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Seed eluates on the germination blotter—a germinability test?

Urlakade frösubstanser på groningspapper — ett grobarhetstest?

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Abstract

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Aged seeds of Scots pine left different eluate spots on the blotter in a germinator after about 24 hours' incubation. Fresh seeds do not give off such eluates. Among the leached substances were found proteins, nucleic acids, amino acids, fats, sugars, etc. The eluate spots are visible on the blotter only after development by simple biochemical methods used in paper chromatography. Possibility of using this eluate-development method as a seed viability test, and other practical aspects, are discussed.

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Contents

1 Introduction

Various metabolic products, e.g. amino acids, proteins, nucleic acids, sugars, etc. have been observed to be given off by some seeds when immersed in water or placed in moist surroundings. The nature of the substances has been studied by many authors from different points of view, viz. Metzner (1930), Kugler (1952), Schroth and Snyder (1961), Schroth, Toussoun and Snyder (1963), Schroth and Cook (1964), Agnihortti and Vaartaja (1968), Ching and Schoolcraft (1968), Abdul-Baki (1969), Koostra and Harrington (1969), Abdul-Baki and Anderson (1970), McDonough and Chadwick (1970), and also in our laboratory. Since the leaching of substances seems to be connected with low viability of the seeds, a considerable number of methods for quick determination of seed quality/germinability, based on the specific properties of the eluates, has been developed. The eluates have mainly been studied in aqueous solution by a) *physicochemical methods,* e.g. by optical measurement of leached materials (Hottes and Huelsen, 1927; Tatum, 1954; Presley, 1958; Zlobin, 1971; etc.) or by measuring electrical conductivity of the water used for steeping (Fick and Hibbard, 1926; Thomas, 1960; Matthews and Bradnock, 1968; Bradnock and Matthews, 1970); by b) *biochemical methods,* e.g. by determination of exudates of sugars (Flentje and Saksena, 1964; Takayanagi and Murakami, 1968 and 1969 a, b), or by colour intensity of KOH solutions in which the seed had been placed (Lesage, 1911; Tolentino, 1924).

The information gained through these studies has been, with some exceptions (see Discussion), valid as an average for the entire seed sample immersed in the solution, leaving the individual seed without evaluation. However, information about individual variations among the seeds, depending on seed age, health, injuries, genetic constitution, etc., may be valuable in practical seed testing. Moreover, too little attention has been paid to other eluates than sugar, e.g. amino acids and proteins, as a possibly useful indicator in a seed viability test. Finally, all the above-mentioned methods have been developed for agricultural seeds, and there is practically no information available about forest seeds.

The aim of this paper is therefore to study by biochemical methods the various leachates from Scots pine seeds of different viability, as well as the applicability of the results in practical seed processing. The tests will be carried out on individual seeds, or more specifically on the eluate-spots which each seed gives off on the blotter when incubated in a germinator.

2 Materials

Seed samples used in these investigations are given in Table 1.

Table 1. Information about seed material used in experiments 1 to 4.

No.	Locality	Year of collection	Latitude °N	Altitude m	Germ. % 21 days	Used in Experiment No.
1	Blyberg	1945	61	350	6	3
2	Norrbotten	1945	63	50	13	3
3	Fällforsån	1945	63	75	6	3
4	Västerede	1945	63	130	0	1
5	Norrbotten	1945	63	250	20	3
6	Ström, Umeå	1945	64	10	67	3
7	Ersmark, Umeå	1945	64	60	51	3
8	Lögda	1945	64	340	43	3
9	Harads	1945	66	185	11	3
10	Nysäter	1949	63	150	9	2, 3
11	Stugun	1950	63	230	5	2, 3
12	Fiskesjö	1968	57	181	98	3
13	Ingabo	1968	58	252	98	2, 3
14	Gölen	1968	58	320	91	3
15	Floren	1968	62	270	99	3
16	Härjeåsjön	1968	62	530	99	3
17	Mossebo	1970	58	200	98	1
18	Hohult	1971	56	175	100	3, 4
19	Ingestorp	1971	58	80	98	3, 4
20	Fjellskäfte	1971	59	50	98	2, 3, 4
21	Ramsberg	1971	59	160	100	3, 4
22	Sörbo	1971	61	210	99	2, 3

3 Methods

3.1 Germination percentage

The test was carried out in Copenhagen apparatus at 22°C temperature and 1000 lux light, constant. In each sample 3×100 filled seeds were tested during 21 days. The filled seeds were selected with the help of the x-ray radiographic method (Simak and Gustafsson, 1953).

3.2 Incubation of individual seeds

The seeds were arranged in rows on the blotters, in order to keep them under individual control, and were then incubated in single Copenhagen apparatus under different conditions of temperature, time, light, etc., as indicated in the respective experiments. The incubation was carried out in a climatechamber. For the determination of substances given off by seeds, blotter No. 1701, ⌀ 75 mm, Grycksbo Pappersbruk, Grycksbo, Sweden was used.

