Microsporogenesis, pollination and potential yield of seed of Larix in NE Scotland

By J. P. HALL and I. R. BROWN

(Received January / August 1976)

Introduction

It is widely known that the yield of seed from Larch seed orchards is poor. KELLANDER (1966) suggested that thirty percent germination could be considered normal in European Larch, Larix decidua (Mill.) and Japanese Larch, L. kaempferi (Sarg.) and MISSLER (1956) found that the percentage of empty seeds in L. decidua ranged from 59.8% to 80.1%. Similar yields of seed from controlled hybrid crosses by the authors have resulted in 20 or fewer full seed per cone which is one third or less of the potential yield.

Seed production in seed orchards is affected by a variety of factors and failure at, or prior to, fertilization, due to either maternal, paternal or environmental effects, with subsequent disturbances in embryogenesis is known to have an important effect on yield of seed (SARGAS 1962, MATTHEWS 1963, ANDERSON 1965 and ERIKKSON et al. 1972). Failure at this stage may be due to variation in quality and/or quantity of pollen leading to non-fertilization or be due to genetic and/or environmental factors which cause early failure of the zygote. Swedish workers have shown that low seed yield may be due to unfavourable climatic conditions during microsporogenesis (ERIKSSON 1968, ERIKKSON et al. 1972).

In Larch, microsporogenesis takes place between October and March (ERIKSSON et al. 1968). Low temperatures during the active stages of meiosis appear to result in chromosomal irregularities, disturbances of cell division and non-functioning pollen. Disturbance of meiosis due to low temperatures has been reported to occur in many conifers for example in Abies sachalinensis (Mast.) MERRY and LESTER 1961, Picea abies L. (KARST), ANDERSON 1965, Pinus edulis (Engel.) Voss, CHIRI 1967 and Larix laricina (K. Koch), CHANDLER and MAVRODINAEV 1965. In some species abnormally high temperatures are also known to disturb meiosis as reported for Picea abies, CHIRI 1965 and Taxus brevifolia (Koch) C. CHIRI 1964. Because of the reported detrimental effects of local climate on microsporogenesis in Sweden it is felt that the location of seed orchards for the production of forest tree seed is an important consideration (ERIKSSON et al. 1972).

Winter temperatures in North-east Scotland are not as low as in Sweden but temperatures below the critical level...
of $-2.5^\circ$ C can occur. An analysis of temperature data for the 5 years prior to this study for areas near Newton showed that each year between December and March periods of 10 to 14 days with frost occurred. Monthly minimum temperatures of $-10^\circ$ C to $-14^\circ$ C occurred in January and February and temperatures of $-6^\circ$ C to $9^\circ$ C in December to March (HMSO 1969–1973). As a first step in a general investigation of factors affecting seed yields in Larch, a study was undertaken during the winter of 1973–74 to determine the effects of local climate on microsporogenesis at two sites in North-east Scotland. Additional studies on pollen viability, estimated by fluorescent staining and distribution of pollen within strobili were also carried out.

The two sites were in Morayshire, North-east Scotland, at Newton Nursery and in Teindland Forest. The seed orchard at Newton is approximately 6.4 km from exposed coastline and is 24 m above mean sea level. The second site at Teindland Forest is 9.7 km inland and 107 m ASL with a more continental climate than at Newton. The two sites are about 13 km apart. At both sites grafted clones of *L. decidua* and *L. kaempferi* were used for the microsporogenesis study. The pollen collection and distribution study was carried out at Newton nursery.

**Methods**

Male strobili were collected at weekly intervals from 20 October 1973 to 27 March 1974. The following grafted clones were sampled at Newton only, *L. decidua*, E-6 and E-120 and *L. kaempferi*, J-5, J-12, J-42, J-50 and J-52. At Teindland twelve year old progeny from crosses between some of these clones were sampled: *L. decidua × kaempferi*, E-6 × J-42 and E-120 × J-42; *L. kaempferi × kaempferi*, J-12 × J-52, J-50 × J-52 and J-12 × J-50. The following clones of Danish Provenance were sampled at both locations, *L. decidua*, E-9000, E-9004 and E-9011, *L. kaempferi*, J-9005 and J-9007.

The strobili were fixed in a mixture of Ethanol : Propionic Acid (3 : 1) saturated with Ferric Acetate and stored at $-12^\circ$ C. For staining the strobili were soaked in a solution of alcoholic carmine for 96 hours. The pollen mother cells were dissected from 10 or more microsporangia and squashed in 45% acetic acid. The pollen mother cells (PMC's) were then examined microscopically to determine the course of development of meiosis and to detect any abnormalities that might occur during microsporogenesis.

