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Pollination in *Betula pendula* Roth. and *Betula pubescens* Ehrh.

Bestøvning af Betula pendula *Roth. og* Betula pubescens *Ehrh.*

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Summary

In order to get knowledge of pollination capacity in *Betula pendula* Roth. and *Betula pubescens* Ehrh. fruit set was investigated in selected clones.

In *B. pendula* 3 clones were placed in couples in greenhouses at low temperatures in January. Investigations have been performed in 1983, 1984 and 1985. In 1983 the clones were handpollinated by brush. In 1984 and 1985 the examined clones were pollinated by artificial wind. Selection of clones in the experiments was based on value of cultivation.

The overall fruit set registered in 1983 was lower than fruit set observed in 1984 and 1985. Fruit set was excellent in 1984 and 1985 except in one combination examined in 1985 where low fruit set was recorded. Low fruit set has also been observed in this combination in 1983 but in 1984 fruit set was nearly 1.00. By examination of cross incompatibility in this combination in 1985 also few pollen tubes were recorded to reach the basis of the style.

In 1985 clones of *B. pubescens* were isolated two by two in greenhouses at different temperatures. Clones grown at 20°C (day) resulted in low fruit set by wind pollination. Clones kept above 0°C in winter months and in totally unheated conditions resulted in excellent fruit set.

High temperature was also seen to affect pollen tube growth in the style. Only few per cent pollen tubes succeeded to reach the basis of the style. Increased incompatibility or decreased pollen viability seems to result from higher temperatures.

Forcing of *B. pendula* and *B. pubescens* in greenhouses at low temperatures seems to ensure earlier pollination without the risk of contamination from plants grown outdoors.

Key words: *Betula pendula, Betula pubescens,* intraspecific cross pollination, fruit set, germination, cross incompatibility, forcing temperature, blooming period, pollination methods.

Resumé

For at opnå kendskab til befrugtningsevnen hos *Betula pendula* og *Betula pubescens* er udvalgte kloner undersøgt for deres evne til at sætte frugt ved krydsbestøvning. Klonerne er udvalgt i forsøg ud fra disses dyrkningsværdi.

Hos *B. pendula* er der i 1983, 1984 og 1985 krydsbestøvet på kloner, der parvis har været isoleret i væksthus. I 1983 blev klonerne håndbestøvet med pensel. I 1984 og 1985 bestøvedes med kunstig vind (støvsuger). Ved håndbestøvning med pensel blev der generelt fundet lavere frugtsætning end ved vindbestøvning. Ved bestøvningerne i 1984 og 1985 blev der fundet rigelig frugtsætning på nær én kom-

bination med lav frugtsætning. Samme kombination viste sig at have lav frugtsætning i 1985, men nær 1,00 i 1984. Pollenvækst i grifler blev fulgt i denne kombination i 1985. Kun en lille procentdel af griflerne havde pollenrør, der havde gennemvokset griffelen. Der kan være tale om semikompatibilitet i denne kombination. Hos *B. pubescens* blev 4 kloner parvis krydsbestøvet ved blæsning med støvsuger i 1985. Effekten af forskellige drivtemperaturer blev undersøgt. Alle kombinationer, der blev drevet ved temperaturer over 0°C eller i totalt uopvarmede huse, resulterede i god frugtsætning. Planter, der blev drevet ved 20°C (dag), gav derimod lav frugtsætning. Frugterne blev dannet, men faldt af efter en tid. Undersøgelse af krydskompatibilitet i den kombination, der blev drevet ved 20°C (dag), viste meget lav pollenrørsvækst i hele griffelens længde i krydset den ene vej og ingen spirede pollenrør i krydset den anden vej. Høje drivtemperaturer ser ud til at ændre kompatibiliteten eller pollenvitaliteten.

Drivning af *B. pendula* og *B. pubescens* gav tidligere blomstring end i de samme arter, der voksede på friland. Det blev derved muligt at hindre kontaminering med pollen udefra.

Nøgleord: *Betula pendula, Betula pubescens,* intraspecifik krydsbestøvning, frugtsætning, spiring, krydsinkompatibilitet, drivtemperatur, blomstringsperiode, bestøvningsmetode.