3.3 Biochemical tests

General biochemical spray and colouring methods were used, as given in the literature by Grassmann and Hannig (1950), Block et al. (1958), Stahl (1969), Stensiö and Ekedahl (1969), Reinhold and Liwschitz (1970). For simplicity we refer in this paper to "the metabolic products" in the spots, instead of using the more correct term "the positive or negative reactions of the respective reagents" (e.g. "amino acids" instead of "Ninhydrin positive"). Thus the *nucleic acids,* though negative to Ninhydrin and Amido black, were visible under 220 V Philips shortwave UV on the dried blotters, the *amino acids* were detected by spraying the blotters with Ninhydrin Reagent, Art. 6758 Merck, consisting of 0.2 % Ninhydrin and 2.5 % Collidin in isopropanol. After spraying with this reagent, the blotters were developed for 5—10 min at 100°C. For *proteins,* Amido black, consisting of 7 g Amido black + 450 ml methyl alcohol + 100 ml glacial acetic acid + 450 ml H_2O was used. After 10 min colouring, the background was washed several times with the same solution as above, but no Amido black was present. A freshly prepared mixture of 0.5 ml anisaldehyde, 9 ml ethanol, 0.5 ml conc. sulphuric acid and 0.1 ml glacial acetic acid was used for developing *sugars,* which appeared on the blotters after the sprayed blotter had been treated for 5—10 min at 90—100°C. A Rhodamin B solution, consisting of 1.5 g Rhodamin B in 100 ml 95 % ethyl alcohol, was used for demonstrating the presence of *higher fatty acids and lipids.* The *pH reaction* of the blotters on which the seeds were germinated was studied by means of Universal Merck pH solution. The seeds were placed on the blotters soaked with the indicator, and the pH changes of the blotters were observed after 24, 72 and 96 hours. Iodine vapors were used to colour *double-bonds* brown on yellow background, as follows: Crystals of Iodine were placed, together with the paper to be developed, into a covered glass container which had been immersed in hot water. The experiments have shown that on the same blotter on which the Ninhydrin test has been carried out, the Amido black treatment may be applied without complications. Thus the exudates from nucleic acids (UV), amino acids and proteins from a seed sample can be studied on the same blotter (triplet-test).

4 Results

Various problems were studied in four experiments:

4.1 Evaluation of different biochemical methods

Experiment 1

Seed samples used: No-s. 4 and 17.

Seed numbers and arrangement: 3 rows of 5 viable seeds (No. 17) alternating with 3 rows of 5 dead seeds (No. 4).

Conditions during incubation: 25°C temperature and 1000 lux light, constant. Incubation time between 1—96 hours.

The results of this experiment may be summarised as follows:

1. Only dead seeds give off eluates on the blotter, which can be identified by the colour reagents described above. No such eluates could be detected in the case of viable seeds.

2. The visibility of the spots depends upon the incubation time, but probably also on other factors, e.g. temperature, quality of the blotters, humidity, etc. For further

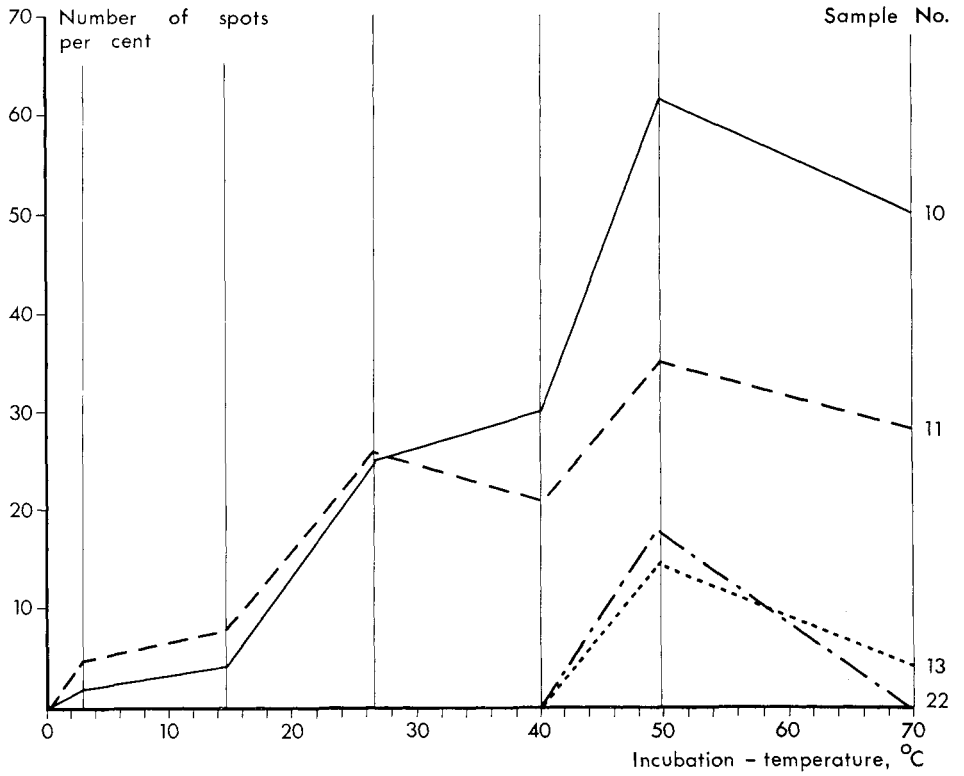
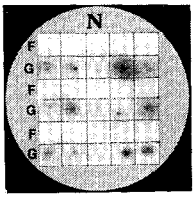
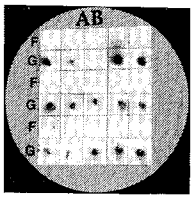
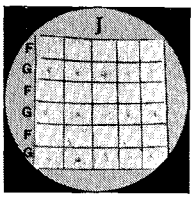


