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X-RAY RADIOGRAPHY OF AGEING SCOTS PINE SEEDS

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1. INTRODUCTION

As far as is known, X-rays were used for the first time to determine the quality of seed about 75 years ago. Prof. LUNDSTRÖM, working at Uppsala University, reported in 1903 that he was subjecting seeds to X-rays in order to determine whether it was time to start cone collection. This was a form of ripening research. He found that some of the seeds were full, others were empty, were bad seeds. In his opinion, however, this method was not suited to practical use because »it would be too laborious and troublesome» (KAMRA 1964). This method was thus forgotten for a quarter of a century. However, X-ray radiography was used in the 1920's to determine whether there were insect larvae in seeds of, for instance, cotton. X-ray method was used in the 1950's in the studys of insect damage in wheat and maize seeds (NICHOLSON et al. 1953).

Twenty five years ago, a method for taking X-ray radiographs of forest tree seed was presented in Sweden (SIMAK and GUSTAFSSON 1953). A number of researchers investigated the use of this rediscovered method in different fields of seed research, initially only with forest trees. It was used to classify seeds according to the size of the embryo (PLYM FORSHELL 1953, SIMAK and GUSTAFSSON 1954). The method was also found to be suitable for forest breeding purpose (EHRENBERG et al. 1955). The effect of radiation on seeds has been studied on a number of occasions (SIMAK and GUSTAFSSON 1953, SIMAK et al. 1961, KAMRA and SIMAK 1965). DOGRA (1967) has compared the applicability of X-ray radiography and microscopy in the study of the embryology of conifers and the degree of ripering of seeds in his extensive study.

The method was further developed by employing stains, such as barium chloride, in the X-ray radiography of Scots pine seeds (SIMAK 1957). A stain containing iodine was used in the 1960's to show up mechanical damage in Norway spruce seed (KAMRA 1963). The stains used were rather difficult to obtain and so were replaced by cheap in organic iodine salts. Potassium and sodium iodides were found to be suitable for spruce (KAMRA 1971). The most recent innovation has been the introduction of volatile organic compouds, such as trichloromethane (SIMAK 1974). However, methods employing these last-mentioned compounds are still in the process of being developed and they are not yet in common use in seed laboratories.

Soft X-ray radiation (Grenz rays) are used in the X-ray radiography of seeds. It is based on the principle that the different parts of the seed, the seed coat, endosperm and embryonic tissues, absorb X-rays to a slightly varying extent. The more absorptive the tissue is, the smaller the amount of X-rays which pass through the specimen and stike the roentgen film. This is evident as light areas on the film. Empty seeds and empty regions in the seed, such as the embryo cavity, appear as dark areas on the film. Normally, there is a distinct border around the embryo in the embryo cavity. However, this is sometimes somewhat unclear.

The proportion of the embryo cavity occupied by the embryo is measured or estimated from the radiographs. The estimations are usually carried out in the longitudinal direction. Surface area projections perhaps give a better idea of the development stage of the embryo and the degree of ripening of the seed.

The seeds are divided up into five embryo classes (0—IV), class II being up into two sub-classes.

Embro class

0: Empty seed; no embryo or endosperm. Expected germination 0 %.

I: Seed lacking an embryo; no embryo but endosperm present. Expected germination 0 %.

II A: Under-ripe seed; largest embryo occupying 1/4 of the embryo cavity. Expected germination 5 %.

II B: Under-ripe seed; largest embryo occupying 1/4—1/2 of the embryo cavity. Expected germination 30 %.

III: Ripe seed (premature seed); embryo occupying 1/2—3/4 of the embryo cavity. Expected germination 88 %.

IV: Mature seed; embryo completely or almost completely fills the embryo cavity. Expected germination 99—100 %.

The so-called, expected germination percentage, has been obtained for each embryo class by germinating the seeds which have been X-rayed. These figures are only valid for completely healthy, undamaged seed. Cone handling and seed extraction or damage caused during storage can effect the results.

Soft X-ray radiation does not affect the germination results even when a dosage one hundred times the normal one is used. Different types of chromosome damage can in fact occur in coniferous seeds when ten-times the normal dosage is used. The most common type of damage is chromosome breakages. This type of damage has not been found when crop plants are given large doses of radiation (KAMRA and SIMAK 1965). This may possible be due to the fact that the cells of conifers have relatively large nuclei and long chromosomes. Much more sensitive film is nowadays used, however, and the radiation dose has been decreased to about a tenth of its original level.

Different forms of X-ray radiography are nowadays in use in a number of seed laboratories. It is most frequently used in cases where information is required about the development, progress of ripening and quality of coniferous seed. The method was introduced in Finland at the end of the 1950's, mainly for studying the development and ripening of Scots pine seed and the seed crop. It has been, and is still being, used in the determination of the limits in North Finland for the collection of Scots pine and Norway spruce cones (so-called ripening service).

