PNFI Technical Reports

Petawawa National Forestry Institute, Chalk River, Ontario K0J 1J0, Canada

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POLLINATION TECHNIQUES I. POLLEN COLLECTION

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INTRODUCTION

In most tree improvement programs, the production of progeny with known pedigree through controlled pollination is an essential component, especially after the first generation. The Tree Genetics and Breeding Project of the Petawawa National Forestry Institute (PNFI) has been producing control-pollinated seedlots for research into tree genetics for more than 40 years.

This is the first of three technical notes describing the procedures used at PNFI in the production of control-pollinated seedlots. Pollen collection techniques are presented here, together with information on the timing of dehiscence (pollen release) for different species, based upon data gathered over the past 40 years. In the text, the scientific terms "microstrobilus" and "megastrobilus" are occasionally referred to using the colloquial terms "pollen flower" and "female flower" respectively. Subsequent notes will describe pollen handling and the pollination process, and the pattern and timing of female flower development.

For more detailed information on pollen and seed biology and ontogeny, the reader is referred to Stanley and Linskens (1974), USDA Forest Service (1974), USDA Forest Service (1981), and Owens and Blake (1985).

POLLEN COLLECTION

Breeding at PNFI has been conducted for experiments mainly involving the genera *Pinus*, *Picea*, and *Larix*. Each genus has different flowering characteristics, which necessitate particular expertise to best obtain the required amounts of viable pollen. Two techniques in particular have been used:

1) the collection of microstrobili prematurely, with final maturation occurring under controlled laboratory conditions;
2) frequent monitoring of microstrobili in the field and collection at the first indication of dehiscence.

Regardless of the genus, the closer to natural dehiscence at collection time the better because there is less chance for the pollen flower to abort, thus allowing for maximum pollen production.

Pinus

Pollen flower clusters are found in the lower part of the crown, mostly on older lateral branches (USDA Forest Service 1974). They are of sufficient size such that the stages of development to maturity are readily recognized (Figure 1). The earliest collection stage is recognized at a point when the microstrobili begin to show flexibility; when squeezed the pollen will form a damp paste which, when rolled between one's fingers, will almost dry to a powder. Collections can be made at this stage, which may be as early as three days prior to natural dehiscence. The collected microstrobili are allowed to mature on wire mesh trays in a controlled laboratory environment as described in part II of this series (Copis 1990). At a later stage of natural development, the microstrobili become swollen and the scales flex easily (Figure 2). This is the best time for collection; if left too long, the microstrobili will shed the pollen when touched (Figure 3). It is critical, therefore, that the pollen flowers be collected before this stage.

Picea

Pollen flowers are generally well distributed in the midcrown. Unlike in the pines, pollen flowers on spruce do not occur in clusters (Figure 4), and much greater effort is required to produce ample quantities of pollen. Although forced development of spruce microstrobili is possible (USDA 1974 - see Table 1), early

removal of microstrobili from branches for pollen extraction has not met with success as in the pines. The microstrobili are much more sensitive and readily abort, unless collected toward the extreme latter stages of development, just prior to natural dehiscence.

Thus it is critical to closely monitor maturation of the pollen flower in the field. The time of collection for any individual tree is very important and ideally should be at the first indication of dehiscence. This generally occurs first in microstrobili located on the southerly side of the upper crown. As the microstrobili mature, they become swollen and increasingly flexible, and appear to form cracks on their surface. The substance formed when the microstrobilus is crushed progresses from a paste to a powder with increasing maturity. The colour of the pollen flower remains purplish and, at dehiscence, the pollen releases from the scales almost without warning (Figure 5 shows pollen flowers after dehiscence has occurred). It is essential, therefore, that as dehiscence approaches, monitoring be increased to determine the optimal time of collection. Even a couple of hours, given the appropriate weather conditions, can make the difference between success or failure.

Larix

Pollen flowers in larches (along with female flowers and short shoots) occur on the twigs and branches throughout the entire crown (Figure 6). *Larix* buds are the first of the conifers to mature sexually and the larches are the most difficult of the genera discussed so far with which to gain any degree of success; often, cut branches placed in a greenhouse environment to force the pollen are the only practical solution (see Table 1). The ideal collection period, however, is at the first indication of dehiscence. Do not be tardy in monitoring because, within a 2-hour period on a sunny and breezy day, clouds of pollen will escape and collection will have been missed.

FACTORS AFFECTING DEHISCENCE

The period of dehiscence will vary for several reasons. The most obvious is the physiological difference in flower development from species to species. As well, annual variation of pollen shed is commonplace and results from differing temperature and weather conditions.

