.K	n.a.a.K	2,4-DK
Promotion of sprout growth	30.000	1.100	?
Promotion of root growth	30.000	4.000	20.000
Incipient inhibition of sprout growth .	10.000	3.500	200
Incipient inhibition of root growth . .	5.000	3.500	3.000
Swelling of hypocotyl	2.300	400	80
Distinct inhibition, all over	2.300	3.500	400
Maximum number of lateral roots . .	100	18	200

Though there is certainly a difference between continuous contact with a growth substance solution and a relatively short administration of growth substance through the seed-coat, there are qualitative features in the reaction that cannot be accounted for by this difference.

These can only be accounted for by presuming that, during the action via the cotyledons, some interaction with other factors has occurred (selective action of the seed-coat, influence of constituents of the cotyledons). However, one should not overlook the fact that i.a.a., for instance, is poorly stable; it might be decomposed in some enzymatic process.

For the sake of comparison, peas were also soaked in *coumarin* solutions. Cultivation of the embryo on a medium containing this inhibitory substance led to restriction of growth, in both the sprout and the root, at concentrations from 10^{-4} mol. upwards (cf. p. 42). Though the concentration was raised to 7×10^{-4} mol. (being the maximum solubility in water) no other effects were seen. The inhibitory action of such coumarin solutions upon the germination of whole peas was not very striking either; only root growth was slightly reduced. In comparison with other types of seeds (e.g., those of the garden cress) the pea is evidently but little sensitive to this substance. It is possibly a question of enzymatic decomposition in the cotyledons, and these findings might be an argument in support of the author's view

that in seed-treatment the substances administered would exercise their influence mainly via the cotyledons.

§ 3. Study on the development of embryos with different quantities of reserve food, isolated from seeds, pre-soaked either in water or in a growth substance solution

It was already stated (p. 30) that the cotyledon-tissue exerts an enormous influence upon the development of the embryo and upon the formation of lateral roots. If one imagines the simplest case, viz. that during pre-soaking in a growth substance solution the cotyledons initially absorb the growth substance and that they pass it on—as such—to the embryo afterwards, an inhibitory influence should increase at a suitable concentration the more cotyledon-tissue is left to the embryo.

After sterilizing, peas of one size (6.0–6.5 mm in diameter) were pre-soaked in water or in sterile solutions of a growth substance, at about 24° C in the dark. Some 16 hours after, the seeds were washed three times with sterile, distilled water and subjected to excision of either embryos or embryos to which varying amounts of cotyledon-tissue had been left (1/4, 2 × 1/4, 1/2, 2 × 1/2, 1 and 2 × 1 cotyledon). Cultivation on the standard nutrient medium took place in the dark at about 24° C. After ten days the test was finished.

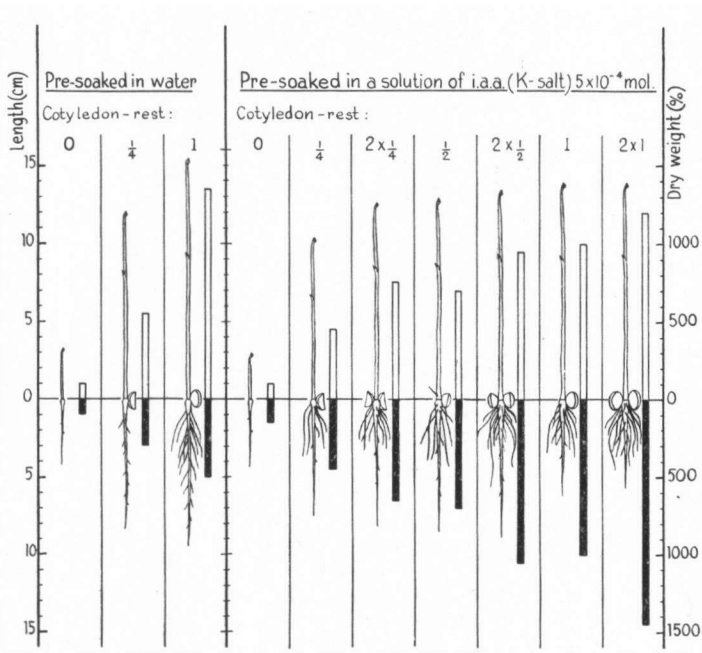


Fig. 9. Development of pea-embryos with different quantities of reserve-food, after the seeds had been pre-soaked in water or in a solution of i.a.a. (K-salt) 5×10^{-4} mol. Results after ten days. For explanation cf. figs. 3 and 4.

a. Pre-soaking in potassium indole-3-acetate

No doubt the concentration chosen for an initial experiment is preferably such as to produce scarcely any effect in cultivating the embryo (i.e. without reserve food). To begin with, a 5×10^{-4} mol. solution of i.a.a.K was used (fig. 9).

Undesired cotyledon-tissue was cut off at a rough estimation (cf. fig. 2, C), and subsequently weighed in the fresh state and after drying. When the test was over, fresh and dry weights of the cotyledon-parts remained could be determined, which revealed the amounts of tissue, having taken part in the growth process.

The development after pre-soaking in water has been outlined already. A small amount of cotyledon-tissue stimulates the growth of sprout and root to the extent that after the ten days' period the majority of the specimens has grown against the cotton plug, the lower parts being pressed down into the agar. Liquefaction of the medium ensues from this. The main roots have a dark hue, lateral roots have developed either as tiny points, or as roots up to 30 mm in length; the root-cap is either hardly visible, or not at all.

The presence of one cotyledon promotes growth still more, as well as the number of lateral roots; the root-cap is clearly visible as a brown tip.

The action of the growth substance answered expectations to a small degree only. As to longitudinal growth, the influence on the embryo is very slight indeed. *In the presence of cotyledon-tissue, however, there is always stimulation of growth.* In comparison with the corresponding, non-treated lots some growth inhibition of sprout and root was noticed, it is true; nevertheless, fresh and dry weights of the root increased in consequence of the treatment (fig. 9). Now, quite remarkable, the lateral roots were implanted chiefly on the swollen hypocotyl and on the upper part of the root.

Another feature was, that two quarters apparently effected the same as half a cotyledon and, likewise, the influence of two halves equalled that of a whole cotyledon. No doubt the differences observed may be ascribed to the circumstance that these amputations were made at random.

The results of another trial with i.a.a.K, at a higher level (10^{-3} mol.) corroborated these findings. Raising the concentration clearly enhanced the inhibition of longitudinal growth of sprout and root; despite of this, fresh and dry weights of the root were on the increase again.

In comparison with the corresponding water controls, the increase of root weight is the larger, the more reserve substance tissue is left to the embryo.

b. Pre-soaking in potassium naphthalene-1-acetate

The results of a ten days' test with n.a.a.K (5×10^{-5} mol.) showed much resemblance to those obtained with 5×10^{-4} mol. i.a.a.K

(fig. 10). On closer examination, however, this does not apply to the inhibition: the combination of reserve substance and n.a.a.K (the dose of which was only one tenth of that in the first test with i.a.a.K) had caused less inhibition of sprout and root. Again, in the presence of more reserve food tissue, the main effect of the growth substance was an augmentation of fresh and dry weights of the roots.

