teres sont nettement intermédiaires, mais il se peut qu'il s'agisse seulement de cas particuliers. Par exemple, la formation de la pousse terminale est intermediaire entre l'orthotropie de P. omorica et la tendance de P. sitchensis a dévier de ce type de croissance. Cela apparait en particulier dans la reaction des divers types a la neige. De même, la sensibilite au fr'oid de l'hybride est intermediaire entre celle de P. sitchensis (sensible aux gelées tardives) et celle de P. omorica (resistant). Aucune conclusion ne peut encore être donnee en ce qui concerne l'allure générale de la croissance.

Literatur

EKLUNDH, C.: Artkorsningar *inom sl. Abies Pseudotsuga., Picea, Larix, Pinus och Chamaecyparis, tillhörande fam. Pinaceae. Svensk* Papperstidning *Nr. 3, 5* och *6 (1943).* — LANGNER, W.: *Kreuzungsversuche mit Larix europaea D. C. und Larix* leptolepis GORD. *Z. Forstgen. 1, 2–18, 40–56 (1951152a).* — LANGNER, W.: *Kreuzungsversuche mit verschiedenen Fichtenarten. Z. Forstgen.* 1, *58–59 (1951152b).* — LANGNER, W.: *Selbstfertilität und Inzucht bei Picea Omorika* (PANČIČ) PURKYNE. *Silvae Genetica 8, 84–93 (1959).* — OKSBJERG, E.: *Om Picea omorika. Skovf.Tidsskr. 38, 179–192 (1953).*

The Processing of Pollen

By R. G. F. WORSLEY

British Forestry Commission, Genetics Section

(Received for publication August 8, 1959)

For some years the work of controlled pollination has been carried out by means of a simple and practical technique. Before they become receptive, female flowers are isolated with greaseproof paper bags. When they are judged to be ready for pollination, this is accomplished with a camel hair brush or a pollen gun (see Figure 1). The pollen is collected either from the selected trees as the male flowers mature, or individual strobile are snipped off into pollen extractors (see Figure 2) and left to mature there. If the pollen is not used immediately it is stored in a cold room until it is required.

In general this procedure worked well enough during the large-scale larch breeding Programme at Newton Nursery, Morayshire, in 1956. It was also employed successfully in smaller operations on Douglas fir, some two-needled pines and birch. However, several factors, particularly failures in some crosses, have led to the study of pollen processing in order that this part of the work should be as soundly based as possible. The experiments summarised below were carried out at the British Forestry Commission's Research Station, Alice Holt Lodge, during 1957 and 1958.

The processing of pollen rnay be divided into six stages, namely collection, ripening, extraction, storage, testing and use. Greatest attention has been paid to ripening, extraction and testing as these presented the greatest problems. Study of the work of DUFFIELD (1954) and others on the storage of pollen suggests that suitable storage techniques are already available: none tested so far is perfect, however, and much work remains to be done in this field.

1. The Collection of Pollen

Male flowers on their branchlets rnay be collected from grafts made two or three years previously or, if these are too small and for that reason are male sterile, the twigs are obtained straight from the parent tree. This is done either with a shotgun firing heavy gauge shot, by using high pruning secateurs or by climbing the tree. Whichever method is adopted it must be stressed that the branchlets, however collected, should have their newly severed ends soaked in water as *soon as* possible after cutting. A supply of water should be carried on field expeditions if none is likely to be available on the site. The material should then be stored in polythene bags with a little water until it is to be used — this time should not exceed three or four hours unless the material is kept moist and very cool.

The male flower bearing branches rnay be collected before the pollen is normally released, but even when the flowers have begun to shed their pollen sufficient is usually left after collection to provide the relatively small amounts necessary for controlled pollination work.

2. Ripening the Male Flowers

This rnay be allowed to proceed naturally but, for the purposes of crossing Programmes, some forcing is usually necessary. For example the female flowers of a given clone rnay become receptive a little in advance of the male flowers even without their being isolated in paper bags, which hastens this development still more. Also one particular clone rnay be well ahead of others both in male and in female flowering. Thus self-pollination or reciprocal crossing with a clone of later flowering rnay be impossible without hastening the ripening of the male flowers or storing the pollen for a season. A further advantage given by forcing the early release of pollen is that collection and testing can be completed before the actual pollination work begins.