Figure 1. The number of spots appearing at different temperatures during incubation. Experiment 2.

Table 2. Experiment 1: F = fresh seeds, No. 17, G dead seeds, No. 4.

"Metabolic products" (cf. 3.3)	Colour reagent	Colour of spots	Stability	Spots in dead seeds	
				incub. time h.	per cent
Amino-acids	Ninhydrin Merck Art. No. 6758	Rose, later violet to blue	Very good	3 15 24	5 95 100
					
Proteins	Amido black 10 B	Dark blue to black	Very good	15 24	90 100
					
Double bonds etc.	Iodine vapours	Dark brown on yellow background	Fading spots can be recoloured	24	100
					
"pH"	Universal Merck pH	Acidic (red) later Basic (blue)	Spots bleach after few hours	24	90
Nucleic acids etc.	Shortwave UV	Dark and light blue	Very good	variable	
Fats and lipids	Rhodamine B	Dark red on rose background	Very good	16	poor visibility
Sugars	Anisaldehyde	Rose on green	Bad	24	poor visibility

investigation, a 24-hour incubation will be used, although this is not necessarily the optimal time under all conditions.

3. Among the eluates tested, proteins and amino acids seem to be promising for further studies. In the following experiments main attention will be paid to the protein spots.

4.2 The influence of temperature during incubation on the visibility of the protein spots

Experiment 2

Seed samples used: No-s. 10, 11, 13 and 22.
Seed numbers: 3 × 33 seeds per sample.
Conditions during incubation: Seeds in-

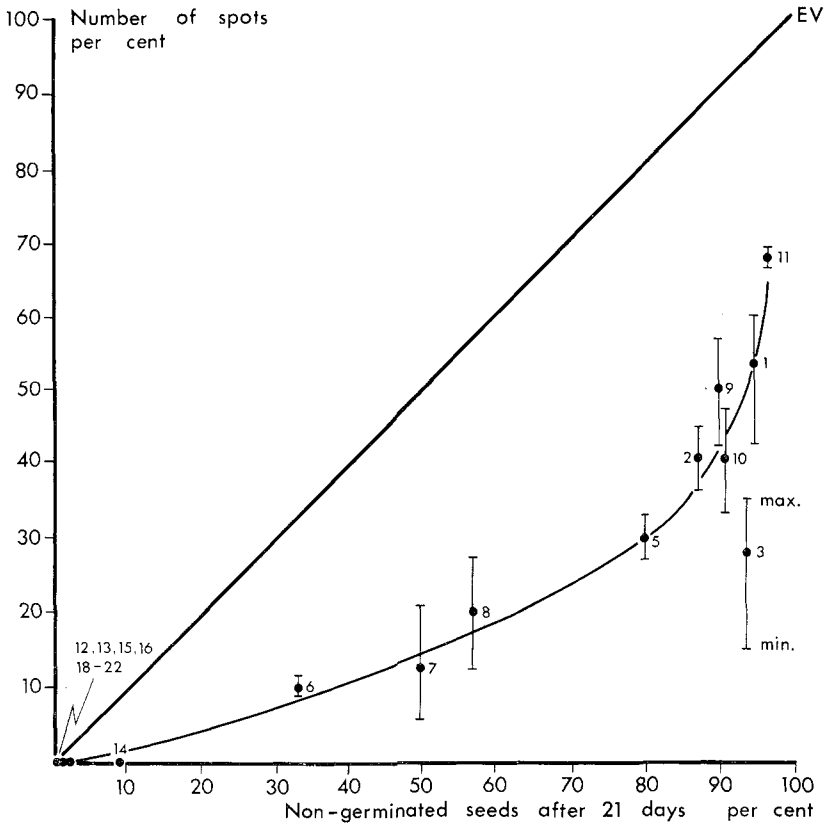


Figure 2. Relationship between the percentages of non-germinated seeds after 21 days and the number of spots of seeds with different viability. The line EV (expected values) marks the direct relationship between the Copenhagen and eluate test. Experiment 3.

cubated 24 hours at 4°C, 14°C, 27.5°C and at 40°C in constant light (1000 lux), also at 50°C and 70°C in darkness.