Other stations consisting of Stevenson screens containing a thermohygrograph and a maximum — minimum thermometer were established at both locations. The station at Newton was approximately 2 m above the ground within the area of male flowering of the parent clones. The station at Teindland was about 5 m above the ground and within the lower crowns of the progeny. Daily temperature records were kept during the winter of 1973–74 at both locations.

Pollen was collected from twigs which had large numbers of flowers. The twigs were collected from six clones of each species before anthesis and laid out for the pollen to shed on sheets of dry paper. Air temperature in the extraction area was maintained at 18–21°C and relative humidity at 70–90%. The pollen was cleaned of foreign matter and stored at 4°C in a desiccator with calcium chloride. Collections of *L. kaempferi* took place between 1 and 17 March and *L. decidua* between 18 and 28 March.

Pollen viability was estimated by staining pollen with fluorescein diacetate and examining it under a microscope according to methods outlined by Paton and Jones (1975). Approximately 600 pollen grains from 12 random samples were examined for each pollen mixture.

Female strobili were isolated in clear tubes of cellulose nitrate, sealed with foam plastic at each end and pollinated with pollen guns consisting of a pollen agitator and bellows. In order to detect and count pollen grains in the ovules it was found necessary to stain the grains before application. The pollen was stained with a 0.5% aqueous solution of methylene blue and redried over calcium chloride. Female strobili on clones *L. decidua* E-1102 and *L. kaempferi* J-12 were each pollinated with stained pollen three times during the receptive period of the strobili. Pollinations were done in late March and strobili collected in mid-May after the micropyles had closed. The strobili were partially frozen and the floral bracts broken off individually and examined under a wide-field dissecting microscope. The occurrence and number of pollen grains in each ovule was determined.

**Results**

The results of the examination of the pollen mother cells are shown in Figures 1 and 2. The temperature data consisting of the daily maxima and minima are shown graphically in Figures 3 and 4. The occurrence of pollen grains in the female strobili is shown in Table 1 and the distribution of pollen grains within the strobili is shown in Figure 5.

(i) Meiosis in grafted clones (Newton) and their progeny (Teindland)

According to previous work in Sweden it is known that there are three distinct periods of meiosis in relation to temperature sensitivity;

(a) the temperature — sensitive pre-diplotene (Interphase 1 to Diplotene) stages;

(b) the temperature — insensitive Diplotene period, and

![Figure 1](image-url)

**Figure 1.** — Times of occurrence of various stages of meiosis in ovule PMC from Interphase 1 to Microspores. Vertical lines indicate sampling points and the figures show the percentage of cells in temperature-sensitive stages. Since the sampling interval was approximately one week, boundaries between different stages were, of necessity, arbitrary. The parent clones were located at Newton (top half), the progeny at Teindland (bottom half).
(c) the temperature — sensitive post-Diplotene to Tetrad (Diakinesis to Telophase I and Prophase II to Telophase II) stages. (Interphase II is considered to be insensitive to low temperatures.) The pre-Diplotene stages in the parent clones occurred over a two week period during the middle of October with little variation in time of occurrence among clones (Figure 1).

Figure 3. — Percentage of pollen mother cells in temperature sensitive stages for clones of Larix at Newton (N) and Teindland (T) during 1972—74.

In the progeny these stages occurred in mid and late October. Results from Teindland were incomplete because the trees produced few flowers. Diplotene lasted for 8—10 weeks in L. kaempferi and about 14 weeks in L. decidua. The post-Diplotene stages, Diakinesis to Telophase II were completed during a two to three week period in each clone. The earliest clone which completed these stages was J-42. Clones J-5, J-12, J-50 and J-52 all passed through these stages about two weeks later. In L. decidua the post-Diplotene stages occurred in late February and early March, after the L. kaempferi clones had completed meiosis.

Data for the progeny were scarce but they show that the post-Diplotene stages in the inter-specific crosses, E-6 × J-42 and E-120 × J-42 occurred between the dates of the corresponding stages in the parents. The intra-specific crosses, J-50 × J-52, J-12 × J-52 and J-12 × J-52 passed through the post-Diplotene stages at approximately the same time as did their parents. On any given date usually less than 30% of the PMC's sampled were in the stages of Diakinesis to Telophase II, the maximum number of cells in these stages was 56% found in clone J-5 on 20 January. This is in contrast to the pre-Diplotene stages which occur almost simultaneously, with all or nearly all the PMC's in sensitive stages at the same time.