Experiments at Alice Holt have shown that it is possible to bring on pollen of many species of coniferous and broadleaved trees by subjecting the developing male flowers to conditions of increased day-length, light intensity and warmth in a highly humid atmosphere. In the first trials of this method twigs of several species bearing male flowers were taken three weeks, one month and two months before they were due to shed pollen naturally and brought to Alice Holt. Some species had to be transported over 80 miles and in all cases the newly-severed ends of the branches were dipped in water; where possible the branch was dipped under water at the point at which it was cut. All branches were then packed in polythene bags until reaching the glasshouse at Alice Holt. On arrival the branches were placed in jars of water or weak nutrient solution (HEWITT, 1952), covered with the polythene bags and placed in the glasshouse. This had been partitioned so that different treatments could be given to the branches as shown in Table 1.

The duration of the treatment varied with species snd how far in advance of normal flowering time the branches had been taken. The treatment time ranged from one to four weeks, the male flowers being closely watched for full ripening; when this stage had been reached the ties holding the polythene bags in position were loosened to lower the

Table 1. — Conditions applied to male flowering branches

Treatments	1	2	3	4
Light intensity	600 watts at 1 m	400 watts at 1 m	200 watts at 1.5 m	60 watts at 1.5 m
Day Length	18 — 22 hours	20 hours	24 hours	24 hours
Air Temperature	Up to 27° C	Up to 25° C	Up to 20° C	Up to 15° C
Humidity	80 — 100% for ripening, 60 — 70% for shedding the pollen in all cases			

humidity gradually (Table 1). The strobile could then be cut off into extractors when they started to shed pollen. Alternatively the branches were arranged with their cut ends in flat dishes of water, under polythene sheet, with their flowers overhanging black paper. Complete pollen shedding generally took from one to three days, but in some species of pine the shedding started immediately and was complete in a few hours.

Treatment 1 was severe and was intended to obtain speedy ripening of pollen. The high light intensity from tungsten filament lamps resulted in high air temperatures and the lamps were controlled thermostatically to cut out if the air temperature rose above 27° C. This only happened in practice when the sun was out. The glass-house used in these experiments was also lined with polythene sheet to cut down heat transfer. The viability of the pollen obtained was tested both by germination and by the vital staining method described in Section 4 of this paper. The size of sample varied a great deal, but was never less than 10 male flower bearing branchlets of diameter not more than 0.7 cm. The response of the various subjects is dealt with by genera.

Abies: The species tried were *A. amabilis, concolor, homolepis, nobilis, nordmanniana, pinsapo* and *veitchii*. All withstood the transport and storage well and under Treatment 1 gave a good release of viable pollen. It is concluded that pollen-bearing branches of *Abies* can be collected three weeks before normal shedding time and ripened off quickly and easily.

Cedrus: The species tried were *C. atlantica, deodara* and *libani*. All species, particularly the latter, produced copious supplies of pollen up to one month ahead of normal flowering time when Treatment 1 was applied.

Chamaecyparis: C. nootkatensis flowers twice during the growing season in Britain, namely in April and October. Flower bearing branches collected three weeks before each time of flowering reacted well to both Treatments 1 and 3 and soon released viable pollen. The other species tried were *C. lawsoniana, pisifera* and *thyoides*.

C. pisifera was as easy a subject as *C. nootkatensis*, but the other two species did not give large amounts of pollen.

Larix: The species tried were *L. decidua, leptolepis, occidentalis* and *potanini*. Male flowers of larch are more difficult to ripen when collected before normal flowering time and branches collected more than three weeks in advance did not produce any pollen. Treatment 1 was too severe and best results were obtained with Treatment 3.

Picea: No difficulty was presented in obtaining viable pollen from the species *P. abies, breweriana, mariana, omorika, sitchensis* and *smithiana*. Treatments 1 and 3 were used successfully and branches were collected up to two months before normal flowering time.

Pinus: The species tried were *P. banksiana, contorta, nigra, peuke, ponderosa, strobus, sylvestris* and *thunbergii*. The result was as for *Picea*, but *Pinus strobus* branches collected more than a month before normal flowering time failed to shed viable pollen.

Pseudotsuga taxifolia: Viable pollen was obtained from branches collected three weeks and one month in advance and subjected to Treatment 1.

Sequoia and *Sequoiadendron:* Both *S. sempervirens* and *S. giganteum* responded when carefully handled under Treatment 4, and the flowers appeared to ripen normally but very slowly. Only *S. sempervirens* produced viable pollen however.

Tsuga: The species tried were *T. canadensis, dumosa, heterophylla* and *mertensiana*. Male flower bearing branches were difficult to obtain, but what there were (with the exception of *T. dumosa*) ripened and produced viable pollen under Treatments 2 and 3. Branches may be collected from these species up to two months before normal flowering time. The failure with *T. dumosa* was due to its extremely early collection — two and a half months early — coupled with drying out on the way back to the laboratory.