The results illustrated in Figure 1 may be interpreted as follows:

1. The viable seeds do not give off protein spots when the temperature ranges from +4°C to +40°C during incubation.

2. The number of spots from the seeds of low viability increases when the temperature ranges from +4°C to +28°C. Between +28°C and +40°C the number of spots is rather constant.

3. Between incubation temperatures of +40°C and +50°C, the number of protein spots increases rapidly in all seed samples. It seems that the viable seeds were injured in this temperature range.

4. At temperatures higher than +50°C, the number of protein spots decreased in all samples; this is probably due to the denaturation of proteins.

5. The number of spots in seeds of low viability never became equal to the number of non-germinated seeds after 21 days (cf. Table 1 and Figure 1).

6. A suitable temperature range for the protein test seems to be between +30°C and +35°C.

4.3 Biochemical test for protein spots from seeds of varying viability

Experiment 3

Seed samples used: No-s. 1—3, 5—16 and 18—22.

Table 3. Different eluate spots on the blotter from artificially damaged seeds. Experiment 4.

Sample No.	Uninjured seeds* G % 21 days	3×33 seeds injured by pressing			
		No. of spots			No. of non-germinated seeds
		Nucleic acids	Amino acids**	Proteins	
18	100	37	69	77	94
19	98	63	87	90	96
20	98	37	82	85	97
21	100	41	65	79	93

* Seeds did not show any eluates on the blotters.
** With reservation, because the spots are in some cases fuzzy.

Seed numbers: 3×33 seeds per sample.
Conditions during incubation: 24 hours at +32°C in 1000 lux light, constant.

The analysis shows the following results:

1. Seed samples of the highest viability (91—100 per cent germination) did not leave any protein spots on the blotter.

2. There is a positive, non-linear correlation between the number of spots and the number of non-germinated seeds after 21 days in Copenhagen apparatus. The number of spots in seed samples of low viability is lower than the expected values (EV) according to the Copenhagen test.

3. Great variations were often observed among the replications of a sample. This indicates a need for the study of other possible factors which may influence the test.

4.4 **Biochemical test on mechanically injured fresh seed**

Experiment 4

Seed samples used: No-s. 18—20 and 21.
Seed numbers: 3×33 seeds per sample and treatment were used for the biochemical test.
Seed treatment and incubation conditions:

a) Undamaged seeds, used as control.
b) Injured seeds: The seed coat was crushed by pressing a pencil against the seed on a hard surface.

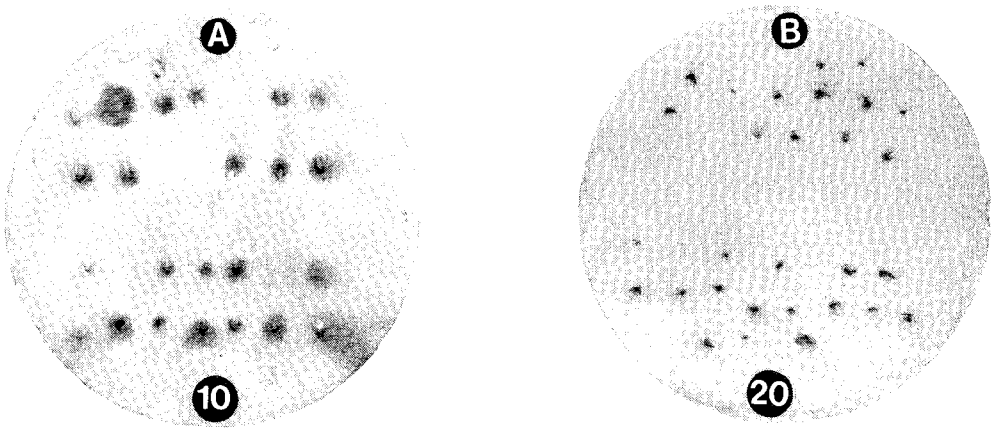


Figure 3. The different appearance of protein spots A = aged seeds, B = artificially damaged seeds. Experiment 4.

3×33 seeds per sample and treatment were incubated at 32°C for 24 hours at 1000 lux light, constant.

Germination test:

a) 3×100 undamaged seeds as control,
b) 3×100 artificially damaged seeds.

In Table 3 are shown the results for the biochemical test regarding nucleic acids (UV+) amino acids and proteins for undamaged and for artificially damaged seeds. All three biochemical tests were carried out on the same seed sample in the same order as mentioned (triplet-test).