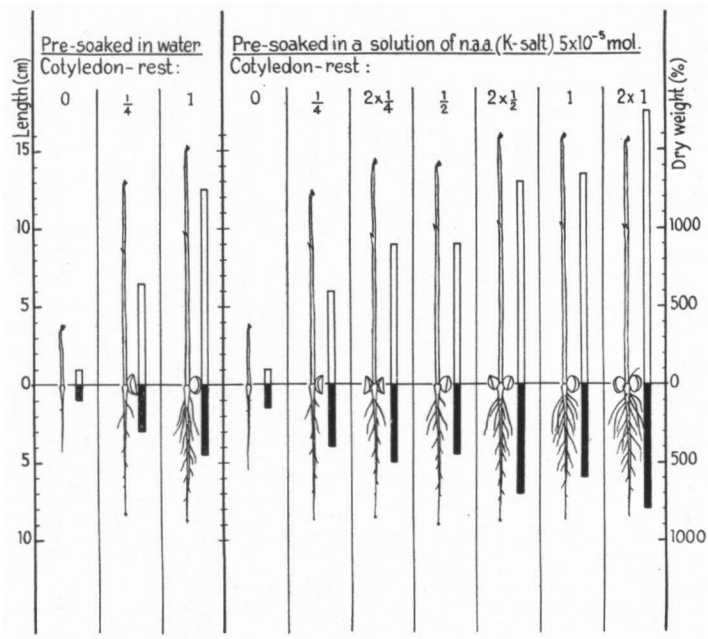


Fig. 10. Development of pea-embryos with different quantities of reserve-food, after the seeds had been pre-soaked in water or in a solution of n.a.a. (K-salt) 5×10^{-5} mol. Results after ten days. For explanation cf. figs. 3 and 4.

c. Pre-soaking in potassium 2,4-dichlorophenoxyacetate

A comparatively low concentration of 2,4-D, viz. 2.5×10^{-6} mol., was much more effective than the two substances mentioned above (fig. 11). Though, in the absence of reserve food hardly any inhibition took place, it was distinctly visible when, after the treatment, embryos with one quarter or a whole cotyledon were compared with the water controls.

The competition of growth promotion by the cotyledon-mass with inhibition by the growth substance was mostly won by the latter. In some specimens from the groups with two quarters, or more, of a cotyledon, inversion of polarity was observed. At first, the sprout grew downward, then it became negatively geotropic. The callous, swollen root-stump pointed upwards, rising above the medium.

Just as in all the previous experiments the treatment strongly affected both hypocotyl and root. An increase of fresh and dry weights was the final result.

A concentration four times higher (10^{-5} mol. 2,4-DK) produced quite another picture. After ten days the embryo without reserve substance was clearly inhibited already; the basal part of the sprout had thickened and the hypocotyl had swollen. In the presence of cotyledon-tissue, inhibition was very strong; here, in comparison with the two other substances, the action was rather a toxic one. Most of

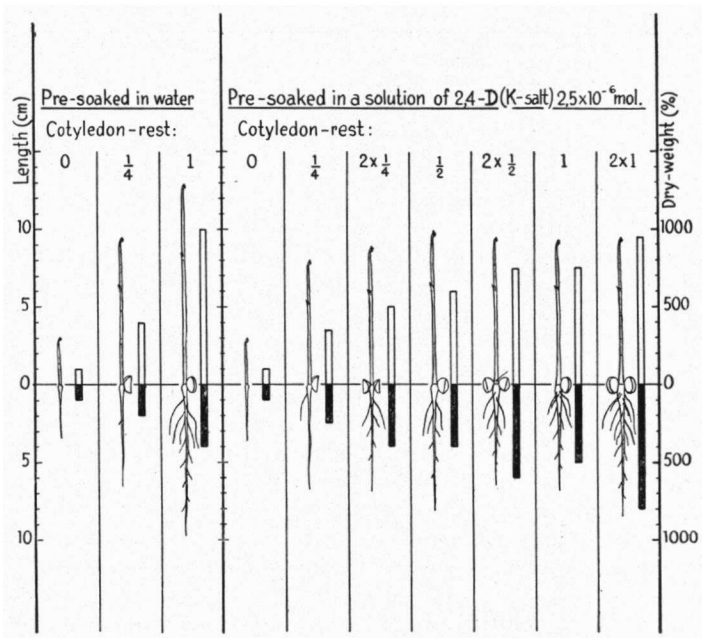


Fig. 11. Development of pea-embryos with different quantities of reserve-food, after the seeds had been pre-soaked in water or in a solution of 2,4-D (K-salt) 2.5×10^{-6} mol. Results after nine days. For explanation cf. figs. 3 and 4.

the specimens had grown into the agar medium sprout downwards, the callous root-mass aloft. Despite of the strong inhibition of longitudinal growth in sprout and root, a considerable increase of fresh and dry weights never failed with the root, including the hypocotyl.

When culture at this high concentration was protracted for over ten days, the sprouts of several specimens were still capable of growing further.

The experiments might be carried out with peas of different size, provided some changes are made. For, in the pea, no mean differences are found in the proportion of embryo-mass to reserve substance, in comparing seeds of unequal size (cf. p. 24). In that case, however, mutual comparison is hampered by disparity of the embryo.

For a clear apprehension of the growth substance action this investigation ought to be extended to tests in which, during pre-

soaking, such concentrations are applied as to finally procure equal amounts of growth substance for the embryos of all groups, after partial amputations of cotyledon-tissue.

The following series of tests was set up:

	Peas, pre-soaked in:	Number of cotyledons, left to embryos under cultivation:
A	water (controls)	0, ¼, 1
B	i.a.a.K. 4×10^{-3} mol.	0, ¼
C	„ 2×10^{-3} „	0, $2 \times$ ¼, ½
D	„ 10^{-3} „	0, $2 \times$ ½, 1
E	„ 5×10^{-4} „	0, 2×1

Barring the lots, consisting of embryos without reserve substance tissue, the concentrations were chosen in such a way as to halve the dose of growth substance on doubling the quantity of cotyledon-tissue. Assuming that the growth substance is uniformly absorbed into the cotyledons and that its action is proportional to the mass of reserve substance tissue, ultimately about the same dose should be present in the following combinations:

1/4 cotyledon and i.a.a.K 4×10^{-3} mol.; $2 \times$ 1/4, or 1/2 cotyledon and 2×10^{-3} mol.; $2 \times$ 1/2, or 1 cotyledon and 10^{-3} mol., and, finally, 2×1 cotyledon and i.a.a.K 5×10^{-4} mol.

Judging from the results of earlier experiments, expectations were not put too high in this case either. The lower the growth substance concentration, the less the embryo (without cotyledons) is inhibited, of course. This was already shown in the earlier pre-soaking tests with i.a.a.K (fig. 4).

The highest concentration, 4×10^{-3} mol., strongly inhibited the development of the embryo with one quarter of a cotyledon, though to a lesser degree than that of the plain embryo. Nearly all of the embryos had turned upside down, i.e., the sprout had penetrated into the agar, whereas the root had grown upwards. Both of the organs bent at their ends finally, thus striving after re-establishment of the polarity. *The less growth substance was administered and the more cotyledon-tissue was left to the embryo, the more growth improved.*

Almost every growth substance treatment led to swelling of the hypocotyls and to accumulation of lateral roots, especially on this part of the plant.

From all these tests the following combinations are available for comparison:

I. embryos with equal quantities of cotyledon-tissue and varied concentrations of growth substances (p. 44);
II. embryos with equal quantities of growth substance administered and variable cotyledon-mass (p. 48);
III. embryos with varied quantities of cotyledon-tissue, together with different doses of growth substances, in such a way as to yield about the same total in every case (p. 52).

It should be borne in mind, however, that the action of the growth substance during pre-soaking is twofold. There is a direct action on the embryo by the permeating growth substance that will increase with increasing concentrations. On the other hand growth substances are absorbed by the cotyledons and, afterwards, exert their influence through these.

It is difficult to form a clear-cut picture of the growth substance action proper, the growth stimulation through the cotyledons interfering with the processes involved in the former. How far the nature of the reserve substances is concerned with the problem, cannot be settled before other types of seeds have been tested, the reserve tissues of which consists mainly of lipids or proteins.

