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On the Extraction of Forest-Tree Pollen from Inflorescences forced in a specially designed house

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When large series of controlled crossings are to be made, there is frequently risk of mixing the pollen from different trees, the catkins of which have been brought indoors at the same time for forcing.

To eliminate this source of error as completely as possible, and to increase the pollen yield, the Plant Breeding Station of the State Forestry, located at Humlebaek near Copenhagen, had in the year 1949 a house built and specially adapted for extraction of pollen.

Description of the Pollen-House

In this pollen-house (cf. *figs.* 1, 2) catkin-bearing twigs from each male are isolated in small, pollentight, glass-walled compartments, which need not be opened until the pollen is ready for collection.

Special arrangements are made for ventilation and renewal of water.

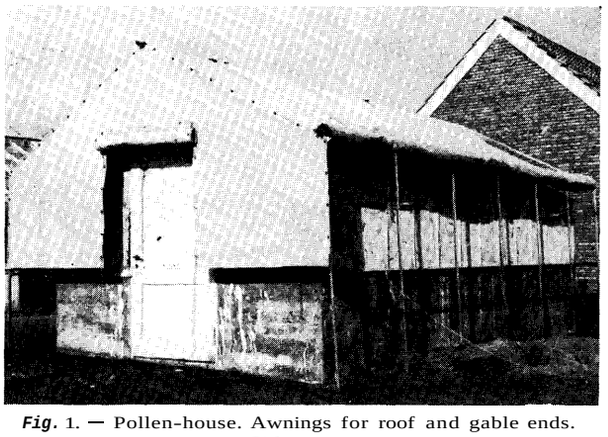

Fig. 1. — Pollen-house. Awnings for roof and gable ends. Side open.

The temperature in the pollen-house, or sections thereof, is regulated by awnings, supported by an iron framework and placed at a suitable distance from the glassroof and -walls to allow air to circulate freely between the awnings and the pollen-house.

The temperature inside each compartment is, within certain limits, controlled by means of infrared lamps.

The house is constructed on the principle of a greenhouse. Its outer base measurements are 3×6 m.

Within, on each side of a 1.5 m. wide central corridor, are 6 small glass-walled compartments, i. e. 12 seperate rooms for forcing and extraction of pollen.

Fig. 2 shows the corridor with compartments on either side. The floor of each compartment measures 103×80 cms. The height of the compartment towards the corridor is 126 cms., towards the outer wall 84 cms. All rabbets of the windows, etc., are sealed with foam-rubber strips.

Each compartment has two glass-doors opening on to the corridor. The lower glass pane of each door may be opened seperately *(fig.* 3). In the outer wall of each compartment is a small glass window for ventilation, cf. *fig.* 1, where the ventilation windows are just discernible.

To ensure ventilation without risk of contamination by foreign pollen, the openings of the ventilation windows, as well as those of the single lower panes of the glass doors, rnay be covered by pollen-proof material (aerotex), stretched on special metal frames.

Two water containers for catkin-bearing twigs are placed in holes in the floor of each compartment. These containers are actually irrigators, each one with an outlet under the floor of the compartment. Through plastic tubes connected to the outlets the containers may be filled or emptied from the corridor without opening the compartments (*fig. 2*).

To facilitate collection of pollen the floors of the compartments are covered with cellophane in which cuts are made for the containers. Foam-rubber strips are pressed around the edges of the cuts and the upper part of the containers to prevent entrance of extraneous pollen.

Use of the Pollen-House

Branchlets with male catkins are cut off at suitable lenghts, the end of the stems slightly pounded and put into the containers. These are then filled with water, the doors are closed and the forcing of the catkins may begin.

When the catkins are fully stretched and the anthers yellow, the forcing is finished, and the next step is to make the anthers discharge their pollen. This is done by a special extraction process.