(ii) Meiosis in grafted clones of L. decidua and L. kaempferi at two sites.

The pre-Diplotene stages occurred during mid and late

Figure 3. — Maximum and minimum daily temperatures at Newton 6 December 1972 to 4 April 1974.

Figure 5. — Average numbers of pollen grains per ovule in L. decidua, clone E-1192 and L. kaempferi, clone J-12. Averages are for 36 ovules — 3 braets each containing 2 ovules and for 3 strobili.

Figure 4. — Maximum and minimum daily temperatures at Teindland 10 November 1973 to 28 March 1974 (Broken lines indicates missing data).
Table 1. — Occurrence and number of pollen grains in strobili after controlled pollination.

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<th>Clone E-1102</th>
<th>Clone J-12</th>
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<tr>
<td>Percent of ovules with 1 or more pollen grains</td>
<td>66.4</td>
<td>79.5</td>
</tr>
<tr>
<td>Average number of pollen grains per ovule in ovules with 1 or more pollen grains</td>
<td>4.1</td>
<td>3.1</td>
</tr>
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</table>

October for both species at both sites. Clone J-9007 at Newton was an exception in that this stage was not completed until some time in early November. The period over which Diplotene occurred was variable between clones and sites. In all L. decidua clones at Newton and in one of these clones at Teindland some PMC’s entered the post-Diplotene stages at various times from mid November to late December–early January. All cells however, had completed Diplotene by late February or early March. One of the L. kaempferi at Teindland had PMC’s which entered post-Diplotene stages in early January and others which did not complete this phase until mid February. In the L. kaempferi clones for which complete data were available, all PMC’s entered post-Diplotene stages in late January to early February.

Given that data was not complete for all clones due to the shortage of flowers it appears that there were no significant differences in microspore development between sites. But, as before, there appear differences between species, in that L. kaempferi entered the final stages of microsporogenesis earlier than L. decidua. These clones differed from the older clones and their progenies in the variable times of occurrence of post-Diplotene stages.

The stages between formation of microspores and anthesis proceeded normally.

(iii) Temperature records from the two sites.

Freezing temperatures at Newton occurred during the first two weeks of December, in mid February for six nights and again during the first three nights in March (Fig. 3—4). Temperatures below —2.5° C only occurred during five nights in December. Maximum temperatures during the day were 5° C or greater throughout most of the period between 23 December and the end of February.

At Teindland, frosts occurred on several occasions during the winter, however, temperatures below —2.5° C occurred only on 25 November and on 13 February. Maximum temperatures of 5° C or greater occurred frequently from mid December to early March.

Mean daily temperatures (not shown) varied from 0° to 10° C throughout the winter and there was little difference between the two locations.

(iv) Pollen viability

The viability of pollen grains of each species as estimated by fluorescent staining was:

(a) L. decidua mean 84% max. 90% min. 80%
(b) L. kaempferi mean 91% max. 97% min. 82%

(v) Occurrence and distribution of pollen within the strobilus following controlled pollination

The percentage of ovules pollinated and the average number of pollen grains in the micropyle was higher in clone E-1102 than in clone J-12 (Table 1). The distribution of pollen grains within the female strobilus of clone J-12 shows that ovules in the middle part of the strobilus had more pollen than those at the bottom or the top (Figure 5). In clone E-1102 larger numbers of pollen grains were found in the top third of the strobilus.

Discussion

The general pattern of meiosis in clones of both species was similar to that previously described (Emrsson 1968). The pollen mother cells passed from Interphase I to Diplotene during October and early November. The high percentages of cells in Pachytene shown in Figure 1 reflect the fact that nearly all PMC’s went through this stage simultaneously in any particular microstrobilus.

Diplotene lasted for about 2 months in L. kaempferi and about 3 months in L. decidua. Emrsson (1968) reported that Diplotene in Sweden was of approximately the same length. Diplotene was interrupted during the winter in 3 clones of L. decidua and in one of L. kaempferi when PMC’s in a few of the microstrobili passed from Diplotene to the Tetrads stage.