§ 4. Comparison of the development of embryos from seeds, pre-soaked either in water or in growth substance solutions, after the cotyledons had been amputated at various stages

Amputation of the cotyledons at various stages (cf. p. 32) has shown that the initial growth process is already considerably favoured if these organs remain connected with the embryo for 1 × 24 hours. After pre-soaking in water or in a growth substance solution, in the dark at about 24° C, the peas were washed three times with sterile, distilled water. The embryos (without cotyledons), from twenty water controls and from twenty peas pre-soaked in the growth substance solution, were transferred to the standard nutrient medium. Two more series were set up, consisting of 5 × 20 peas each, from which only the seed-coats had been removed, after pre-soaking in water or in the growth substance solution, respectively. These embryos with cotyledons were also put into tubes, containing the same medium. Hereafter, they were cultivated in the dark at 24° C, just as the others.

From a water control group and from a treated group, of 20 specimens each, the cotyledons were amputated under aseptic conditions at 24 hours' intervals. From each series a group of 20 specimens was left intact (embryos with cotyledons). The experiment was finished after ten days.

a. Pre-soaking in potassium indole-3-acetate

In the first test with i.a.a.K a 3×10^{-4} mol. concentration was chosen. The result is found in fig. 12: A, B. As to the *water controls*, again the development of both the sprout and the root was favoured during the first 24 hours, reckoning from the moment pre-soaking was concluded. Development improved and lateral roots increased both in number and in length, as the cotyledons remained attached for a longer time. At a certain stage the development of the root-cap became manifest. Moreover, in the groups 3 to 6, inclusive, the agar liquefied.

After pre-soaking in the growth substance solution some inhibition was seen in the development of the embryo without reserve substance. The same effect was observed in young plants, to which the cotyledons had been left for a longer time. Amputation after 2 × 24 hours resulted

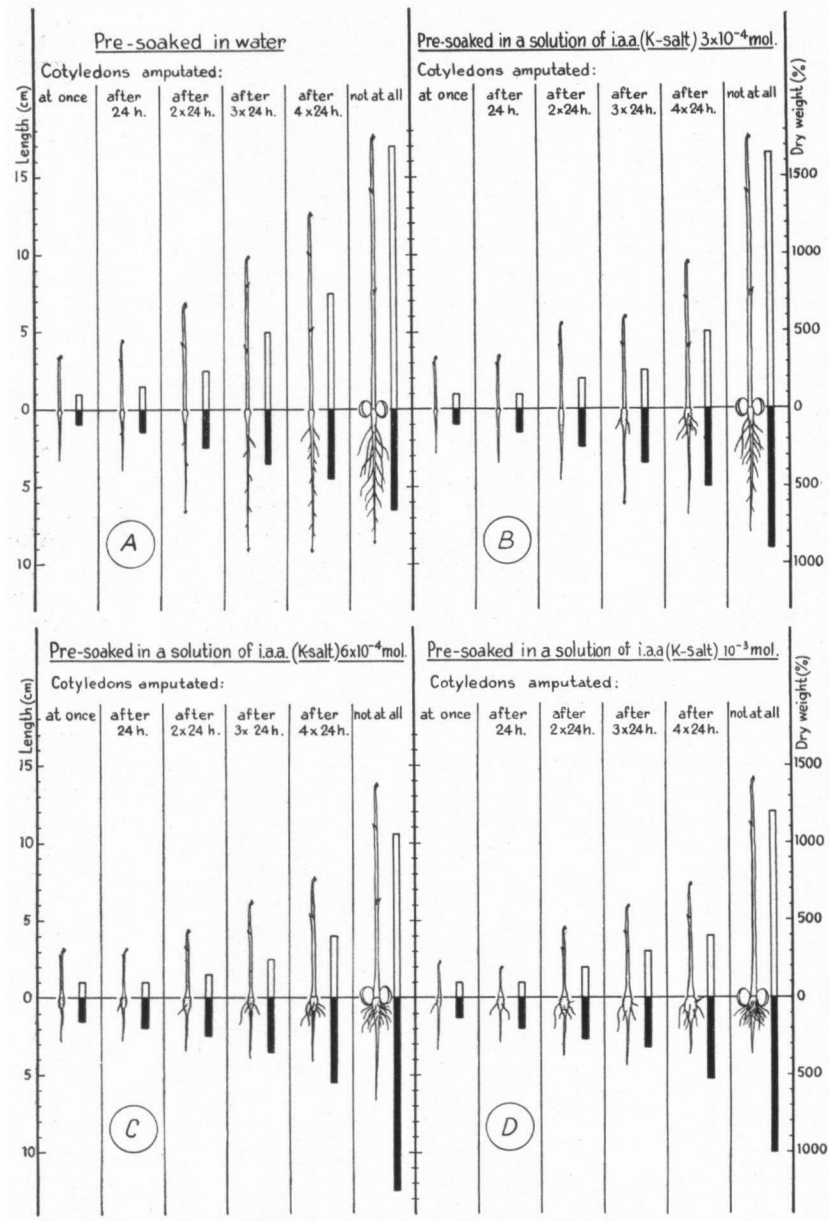


Fig. 12. Comparison of the development of pea-embryos from seeds pre-soaked in water or in a solution of i.a.a. (K-salt) after the cotyledons had been amputated at different stages after pre-soaking.

A. water-control groups (controls of B);
B. influence of i.a.a.K 3×10^{-4} mol.;
C. „ „ „ 6×10^{-4} mol.;
D. „ „ „ 10^{-3} mol.

Results after ten days. For explanation cf. figs. 3 and 4. The data of C and D are reduced to the relative water-controls omitted in the figure.

in roots slightly thicker than in the water controls, when the test was finished. The next groups showed the picture, typical of growth substance action, viz. swelling of the hypocotyl, and, on to it, the location of lateral roots. Although, in the two remaining groups, growth inhibition of sprout and root continued, the average fresh and dry weights of the root had risen.

Exclusive of the last group, the number of lateral roots had been diminished by the growth substance treatment. Sometimes root fasciation was found.

Another experiment was made with double the concentration of i.a.a.K, so 6×10^{-4} mol. (fig. 12, C). As compared with the preceding test, inhibition by the growth substance was stronger. It was evident already in the instance of the young plant, deprived of its cotyledons at the very beginning. Sprout growth was hampered in particular; moreover, a callous thickening of the hypocotyl was seen. Fresh and dry weights of the root had increased.

Development would slightly improve as the cotyledons were left to the plant for a longer time, though as a whole it was considerably inhibited in comparison with the water controls. In the hypocotyl the treatment, as it were, has caused an accumulation of substances necessary to the development of lateral roots, hence they will develop mainly in this region. Here, too, root fasciation was frequent.

A dose of i.a.a.K even higher (10^{-3} mol.) had chiefly increased the inhibition of root growth (fig. 12, D). It looked as if the working hypothesis would become realized, viz. that the inhibitory action by the growth substance would be the stronger, the longer the cotyledons remain attached. However, amputation after 2×24 hours again resulted in growth stimulation in respect of the preceding group. There was an all-round rise of fresh and dry weights in the root-system including the hypocotyl. The lateral roots, in comparison with the controls, had diminished in number and, as to the groups with prolonged retention of the cotyledons, also in length. The crowding together of a large number of lateral roots in a small area, probably favours root fasciation, which was often observed.

b. Pre-soaking in potassium naphthalene-1-acetate

The influence of n.a.a.K was studied at a concentration of 5×10^{-4} mol. (fig. 13). The overall picture resembled that produced by i.a.a.K, yet, the action was much stronger than that exercised by the more concentrated solution of the latter (10^{-3} mol.). The plant, grown from an embryo isolated at the beginning of the test, shows a hypocotyl amply swollen. Whereas the root, together with the hypocotyl, was shorter than the corresponding part in the control, its dry weight had trebled.

When the cotyledons were amputated later on, inhibition kept going on at first, thus coming up to expectations. Thereupon, however, the beneficial influence of the cotyledons themselves obviously surpassed the effect, so that the longitudinal growth of sprout and root continued

anew. Nevertheless, both organs were inhibited in comparison with the water controls.