The extraction process is best accomplished when the air-humidity is slightly reduced, but not so much that the anthers may shrink and their opening mechanism be destroyed. In order to reduce air-humidity the water in

Fig. 2. — Central aisle in pollen-house. Compartments on both sides for pollen forcing. — *a.* Two panes opened to permit insertion and removal of catkin-bearing twigs (cf. *fig. 3*). — *b.* Plastic tubings, through which water containers can be filled or emptied without opening compartments. — *c.* Vacuum cleaner used for collecting (sucking up) pollen.

the irrigators is first drawn off. If this does not suffice, the small infrared lamp is lighted, and at the same time the little window, seen on the right in *fig. 3* is opened.

When humidity is thus sufficiently reduced, the majority of the anthers open and the pollen falls on the cellophane at the floor of the compartment. The little window seen on the left, *fig. 3,* is then opened and the branchlets lightly shaken to release the remaining pollen.

The pollen is collected through the two small windows. It is sucked up by means of a special tube *(fig. 6),* connected by rubber tubing to the mouthpiece of a vacuum cleaner (D. Lewis, 1944). The arrangement is shown in *figs. 5 and 6.* Pollen is collected (sucked up) by slowly moving the mouthpiece with its gauze filter across the cellophane. This process is quick and effective.

When collecting is finished, the pollen is left to dry for a couple of hours in the open tube. The pollen can thereafter be stored in the tube, both ends of which must be tightly sealed.

If pollinators with very fine nozzles are used, an extra filtration of the pollen may prove necessary.

When the pollen has been removed from a compartment, the doors are closed, and before another compartment is opened for pollen collection, both doors at the ends of the pollen-house are opened for airing and the corridor sprayed with water to fix stray pollen.

As an additional precautionary measure to prevent access of extraneous pollen, the pollen may be collected through a funnel-shaped piece of plastic, fastened to the frame of one of the small windows before the forcing is started (cf. *fig. 5*).

Experiments on the Technique of Forcing and of Pollen Extraction

In addition to maximum security against contamination by foreign pollen, it is often of importance to get the greatest possible yield of pollen, f. inst. from young trees with few male inflorescenses, or from trees whose flowering for other reasons is scant.

From a practical point of view, it is also important to know how early catkins can be brought in for forcing, and how many days before pollen-shedding starts naturally on the trees, shedding can be achieved artificially in the pollen-house.

We have, therefore, tried to gain some more information on the following points:

1. The most suitable air-humidity and air-temperature during forcing and pollen-shedding in the house.
2. Treatment of the flower-bearing twigs during the above processes.
3. How early the forcing of the flower-bearing twigs may be started.
4. Time of pollen-shedding in the house as compared with outdoors.

In this connection experiments with different species of forest trees have been made in the pollen-house as well as in the laboratory.

Although these experiments are merely preliminary, we deem it expedient to state the results so far obtained.

Alnus incana

1. Air-Humidity and Temperature

In the laboratory, where the relative air-humidity was 30—60%, forcing was impossible unless the catkins were

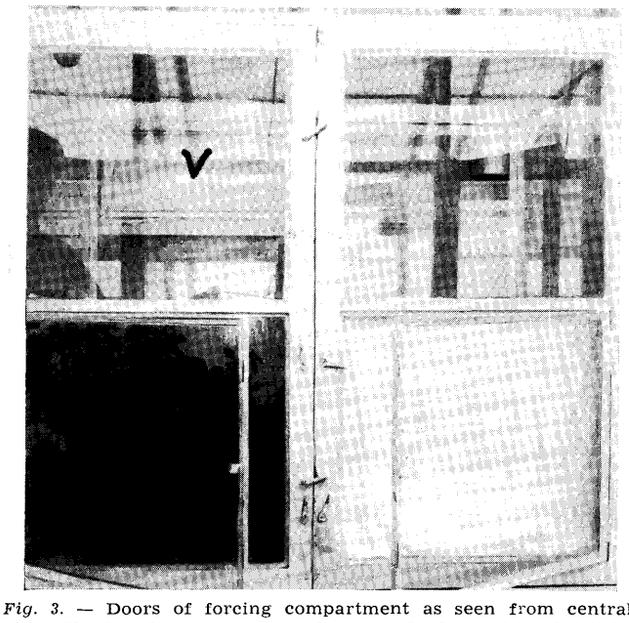

Fig. 3. — Doors of forcing compartment as seen from central aisle. The two lower panes can be opened; the one on the right is screened with pollen-proof material. — *V.* Ventilation pane in the outer wall.