Other conifers: Specimens of *Cryptomeria japonica, Cunninghamia lanceolata, Cupressus arizonica* and *C. goveniana, Taxus baccata, Thuja occidentalis, plicata,* and *standishii* were tried on a smaller scale than those given above. Treatments 1, 2 and 3 were given to all of them, *Cunninghamia* being the only species not to yield viable pollen.

Broadleaved species: The species tried were *Alnus glutinosa, Betula jacquemontiana, B. pubescens* and *B. verrucosa, Fagus sylvatica* and *Fraxinus excelsior*. *Alnus* and *Fraxinus* gave copious supplies of pollen, *Betula* a fair quantity and *Fagus* only a very small quantity all under Treatment 1.

3. The Extraction and Cleaning of Pollen

When the pollen sheds it can either be tapped off the black paper into a pollen extractor (Figure 1) or, as this tends to spread pollen about in the glasshouse, picked up and cleaned by the apparatus shown in Figures 2a and 2b. In this the suction is supplied either by a water pump or by an electric motor; the apparatus ensures that little or no pollen is wasted, and no contamination occurs as there is so little movement of air. Type 2a can be used for rare or small quantities of pollen, type 2b for collection in bulk where the pollen is to be used in seed orchards.

During the work of controlled crossing, in any one season, the extractors may have to be used more than once for different clones of the same species: thus sterilization of the interior is necessary. Hodgkins (1952) and Watanabe (1953) have used heat as a pollen sterilant but this is not practicable in the extractors used at Alice Holt because of the wax used in their construction. Similarly alcohol or acetone are unsuitable as not only do they attack the adhesives in the extractor but unless it is completely dismantled before cleaning some small amount of chemical seems to remain in the cracks and this damages the next lot of

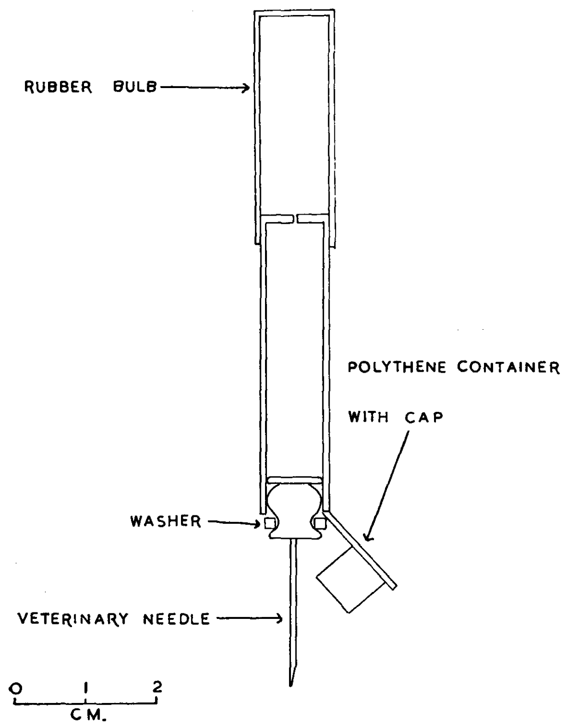

Figure 1 a (on the left): Pollen gun*).

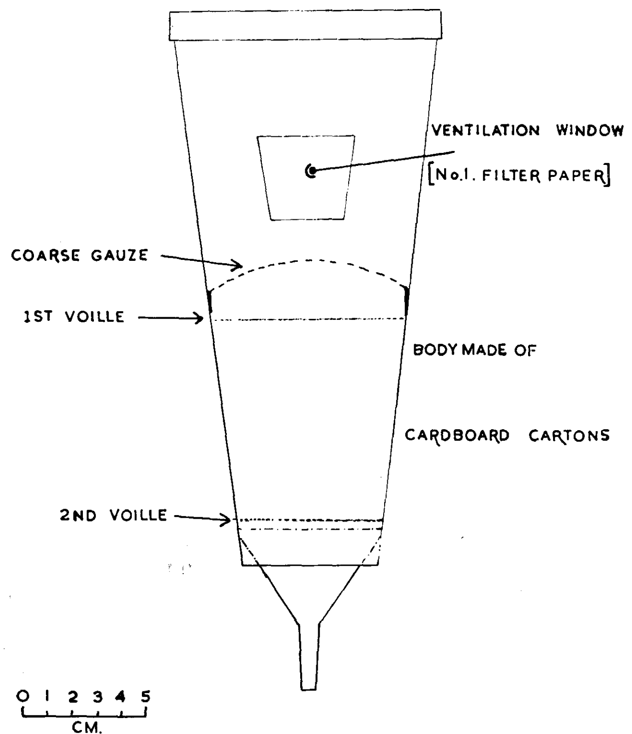

Figure 1 b (on the right): Pollen extractor.