The treatment had lowered the number of lateral roots; the tight packing on the hypocotyl led to fasciation.

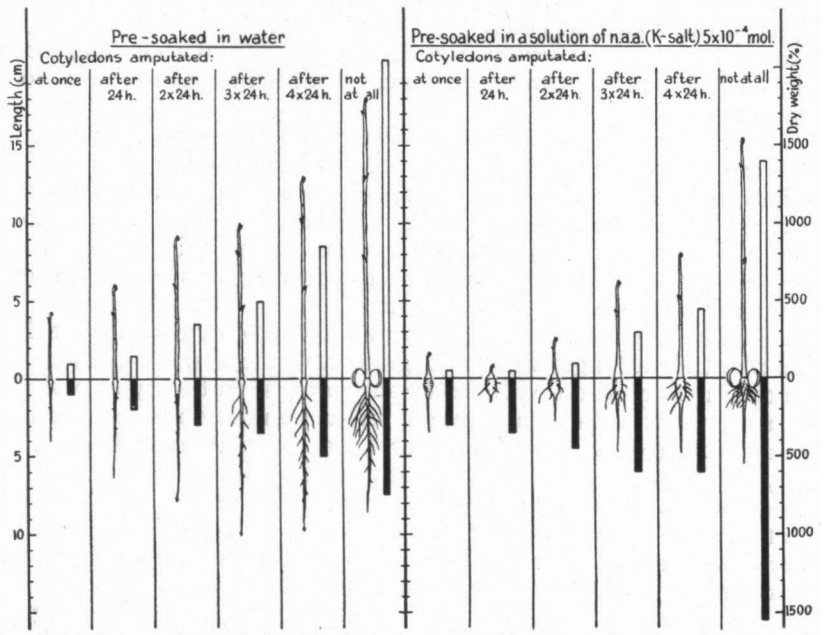


Fig. 13. Comparison of the development of pea-embryos from seeds pre-soaked in water or in a solution of n.a.a. (K-salt) 5 × 10^{-4} mol. after the cotyledons had been amputated at different stages after pre-soaking. Results after ten days. For explanation cf. figs. 3 and 4.

c. Pre-soaking in potassium 2,4-dichlorophenoxyacetate

The results of an experiment with 2,4-DK, at a concentration of 10^{-5} mol., are summarized in fig. 14. Roughly speaking, the issue was like the situation to be imagined in the event of a simple passing on to the embryo of the growth substance absorbed by the cotyledons, without any growth-stimulating action of their own coming into play. The divergence from the other results described in this paragraph probably must be explained by a toxic action, interfering with the normal, enzymatic processes.

In the water controls, the longer the cotyledons remained attached, the more growth increased, both of sprout and root. *In the growth substance groups, it was just the reverse: development was inhibited more and more, when amputations were done at a later stage.*

The final group, in which no amputation was applied, differed somewhat from the others, in that the growth-promoting influence of the cotyledons was predominant, so that sprout and root would develop a little further.

A comparison of the water controls with the corresponding groups from the growth substance series (mainly those with i.a.a.K) leads to the following conclusions:

1. Longitudinal growth, both of the sprout and the root, is inhibited the stronger, the more the growth substance concentration is raised; at the same time fresh and dry weights of the sprout *diminish*, whereas those of the root (with the hypocotyl) *increase*.

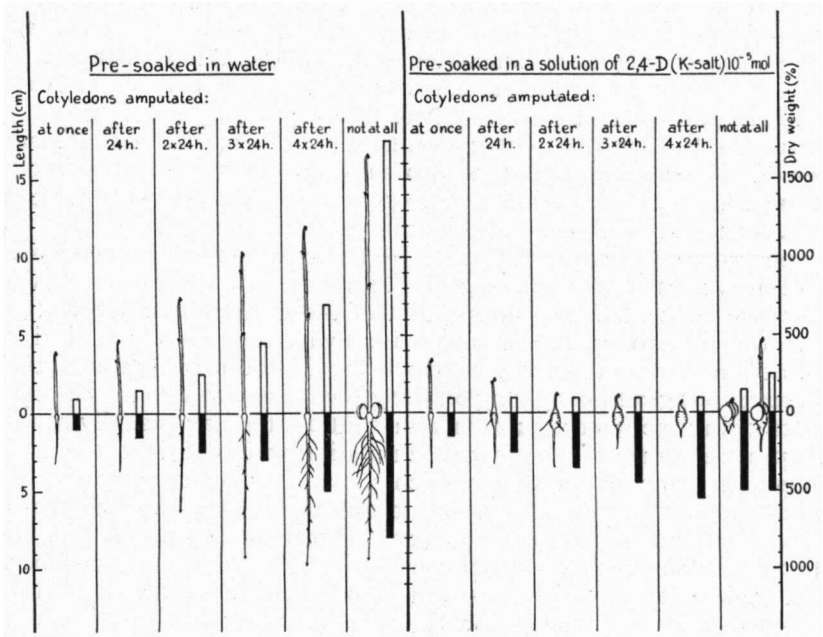


Fig. 14. Comparison of the development of pea-embryos from seeds pre-soaked in water or in a solution of 2,4-D (K-salt) 10^{-5} mol. after the cotyledons had been amputated at different stages after pre-soaking. Results after ten days. For explanation cf. figs. 3 and 4.

2. As the growth substance concentration becomes higher, the period during which the cotyledons remain connected with the embryo will have to be reduced ever more, in order to attain a maximum inhibition of *sprout* growth. The *root* is not affected to such an extent: the strongest inhibition is usually found with the groups in which amputating of cotyledons was done after some 3 × 24 hours. This is probably linked up with the fact that, in the water controls, maximum longitudinal growth is already achieved after a 3 × 24 hours' connection (cf. fig. 12, A and table VI).

3. The point of time at which cotyledons should be amputated in order to ultimately raise fresh and dry weights of hypocotyl plus root *above* those of the controls, falls the earlier, the higher the concentration applied.

4. From 2 and 3 it follows that the effect of a high dose of growth substance is passed on to the embryo sooner than that of a low one.

5. At similar concentrations the influence of n.a.a.K and 2,4-DK is stronger than that of i.a.a.K.

SUMMARY

The effect of the three growth substances mentioned above, administered during pre-soaking, is largely comparable to that exercised on the isolated embryo, by way of the nutrient medium.

From the ratio of the optimum concentrations required for certain, striking phenomena, induced either via the medium or via the cotyledons, it might be concluded that, in the latter instance, an interaction with other factors has taken place (selective action of the seed-coat, influence of constituents of the cotyledons).

From tests with different concentrations of i.a.a.K, n.a.a.K and 2,4-DK it appears that the growth substance acts not only directly on the embryo, but also in an indirect way, via the reserve substance mass. The latter influence also manifests itself afterwards.

The growth substance taken up by the cotyledons is passed on to the embryo, as it were, at a gush. Thus, at an appropriate growth substance concentration, amputation of the cotyledons after 1×24 hours (or, sometimes, after a longer time) will cause merely inhibition. The action of the cotyledons, therefore, should not be compared to that of a reservoir, gradually giving off the growth substance to the embryo.

Removing the reserve substance mass at a later stage usually results in a predominance of the growth-stimulating action of the cotyledons, though, in comparison with the corresponding water controls, not without some inhibition. Both sprout and root develop further, which is accompanied by an action typical of growth substances, viz. the lateral roots being crowded together on the callous, swollen hypocotyl.

In general it may be observed that, in spite of the inhibitory influence exerted on the region below the insertion of the cotyledons, the fresh and dry weights of the root (including the hypocotyl) have considerably increased in consequence of the treatment.

A growth substance dose not too high will not cause any serious loss of the total amount of dry matter, but merely a different distribution.