protected against drying out. This was ensured by covering the catkin-bearing twigs with an airtight cellophane or plastic bag, firmly fastened with a rubber band around the container in which the twigs are placed, and thus forming a high-humidity chamber. The bags used must be roomy enough to permit the catkins to develop freely, but not so large as to preclude the required high-humidity. A temperature of 10—16° C. seems to be favourable for forcing in a humidity chamber. The pollen yield from catkins forced at a temperature of 24° C. was only one third of the yield from those forced at a temperature of 16° C. If relative humidity during forcing is kept permanently above 80%, no pollen will be released.

When the catkins are fully stretched and the anthers yellow, the bag is removed. Discharge of pollen was best at a temperature of about 15—18° C., and a relative air-humidity of 40—60%.

100 catkins, treated as above described, yielded 4 cubic cms. pollen.

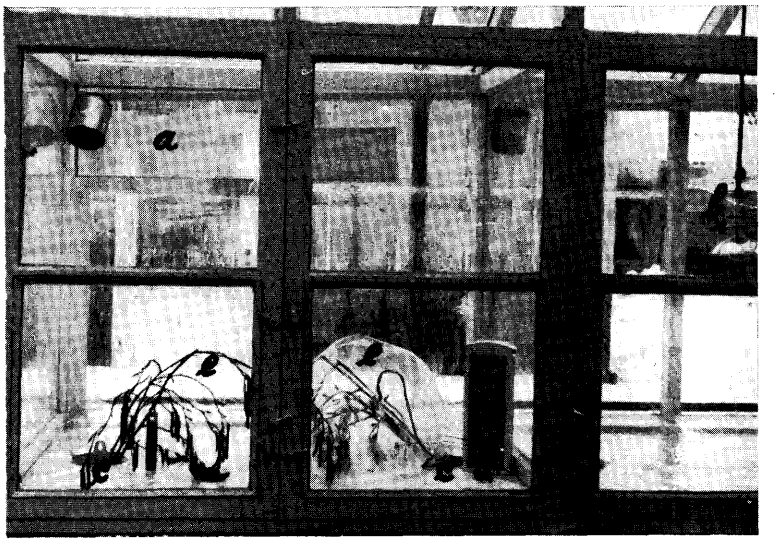

Fig. 4. — Forcing compartment seen from central aisle. The two doors on the left belong to the same compartment. — *a.* Ventilation pane in the outer wall. — *b.* Infrared lamp. — *c.* Water containers for twigs. — *d.* Psychrometer. — *e.* Catkin-bearing Alder twigs: on the right side in plastic bag used as humidity chamber, on the left without plastic bag.

Fig. 5. — Pollen from oak catkins is sucked up from the cellophane by means of a vacuum cleaner (cf. description page 3 and diagram *fig. 6*). The mouthpiece is operated through a plastic funnel to prevent contamination by foreign pollen.

In the pollen-house, during the period Feb. 21—April 1, it was possible to maintain the temperature between 8° C. at night and 15—20° C. at noon. The relative air-humidity in the house during this period varied between 60 and 80% but most of the time it was 70—80%.

As shown in *fig. 4* the forcing of catkin-bearing twigs in the pollen-house was effected both in and without plastic bags. The pollen quantities collected were in both instances approximately equal, i. e. 4—6 cubic cms. from 100 catkins, and it would therefore appear that the air-humidity and temperature in the house were suitable for forcing and shedding of *Alnus* pollen.