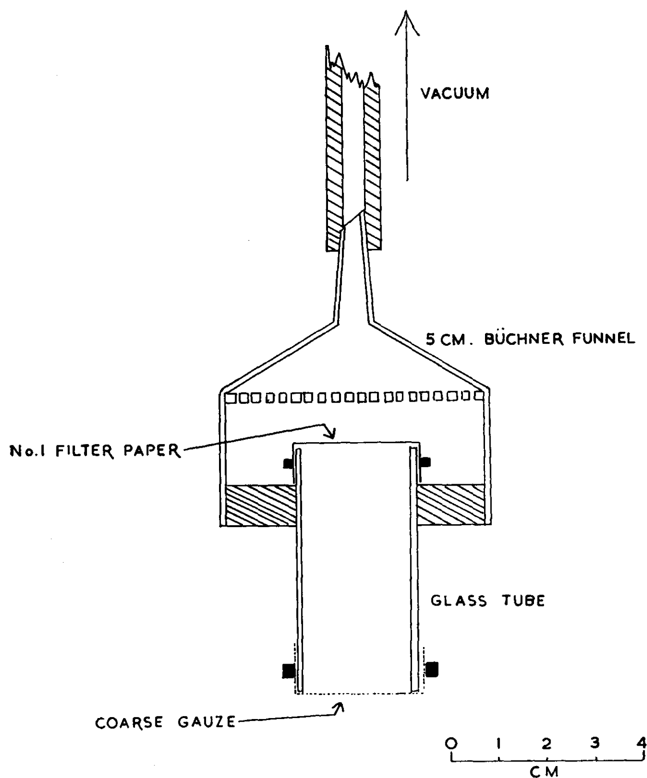

Figure 2 a (See text).

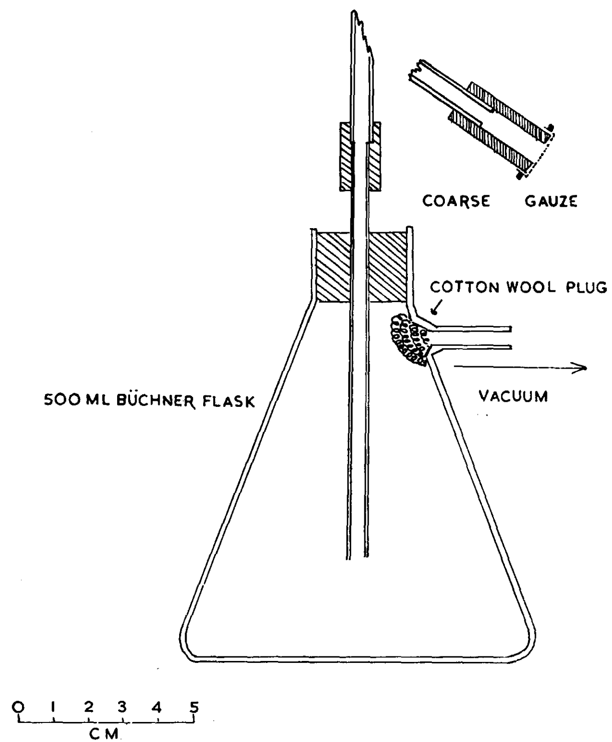

Figure 2 b (See text).

pollen passed through. The chemical now used for sterilization is 1—2 Epoxypropane, a volatile liquid boiling at 35° C. The extractors, with their tops removed, are each put into a polythene bag and a small swab of cotton wool bearing app. 0.5 ml. of the chemical introduced. The top of the bag is sealed with a plug of cotton wool and a plastic tie to prevent the extractor being recontaminated After two days the extractor may be used, but not before it has been aired well in a pollen free atmosphere.

This method was tested by contaminating some extractors with pollen of known viability and carrying out the above

*) Although it is not strictly within the scope of the present paper, the diagram of the pollen gun used at Alice Holt is included as it may help other workers in this field.

sterilization procedure. After two days all the grains had been killed, even those in the most obscure crevices. The extractors were then aired and recontaminated with fresh pollen: this showed no loss of viability in three days, indicating that all harmful vapour had dispersed.

This method has the advantages that the extractors may be kept clean in the bags between seasons, they may be sterilized during the pollination programme and are not attacked by insects during storage: a large number of untreated extractors were damaged by the False Clothes Moth *(Hofmannophila pseudospretella* STAINTON) during a winter's storage in warm, dry conditions. The insect attacked the acetate rayon voille filters, rendering them useless.