The method has been used in Finland in its original form, without any staining. Quite the same information about the number of living and dead seeds is obtained. The seeds are classified on the basis of their anatomical quality only, according to the size of the embryo. No information is obtained about the physiological condition of the seed. In addition to X-ray examination, germination percentage is also determined in a Jacobsen germinator in seed analysis. The procedure is laborious and time-consuming: germination alone takes three weeks.

X-RAY CONTRAST RADIOGRAPHY

One characteristic of living cells is that the cell membranes are semi-permeable. If the physiological condition of the cells is sufficiently weak, the activity of the cell-membranes is disturbed. If dead cells or ones which have damaged cell membranes are placed in a stain solution, passage of the stain into the cells takes place rather freely. This phenomenon is exploited in X-ray contrast radiography. The seeds under study are placed in an aqueous solution of a salt which strongly absorbs X-rays, such as barium chloride. The more seriously the cell membranes are damage,

the more stain passes into the cells. The areas in the seed to which the barium ions have passed now absorb X-rays and show up as light patches on the film. It has been demonstrated experimentally (Simak 1957, Kamra 1963, Kamra 1971) that none of the stain enters the embryos of seeds which are capable of germinating. The area in the seed endosperm which has been stained must not be more than 1/4 of the area of the endosperm projected on the film if the seed is to germinate whith any certainty.

MATERIAL AND METHODS

Methods for determining the quality of stored Scots pine seeds have been studied at Kolari Research Station, Finnish Forest Research Institute. The oldest seeds had been stored for 16 years and the youngest for five years. The seed had been stored in plastic bottles sealed with wax in a cold room (+2° C). The first method to be compared was X-ray radiography without staining, which is normally used in germination service. The second was X-ray contrast radiography. The seeds were kept overnight in sterile water. In the morning the surface of the seeds was carefully dried with filter paper. The seeds were then immersed in a 20 % solution (w/v) of $BaCl_2$ for 45 min. Following rinsing with water (2 min.), the seeds were left to dry overnight on filter paper at +20° C. The normal procedure used in X-ray radiography was then followed: the seeds were placed on a photographic plate and X-rayed.

The seeds were X-rayed using an FKA_2 seed X-ray machine (Oy Havemann Ab, Helsinki), focal distance f = 50 cm, a voltage of 18 kV, a current of 10 mA and an exposure time of 0,4 s. Kodak Industrex M film was used. The films were developed immediately after exposure.

The comparison method was germination in a Jacobsen germinator according to ISTA norms. The germination results were calculated after 5, 7, 10, 14, 21 and 30 days. Each treatment was carried out with four replications, each consisting of 400 seeds. The results are presented as the mean values of the replications.

RESULTS AND DISCUSSION

Normal X-ray radiography

Only one seed lot — Muonio 69 (Table 1) — gave similar results with normal X-ray radiography as with germination in a Jacobsen germinator (21 days). The results for seed lots which contained a lot of under-ripe seed (Muonio 71 and Kolari 71), obtained with X-ray radiography, differed the most from the germination results. The expected germination percentage calculated from the results of X-ray radio graphy on seeds which had been stored for a long time (Sodankylä 62) was almost twice as high as the actual germination percentage. The large error for this seed lot may be at least partly due to the fact that it contained rather many seeds classified as belonging to embryo classes II and III. It has been known for a long time that the germination capacity of under-ripe seed rapidly deteriorates during storage (e.g. Huss 1967). Changes which are revealed by normal X-ray radiography were not found, however, in such seeds.

It is apparent from the results (Table 1) that »almost reliable» results are only obtained when normal X-ray radiography is carried out on seeds which are fully-ripe and which have recently been collected in an undamaged condition. The expected germination results obtained from under-ripe seeds or those which have been stored for a long time are too high in comparison to the actual germination percentages.

Table 1. Expected and actual germination values for different Scots pine seed lots. Each treatment 4 × 400 seeds.

Seed lot (seed class B 4)	Weight of 1000 seeds, g	Actual germination percentage (Jacobsen germinator)						Expected germination percentage on basis of X-ray radiographs	
		5 days	7 days	10 days	14 days	21 days	30 days	Normal	Treated with $BaCl_2$
G3—71—094 Muonio	3,90	0,4	1,5	2,2	2,9	3,4	4,1	24,0	10,2
G3—71—108 Kolari	3,94	3,2	9,4	12,9	14,3	15,1	15,6	36,6	23,2
M2—62 Sodankylä	3,76	5,7	15,9	24,1	27,5	28,8	29,6	64,5	26,5
T14—66—2 Kuhmo	3,98	20,1	46,8	60,2	63,6	64,9	66,2	77,8	56,4
G3—69—027 Muonio	3,60	24,1	52,7	61,9	63,9	65,0	68,7	66,0	67,3
M9—61—29 Kemijärvi	4,33	23,1	54,7	75,6	83,2	85,1	86,0	95,2	82,8
M22—61—1 Vaala	4,38	28,0	63,1	79,6	83,6	84,8	86,1	96,7	81,4
G3—72—014 Kolari	4,96	77,8	91,1	94,2	94,3	94,4	94,5	90,3	93,6
G3—72—012 Muonio	4,83	83,3	95,1	96,8	96,8	96,8	97,1	94,3	96,0