Emrsson (1968) also found interruptions in Diplotene in 2 of the 3 clones of L. decidua he studied. Interruptions in Diplotene were preceded by mean daily temperatures in the range of 2—7° C. These temperatures occurred frequently throughout the winter and probably, did not stimulate the PMC’s to pass from Diplotene to the post-Diplotene stages. Only a small proportion of the microstrobili contained PMC’s which passed Diplotene and the PMC’s in the remainder of the microstrobili completed meiosis later in the year.

The post-Diplotene stages were completed within a 3 week period in both species while in the hybrids up to four weeks was required for completion. This is in contrast to the case in Sweden where a more extended post-Diplotene period was observed, lasting, in some cases up to 8 weeks in L. decidua (Emrsson 1968). At any given sampling date a small proportion of the PMC’s were in post-Diplotene stages — a maximum of 56% was recorded in clone L. kaempferi — 5 sampled on 20 January. Similar results have been reported for Sweden (Emrsson 1968).

There was very little difference in the development of meiosis within the same clones at the two sites. The site at Teindland had greater extremes of maximum and minimum temperatures but there was very little difference between the mean daily temperatures at the two sites.

Temperatures below 0° occurred rarely and only once when there were any PMC’s in the post-Diplotene stages. The temperatures dropped to —2.5° C on 13 February while some PMC’s were in the post-Diplotene stages in the hybrid clone E-120 × J-42. No chromosomal irregularities however were observed in any of the PMC’s. In Sweden, PMC’s in post-Diplotene stages and below freezing temperatures frequently occurred together and chromosomal irregularities were observed shortly after, which were believed to result in almost complete pollen sterility in some clones (Emrsson 1968).

Low temperatures during pollen mitosis are also believed to cause chromosomal irregularities (Emrsson et al. 1968), however none were observed in any of the clones at either site investigated in north-east Scotland. Emrsson et al. (1966) reported that 0.5% of pollen grains were “giant pollen grains” (double the average volume) under normal
conditions and about the same proportion was observed in this study.

Since winter minimum temperatures in North-east Scotland are not nearly as low as those in Sweden it is not surprising that no chromosomal irregularities were observed if a causal relationship does exist.

Our findings did not demonstrate a causal relationship between the different stages of meiosis and the air temperature in the crowns where meiosis was occurring. If the PMC's in Diplolene need a “cold period” to break dormancy as suggested by EKSSON (1968) then the low frequency of such periods in North-east Scotland would suggest a longer Diplolene than in Sweden which is not the case. If short fluctuations of temperature around 0°C did indeed stimulate post-Diplolene stages, it would have been expected that the PMC's of Larch in North-east Scotland would pass through Diplolene and complete meiosis during the winter. This, of course, did not happen. If, as has been suggested by Swedish workers, meiosis is controlled by air temperatures it is surprising that the timing of meiosis is broadly similar in both areas where temperatures are very different. It seems reasonable to suggest that the timing of meiosis might be controlled by photoperiod which is broadly similar in Sweden and North-east Scotland.

In assessing the possibility of the occurrence of damage to pollen in a seed orchard several factors must be considered. At any given time in any given clone there is only a proportion of PMC’s in the temperature sensitive post-Diplolene stages. There is variation in the time of meiosis within a single strobilus as well as among strobili on the same branch, within the crown and among ramets of the same clone (EKSSON 1968). There is a point at which the highest proportion of PMC's are undergoing meiosis and this point is different for each clone. Results from this and other similar studies indicate that the temperature sensitive periods Diakinesis to Telophase I and Prophase II to Telophase II, are of very short duration compared to the stages Interphase II and Tetrads, which are much less susceptible to damage from low temperatures. Thus the probability that an entire clone (much less several clones) would be rendered pollen sterile due to low temperatures must be considered as the product of several probabilities — the probability of temperatures below 2 or 3°C; the probability of the PMC’s in that clone being in post-Diplolene stages and the probability of those stages being the temperature sensitive ones discussed above. It can be seen that the final probability of the pollen crop in any given year being significantly affected is extremely small. It must also be stressed that some of the more common temperature-induced irregularities in the chromosomes may heal and viable pollen be produced (EKSSON 1968).

Results of the pollen viability test indicate that viability was high. The fluorochromatic reaction is a test of the integrity of the plasmalemma of the vegetative cell which is thought to be closely related to the ability of the pollen grain to fertilise an archegonium (HESLOP-HARRISON, J. and HESLOP-HARRISON, Y. 1970). Thus, a positive reaction is not a certain measure of viability, but a negative reaction almost certainly indicates a non-viable pollen grain.