CHAPTER VI

PRELIMINARY EXPERIMENTS ON WATER ABSORPTION IN SEEDS AND ITS AFFECTION BY A GROWTH SUBSTANCE

§ 1. INTRODUCTION

Provided its internal conditions and the temperature are suitable, a seed brought into contact with water will absorb it in a measure. At first the absorption may be largely, or entirely, due to imbibition; as more water is absorbed, the consequent production of osmotically active substances from the reserves may promote the osmotic absorption.

It is within the range of possibility that the initial process is influenced

on treating the seeds with growth substances. The seed-coat, as a *selectively permeable membrane*, may or may not play a part in that case.

VAN DER MAREL (241), in 1919, demonstrated that, as to the pea, the seed-coat is *not* selectively permeable, aqueous solutions passing freely through it. The solute then might influence the embryo quite unhampered.

Interesting data on swelling during the water uptake of the seed are to be found in the papers by PRINGSHEIM (229), BROWN (219) and EYSTER (220). In several cases the technique applied, from modern standards, was rather lacking in perfection, however. As, for instance, the duration of the experiments gave rise to bacterial contamination, the interpretation of the results was not unambiguous. So, a sensitive and more accurate registration-method might give better information about these processes. Especially the examination of the response of swelling seeds to growth substances was deemed of interest.

§ 2. WATER ABSORPTION IN NORMAL SEEDS

The water uptake was studied with the aid of small (15-ml) flasks, connected with a glass capillary (1 mm wide and some 400 mm long) by means of a ground joint. The requisite number of seeds, after weighing, was put into a flask which, thereupon, was made up with distilled water, care being taken to expel any adhering air bubbles by shaking. The capillary, greased with a little Apiezon-L for the sake of a tight fitting, was placed on to the flask, while its other end was provided with a piece of para rubber tube.

Eventually, a number of flasks thus filled was ranged horizontally on a frame, each capillary alongside of a rule. The whole was put into a water-filled thermostat, the rubber tubes allowing the capillaries a free communication with the air.

All the materials, the air-dried seeds as well, were conditioned at a temperature of 26° C for some 24 hours, in the same room to be used for the observations. The process of swelling was judged by the displacement of the meniscus in the capillary. Illumination by means of a 15 watt bulb was maintained throughout the experiment.

According to PRINGSHEIM (229), DETMER, as early as 1880, would have applied a flask, filled with water and equipped with an upward glass tube, in studying the water absorption in peas. Apparently, too little allowance has been made for the temperature, so that the apparatus acted chiefly as a thermometer.

No doubt the present method is liable to errors too. For instance, swelling takes place in the absence of air. It was presumed, however, that the gaseous exchange probably would play little part in the initial process of germination, viz. the water absorption, and that the intramolecular respiration would predominate. Moreover, the experiment took only seven to eight hours. All the methods, practised so far, are more or less inadequate and are lacking in accuracy to afford an insight into the subtle mechanism of the very first stage of germination.

The graphs in fig. 15 show the displacement of the menisci in the capillaries, brought about by various seeds. Although fairly large

fluctuations may be found between duplicate determinations, each kind of seed proved to yield a characteristic graph. Of course the size and the number of the seeds, thus the total amount of waterabsorbing material, will determine the ordinate value of the graphs.

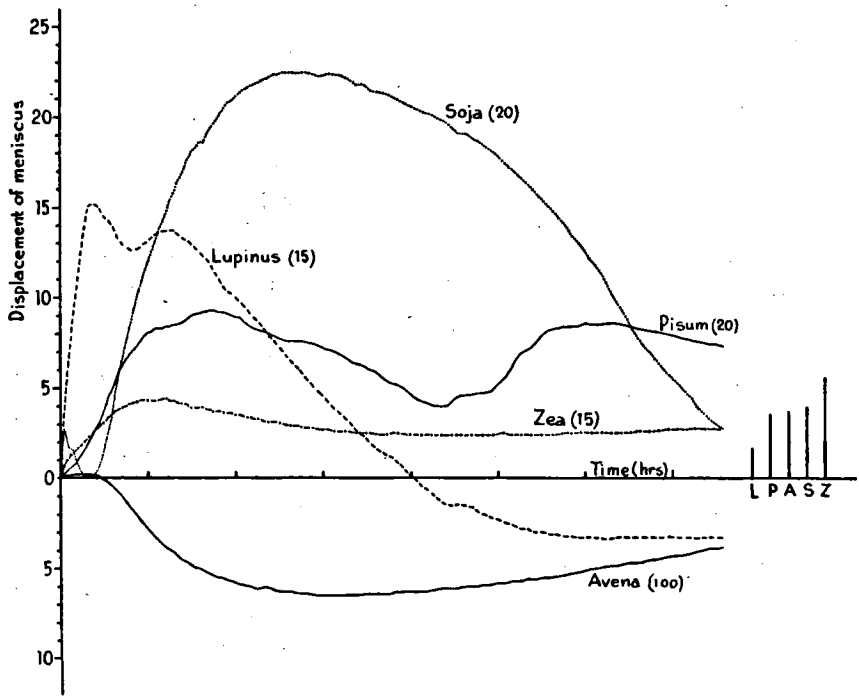


Fig. 15. "Swelling graphs" of five kinds of seeds; numbers in brackets. The displacement of the meniscus is rendered in centimetres along the ordinate; an increase in volume by a positive direction, and conversely. The verticals on the right represent a displacement corresponding with a change in volume by 1%, calculated on dry seed volume at the beginning of the test.

At first, it was tried to find some relation between the shape of the graphs and the observations both by PRAT (221–223) and by PRAT and CALVET (224–228), who studied the initial water absorption of seeds by means of a specially designed microcalorimeter. PRAT (223) arrived at the following conclusion: "immediately upon the contact between the dry seed and water there is a quick, then a falling off and then a more slowly developing production of heat. The initial phase of rapid rise and fall in the curve is common to both living and dead seed and is ascribed to *physico-chemical thermogenesis*. Then follows a phase of depression, often endothermic or weakly exothermic that was named "dead time" and finally the curve presents an increasing thermic flux ("*biological thermogenesis*") which corresponds to the beginning of the growth of the seedling, involving a progressive rising of its respiratory activity."

On closer examination of his own observations (checking the temperature during swelling; determining the rise of temperature required in obtaining a similar displacement by heat only) the present author was not able to ascertain any effect due to the evolution of heat. It is remarkable indeed that also these curves (fig. 15) were twin-peaked curves, whereas only the first part of a graph (as far as the pea is concerned, see below) could be reproduced after killing the seeds.

Some features of the water absorption in the pea are given here. Preliminary experiments with peas of one size soon revealed the presence of two different kinds in a given lot: *smooth* seeds and slightly *wrinkled* ones, behaving differently in soaking. There was much similarity in the amount of water absorbed, yet, the course of the process was different.

Smooth peas, in contact with water, developed local wrinklings on their seed-coat, spreading gradually over the surface until they covered the whole of the seed-coat. The final phase was marked by smoothing, the pea then being soaked with water.

Peas, somewhat *wrinkled* in the dry state, were seen to absorb the water more quickly at the whole of their surface. Here, absorption obviously took place with much more ease, so that nearly all the specimens had smoothly swollen when the test was finished. (The *smooth* peas, on the other hand, often contained specimens that would not swell beyond the wrinkled stage, or even such, remaining hard after the usual lapse of time).

The difference in behaviour between wrinkled peas and smooth ones also finds expression in the shape of the graph rendering the course of the water absorption; probably some diversity in the reserve substances must be held responsible for it. Wrinkled peas are lower in density than smooth ones, their water content is much the same.*)

In the water absorption curve of wrinkled peas the twin-peak is more pronounced than in that of smooth ones. The different slope clearly represents the quicker absorption in the former.

The descent following the first maximum corresponds to the gradual change from the wrinkled stage to that of complete swelling. Since, at any given moment, the seeds will not have swollen to the same degree, the results obviously will be variable.

Uniformity was favoured by using material of 6.0–6.5 mm diameter and of the same crop; as a rule the flasks contained twenty seeds.