Introduction

Both *Betula pubescens* and *Betula pendula* commonly occur in Denmark. *Betula spp.* have unisexual flowers male and female catkins being born on the same tree. When growing wild they are pollinated by wind. The fruit is a winged nut containing the seed (2).

Propagation of *Betula pubescens* and *Betula pendula* by seed often shows considerable variation when the fruits collected are results of free pollination (3).

Hybrid formation has been observed between *B. pubescens* and *B. pendula* both in natural habitats and in controlled experiments (3,5,7). Several intermediate forms have been described between the diploid *B. pendula* Roth. (2n=28) and the tetraploid *B. pubescens* Ehrh. (2n=56), the gene flow probably proceeding from the diploid level to the tetraploid level (4).

To obtain seeds with desired qualities, controlled seed production is needed, e.g. in seed orchards.

Before planting out of clones with desired characteristics for seed production it is neccessary to obtain information about pollination capacity in the plant material.

In these investigations controlled pollination experiments were carried out in 1983–85. Selection of clones has been performed in experiments based on value of cultivation.

B. pubescens and *B. pendula* are reported to be highly self incompatible (5). No efforts have been made to self fertilize the plants included in these experiments. Tests are limited to examination of intraspecific incompatibility/inviability.

Material and methods

3 clones of *Betula pendula* (clone no. 9281-1, 9282-2, 9283-3) and 4 clones of *B. pubescens* (clone no. 1033-1, 1033-2, 9506, 9507) were included in the investigations in 1983–1985. The clones were isolated in couples in greenhouses at different temperatures. *B. pendula* was kept at 2 different conditions. *B. pubescens* was kept at 3 different conditions. In 1985 the flowering season was registered and compared to flowering season in *Betula pendula* and *Betula pubescens* grown outdoors.

In 1983 the female flowers were handpollinated transferring pollen by a small brush. In 1984 and 1985 the clones were pollinated by blowing with a vacuum cleaner. Pollination was repeated 2–6 times during the flowering season. On selected branches the development of female catkins was followed from blooming to fruit set. Each combination was registered on two or more

mother plants using 3–4 branches with flowers at the same stage. The fruits were harvested when the catkins became brown and the the scales of the individual flowers began to loosen.

The seeds were stored at 3–5°C. Germination of seeds was registered in seeds from crosses in 1985. The seeds were sown in boxes in December 1985 and kept at 8°C in a greenhouse for 2–3 months. 1 g seed per combination was sown.

Cross compatibility was examined in 1985 in 4 clones. Female catkins were fixed in the fixative FAA (formalin, acetic acid, ethanol) 1:1:7.5 nine days after the first pollination. Fixation time was 1–2 hours. The fixed catkins were stored in 70% ethanol at ÷18°C. At least 4 catkins and 160–570 styles were examined for each combination. The styles were macerated in 4 N NaOH for 45 min. at 60°C. The styles were washed and squashed in 0.1% Analine-blue in 0.1 N K_3PO_4. In UV-microscope flourescence of the pollen tubes is followed. Pollen tubes grown to the basis of the style were used as indication of compatibility.

Table 1. *Betula pubescens.* Blooming period of plants placed in different greenhouse conditions in 1985.

Female plant	Male plant	Blooming period	Greenhouse conditions
1033-1	1033-2	19/4–5/5	1
1033-2	1033-1	19/4–5/5	1
1033-1	1033-2	20/3–29/3	3
1033-2	1033-1	20/3-29/3	3
1033-1	1033-2	16/4–24/4	2
1033-2	1033-1	10/4–24/4	2
9506	9507	1/4–19/4	2
9507	9506	4/4–19/4	2

[1] No temperature regulations, temperature in Jan.–Feb. often below 0°C.
[2] Temperatures kept above 0°C in winter.
[3] Temperatures from the first of Feb. day about 20°C.

Table 2. *Betula pendula.* Blooming period of plants placed in different greenhouse conditions in 1985.

Female plant	Male plant	Blooming period	Greenhouse conditions
9281-1	9282-2	18/3–27/3	2
9282-2	9281-1	18/3–27/3	2

[2] Temperatures kept above 0°C in winter.

Results

In *Betula pubescens* and *B. pendula* plants growing outdoors in 1985, the male flowers in the catkins started pollination 7 May.