2. Treatment of the Flower-bearing Twigs

Normally the branchlets are cut off clean and the bark and stem pounded slightly at the end before they are placed in water in the pollen-house. In a few experiments the stems were cut and pounded and the water in the receptacles changed daily, while others were left untreated, and the water unchanged. There was no response in favour of daily treatment; nor was there any significant difference in yield of pollen from short twigs (20—30 cms) as compared with the yield of pollen from longer twigs (60—70 cms).

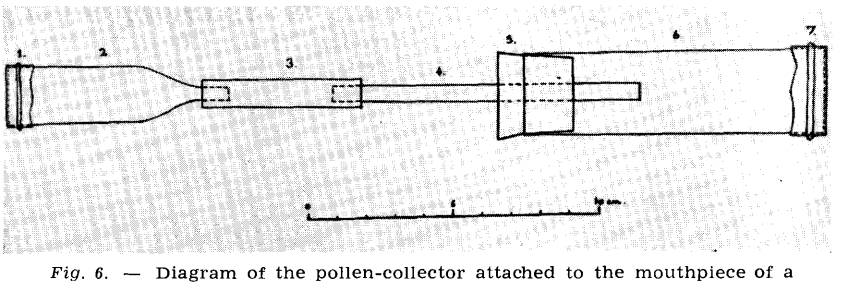

Fig. 6. — Diagram of the pollen-collector attached to the mouthpiece of a vacuum cleaner (cf. *fig. 5*). — *(1)* Gauce cover for filtering the pollen. — *(2)* Glass funnel, inside diameter 1.6 cms. — *(3)* Rubber connecting-tube. — *(4)* Glass tube, inside diameter 0.4 cms. — *(5)* Rubber stopper. — *(6)* Glass containers for sucked up pollen, inside diameter 2.5 cms. — *(7)* Pollen-proof material held in position by a rubber band. — *Note:* In *fig. 5* the funnel *(2)* is inserted directly in the rubber stopper *(5)* without the connecting links *(3)* and *(4)*.

3. Data Concerning the Beginning of Forcing and the Discharge of Pollen in the Pollen-House

In 1956 catkin-bearing twigs were taken in for forcing as follows:

Forcing began	Pollen Discharge started	Pollen Discharge finished
Feb. 21	Feb. 25	Feb. 28
March 1	March 4	March 7
March 17	March 19	March 24
March 28	March 30	April 1

4. Time of Pollen Discharge in the Pollen-House and under outdoor Condition

The trees used in the experiment discharged their pollen in their natural surroundings from March 28—April 4. From section 3 it will be seen, that in the pollen-house after forcing, pollen was discharged a month earlier than out of doors. This pollen was used in controlled pollinations and proved fully fertile. The branchlets brought first into the pollen-house yielded just as much pollen per 100 catkins, as those brought in last.

Under climatic conditions in Denmark, catkins of *Alnus incana* are frequently damaged by frost after stretching has started. If there is risk of such damage, it is advisable to take catkin-bearing twigs in for forcing before the catkins have started to stretch.

Larix decidua and Larix leptolepis

1. Air-Humidity and Temperature

In the laboratory where relative humidity was 60% and temperature 20° C., flower-bearing twigs, cut from the same tree were divided between two receptacles for forcing. The twigs in one of the receptacles were enclosed in a plastic bag as described above, the twigs in the other left free. The experiment, repeated several times, demonstrated that if flower-bearing twigs of *Larix* were brought in before the anthers were quite yellow and nearly ripe, the anthers would dry up, unless protected by the plastic bag. Inflorescenses with entirely unripe anthers (small and green) cannot normally be forced, even when covered by a plastic bag.

A similar series of tests was made in the pollen-house. Here too, it proved impossible to force unripe anthers to the stage of pollen discharge. Only when the catkins were nearly ripe this could be done successfully.

Consequently, we have so far only been able to complete the ripening process of the anthers, already started on the tree, and accelerate the pollen discharge.