*) The divergencies observed in the swelling-tests between smooth peas and wrinkled ones was an incentive to study, as yet, the question whether the embryos from both types would develop differently.

Wrinkled peas, after pre-soaking, contain a higher percentage of specimens unfit for use than smooth seeds; many of them have a "marbled" appearance (perhaps owing to virus attack?). After a three weeks' culture the average lengths of the sprout and of the root plus hypocotyl in plants, grown from wrinkled seeds, were somewhat behind those obtained from smooth peas.

As, in the first part of this study, this fact was not taken into account a still more rigorous selection after sieving might contribute to lessen variability.

§ 3. Role of the seed-coat; water uptake by killed seeds

The shape of the water absorption curve, pictured in fig. 15, has raised a number of problems unsolved till now. Though, initially, water will be absorbed, the total volume was shown to increase; absorption is apparently attended by swelling. The decline of the curve probably corresponds to the penetration of water, after the seed-coat has been passed. Finally the peas, wrinkled by taking up water, changed into smoothly swollen ones; the increase in volume was distinctly visible and, judged from the curve, it surpassed the water uptake.

The resolution of these problems would require a separate investigation beyond the scope of the present work and, possibly, of a colloidochemical nature as well.

Some experiments on the role of the seed-coats in water absorption should not be omitted here. For that purpose, peas *without a seed-coat* were tested. Those with intact cotyledons produced already quite another curve (fig. 16, nr 2): after a slight decline a maximum appeared, the further course being nearly flat. Still less effect was produced when the cotyledons were isolated from the same number of peas (viz., twenty), so that forty loose organs were subjected to the swelling test (fig. 16, nr 3).

The water absorption by an adequate amount of small pieces of seed-coat was characterized by a curve being the inverse of nr 3 (fig. 16).

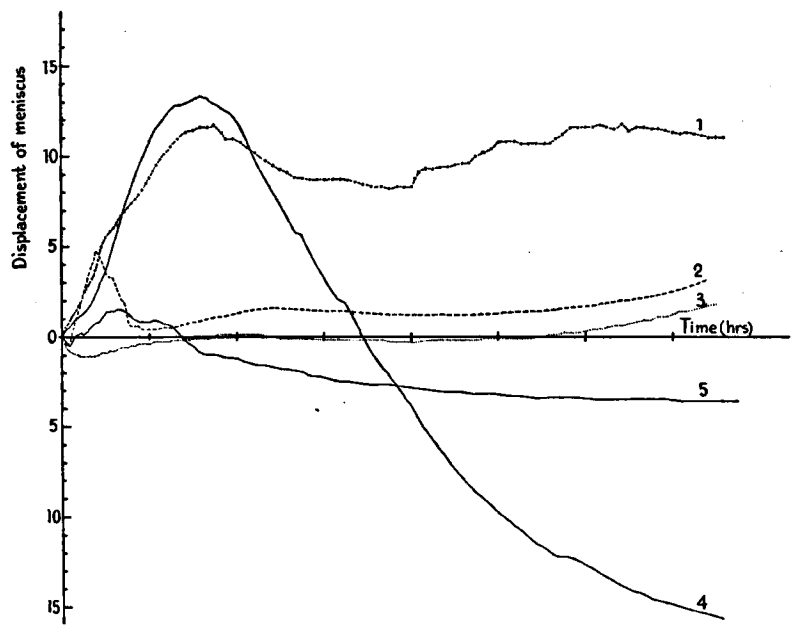


Fig. 16. Water uptake by smooth peas (20 specimens, 6.0 - 6.5 mm in diameter).
Graphs: 1. intact, normal seeds;
2. seeds without the seed-coats;
3. 40 loose cotyledons from similar peas;
4. intact peas, after killing (heating for 30 minutes at 100° C);
5. similar to 4, deprived from seed-coats after heating.

The circumstance that the curve changed completely when the seed-coat was removed proved the action of the whole seed to be different from that of the sum of its component parts. This suggested the presence of an ample amount of gases in the seed, which would determine the behaviour during water absorption. The above shows that, in the intact seed, the seed-coat may play an important part.

When it had been ascertained that dry peas, after heating at 100° C for half an hour, had lost their germinative power, this material was tested also (fig. 16, nr 4). The ascent of the curve, at first very similar to that of normal peas, was followed by a steady descent. So, there was a continuous absorption of water, whereas the total volume diminished. When the seed-coat was removed after killing the peas (fig. 16, nr 5) much of the effect was lost, just as with the viable seed.

§ 4. Excretion

In Chapter III (p. 26) there was mention of substances, given off by peas during pre-soaking in water. After the above swelling tests the soaking liquids were always evaporated to dryness on a steam bath. The residues, expressed in milligrams produced by 1 gram of air-dried starting material, were compared, and allowed of the following conclusions.

Wrinkled peas, in comparison with *smooth* ones, excrete nearly double the amount of matter (table XI). Killed seeds excrete *more* than viable ones, in both categories the wrinkled seeds again surpass the smooth ones. Finally, *maximal* excretion is found in *peas, deprived of their seed-coats*: twelve to seventeen times as much as in intact seeds, the wrinkled ones holding the record once more.

Excretion is enhanced by adding potassium hydroxide, whereas water saturated with carbon dioxide effects the reverse, the water absorption getting delayed.

From the observations that killed, intact peas excrete larger amounts of matter than viable ones, and even more in the absence of seed-coats (the distinction between viable and killed seeds having nearly vanished in that case) it might be inferred again that the seed-coat of *viable* seeds is important to the initial processes of germination.

§ 5. Influence of a growth substance

Both smooth and wrinkled peas were subjected to similar swelling tests in solutions of potassium indole-3-acetate, at concentrations of 10^{-4}, 3×10^{-4}, 10^{-3} and 5×10^{-3} mol. Except for the usual fluctuations, also found in the experiments with distilled water, no particular influence whatever of i.a.a.K was to be perceived. So, in seed treatment of the pea the growth substance probably will play little, if any, part in the initial phase of water absorption.

Summary

A new, sensitive method was applied in testing the initial water uptake in five kinds of seed. Each of them proved to yield a characteristic "swelling curve."

TABLE XI

Average values of water absorption, change in volume, and excretion, in *peas*, both smooth and wrinkled, under various conditions. Each test, lasting 7 to 8 hours, comprised 20 seeds, 6.0 - 6.5 mm in diameter.

Type of pea		Milieu	Weight (g)			Volume (ml)			Increase in volume, by displacement of meniscus in the capillary (mm^3)	Number of seeds swollen	Total amount of matter excreted (mg)
smooth	wrinkled		outset	end	increase	outset	end	increase			
	+	air	3.6	3.6	—	2.8	2.8	—	—	—	—
	+	water	3.6	7.0	3.4	2.8	6.2	3.4	48.0	19	38
+		air	3.8	3.8	—	2.9	2.9	—	—	—	—
+		water	3.8	6.7	2.9	2.9	5.9	3.0	75.9	16	20
+		water + CO_2	3.8	5.8	2.0	2.8	5.0	2.2	—	4	10
+		HCl 10^{-4} N	3.8	7.2	3.4	2.9	6.5	3.6	76.2	20	16
+		HCl 10^{-3} N	3.8	6.8	3.0	2.9	6.0	3.1	88.8	18	21
+		KOH 10^{-2}N	3.8	7.1	3.3	2.8	6.2	3.4	24.4	20	30

On the basis of more or less detailed observations, taken with the pea—in various states—it would seem that the first part of the graph is related to physico-chemical processes during water absorption, whereas the other part probably bears on physiological phenomena of the organism.

The seed-coat must be considered important, particularly to the excretion of certain substances by the seed. In view of the above tests, the question whether the initial absorption of water in peas would be influenced by i.a.a. during seed-treatment, has to be answered in the negative.