The results are as follows:

1. The artificially damaged seed loses large amounts of protein, amino acids and nucleic acids. The UV-spots and the Amido black spots are relatively small, with very sharp margins in damaged seeds, as compared with the spots from aged seeds (cf. Figure 3).

2. Not all seeds which show UV and amino acid spots have protein spots. This may possibly indicate some differences in the degree of damage. Analysis of the seeds from this point of view will be continued.

3. The number of non-germinated seeds is on the average about 12 per cent higher than the number of protein spots.

5 Discussion

This investigation had a practical aim, namely to discover whether the biochemical changes typical of aged forest seeds may be applied as a viability test. As regards the biochemical processes in aged agricultural seeds, there is a great deal of information available. However, in forest seeds many fundamental problems still await clarification before the practical questions may be approached. Some research on forest seeds in this connection is being carried out in our laboratory. Dead Scots pine seeds can be detected in Copenhagen apparatus as early as 15—24 hours after the start of incubation. Under suitable conditions of humidity, etc., on the surface of the dead seed a crystal clear droplet not larger than the head of a pin may be observed. This "necrotic droplet" consists of many breakdown products from the seed, e.g. proteins, sugars, amino acids, fats, nucleic acids, etc.

By using an automatic amino acid analyser, we identified in the droplet a minimum of 16 amino acids, with the highest amounts of alanine, threonine and phenylalanine (Pehap, unpublished). Also the biochemical analyses of various parts of Scots pine seeds (e.g. seed coat, various parts of the embryo and endosperm) showed differences between fresh and old seeds. Preliminary results suggest that the amounts of degradation products originating from DNA and RNA are present in considerably higher amounts in aged seeds than in fresh seeds (Pehap, unpublished). This suggests that seed ageing is the result of the denaturation of long-lived messenger RNA, the presence of which is of importance in the early stages of germination (cf. Roberts, 1972, intrinsic theories classified to account for a loss of seed viability). RNA-degradation, as demonstrated by biochemical analyses in our laboratory, agrees also with observations from electron microscopic studies of polysomal RNA-destruction (Altschul, Yatsu, Ory and Engleman, 1966; Öpik, 1968; Mossé, 1968). All these and other decay products leach out from the seeds when the cell membranes weaken and permeability increases in aged seeds.

Most of the viability tests mentioned in the introduction are based on the physiochemical properties of the eluates in water (conductivity, turbidity test, etc.). As regards the biochemical eluate test as an indicator of viability of individual seeds, there is a lack of information, and only two tests seem to be of interest in connection with our investigation. Kugler (1952) tested some *Cruciferae* seeds on moist paper, and found a relationship between the number of fluorescent spots left by the seeds on the blotter, and the germination percentage. Takayanagi and Murakami (1968) tested the sugar eluates in water from individual seeds of *Brassica napus*. Different viability of each seed was estimated by these authors by means of a paper indicator commonly used for the urine-sugar test in medical practice. The differences in the coloration of the test-strip express the relative seed viability. The method is discussed in Roberts (1972). The authors also carried out a mass test for sugar eluates from a whole seed sample. However, the individual test—if well standardised and technically simplified—can give more information about seed viability in a sample than a mass test, where many uncontrolled factors may influence the results.

The decomposition processes in the ageing seeds occur successively, which seems to be reflected in the biochemical differences of the eluates. According to the result in Experiment No. 3 (1), the negative protein test is a good indicator of seed of high germinability (e.g. the ten samples harvested

in the years 1968 and 1971—all with a germination percentage of about 100). However, the five seed samples from the year 1968 left more UV-positive eluates on the blotter than another five seed samples harvested in the year 1971 (not presented in Results). It may be concluded—judging from the UV-positive reactions—that the ageing processes in these seed samples stored for three years have already started. However, the UV-positive reactions in this case are not connected with a lower germination of seeds, as in the experiment by Kugler, who states that the number of fluorescent spots on the blotter fitted well with the number of nongerminated seeds. This may possibly indicate some interspecific difference in the aged seed metabolism, or biochemical differences in the leached UV-positive substances.*

Of the substances which we have tested, the proteins seem to have a promising diagnostic value for estimation of seed viability; the large protein molecules are less mobile than other leached substances. Consequently, the protein spots on the blotter become very distinct and concentrated. As against this, the amino acids have higher Rf-values than the proteins, which results in fuzzy spots. Not all seeds which show protein spots leave amino acids spots on the blotter, and *vice versa*. Whether this is due to shortcomings in the technique or whether it is bound up with a biological phenomenon, must be further clarified with the help of additional analyses. The correlation between the germinated seeds and eluates will be much improved if many substances in a spot are tested. Such a multiplet-test was not used in our investigation because of the difficulties, already mentioned, in judging correctly the fuzzy spots of amino acids. However, there is good reason to believe that these disadvantages can be eliminated with a better incubation technique. As with other tests, it seems that even the iodine-test may be used successfully after some modification.