Blooming of the isolated plants of *B. pubescens* and *B. pendula* was hastened when plants from January onwards were placed in greenhouses in containers, table 1 and table 2. Plants placed in 20°C (day temperature) were blooming 20–29 March whercas almost unheated (condition 2, table 1) and totally unheated conditions (condition 1, table 1) resulted in blooming from 1–24 April, 19 April – 5 May respectively.

Table 3. *Betula pendula.* Cross pollination – estimated by fruits per pollinated flower.

Female plant	Male plant	Fruits per pollinated flower	Experimental year
9281-1	9282-2	0.33	1983
9281-1	9283-3	0.55	1983
9282-2	9281-1	0.67	1983
9283-3	9281-1	0.71	1983

Pollinated by a small brush.
Plants kept in greenhouses without temperature regulations.

Table 4. *Betula pendula.* Cross pollination – estimated by fruits per pollinated flower in two experimental years.

Female plant	Male plant	Fruits per pollinated flower	Experimental year
9281-1	9282-2	0.99	1984
9281-1	9282-2	0.25	1985
9281-1	9283-3	0.83	1984
9282-2	9281-1	1.00	1984
9282-2	9281-1	0.94	1985
9283-3	9281-1	1.00	1984
LSD		0.24	

Pollinated by blowing with a vacuum cleaner.
Plants kept in greenhouses without temperature regulations or with temperatures just above 0°C in winter.

Betula pendula

Cross pollinations in four combinations investigated in 1983 resulted in relatively low fruit set (table 3) compared to fruit set in the same combinations examined in 1984 and 1985 (table 4) except in the case with lowest fruit set. The lowest fruit set was found in the combination 9281-1 mother plant pollinated by 9282-2, the value being 0.33.

The mother plant 9281-1 pollinated by 9282-2 investigated in 1985 resulted in 0.25 fruits per pollinated flower whereas in the same combination 0.99 was measured in 1984.

Fruit set in the other combinations examined in 1984 and 1985 ranged from 0.83 to 1.00 and in 1983 from 0.55 to 0.71 respectively.

Intraspecific incompatibility was examined in 1985 in the reciprocal cross 9281-1 × 9282-2. In 49% of the investigated styles, germinated pollen tubes reached the basis of the style in the combination with 9282-2 as mother plant. Only 19% did so with 9281-1 functioning as mother plant (table 5). In the other styles examined, pollen tubes grew through part of the style or pollen tubes failed to germinate on stigma.

Germination of seeds was observed in both 9281-1 and 9282-2 in crosses from 1985.

Table 5. *Betula pendula.* Cross pollination – Cross incompatibility – estimated to what extent (%) germination of pollen occurred in the style.

Female plant	Male plant	Pollen tubes to the basis of style	Pollen tubes in part of style	No pollen tubes
9281-1	9282-2	19%	7%	74%
9282-2	9281-1	49%	9%	42%

Betula pubescens

The different temperatures in the greenhouses during flower development influenced the results of fruit set in the reciprocal cross 1033-1 × 1033-2 examined in 1985.

In greenhouses unheated (condition 1, table 6) or temperatures kept just above 0°C (condition 2, table 6) in winter the yield was 0.81 – 0.96 fruits per pollinated flower.

Table 6. *Betula pubescens.* Cross pollination – estimated by fruits per pollinated flower.

Female plant	Male plant	Fruits per pollinated flower	Greenhouse conditions
1033-1	1033-2	0.92	1
1033-1	1033-2	0.91	2
1033-1	1033-2	0.38	3
1033-2	1033-1	0.81	1
1033-2	1033-1	0.96	2
1033-2	1033-1	0.32	3
LSD		0.54	

[1] No temperature regulations, temperatures in Jan.–Feb. often below 0°C.
[2] Temperatures kept above 0°C in winter.
[3] Temperatures from the first of feb. day about 20°C.

Plants kept at temperatures of about 20°C (condition 3, table 6) resulted in low fruit set, 0.32 and 0.38 fruits per pollinated plant were recorded.

In the combinations 1033-1 × 1033-2 kept at 20°C (condition 3, table 6), intraspecific cross incompatibility was investigated. The results in table 8 indicate that few pollen tubes succeeded in reaching the basis of the style. 1033-1 functioning as mother plant, no pollen tubes were observed at all.