CHAPTER VII

SURVEY OF THE LITERATURE ON THE DISTRIBUTION OF NATURAL AUXIN IN SEEDS, DURING DEVELOPMENT, DORMANCY AND GERMINATION

The isolation of the auxins *a* and *b* from germinating *barley* and from *maize* seed oil, performed in 1934 by KÖGL, ERXLEBEN and HAAGEN-SMIT (162) on the one hand, the discovery of the importance of the aleurone layer to seeds of various *Gramineae* by SCHANDER (178) on the other underlie CHOLODNY's hypothesis (149). Immediately after taking up water, the endosperm would start forming a growth hormone ("Blastanin") as an enzymatic process, coupled with the hydrolytic degradation of amylum. Initial growth and subsequent development of the plant would then depend largely on the "hormone charge" received at the beginning of its life cycle.

Starting from this hypothesis, and on the assumption that the acceleration of development—to be observed after the vernalization of seeds—would result from an increasing amount of growth hormone in the meristematic parts of the embryo (150), CHOLODNY, in 1936 (25, 26), was the first to check whether normal plant growth might be influenced by administering a synthetic growth substance. This has induced the numerous investigations described in Chapter I.

On the basis of data from the literature it will be examined now as to what extent the above concept is supported by the findings of other workers and, merely on the score of theoretical considerations, how far any success is to be expected of treating seeds with synthetic growth substances. As to terminology, that from the original papers is retained as far as possible.

Experiments with the *Avena* coleoptile as a test-object raised a surmise that auxin would be present in the endosperm (171). In *maize*, the centre of growth hormone production would reside in the yellow, horny layer enclosing the endosperm, whereas in *Helianthus* the growth-stimulating substance is stored by the cotyledons (163). CHOLODNY's hypothesis, as such, cannot hold good for all cases, because

some growth hormone is already present in *dry* oat meal, while its formation starts soon after fertilization (163).

On the other side, DRABKIN (see under 233) demonstrated that the endosperm of vernalized seeds, as opposed to that of non-treated ones, fails to bring about any curvature in the Avena coleoptile. This suggests that the embryo absorbs the hormones of the endosperm in the initial developmental phase and provides additional evidence for CHOLODNY's hypothesis.

The use of non-specific test-methods (151, 170) has certainly given cause for a good deal of confusion and contradiction. POHL (172), after injuring the seed-coat and the aleurone layer, was able to remove the growth hormone from the endosperm by means of water or by applying a potential difference; eventually, he identified "Blastanin" with the growth hormone of the coleoptile. Thus, in his opinion heteroauxin and phenylacetic acid, which are unfit for stimulating the growth of coleoptiles from extracted seeds, should not be regarded as growth substances.

In *maize*, the active growth hormone of the endosperm would be converted into an inactive form during the passage through the scutellum. This substance might then be easily transported towards the top of the coleoptile, where re-activation to the auxin would take place. Some inhibitory substance (or growth substance antagonist) regulating the capability of the cells to react with the growth hormone, is very important to germination (172, 183, 184).

RUGE (176) completely shares the view of VON VEH and SÖDING (182), in that the growth hormone does not take part in the germination proper, that is, the change from dormancy to growth. The elongation-promoting hormone which does take part in primary development, therefore, should not be regarded as a *germination-hormone*.

AVERY, et al. (141, 142, 146), have compared various extraction methods when determining growth hormones in *maize*-endosperm during primary development. In dormant *maize*-endosperm, 90 per cent of the total auxin content exists as a physiologically inactive compound (in the Avena test), i.e. as a "precursor", which becomes auxin only after hydrolysis. Immediately after fertilization there is a rapid rise in auxin content; the maximum is attained in one to three weeks, after which a steady decrease sets in. There was *no relationship between vegetative vigour of hybrids and the amount of auxin stored in the kernels produced by them*, nor was there any relation between the auxin content of normal and polyploid forms (144). *Maize* seeds, the endosperm of which contains sugar, show a higher auxin content than those containing starch (145).

In *wheat* no relation was to be found between protein and auxin content. Probably two precursors are present, hydrolysis yielding two auxins distinguishable by their stability towards alkali (143).

In some respects the above meets the results obtained by HATCHER and GREGORY in their study of the auxin relations in *rye* varieties. The Avena test showed that, as soon as the presence of free auxin can be demonstrated (i.e., from three weeks after fertilization), here too the

auxin content increases during the next month, whereas it falls off during ripening. A maximum is found some five or six weeks after fertilization: the stage of complete differentiation of the embryo (160). In *rye*, auxin production would take place in the aleurone layer, close to the embryo (158; compare 151, 178).

According to HATCHER (159) the term "precursor" is misleading, since there is no evidence to date of its formation previous to the formation of auxin. It is quite possible that no specific, inactive substance is formed at all, but rather a protein-bound auxin. GORDON (153), after examining *wheat* grains, expresses himself in the same sense. During germination adequate amounts of growth hormone would then be set free in proteolysis.

Investigations by the school of GREGORY, by KONOVALOV and by SEN and CHAKRAVARTI (see under 237 and 238) demonstrated the possibility of vernalization of isolated embryos, independent of endosperm, aleurone layer or cotyledons. Thus, CHOLODNY's hypothesis in its original form has become untenable and it is even a question whether the hormone-metabolism is influenced by vernalization (159). It is worth mentioning that the loss of total auxin in ripening is relatively less in the earlier harvested ears. For that reason the highest auxin concentration is found in the most dwarfed grains (159).

A new era in the history of this plant hormone research was ushered in when, in 1941, HAAGEN-SMIT, LEECH and BERGREN (156) succeeded in isolating the growth hormone from fresh corn-meal (*Zea mais*) and, after identifying it with i.a.a., were the first to prove this to be a constituent of higher plants. From that time on it has been emphasized, anyhow, that what is usually called "auxin" may consist of more than one substance in the plant. It depends on the extraction-technique which of these is obtained (157).

One will have to take full account of the possibility that growth inhibitors may mask growth promotors, and reversely. The way in which this finds expression will depend on the test-method (152).

The free growth hormone of unripe *maize*-kernels, which attains its optimum concentration ten or fifteen days after fertilization, proves to be i.a.a. (155, 175). BERGER and AVERY (147, 148) make an attempt to elucidate the chemical character of the "precursor" in the resting *maize*-grain. Alkaline hydrolysis of this protein-bound substance yields i.a.a. as well.

VON GUTTENBERG and LEHLE-JOERGES (154) are also able to show that, besides auxin (*a* and *b*-type), several seeds contain an alkali-resistant substance, probably i.a.a. Extraction of swollen, germinating seeds shows an increase of the content after 24 hours, followed by a decrease after one or two days. *Leguminosae* (*Lupinus*, *Phaseolus*, *Pisum* and *Vicia*) give negative results.

From an investigation by MIROV (167) it would appear that resting seeds of *Pinus* do *not* contain any *active auxin*. This is only formed during stratification in cold storage; parallel with its appearance, the seeds are able to germinate. This speaks well once more for CHOLODNY's view and so does JUEL's paper (161), giving evidence of the relationship

between the decline of germination and the fall of the auxin content in seeds of *maize* and of *Phaseolus*.

In the germination of *Gramineae* root growth depends on the growth hormone content of the endosperm (169). The beneficial effect, or otherwise, of treating seeds with growth substances might be connected with this.

Some investigators imagine that the hormones in developing seed would act in the control of fruit drop rather than in further development (164).

In the more recent papers attention is paid not only to growth-promoting hormones but also to inhibitors, their mutual relations being considered as well (186–189). POHL and TEGETHOFF (173, 174, 181) isolated an inhibitory substance from *maize*-scutellum by electrodialysis. With the aid of MOEWUS's cress root test they examined the simultaneous action of inhibitor, active growth substance and inactive growth substance. The inhibitor of the endosperm, probably non-proteinaceous by nature, is able to inactivate both i.a.a. and the growth substance of the endosperm. The latter, in that case, gives rise to another substance, probably an inactive growth substance (181).