The seeds with damaged coats lose different substances, as has been shown in our Experiment 4. Schroth and Cook (1964), using the filter paper method, also observed an increased leaching of exudates (silver nitrate positive and Ninhydrin positive reactions) from bean seeds with cracked coats. Higher amounts of sugar exudates have been found from artificially damaged pea seeds (Flentje and Saksena, 1964). Seeds of *Pinus* sp. can easily be damaged during processing, especially during dewinging. The damage is often very slight and inconspicuous. It may not decrease the germinability of the seeds, if these are tested in the laboratory immediately after the injuries have been caused, and if the damage is not too serious. However, such seeds are unsuitable for storage, because they will lose their viability very soon (Huss, 1956). This indicates that it is necessary to pay close attention to mechanical damage to seeds, and that the germination test in Copenhagen apparatus alone is not always sufficient for this purpose. An eluate test, together with the germination laboratory test, would therefore be useful in all cases where a seed sample is intended to be stored for any length of time.

From the results of our experiments it is evident that the aged or injured Scots pine seeds release different substances to the blotter, which are detectable by relatively simple and crude chemical methods. The method is attractive, since the relationship between germinability and the eluates can be studied directly on the same seed. Differences in eluates could be used for analytical purposes, if it could be proved that they reflect differences in viability of the seeds. In this connection, a comparison between the differences in eluates and $BaCl_2$-impregnation on the same seed by the use of the XC-method (regarding the XC-method see Simak, 1957) could provide valuable information about the location of the deterio-

* Our recent experiments in which fresh and aged seeds were eluated, one by one in 100 microliter water in Pasteur pipettes for 24 hours and the eluate chromatographied, showed that UV positive substances—though perhaps qualitatively different—were present in the eluates of most investigated seeds, irrespective of whether these were aged or fresh.

rated parts in a particular seed. However, the improvement and standardisation of the eluate test is necessary. First and foremost, it is desirable to work out a test method in which the number of spots corresponds directly with the germination test in Copenhagen apparatus. In Expt. 2 we have shown how the number of spots in a seed sample depends upon the temperature during incubation. Similar relations may also exist for other factors, e.g. humidity, pH of the substrate, incubation time (cf. McDonough and Chadwick, 1970), etc. In particular, a better quality of blotter should be chosen (possibly chromatography paper). The seeds should have a better contact with the underlay. An incubator different from the Copenhagen apparatus should be used, to avoid the loss of eluates to the underside of the blotter, etc.

6 Practical aspects

The leaching of eluates from aged or damaged seed leads also to other practical considerations. The eluates are very attractive to fungi and probably also to bacteria, judging from the pH changes in Experiment No. 1. The explosive development of fungi on aged seeds can be well observed in Copenhagen apparatus after a few day's incubation. In soil the seed eluates often become a nutritional substrate for many microorganisms which may attack the seeds and seedlings. In agriculture there are some good examples of this, viz. Schroth and Cook (1964) on beans, Matthews and Whitbread (1968) in peas, Presley (1958) in cottons. As far as forest seeds are concerned, Agnihortti and Vaartaja (1968) examined the exudates from *Pinus resinosa* seedlings in relation to germination and the growth of zoospores of *Pythium afertile,* and found that some substances stimulated and other inhibited zoospore growth.

Nurserymen often complain of the difficulties of relating the laboratory germination test to the field test. Although there is no chance of finding a test which is universal to all kinds of field conditions, the eluate test, together with the germination test from laboratory analyses, can give a better picture of seed viability than the laboratory test alone. Bradnock and Matthews (1970) suggested, for instance, a method for predicting field emergence potential, by using conduction measurement and seed weight in peas. The conduction test correlates better with the field test than does the laboratory test. Finally, if one has good information about the biochemical processes in ageing seeds, steps may be taken to enhance the quality of stored seed—for instance, the use of the ethylene treatment (Takayanagi and Harrington, 1971). By the use of some chemical pretreatment of seeds to be stored the deterioration processes can be inhibited (cf. Crocker and Barton, 1957).

Although the results in this paper are based only on pilot studies, it is to be expected that further investigation in this field will provide useful help to the practical testing of forestry seeds.

7 Summary

The eluates from aged and injured seeds of *Pinus silvestris* L. were studied in relation to the viability of the seeds and to some practical considerations. The results can be summarised as follows:

1. Fresh, highly viable seeds do not lose any of the investigated substances to the surroundings during the pre-emergence stage.

2. The aged seeds and fresh but damaged seeds, lose during the pre-emergence stage various substances (nucleic acids, amino acids, proteins, sugars, fats, etc.) to the surroundings.

3. These substances can be identified by simple biochemical methods directly on the blotter on which the seeds have been incubated for about 24 hours.

4. Some of these tests, especially amino acids and proteins, when standardised and in combination with the germination test in laboratory, promise to be a useful viability test for aged and damaged seeds. It is desirable to develop a test in which more than one of the substances in a spot of a seed is considered.