4. The Storage of Pollen

DUFFIELD (1954) and SATO and MUTO (1955) describe methods which have been tested for storing pollen for fairly long periods. None of them seems entirely satisfactory, however, and the technique described below is merely a slight modification which will be used until a better and more reliable method is perfected. The method consists of storage at approximately 0° C. at low relative humidities ranging from 10% to 50% controlled by sulphuric acid of the correct specific gravity. In general it was found that 10% and 25% R.H. gave the best results, pollen stored at 50% relative humidity not maintaining its viability for so long. Using this method pollen of *Pinus sylvestris* has been kept alive for three years with no loss in viability (10% R.H., 0° C. storage temperature), and that of *Cedrus atlantica* for two and a half years by the same method.

Pollen of several species was collected in spring, 1958, and stored at 0° C. and humidities of 10% and 25%. Germination tests and viability counts indicate that pollen of *A. amabilis, A. concolor var. lowiana, Larix leptolepis, Picea breweriana, P. smithiana, Pinus banksiana, P. contorta, P. mugo, P. nigra, P. peuke, P. ponderosa, P. strobus* and *P. sylvestris* may be stored by the above method without appreciable loss in viability. On the other hand pollen of *Araucaria araucana, Chamaecyparis nootkatensis, Cryptomeria japonica,* and *Pseudotsuga taxifolia* did not store well, particularly at the lower humidity. The viability in these species was reduced by up to 50%, although *P. taxifolia* stored well at 25% R.H.

When the pollen has been extracted it should be desiccated and stored as soon as possible. Pollen which is left for a little time at room temperature may become infested with dust lice or thrips of the order *Thysanoptera*. Many species of pollen have been attacked in this way when they have been allowed to stand for a few days prior to storage.

The procedure now adopted at Alice Holt for pollen storage is as follows. The pollen is placed in test tubes 12 × 2 cm., to a depth of not more than 4—5 cm.

These are then put into a Kilner preserving jar 14 × 11 cm., in which 14 — 15 tubes can be accommodated. The jar is then filled with sulphuric acid of the required specific gravity to a depth of about 3 cm. The use of cotton wool plugs in the tops of the tubes does not seem to make any difference to the performance of the desiccant, although they should be as small as possible. If a large quantity of one clone is to be stored, a jar of dimensions approximately 7 × 10 cm. is used instead of the individual test tubes. This should not be filled above the level recommended for the test tubes, however, as greater depths of pollen do not give the desiccant a chance to dry the bottom layers, which invariably suffer a loss in viability.

Other work in the field of pollen storage involves lines of research in freeze-drying under vacuum and a method of concentrating the viable grains by fractionation. With the latter there has been some success already, and it is the subject of another paper.

5. Determination of Pollen Viability

Pollen viability can be assessed in two ways, either by germinating the pollen in water or in a nutrient solution, or by staining the nucleus of the grains to distinguish which are viable. DUFFIELD (1954), DILLON and ZOBEL (1957), ECHOLS and MERGEN (1956), MAURINJ and KAUROV (1956), RIGHTER (1939) used various modifications of the germination method. Although several other methods were tried, the one used at Alice Holt is that described by DUFFIELD with only very slight modifications.

Vaseline circles are stamped on the smaller half of a Petri dish (15 cm. diam); the circles are 1 cm. in diameter and about 3 cm. apart. The pollen is then sprinkled onto the vaseline either with a camel hair brush or with a wisp of cotton wool which is then discarded; the latter method is preferable as it obviates the need to sterilize the brush between each batch of pollen. If it is felt that some contamination might occur when testing different pollens on the same plate, a piece of paper with a hole 1 cm. in diameter held over each circle as it is being dusted will eliminate this danger entirely. Only the very smallest pollens have the tendency to drift in these conditions (e. g. *Chamaecyparis nootkatensis*) — in practice no contamination was discovered during the whole series of trials of this method, provided sufficient care was taken with the wisp of cotton wool containing the pollen. The completed plate is then inverted over the larger half of the Petri dish in which is a little distilled water.

The completed dish is then put onto a hotplate and kept at a temperature of 25° — 30° C. for two to three days, when a count of germinated grains is possible.

The method has a number of disadvantages; in particular the use of a hotplate and the closely controlled conditions rules out its use in the field during actual pollination work. (An apparatus using a vacuum flask with 12 volt heaters and a thermostat was, in fact, constructed for employing this test on tour. It was very fragile due to the thin glass in the flask, and had to be carried with great care when grains were under treatment.) A second disadvantage is that, either in the field or the laboratory, the plates are not permanent — mistakes cannot be checked by a recount, slides of one season's pollen cannot be compared with slides of the next. Then again, the grains may be killed by minute amounts of alcohol left over after sterilizing the dish or brushes — this must be done quickly and thoroughly or the pollen is soon swamped by mould and rendered uncountable.