Here, the question arises whether, with the aid of this method, these separate actions can be specifically distinguished at all, considering the impossibility of doing so in determining the content of certain growth-promoting substances in *sunflower* seeds (176).

In connection with the problem of vernalization, SIRCAR and DAS (179) collected some data about the hormone content of *rice*; inhibitory substances would be absent. Likewise, LUCKWILL (165) did not find any relation between the germinative power of embryos isolated from *apple* seeds and their inhibitor content. The conclusion was that the formation of growth-producing substances rather than the disappearance of growth-inhibitors, may be necessary to break the dormancy of the seed.

MOEWUS and his co-workers (168), in reliance upon the results obtained with the well-known cress root test, are inclined to conclude that, in *Lepidium sativum*, two growth substances are present, viz. i.a.a. and, probably, phenylacetic acid. The i.a.a. content of fresh seeds, harvested in 1950, proved some 18 times as high as that of aged ones, of 1944 and 1947.

YAMAKI and NAKAMURA (185) applied the paper partition chromatography when determining tryptophane, indoleacetic acid and indoleacetaldehyde in germinating *maize*. Enzymes or enzyme systems which convert tryptophane, indole-3-ethylamine and indole-3-acetaldehyde into indole-3-acetic acid were found in the embryo of *Zea mais*. The genetic connections of tryptophane and i.a.a. in the embryo and endosperm were discussed, resting on the experimental results. The "bound" indole-3-acetic acid in the endosperm is considered as one of the decomposition products produced by hydrolysis with alkali and to be of very little importance from the physiological point of view.

DISCUSSION

From the literature cited in the foregoing chapter it is gathered that, generally, investigators agree that seeds contain growth-promoting substances, which are essential to development. There is no denying either that inhibitory substances play an important part. Perhaps one should concur in SÖDING's idea, viz. that the rest-period of the seed is generally caused by the presence of inhibitors. As they disappear, growth gradually starts, which requires growth hormones in an increasing measure. So, for the time being there is no sense in distinguishing special germination hormones.

Data about a diminishing content of the seed in aging and its relation to changes in germinative power are often contradictory. Thus, CHOLODNY's theory in its original form will not hold any longer.

The author, however, would like to stress that a serious mistake is made by trying to immediately generalize the results obtained on a single type. It should be properly realized that in this respect too, Nature is multifarious and that the interpretation of some data is accurate only under the set of conditions which prevailed during a given experiment.

As a whole, research on occurrence, isolation and identification of both growth-promoting and growth-inhibiting substances is far from complete; especially the *dicotyledons* have received very little attention on that score. Such experiments entail two main difficulties. First, during isolation (e.g., extraction) i.a.a. or some other growth substance may be formed owing to chemical action; second, in testing, the inhibitors present may mask the effect of growth-promotors, owing to the choice of the test-method (152). The application of modern techniques, e.g. chromatography (166, 185), certainly will conduce to new findings and to the elucidation of various, unexplained matters.

The administration of synthetic growth substances under certain circumstances will affect germination, growth and development, on the understanding that favourable results are obtained only incidentally (several instances are to be found in table II). This shows the details of the mechanism to be hidden still. The penetration of the growth substance administered, its transport, its accumulation in certain parts of the seedling and also further particulars will have to be studied with the aid of isotopic tracers. This is all the more desirable as it is still uncertain whether a beneficial effect of the synthetic growth substance is connected with an influencing of the hormone-metabolism of the seed. Other processes involved in germination may be influenced as well, favourably or otherwise: enzymatic processes in the developing embryos have been inadequately studied as yet. In this connection the recent investigation by MARRÈ and MURNEEK (242) is of consequence. They detected a considerable degree of resemblance between the natural growth hormones (unfolding their activity after fertilization) and the growth-regulators administered, as to their influencing the carbohydrate and hexose phosphate metabolism during the initial stages of seed formation.

The present author is fully aware of several shortcomings in this study. Among other things, the influence of light was eliminated in order to avoid complications. Therefore, the phenomena observed will not agree in every respect with those appearing under natural circumstances. A possible intervention of other substances, being of similar importance to the development of the young plant, was not considered either (233).

As to the penetration of a growth substance into the seed, any investigations whatsoever on the mode and the extent seem to be wanting. Though PRAT and CALVET (221–228) would have demonstrated that the "biological thermogenesis" is increased by indole-3-acetic acid at low concentrations, it follows from the present author's experiments on the initial water uptake in the pea that a growth substance (at least, i.a.a.) has little, if any, effect. So, its action probably will manifest itself mainly afterwards, i.e. after the pericarp, or the seed-coat or both of them have been passed. Interesting facts might come to light if other types of seed would be subjected to this test.

The distinction found between smooth peas and wrinkled ones is probably due to diversity in the reserve food. A varying ergon content may be connected herewith, which, in *maize*, was shown by AVERY, BERGER and SHALUCHA (145) and recently, by TEAS, CAMERON and NEWTON also (180). The divergent behaviour of varieties with regard to seed-treatment thus becomes more intelligible and future research will have to avail itself of carefully selected material in particular.

The present investigation has also shown that synthetic growth substances, administered to the seed, may unfold not only a direct action on the embryo, but also an indirect one, *via* the reserve substance mass. The existence of different types of seed — being distinguished by their reserve food — and the considerable variations in the ratio embryo-size/volume of reserve substances may be advanced as possible sources of the contradictory results obtained in seed-treatment.

One of the most outstanding responses of the embryo to growth substances, often shown, was a swelling of the hypocotyl attended with an increase of the number of lateral roots. Any appearing of larger yields on application in practice, *under certain circumstances* (condition of the soil, manuring, humidity, etc.: see p. 8) might be connected with this phenomenon.

For the sake of co-ordinating the activities of various workers it will be advisable to restrict future research to a well-grounded investigation on a few kinds of plants only. The desirability of commanding reliable methods by the aid of which the treatment of seeds with synthetic growth substances can be practised, also in the light of present-day endeavours to improve the world's food situation, certainly will justify such a fundamental investigation.

SUMMARY

In the treatment of seeds for the purpose of stimulating growth and development, the application of synthetic plant growth substances in

practice has not yet produced the desired result. This will be easily understood, considering the strongly divergent ways in which experiments have been made on a large number of different objects: the results, often contradictory, did not admit of any valuable conclusions.

A more profound knowledge of the process of germination and of the mechanism of growth substance action during the initial stages of development is deemed necessary if advance is to be made in this domain.

An investigation aiming at this purpose was carried out on the pea. Standardized, sterile conditions were maintained during the *in vitro* study of normal correlation-phenomena of the developing embryo and the influence exerted on it by various factors.

The effect of a growth substance (indole-3-acetic acid, naphthalene-1-acetic acid, 2,4-dichlorophenoxyacetic acid) can be clearly distinguished from that of other influences.

It is presumed that the obtaining of contradictory results in seed-treatment may be due chiefly to:
a) variability of the starting material; *b*) selecting action of the seed-coat, and *c*) the manner in which the growth substance is passed on to the young plant by the cotyledons or by the endosperm (filled with different reserve substances in different types of seed).

By means of various experiments it was ascertained that the growth substance administered acts upon the embryo not only directly, but also in an indirect way, like a thrust via the reserve substance mass. So, the role of the cotyledons would *not* be comparable to that of a reservoir gradually giving off the growth substance absorbed.

With the aid of a new, sensitive technique the initial water uptake was studied in five kinds of seed. The results could be recorded in characteristic swelling-curves. Although in this process and also in the exuding of certain substances the seed-coat plays an important part, no influence of indole-3-acetic acid on these processes in the pea could be ascertained.

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B. *Seed germination and early growth responses after the application of synthetic growth substances as selective herbicides*

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C. *Natural auxin distribution in seeds during development, rest-period and germination*

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