5. The fundamental biochemical processes in the aged forest seeds should be studied more intensively, to improve many procedures during practical seed processing (storage, sowing, etc.).

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References

Abdul-Baki, A. 1969. Relationship of Glucose Metabolism to Germinability and Vigor in Barley and Wheat Seeds. — Crop Science 9, 732—737.

Abdul-Baki, A. & Anderson, J. D. 1970. Viability and Leaching of Sugars from Germinating Barley. — Crop Science, 10, 31—34.

Agnihortti, V. P. & Vaartaja, O. 1968. Seed exudates from *Pinus resinosa* and their effects on growth and zoospore germination of *Pythium afertile*. — Canadian Journal of Botany, 46, 1136—1141.

Altschul, A. M., Yatsu, L. Y., Ory, R. L. & Engleman, E. M. 1966. Seed Proteins. — Annual Review of Plant Physiology, 17, 113—136.

Block, R. J., Durrum, E. L. & Zweig, G. 1958. A Manual of Paper Chromatography and Paper Electrophoresis. — Academic Press Inc. Publ., New York, 1958.

Bradnock, W. T. & Matthews, S. 1970. Assessing Field Emergence potential of Wrinkled-Seeded Peas. — Horticultural Research, 10 (1), 50—58.

Ching, T. M. & Schoolcraft, I. 1968. Physiological and Chemical Differences in Aged Seeds. — Crop Science, 8, 407—409.

Crocker, W. & Barton, L. V. 1957. Physiology of Seeds. — Chronica Botanica Company, Waltham, Mass., (1957).

Fick, G. L. & Hibbard, R. P. 1926. A Method for Determining Seed Viability by Electrical Conductivity Measurements. — Michigan Academy of Science Art and Letters, New York, 95—103.

Flentje, N. T. & Saksena, H. K. 1964. Pre-Emergence Rotting of Peas in south Australia. — Aust. J. Biol. Sci., 17, 665—675.

Grassmann, W. & Hannig, K. 1950. Ein einfaches Verfahren zur Analyse der Serumproteine und anderer Proteingemische. — Die Naturwissenschaften, 37 (21), 496—497.

Hottes, C. F. & Huelsen, W. A. 1927. The Determination of Quality in Sweet Corn Seed by Means of the Optical Measurement of Leached Materials. — Journal of Agricultural Research, 35 (2), 147—166.

Huss, E. 1956. Om barrskogsfröets kvalitet och andra på såddresultatet inverkande faktorer. (On the quality of forest tree seed and other factors affecting the sowing result.) — Medd. fr. Statens Skogsforskningsinstitut, 46 (9), 1—59.

Koostra, P. T. & Harrington, J. F. 1969. Biochemical Effects of Age on Membranal Lipids of *Cucumis sativus* L. Seed. — Proc. Int. Seed Test. Ass., 34 (2), 329—340.

Kugler, I. 1952. Keimfähigkeit und Abgabe fluoreszierender Stoffe bei Samen. — Naturwissenschaften, 39 (9), 213.

Lesage, P. 1911. Sur l'emploi des solutions de potasse à la reconnaissance de la faculté germinative de certaines graines. — Comptes Rendus des Séances de L'Académie des Sciences, Paris, V 152, (10), 615—617.

Matthews, S. & Bradnock, W. T. 1968. Relationship between Seed Exudation and Field Emergence in Peas and French Beans. — Hort. Res. 8, 89—93.

Matthews, S. & Whitbread, R. 1968. Factors Influencing Pre-emergence Mortality in Peas, 1. An Association between Seed Exudates and the Incidence of Pre-emergence Mortality in Wrinkle-seeded Peas. — Pl. Path., 17, 11—17.

McDonough, W. T. & Chadwick, D. L. 1970. Pregerminative Leaching Losses from Seeds. — Plant and Soil, 32, 327—334.

Metzner, P. 1930. Über die Abgabe floureszierender Stoffe durch quellende Samen und Früchte. — Biochemische Zeitschrift, 224, 1—3.

Mossé, J. 1968. Les protéines des céréales. — Progrés en chimie Agricole et alimentaire. Hermann, Paris, 47—81.

Öpik, H. 1968. Development of Cotyledon Cell Structure in Ripening *Phaseolus vulgaris* Seeds. — Journal of Experimental Botany, 19 (58), 64—76.

Presley, J. T. 1958. Relation of Protoplast Permeability to Cotton Seed Viability and Predisposition to Seedling Disease. — Plant Disease Reporter, 42 (7), 852.

Reinhold, L. & Liwschitz, Y. 1970. Progress in Phytochemistry. — Volume 1 & 2. Interscience Publ. a division of John Wiley & Sons. London, New York, Sydney, Toronto, (1970).

Roberts, E. H. 1972. Viability of Seeds. — Chapman and Hall Ltd. London, (1972).