The temperature in the pollen-house during forcing was 12—18° C., the relative humidity 85—95%. During pollen discharge, the temperature was 15—22° C. and relative humidity 80—90%. It is our impression that if the anthers are fully ripe, pollen is shed satisfactorily, even when the humidity is 80%.

About 1.0—1.44 cubic cms. pollen per 100 catkins was obtained.

2. Treatment of the Flower-Bearing Twigs

At the State Arboretum at Hoersholm, the following method has been in use for many years: Flower-bearing twigs are cut and taken indoors when the anthers are nearly ripe. They are placed in water for forcing in a heated room (about 20° C.). Just before pollen discharge begins, the twigs are cut into lenghts of 10—20 cms. and placed in pergamyne bags. These are sealed and strung up on a line in a room with temperature about 25° C. Pollen is then discharged in the bags.

If the anthers are nearly ripe when placed in bags, the method is satisfactory and the pollen yield is as large as in the pollen-house. If they are insufficiently ripe, the pollen yield is, however, not quite satisfactory.

In a few tests with unripe catkins using the Arboretum method, the yield was about 0.8 cubic cms. pollen per 100 inflorescenses, while if using the pollen-house, the yield was 1.2 cubic cms. per 100 inflorescenses.

No comparison of the viability of the pollen extracted by the two methods has so far been carried out.

3. Time for Beginning of Forcing; Beginning of Pollen Discharge

As mentioned before, we have not succeeded in forcing entirely immature inflorescenses, i. e. inflorescenses taken indoors earlier than 5—8 days before pollen discharge on the tree. It is, however, difficult to determine the stage of maturity of the inflorescenses, and the inflorescenses on the same twigs are, furthermore, not always at the same stage. We have, therefore, found it most expedient to postpone to take in flower-bearing twigs for forcing until most of the anthers are distinctly yellow and discernible with the naked eye. Consequently it has seldom been possible to force inflorescenses of Larch to pollen discharge more than 2 or 3 days before pollen discharge on the tree, except when the latter was delayed by inclement weather conditions.

Fagus silvatica

The extraction of beech pollen has only been studied one year, viz. 1956. The experiments were all carried out in the pollen-house.

1. Temperature and Air-Humidity

At the State Arboretum at Hoersholm, the experience of several years has proved the importance of a relatively high degree of humidity during forcing (P. Chr. Nielsen and M. Schaffalitzky, 1954).

The conditions of temperature and air-humidity in the pollen-house here described are shown in the following table:

Date	Hour	Temperature C°	Air-Humidity %
May 12	9	15	92
— —	13	12	89
May 14	13	18	82
— —	17	14	90
May 15	9	15	90
— —	13	21	83
May 16	13	18	91
— —	17	18	82
night		18	
May 17	9	14	90
— —	13	15	90
night		6	
May 18	9	11	90
— —	13	12	90
May 20	12	12	90
May 22	12	20	83

Flowering twigs from a single tree were taken into the pollen-house for forcing on May 11th., 15th. and 18th. respectively. The pollen yields from all three batches were good and varied between 1.0 and 1.6 cubic cms. per 100 inflorescenses. It is our impression that pollen was discharged without difficulty at air-humidity as high as 80%. As newly extracted beech pollen has a tendency to stick together in clumps, it is very important to give it an extra

drying. This has been done by using the infrared lamps above the pollen, while it is spread on the cellophane at the bottom of the pollen-chamber. After the pollen had been collected in a test tube, the open tube was left to dry for a couple of hours and thereafter hermetically sealed: no further tendency to clumping was observed.

2. Treatment of the Flower-Bearing Twigs

If no pollen-house is available, or any other room where relative air-humidity can be maintained above 80% during forcing, it is recommended to enclose the twigs in a plastic bag as described for *Alnus incana*.