X-ray contrast radiography

In cases where the germination percentage has been over 20 % (Table 1), the »expected germination percentage» calculated from the results of X-ray radiography in which barium chloride was used as the stain, have closely followed the germination results. The worst germination percentage for under-ripe seeds has been under 20 %, and too high expected germination values have been obtained with this method.

The expected germination percentage for seeds which had been stored for the longest time (Kemijärvi 61, Vaala 61, Sodankylä 62 and Kuhmo 66) were smaller when X-ray contrast radiography was used than those obtained in the germination tests. This would suggest that the cell membranes of these seeds have degenerated. However, the physiological condition has obviously been so slightly affected that it has not affected the germination results. Germination vigour (germination within 10 days) has in fact already become weaker as the age of the seeds increase. The germination results improved considerably more, in the latter stages of germination, that with fully-ripe seeds which had been stored for only a short time (Kolari 72, Muonio 72). The conditions in a Jacobsen germinator are ideal for germination. The results which are obtained are therefore too optimistic in comparison to the germination percentage to be obtained under field conditions (Nordström 1953). Seeds which are diseased or damaged in different ways can, in many cases, germinate normally in a germinator. The reduction in vigour usually only becomes apparant when the seeds are subjected to stress, such as overlong storage or sowing in the ground (Simak 1974). When analysing such seed by means of X-ray contrast radiography, even more reliable results about the actual germination capacity of the seed may be obtained than through germination under optimum conditions.

X-ray contrast radiography of under-ripe seeds or those which have been stored for a long time gives a more reliable prediction of the actual germination percentage of Scots pine seeds than normal X-ray radiography does. When analysing seeds

which have been stored for a long time, normal germination tests should also be carried out. The use of X-ray contrast radiography in the study of seed ageing during storage will be studied further.

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SELOSTE:

VANHENEVIEN MÄNNYN SIEMENTEN RÖNTGENKUVAUS

Metsäpuiden siementen tutkimiseen käytettiin röntgenkuvausta ensi kerran Ruotsissa jo n. 75 vuotta sitten. Silloin sitä pidettiin käytäntöön liian työläänä. Menetelmä esitettiin pitemmälle kehitettynä uudelleen 1950-luvulla. Nykyään se on rutiinimenetelmänä siellä, missä tarvitaan tietoja siementen tuleentumisesta ja laadusta.

Selvitettäessä siementen vanhenemista varastoinnin aikana tutkittiin eräitä n. 15 vuotta vanhoja siemeniä. Normaalin röntgenkuvauksen ja idätyskokeiden tulos oli likipitäen sama vain yhdellä, Muonio —69 -siemenerällä. Suurin ero tuli Sodankylä —62 -siemenerällä. Tällä menetelmällä saatiin »lähes luotettavia» tuloksia vain äskettäin kerätyistä, täysin tuleentuneista ja vahingoittumattomista siemenistä. Kauan varastoiduista tai vajaasti tuleentuneista siemenistä saatiin idätyskokeeseen verrattuna liian hyviä odotetun itävyyden arvoja. Normaalin röntgenkuvauksen avulla ei saada käsitystä siemenen fysiologisesta tilasta. Kuolleesta ja elävästä saadaan samanlainen kuva.

Kun kuvauksissa käytettiin varjoainetta ($BaCl_2$) saatiin idätyskokeeseen rinnastettava tulos, kun todellinen itävyys oli yli 20 %. Kauimmin varastoiduilla siemenillä (Vaala ja Kemijärvi —61, Sodankylä —62) röntgenvarjoainekuvasta lasketut odotetut itävyydet olivat alempia kuin todetut. Tämä viittaa siihen, että näissä siemenissä solukelmut ovat varastoinnin (tai käsittelyn) aikana vaurioituneet, ja varjoainetta pääsi sisälle soluihin. Vauriot ovat olleet kuitenkin niin vähäisiä, etteivät ne ole vaikuttaneet itävyyteen ihanneoloissa laboratoriossa.