In the reciprocal cross 9506 × 9507 (condition 2, table 7) excellent fruit set was observed, 0.87 and 0.93 respectively. Germination of seeds was observed in all the crosses investigated but one. In the reciprocal cross 1033-1 × 1033-2, where the plants were kept at 20°C during flower development, no germination had occurred by the end of March 1986.

Table 7. *Betula pubescens.* Cross pollination – estimated by fruits per pollinated flower.

Female plant	Male plant	Fruits per pollinated flower	Greenhouse conditions
9506	9507	0.87	2
9507	9506	0.93	2

[2] Temperatures kept above 0°C in winter.

Table 8. *Betula pubescens.* Cross pollination – Cross incompatibility – estimated to what extent (%) germination of pollen had occurred in the style.

Female plant	Male plant	Pollen tubes to the basis of style	Pollen tubes in part of style	No pollen tubes
1033-1	1033-2	0%	0%	100%
1033-2	1033-1	3%	72%	25%

Discussion

In *Betula pendula* and *B. pubescens* the examined intraspecific combinations have shown excellent fruit set with few exceptions.

In *B. pendula* the mother plant 9281-1 pollinated by 9282-2 seems to differ in fruit set every second year. The low fruit set observed in 1985 was supported by observation of a low degree of pollen tubes grown to the basis of the style in this combination. Intraspecific cross incompatibility and semicross incompatibility have been observed in intraspecific crosses of *B. pubescens* (5). Incompatibility reactions in *Betula spp.* have been suggested to be under gametophytic control (5,6). Further examinations of the combination of *B. pendula,* the mother plant 9281-1 pollinated by 9282-2 are needed with respect to pollen tube growth and fruit set before anything certain can be concluded about cross incompatibility. The observation of fewer pollen tubes registered at the basis of the style in the mother plant 9282-2 compared to fruits per pollinated flower, is in agreement with the fact that only a certain percentage of the fruits produced contain filled seeds (1).

In *B. pendula* high temperatures have been seen to influence the compatibility system, the incompatibility reaction being increased at high temperatures (5).

Results found in these experiments with low fruit set and few pollen tubes grown to the basis of the style in *Betula pubescens* plants placed at 20°C during flower development and flowering, correspond well with those earlier observations (5). No efforts have been made to study pollen viability. A decrease in pollen viability at high temperatures may also explain the results found here.

In plants kept at lower temperatures, no negative effects of temperatures can be measured. Temperatures at 10–12°C are said to favour fertilization (7).

Forcing of flower development by higher temperatures seems to secure controlled crosspollination of desired combinations without the risk of pollen contamination from plants grown outdoors. Pollination of *Betula spp.* seems to be more successful when using artificial wind than when using a brush. Probably it is difficult to leave as much pollen in the flowers in the latter case as in the former.

Allthough the blooming period is registered from few days to 25 days, it is important to follow the onset of flowering because female flowers are reported to be fully receptive one day before the male flowers. The stigmas remain receptive for a few days, by day no. 6 they usually blacken and die (7).

Germination was observed in most of the investigated clones from 1985. In the clones kept at 20°C during flower development, none of the seeds had germinated by the end of March, probably because of insufficient pollination in the clones.

Germination per cent has not been registered in these experiments. In future research it would be valuable to obtain knowledge of germination per cent. Only 15–20 per cent of seeds sown are recorded to produce 1–0 seedlings (1).

Conclusion

The conclusions drawn here only concern results in pollination studies.

The investigated clones of *Betula pubescens* resulted in excellent fruit set under optimal conditions and the examined clones seem to be suitable for controlled seed production. However, variation in fruit set year by year has not been investigated.

In *Betula pendula* the reciprocal cross 9281-1 × 9283-3 clones resulted in excellent fruit set and seem usable for controlled seed production.

Further investigations should be made with the mother clone 9281-1 to clarify incompatibility reactions when 9282-2 is used as pollen donor.

By isolating clones of *Betula pubescens* and *B. pendula* in greenhouses to obtain controlled seed production, careful measurements should be made to keep the temperature well below 20°C during flower development and pollination period. The optimal temperature is reported to be 10–12°C. Examination of the progeny from the crosses involved in these experiments is still proceeding. The finally selection of clones for controlled seed production has to await results from progeny testing.

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