A new approach has been made in this technique by the use of dilute solutions of hydrogen peroxide, which are put directly on the pollen sample in a hanging drop method (RIGHTER, 1939). Preliminary experiments indicate that, in solutions of strengths ranging from 0.1% to 0.01% hydrogen peroxide (that is 1.13 to 0.11 gm. H_2O_2 per litre)*),

*) The strengths given in the text refer to "0.1%" and "0.01%" solutions of hydrogen peroxide. These were made up assuming that a new bottle of 20 volume hydrogen peroxide would contain 6.0% w/v. H_2O_2. The diluted solutions were standardised using potassium permanganate, and it is on these figures that the amounts in gms./litre H_2O_2 are given.

the germination of pollen is greatly accelerated: it may even take place at temperatures normally considered impossible for that species. Thus some pollen of *Pinus ponderosa* was found to have germinated after eight days at 3^0 C., while the controls in distilled water at that temperature had not germinated after one month. At room temperature (about 15^0 C.) results showed that pollen of *Pinus ponderosa, P. sylvestris, P. strobus, Pseudotsuga taxifolia* germinated readily in two days, while at the highest temperatures used in this experiment (30^0 C.) some grains had begun to form tubes after only six hours in the peroxide solution. Later observations at this temperature showed that this early stimulation did not last and that finally it was identical in germination percentage with the control (distilled water at 30^0 C.). Fungal growth was inhibited in all cases where peroxide solutions were used, and the pollen tubes were longer and straighter.

Figure 3 shows the hanging drop chamber used for the peroxide experiments. It is constructed by cementing a ring cut from a test-tube onto a microscope slide.

The pollen is dusted onto a coverslip which has first been lightly smeared with vaseline: a drop of hydrogen peroxide solution is then put on the pollen and the slip is inverted over the cell, which has had a small quantity of distilled water added to maintain the humidity. Pollen counts and observations can be made without dismantling the apparatus, as with the standard Petri dish.

One or two species of pollen (the most important of which were *Larix* and *Betula* species) did not germinate under any of the above conditions. As it was felt in any case that a less complex method of viability determination was desirable several staining methods were tried out. Tetrazolium bromide did not give consistent results when compared with the germination method and some cytoplasmic stains such as basic fuchsin were not specific for live grains as such, but appeared to work only when the grains were about to germinate. The method now adopted is a vital staining technique using methyl green and phloxin, with glycerine jelly as a base (OWCZARZAK, 1952). With this method live grains have a fully expanded cytoplasm which is stained red, the exine and intine being stained green. Dead grains are either green with collapsed cytoplasm or expanded slightly and stained a mottled red, depending on how long they have been dead (i. e., on the amount of non-autolysed cytoplasm present at the time of mounting). The slides are semi-permanent and have lasted for more than a season without loss of colour: they also have the advantage that they can be prepared in the field during actual pollination work, and analysed later.

The technique is a follows: —

Preparation of the solution

The solutions required are: —

A: Methyl Green, saturated solution in 50% alcohol.
B: Phloxin, saturated solution in 50% alcohol.
C: Glycerine Jelly . . . . 50.0 ml.
Solution A . . . . 2.5 ml.
Solution B . . . . 2.0 ml.

The glycerine is melted on a water bath, then measured off into a preheated bottle, to which solutions A and B are added and shaken in. The solution should be a deep wine colour, if it is not, more of solutions A or B are added to produce this result.

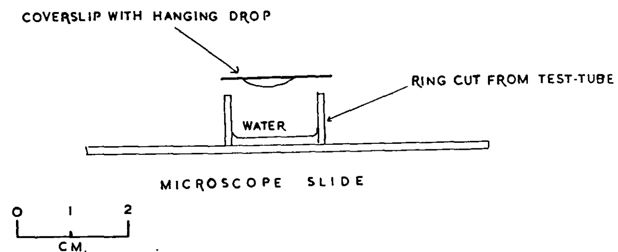

Figure 3: Hanging drop chamber.

Technique

1. Put a small quantity of pollen on a clean slide, then wash it with 70% alcohol until no more oils and resins appear from the pollen in the form of a greyish sludge round the edge of the solution. If this sludge appears it should be removed with a piece of filter paper moistened with 70% alcohol, then the washing repeated until the pollen is clean.
2. After the final wash, and just before the pollen has dried off, add a drop or two of the stain, which should be warmed to about 60^0 C. before it is used; then the pollen is stirred well into it.
3. Drop a clean, warmed coverslip gently onto the stain and allow it to reach the edges of the slip, heating gently the whole time. Do not overheat or apply any artificial pressure or many of the grains will be ruptured. Allow the slide to stand for a few minutes, then warm, remove the first coverslip and replace it with a clean, flamed one.
4. The slides should be allowed to mature for one or two days if there is no urgent hurry for the results, as the phloxin takes some time to act fully.