Varjoaineiden käyttö siementen röntgenkuvauksessa näyttää parantavan menetelmän luotettavuutta. Se ei saisi silti korvata idätyskokeita etenkään pitkään varastoitujen tai vajaasti tuleentuneiden siementen laatua tutkittaessa. Röntgenvarjoaineiden käyttöä ei toistaiseksi ole vielä hyväksytty standardimenetelmäksi. Menetelmässä on vielä kehittämistä ja sen käyttökelpoisuutta siementen pitkäaikaisen varastoinnin aiheuttamien vaurioiden tutkimisessa jatketaan.

KAUPUNKIMETSIEN HOIDON OPASKIRJANEN ILMESTYNYT

Vaikka metsänhoidon oppi- ja käsikirjatilanne ei maassamme ole mitenkään kehuttava, yhtä ja toista on sentään kirjoitettu talousmetsiemme hoidosta ja käytöstä. Sen sijaan yhä tärkeämmäksi nouseva erikoisalue, kaupunkien lähiöiden ja virkistysmetsien hoito sekä uusien asuntoalueiden moninaiskäytöllinen suunnittelu, on jäänyt selvästi vähemmälle huomiolle.

Tervetullut uutuus onkin metsien virkistyskäytön tutkijan, dosentti Seppo Kellomäen yhdessä nuoremman kolleegan, metsänhoitaja Aura Lakan kanssa laatima opaskirja[1]) — ilmeisesti ensimmäinen laatuaan Suomessa. Työ muodostaa osan Suomen ja Neuvostoliiton välisen kaupunkisuunnittelun yhteisprojektista, NEKASU:sta, ja se on yhteensä kymmenosaiseksi suunnitellun teossarjan kolmas julkaisu. Tämä selittää kirjan julkaisusarjan.

Kuten jo julkaisuyhteys osoittaa, kirja on tarkoitettu ennen kaikkea kaupunki- ja yhdyskuntasuunnittelun parissa työskenteleville arkkitehdeille ym. ns. fyysisen suunnittelun ammattimiehille, joiden tiedot metsistä ja puista saattavat joskus olla perin ylimalkaisia. Mikään metsänhoidon alkeisoppikirja Kellomäen ja Lakan teos ei silti ole, vaan se edellyttää lukijalta joltistakin metsikköekologian ja dendrologian tuntemusta.

Kirjan alku tuntuu metsänhoitoon jo aikaisemmin perehtyneestä helpolta ja luonnolliselta: onhan metsien vaikutusta erilaisiin mikroilmastotekijöihin ym. käsitelty metsäalan julkaisuissa runsaasti. Mutta pian siirrytään kirjassa alueille, joissa normaalin metsänhoitajan perustiedot eivät riitä pitkällekään: metsien käyttöön ilman epäpuhtauksien ja yhdyskuntamelun heikentäjänä. Oma lukunsa on omistettu myös metsien merkitykselle kaupunkilaisväestön ulkoiluympäristönä. Kirjoittajat pyrkivät tässäkin perustelemaan väitteitä ja toteamuksia kyselyjen ja haastattelujen tuloksilla, eivätkä vain artikkelityyliin tyydy korostamaan hoidettujen metsien suurta sopivuutta (tai sopimattomuutta) erilaisiin ulkoilutoimintoihin.

Rakentamisen vaikutusta puuston ja sen menestymiseen käsitellään teoksessa monipuolisesti, samoin metsien hoitoa rakennetuilla alueilla. Erityistä huomiota ansaitsee mallina käytetty Malminkartanon uusi lähiöalue Helsingin lähistöllä. Tämän esimerkin avulla käydään vaihe vaiheelta läpi, mitä kaikkia tietoja metsistä voi ja tulee saada tehokasta suunnittelua varten. Tavallisten ikäluokka-, puulajisuhde- ja kuutiomäärätietojen ohella on laadittu puuston pituutta, puiden ja pensaiden tiheyttä, suodatuskapasiteettiä, melunvaimennus- ja sopeutumiskykyä, metsien ulkoilumahdollisuuksia ja pintakasvillisuuden kulutuskestävyyttä esittelevät karttakuvat, joiden pohjalta varsinainen suunnittelu siten etenee. 134-sivuisen kirjan päättää lyhyt luettelo rakennettavalla alueella kasvavia metsiä koskevia suosituksia, laaja kirjallisuusluettelo ja lyhyt englanninkielinen tiivistelmä.

Vaikka kirja pohjautuu ennen kaikkea

[1]) Kellomäki, Seppo & Lakka, Aura. 1979. NEKASU: Luonnon olosuhteiden huomioonottaminen uusien asuntoalueiden suunnittelussa. METSÄT. Summary: Utilizing of forests in urban planning. Yhdyskuntasuunnittelun jatkokoulutuskeskuksen julkaisuja B 25, HTKK. 02150 Espoo 15.