The variation in timing of dehiscence for seven species of conifers, based upon data collected over the past 40 years at PNFI, is presented in Table 2. Annual variation is clearly evident, as is the fact that despite such variation, the species tend to shed pollen in the same relative order from year to year. The years 1956 and 1964 represent the extremes of late and early pollen release, respectively, for the various species pollinated in those years. Temperature information for those years (Table 3) indicates that 1956 experienced an unusually cold spring (May in particular), whereas the spring of 1964 was atypically mild.

Wet conditions associated with seasonable temperatures do not seem to slow development. Pollen flowers continue to mature under these conditions, but do not release their pollen until the first occurrence of warm, dry weather. If continuous rains occur after the microstrobili mature, pollen release may occur in pines (Stanley and Linskens 1974). Given continuous wet or humid conditions, the matured microstrobili should be collected and allowed to dry under controlled laboratory conditions.

Within clones, the development of the microstrobili is very uniform. More variation occurs within families of full-sib and half-sib origin and the monitoring process must be increased accordingly because there is genotypic variation that may be reflected in the maturation process of the microstrobili.

Dehiscence may occur over a span of time ranging from a few hours to several days, depending on the family and the degree of relationship (full-sib or half-sib) within the family. This can result in problems; for example, receptive females from one family cannot be pollinated by another family whose pollen has yet to mature.

In addition, pollen flowers will mature at different rates depending on their position on a tree. For example, those with a southerly exposure will generally be the first to shed pollen. At the first indication of dehiscence, aided by a flick of the finger, collection must be made usually for the whole tree. This is the most critical period and monitoring must be conducted several times a day to guarantee results. At this stage, the microstrobili will generally release their pollen within 24 hours in a regulated laboratory environment.

For a variety of reasons such as the variability in dehiscence, difficult field conditions, or collection from unpredictable genera, specific crosses may necessitate that microstrobili be collected and forced prematurely to meet the female's period of receptivity. Branches have then to be cut and placed in buckets of water in a warm greenhouse. The viability of pollen produced this way is generally as good as that occurring under natural con-

Table 1. Conditions for forcing male strobili for different genera.

Genus	Air temperature (°C)	Tungsten filament light			Collection timing[a]
		Power (W)	Distance (m)	Exposure time per day (h)	
Larix	20	200	1.5	24	3
Picea	27	600	1	20	8
Pinus	27	600	1	20	4

Source: USDA Forest Service (1974).
[a]Weeks before anticipated natural dehiscence.