This schedule has been carried out on all the species of pollen mentioned in this paper, together with a germination test using distilled water where this was practicable. In all cases where the two methods have been carried out side by side, no significant difference has been found in the results of viability given.

6. Preparation of the Pollen for Use

If the pollen has been stored at low humidities it is best to re-humidify it just before use in a moist atmosphere, letting it attain room temperature gradually. The relative humidity should, for this purpose, be around 60 — 70%. It is most inadvisable to leave the pollen at room temperature for any length of time when in the low-humidity storage jars as this seems to lower the viability very rapidly.

Future Work

As a result of the preliminary experiments in a small glasshouse, five controlled condition chambers are being built in the genetics section laboratory at Alice Holt Lodge with independent variation of humidity, temperature, light and day length. A simpler vital staining technique is under test, as well as the vacuum drying and fractionation experiments mentioned in Section 4 above.

With these and other improved techniques as they come along it is hoped to eliminate guesswork in this vital stage in tree-breeding.

Summary

Methods of collecting, ripening, extracting, storing and testing pollen are described, with the accent on ripening and testing. Species of *Abies, Cedrus, Chamaecyparis, Cunninghamia, Cupressus, Cryptomeria, Larix, Picea, Pinus,*

Pseudotsuga, Sequoia, Sequoiadendron, Thuja, Tsuga, and *Taxus* have undergone artificial stimulation to ripen early. Pollen has been tested by two broad groups of methods, those of germination in various media, and vital staining. In the former method solutions of hydrogen peroxide of strengths from about 1.1 gm/litre to 0.1 gm/litre H_2O_2 were found to stimulate pollen to germinate quickly under adverse conditions. A technique is described for determining the viability of pollen by the vital staining method which is simple and can be used in the field.

Zusammenfassung

Titel der Arbeit: *Über die Behandlung von Pollen*

Besonders im Zusammenhang mit dem Reifen und Prüfen von Pollenproben werden Methoden für das Sammeln, Reifen, Extrahieren, Aufbewahren und Testen von Pollen beschrieben. Arten der Gattungen *Abies, Cedrus, Chamaecyparis, Cunninghamia, Cupressus, Cryptomeria, Larix, Picea, Pinus, Pseudotsuga, Sequoia, Sequoiadendron, Thuja, Tsuga* und *Taxus* sind zwecks Frühreife einer künstlichen Stimulation unterzogen worden. Der Pollen ist methodisch auf zwei Weisen getestet worden, nämlich in Keimversuchen in verschiedenen Medien und durch Vitalfärbung. Bei den erstgenannten Methoden war gefunden worden, daß Lösungen von Wasserstoffsuperoxyd in Konzentrationen von 1.1 g/Liter bis 0,1 g/Liter die Pollenkeimung stimulieren, so daß sie auch unter schlechten Bedingungen rasch erfolgt. Ferner wird eine Technik beschrieben, um auch die Lebensfähigkeit von Pollen mit Hilfe von Vitalfärbemethoden bestimmen zu können, die einfach zu handhaben und als Feldmethoden zu verwenden sind.

Résumé

Titre de l'article: *Le traitement du pollen.*

L'article décrit des techniques de récolte, de maturation, d'extraction, de conservation et d'essais de viabilité de pollen en insistant sur le maturation et les essais de viabilité. Des traitements en vue d'obtenir une maturité précoce ont été effectués sur diverses espèces d'*Abies, Cedrus, Chamaecyparis, Cunninghamia, Cupressus, Cryptomeria, Larix, Picea, Pinus, Pseudotsuga, Sequoia, Sequoiadendron, Thuja, Tsuga* et *Taxus.* Deux grands groupes de techniques ont été utilisés pour les essais de viabilité: germination dans des milieux variés et colorants vitaux. Pour les premiers, on a montré que des solutions d'eau oxygénée de 0,1 à 1,1 gr. par litre stimulaient la germination du pollen, même dans des conditions défavorables. Une technique pour la détermination de la viabilité par les colorants vitaux a été mise au point; cette technique est simple et peut être employée sur le terrain.