Schroth, M. N. & Cook, R. J. 1964. Seed Exudation and Its Influence on Pre-Emergence Damping-Off Bean. — Phytopathology, 54,

670—673.

Schroth, M. N. & Snyder, W. C. 1961. Effect of Host Exudates on Chlamydospore Germination of the Bean Root Rot Fungus, Fusarium Solani F. Phaseoli. — Phytopathology, 51, 389—393.

Schroth, M. N., Toussoun, T. A. & Snyder, W. C. 1963. Effect of Certain Constituents of Bean Exudate on Germination of Chlamydospores of Fusarium Solani f. Phaseoli in Soil. — Phytopathology, 53, 809—812.

Simak, M. 1957. The x-ray contrast method for seed testing Scots Pine-*Pinus silvestris*. — Medd. fr. Statens Skogsforskningsinstitut, 47 (4), 1—22.

Simak, M. & Gustafsson, Å. 1953. X-ray Photography and Sensitivity in Forest Tree Species. — Hereditas, 39, 458—468.

Stahl, E. 1969. Thin-Layer Chromatography. — Springer-Verlag Berlin, Heidelberg, New York, (1969).

Stensiö, K.-E. & Ekedahl, G. 1969. Kromatografi och elektrofores. — P. A. Norstedt & Söners förlag, Stockholm, (1969).

Takayanagi, K. & Murakami, K. 1968. Rapid Germinability Test with Exudates from Seed. — Nature, 218, 493—494.

— 1969 a. Rapid Method for Testing Seed Viability by Using Urine Sugar Analysis Paper. — JARQ (Japan Agricultural Research Quarterly), 4 (2), 39—45.

— 1969 b. New Method of Seed Viability Test With Exudates from Seed. — Proc. Int. Seed Test. Ass., 34 (2), 243—252.

Takayanagi, K. & Harrington, J. F. 1971. Enhancement of Germination Rate of Aged Seeds by Ethylene. — Plant Physiol., 47, 521—524.

Tatum, L. A. 1954. Seed Permeability and "Cold-Test" Reaction in *Zea mays*. — Agronomy Journal, 46, 8—10.

Thomas, C. A. 1960. Permeability Measurements of Castor-Bean Seed Indicative of Cold-Test Performance. — Science 131, 1045—1046.

Tolentino, A. 1924. A Viability Test for some Tropical Seeds. — The Philippine Agriculturist. Vol. XIII. No. 3, 129—141.

Zlobin, J. A. 1971. Ispolzowanije dialysa dla ocenki ziznjesposobnosti semjan chwojnych. — Lesnoje choziajstwo, nr 24, 36—37.

Sammanfattning

Urlakade frösubstanser på groningspapper — ett grobarhetstest?

Ur tallfrö med låg vitalitet urlakas vid groningen olika substanser (s.k. eluater, t.ex. aminosyror, proteiner, nukleinsyror, fetter, socker etc.). Hos färskt, vitalt frö har förekomsten av sådana produkter ej observerats. Det har visat sig att dessa substanser kan analyseras på groningspapper, på vilket fröet har inkuberats under ca 24 timmar i Jacobsens apparat. De färglösa eluaterna kan färgas med olika reagenser, vilka vanligen används vid papperskromatografiska undersökningar.

Eluaternas egenskaper har studerats i fyra experiment. Följande resultat har erhållits:

Experiment 1 (tab. 2): De bästa färgreaktionerna hos eluatfläckar har åstadkommits med amidosvart (proteiner) och med ninhydrin (aminosyror).

Experiment 2 (fig. 1): Den kvantitativa och kvalitativa framkallningen av proteinsubstanser är beroende av inkubationstid, temperatur etc.

Experiment 3 (fig. 2): Bevis har erhållits för att några proteinsubstanser ej lakas ur tallfrö med hög grobarhet. Frö med låg vitalitet lämnar däremot alltid proteinfläckar på groningspapperet. Antalet framkallade färgfläckar är emellertid lägre än antalet "ej grodda frön" i de olika fröprov som har testats i experimentet. Korrelationen kunde eventuellt ha förbättrats om varje eluatfläck hade underkastats flera olika test (t.ex. med UV, ninhydrin, amidosvart).

Experiment 4 (fig. 3 och tab. 3): Artificiellt, mekaniskt skadat frö med hög grobarhet lämnar också eluatfläckar på groningspapperet, men fläckarna skiljer sig i vissa fall kvalitativt från dem som lämnas av normalt frö.

Dessa orienterande försök visar att det vore värt att mera utförligt och systematiskt studera sambandet mellan eluategenskaper och fröets vitalitet. En praktisk metod för bestämning av fröets vitalitet skulle kunna utarbetas. I diskussionen har även omnämnts andra aspekter på vad ett sådant vitalitetstest skulle betyda för den praktiska hanteringen av skogsfrö.