If forcing may be expected to require several days, the stem ends of the twigs should be cut daily and the water in the receptacles changed. As during pollen discharge much pollen is caught on leaves and catkins, we have preferred to remove the catkins when the anthers are beginning to open, and place them on a fine wire netting screen in a compartment in the pollen-house, where temperature and air-humidity is suitable. When the anthers open, tapping the screen lightly will cause the pollen to fall down to the cellophane. From there it may be collected by suction.

A considerably larger yield of pollen is obtained by this method than by allowing the catkins to discharge pollen while on the branchlets. It is preferable to the method described for the larches, in which twigs bearing ripe inflorescenses were cut in pieces and put in bags.

3. Dates on which Forcing was begun and Pollen Discharge started and terminated in the Pollen-House

We can only give results from 1956, referring to a single tree from which, as stated, branchlets were cut and taken indoors three times:

Forcing began	Pollen Discharge started	Pollen Discharge finished
May 11	May 16	May 19
May 15	May 19	May 23
May 18	May 22	May 25

4. Time of Pollen Discharge in the Pollen-House and outdoors on the Tree

The above mentioned tree began to shed pollen on May 21-22, i. e. 0-5 days after the pollen discharge started in the pollen-house from forced catkins.

It was thus possible to obtain a good yield of fully fertile pollen (tested by controlled pollination) from inflorescenses taken indoors 10, 6 or 3 days respectively before pollen discharge outdoors began.

It is, however, possible that the pollen discharge outdoors was somewhat delayed by the cold weather prevailing during the month of May 1956, and that the comparatively early pollen discharge, obtained in the pollen-house, was partly due to this climatic condition.

Quercus robur and Quercus petraea

In connection with controlled crossings between these two oak species we have for several years practised forcing of catkin-bearing twigs and extraction of pollen. No actual experiments have been made, but our observations may be summarized as follows:

When the catkins began to stretch and were pale yellow, catkin-bearing twigs were placed in the pollen-house for forcing. If taken in too early, the stretching will usually stop. It has been possible to force catkins to pollen discharge 2-5 days before pollen discharge outdoors, dependent on the weather conditions.

It is very important that relative humidity is kept high (80-90%) during forcing. The temperature, therefore, should not be too high, maximum 20-22° C.

Evaporation from the rather large number of leaves, which develop on the branchlets in the forcing compartments, will often produce the desired humidity, but sometimes it may be so high, that pollen discharge is impeded or prevented, and if pollen is discharged, a large part of it will stick to the leaves.

These difficulties may be avoided if most of the leaves and buds are removed before the catkin-bearing twigs are placed in the compartments. The results are satisfactory, but the procedure is rather time-consuming.

The best results have, however, been obtained by forcing in high-humidity and thereafter removing the fully stretched and ripe catkins. After removal the catkins are placed on fine wire nettings as described in the section concerning beech; pollen discharge follows promptly, and the pollen may easily be sucked up.

Species of Picea and Pinus

Pollen extraction from *Picea* and *Pinus* does not as a rule involve any serious problem.

If the male catkins of these species are not fully ripe, we usually place the twigs in water for forcing as described above. If, however, the catkins are ripe, the catkin-bearing twigs are simply placed on the cellophane on the floor of the compartments, or, following the method used at the Arboretum (cf. p. 22), they are placed in bags, where the pollen is usually discharged without difficulty.

Summary

As far as can be ascertained at the present moment the pollen-house fulfills the requirements for protection against contamination by foreign pollen, and the facilities for adjustment of temperature and air-humidity have considerably increased the pollen output. Furthermore the described technique for collecting the discharged pollen by suction is effective and labour-saving.

The experiments here described have shown the importance of maintaining a high air-humidity and a relatively low temperature (max. 15-20° C.) during the forcing period.

As the pollen-house has no installation for maintaining a definite, desirable degree of humidity and temperature, it has not been possible to make experiments in which these two factors were varied systematically. The pollen output, as quoted by us, cannot therefore be assumed to be the maximum obtainable, but it must be considered satisfactory for practical purposes.