References

DUFFIELD, J. W.: Studies of extraction, storage and testing of pine pollen. Z. Forstgenetik 3, (2): 39—45 (1954). — DILLON, E., and ZOBEL, B. J.: A simple test for the viability of pine pollen. J. Forestry 55 (1): 31—32 (1957). — ECHOLS, R. M., and MERGEN, F.: Germination of slash pine pollen in vitro. Forest Sci. 2 (4): 321—327 (1956). — HEWITT, E. J.: Sand and water culture methods used in the study of plant nutrition. Tech. Commun. Bur. Hort. E. Malling. No. 22, Sect. II, 1952. — HODGKINS, E. J.: Effect of different heat treatments upon the viability and vigor of pine pollen J. Forestry 50 (6): 450—452 (1952). — MAURINJ, A. M., and KAROV, I. A.: Comparison of methods of determining viability of tree pollen. Bot. Z. 41 (1): 81—84 (1956). — OWCZARZAK, A.: A rapid method for mounting pollen grains with special regard to sterility studies. Stain Technology 27 (5): 249—251 (1952). (Phloxin/methyl green method also mentioned from this reference in Medical and Biological Staining Techniques, E. GURR, pub. Hill, London, 1956.) — RIGHTER, F. I.: A simple method of making germination tests of pine pollen. J. Forestry 37: 574, 576 (1939). — SATO, Y., and MUTO, K.: On the viability of forest tree pollen. Res. Bull., Col. Exp. For., Hokkaido Univ., 17 (2): 966—979 (1955). — WATANABE, M.: The effect of heat on pollen viability of Japanese Black Pine and Japanese Red Pine. J. Japan. For. Soc 35 (8): 248—251 (1953).

Berichte

Organisatorische und technische Voraussetzungen zur Prüfung der Nachkommenschaften der Plantagenklone*)

Ein gesicherter züchterischer Erfolg ist nur über die Prüfung des Erbwertes der Ausgangsbäume und über die Prüfung der Kombinationseignung der Klone zu erreichen. Dazu haben wir heute zwei Verfahren: *die Klonprüfung und die Prüfung generativer Nachkommenschaften.* Die Langlebigkeit der Waldbäume, später Beginn der Mannbarkeit, lange Zeitspanne einer Generation und großer Flächenbedarf für Feldversuche stellen die Forstpflanzenzüchtung vor andere und z. T. schwierigere Probleme, als den Züchter landwirtschaftlicher Kulturpflanzen. So kann in der Forstpflanzenzüchtung mit ersten Ergebnissen erst in 30 bis 40 Jahren gerechnet werden. Da nur mit der Stetigkeit genauer und intensiver Bearbeitung später brauchbare Ergebnisse zu erwarten sind, muß man sich vor Beginn solch langwieriger und aufwendiger Arbeiten über Anlage, Durchführung und den Umfang der Arbeiten im Klaren sein. Zur rationellen und sinnvollen Durchführung ist die Aufstellung eines Arbeits- und Zeitprogramms unerläßlich. Ein solches Programm wurde für die Nachkommenschaftsprüfung von rund 400 Klonen in 10 Plantagen aufgestellt. Ausgegangen wurde von den Verhältnissen in Bayern. Alle Klone der wichtigen Plantagen sind damit zur Nachkommenschaftsprüfung erfaßt.

Zur technischen Durchführung: Die Klonprüfung ist relativ einfach, der Flächenbedarf gering, für einen Klon etwa 150 qm. Schwieriger und aufwendiger ist die Prüfung der generativen Nachkommenschaften. Als das geeignetste Verfahren, um die Kombinationseignung abschätzen zu können, erscheint die $N_1 \times N_2$ Testkreuzserie. Bei der Anordnung der Feldversuche mit den erhaltenen Nachkommenschaften wird v. a. darauf zu achten sein, daß die Parzellen so groß gewählt werden, daß die Individuenzahl im Alter des Versuchsabschlusses (30 bis 40 Jahre) 20 bis 30 beträgt. Es errechneten sich für die einzelnen Baumarten Parzellengrößen von 150 bis 256 qm pro Nachkommenschaft. Um Vergleiche über die Wuchspotenz und die Flächenproduktion der einzelnen Nachkommenschaften zu erhalten, wird vorgeschlagen 4 Nachkommenschaften ein und desselben Plantageklons in sogenannten Sekundärparzellen zusammenzunehmen. Jeder Klon erscheint auf diese Weise in einer Anzahl Sekundärparzellen zwischen 600 und 1025 qm. Damit wird eine sichere Berechnung der Massenleistung ermöglicht. Für die oft sehr großen Prüfanlagen empfiehlt sich die Planung mit unvollständigen Blocks, aber vollständigen Wiederholungen. Um Einblick in die Interaktion der Klone zum Standort zu bekommen,

*) Autorreferat über einen Vortrag, der anläßlich der 6. Tagung der Arbeitsgemeinschaft für Forstgenetik und Forstpflanzenzüchtung in Weinheim/Bergstraße gehalten worden ist (vgl. *Silvae Genetica* 8, Seite 123, 1959).