Figure 1

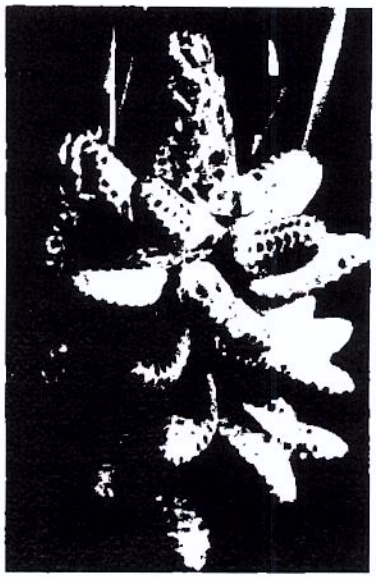


Figure 2

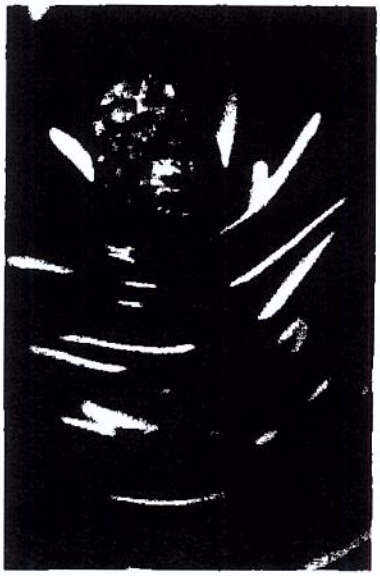


Figure 4

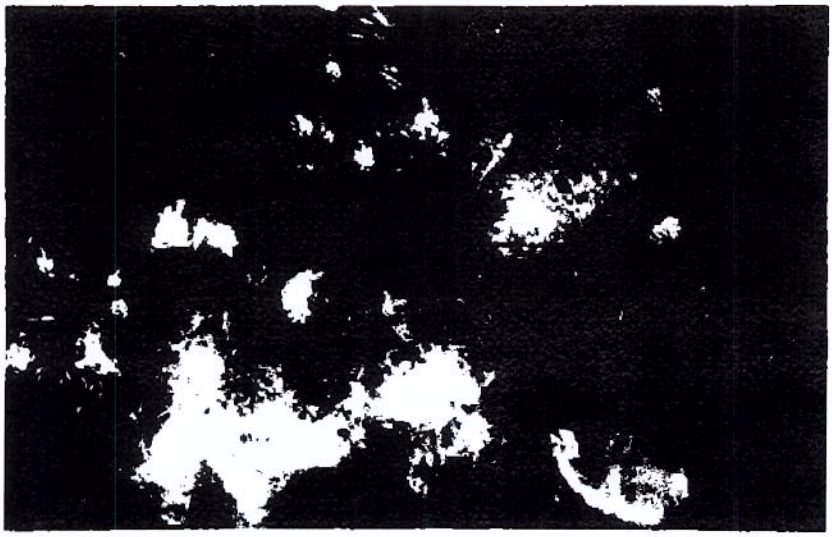


Figure 3

Figure 5

Figure 6

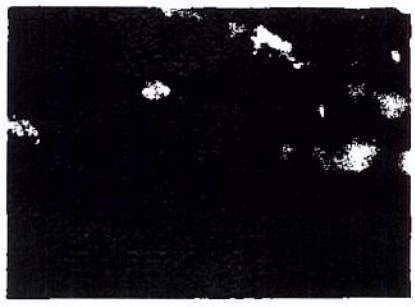
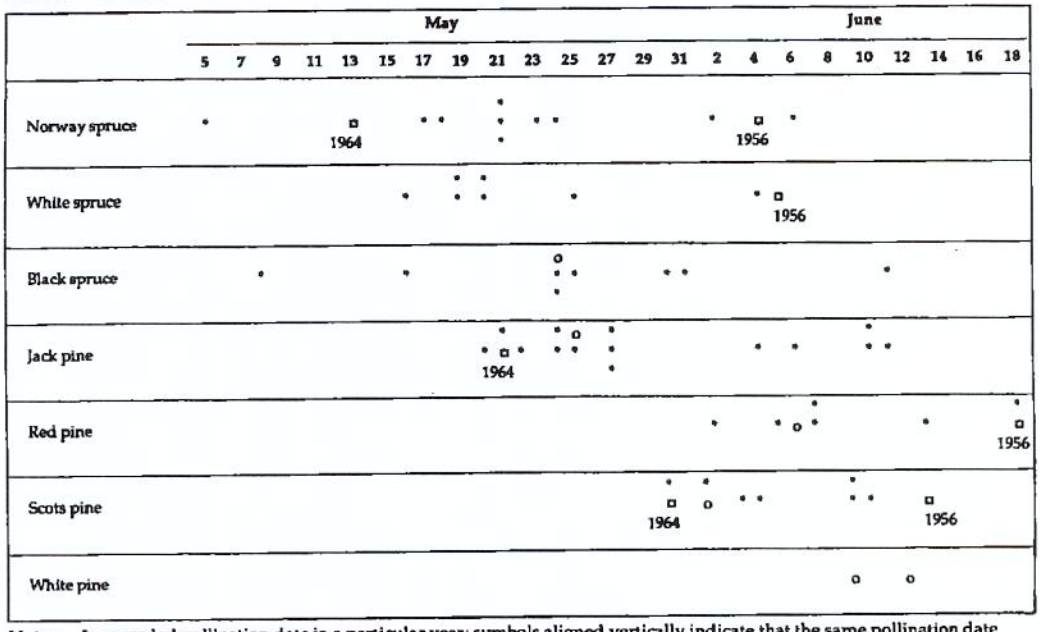


Table 2. Timing of natural dehiscence for seven conifer species at PNFI.



Notes: * recorded pollination date in a particular year; symbols aligned vertically indicate that the same pollination date was recorded for different year.
o observed date of pollen release in a particular year.
□ recorded pollination date showing extremes of late and early pollen release for the various species pollinated in the extreme years 1964 and 1956.

Table 3. Temperatures (°C) in 1956 and 1964.

	April		May		June	
	1956	1964	1956	1964	1956	1964
Mean	2.9	4.5	7.9	13.6	17.3	16.1
Mean maximum	7.9	10.4	14.3	21.0	23.9	23.4
Mean minimum	-2.1	-1.4	1.4	6.2	10.6	8.8

ditions (Stanley and Linskens 1974). Conditions for forcing flowers on cut branches to shed pollen are summarized in Table 3.

The greenhouse situation with branches and buckets makes for awkward retrieval. To improve conditions it is preferable to collect the pollen flowers when they are close to shedding the pollen, and place them on the extraction trays. Again, it is necessary to frequently monitor flower development so that the optimum collection period is not missed. Forcing microstrobili under these conditions is never easy. Storing pollen from good flowering years in anticipation of future breeding requirements would alleviate problems associated with timing and acquisition.

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Canada 1990
Forestry Canada Forêts Canada
ISSN 1180-2618 ISBN 0-662-17944-7 Catalogue No. Fo29-26/4E