The ideal solution would of course be a pollen-house with installations for complete control of temperature and humidity, but it is questionable whether the expected increase in pollen output would justify the considerable increase in costs.

If plant breeding institutions dispose of greenhouses with automatic humidity control, it is possible that the cheapest solution would be to make transportable, pollen-proof cages for forcing. These cages could be kept in the greenhouse during forcing and then moved to a room with temperature and air-humidity suitable for pollen discharge.

Zusammenfassung

Titel der Arbeit: *Über die Gewinnung von Waldbaumpollen aus in einem Spezialgewächshaus angetriebenen Blüten.* —

Soweit dies im Augenblick zu beurteilen ist, erfüllt das Pollenhaus alle Voraussetzungen zur Sicherung gegen Verunreinigung mit fremden Pollen und bewirkt durch leichte Regulierbarkeit der Temperatur und Luftfeuchtigkeit eine Erhöhung des Pollenertrages. Außerdem ist die beschriebene Aufsaugtechnik zum Einsammeln des ausgestäubten Pollens wirksam und arbeitssparend.

Die beschriebenen Experimente zeigen die Bedeutung der Erhaltung hoher Luftfeuchtigkeit und relativ niedriger Temperatur (Max. 15-20° C) während der Antreibezeit.

Da das Pollenhaus keine Einrichtung besitzt, Temperatur und Luftfeuchtigkeit konstant zu halten, konnten keine Versuche mit systematischer Variierung dieser beiden Faktoren unternommen werden.

Es kann daher nicht angenommen werden, daß die von uns erhaltene Pollenmenge das mögliche Maximum ist. Sie genügt jedoch für die praktischen Zwecke.

Die Ideallösung würde zweifellos ein Pollenhaus mit Einrichtungen für eine vollständige Kontrolle der Temperatur und Luftfeuchtigkeit sein, aber es ist fraglich, ob der zu erwartende Anstieg des Pollenertrages die damit verbundenen beträchtlichen Mehrkosten rechtfertigen würde.

Für Züchtungsinstitute mit temperatur- und feuchtigkeitsgesteuerten Gewächshäusern würde die billigste Lösung darin bestehen, transportable Pollengewinnungskabinen zum Antreiben anzufertigen. Diese Kabinen könnten während des Antreibens in das Gewächshaus gestellt und dann in Räume mit einer der Pollenentleerung günstigen Luftfeuchtigkeit und Temperatur gebracht werden.

Résumé

Titre de l'article: *Extraction du pollen des arbres forestiers à partir d'inflorescences forcées dans une chambre spéciale* —

Autant qu'on puisse en juger à l'heure actuelle, cette chambre à pollen répond aux exigences voulues pour la protection contre la contamination du pollen étranger, pour la commodité du réglage de la température et de l'humidité : elle a permis d'augmenter de façon considérable la production du pollen. En outre, la technique décrite pour la récolte par succion du pollen disséminé est efficace et rapide.

Les expériences décrites ont montré l'importance du maintien d'un état hygrométrique élevé et d'une température relativement basse (15-20° C) pendant la période de forçage.

La chambre à pollen ne possédant pas de dispositif pour le maintien à un degré donné de la température et de l'humidité, il n'a pas été possible d'entreprendre des expériences en faisant varier ces deux facteurs. La production de pollen que nous avons obtenue ne peut donc être considerée avec certitude comme un maximum, mais simplement comme satisfaisante pour les besoins de la pratique.

La solution idéale serait évidemment une chambre à pollen avec un contrôle complet de la température et de l'humidité, mais is est douteux que l'accroissement de la production de pollen qu'on pourrait en attendre compense l'augmentation importante du coût de l'installation.

Si les stations d'amélioration possèdent des serres avec contrôle automatique de l'humidité, il est possible que la solution la moins coûteuse consiste à réaliser des cages étanches, transportables, pour le forçage. Ces cages pourraient être placées dans la serre pendant le forçage et ensuite transportées dans une pièce à température et humidité convenables pur la dissémination du pollen. —

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