Ovules containing one or more pollen grains were found mostly in the mid-section of the strobili (where nearly all of the ovules were pollinated with one or more pollen grains). Since the estimated pollen viability is high a high proportion of ovules will contain a viable pollen grain. The proportion of ovules pollinated with at least one viable pollen grain is the product of the percentage of ovules pollinated and the probability of one viable pollen grain in that ovule. This has been calculated for combinations of one, two and three pollen grains per ovule. The probability of one or more viable pollen grains per ovule in those which receive more than three pollen grains is so close to 1.00 as to make calculations for these combinations unnecessary. The estimated proportion of pollinated ovules in each species is shown in Table 2. In clone J-12 ovules with 1 or more pollen grains had an average of 3.10 pollen grains per ovule; in clone E-1102 the corresponding figure was 4.13.

The effect of pollen viability was quite small and only reduced the potential number of fertilized ovules by 1.7 and 2.5 percent for J-12 and E-1102 respectively. At this stage of development it could be expected that 77.8 and 63.9 percent of ovules in the two clones would contain at least one viable pollen grain. The results also indicate that a relatively high proportion of non-viable pollen would be needed in order to affect the number of ovules pollinated with one viable pollen grain when the average number of pollen grains per ovule is two or more.

Under conditions of artificial pollination the effect of pollen viability is small. If there were fewer pollen grains present, for instance, with wind pollinations then pollen viability would have a greater effect.

**Summary**

The general pattern of meiosis in *L. decidua* and *L. kaempferi* was similar to that described previously with Diplolene beginning in October and early November and lasting for 2-3 months and meiosis being completed in the early spring over a period of 1-4 weeks. The occurrence of meiosis on the hybrid progeny was intermediate between the times of meiosis in the parent trees, and in non-hybrids meiosis occurred at the same time as the parents. No disturbance in meiosis attributable to low temperatures was observed.

It is perhaps surprising that Larch, originating from countries with continental climates, should not suffer some disturbance of meiosis in the variable winter climate of

<table>
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<tr>
<th>Table 2. — Percent of Ovules Pollinated with at least 1 Viable Pollen Grain in Clones J-12, E-1102.</th>
<th>Percent of ovules with 1, 2, 3 and 4+ pollen grains*</th>
<th>Probability of 1 viable PG present in ovules with more than one pollen grain**</th>
<th>Percent of ovules with 1 or more viable pollen grains</th>
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<tr>
<td></td>
<td>J-12</td>
<td>E-1102</td>
<td>J-12</td>
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<tr>
<td>% w. 1 PG</td>
<td>16.4</td>
<td>13.1</td>
<td>.91</td>
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<tr>
<td>2</td>
<td>16.4</td>
<td>9.3</td>
<td>.99</td>
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<td>3</td>
<td>17.9</td>
<td>12.6</td>
<td>1.00</td>
</tr>
<tr>
<td>4+</td>
<td>28.8</td>
<td>31.4</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td>72.3</td>
<td>66.4</td>
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* Determined by examination of ovules under dissecting microscope.
** Calculated from the results of the fluorescent staining.
North-east Scotland at as least as great a degree as was reported from Sweden. Although minimum winter temperatures were mainly above the reported threshold of -2.5° C, warm winter periods did not appear to stimulate the progress of melosis as has been reported in Sweden. It should be noted that the winter of 1973-74 was considerably milder than normal. The fact that melosis in parent clones at Newton and their progenies at Teindland was comparable indicates that genetic control and photoperiodic control of melosis is stronger than any temperature effects experienced in North-east Scotland.

A relatively high proportion of ovules were pollinated with viable pollen and the viability of pollen had a very small effect on the potential yield of seed in controlled crosses.

The low yield of seed in hybrid larch seed orchards was not attributable to temperature conditions during microsporogenesis and work is continuing on the post-pollination stages to determine the causes of empty seed.

Key words: Meliosis, Winter temperatures, Larix decidua Mill., Larix kaempferi (Lamb.) Carr.

Zusammenfassung


Literature Cited


Spontaneous chlorophyll mutations in Bombax L.

By C. S. Venkatesh and C. J. S. K. Emmanuel

Forest Genetics branch, Forest Research Institute, Dehra Dun, India

(Received June / August 1976)

Introduction

Spontaneous chlorophyll mutations have been reported in several temperate tree species (McKay, 1956; Franklin, 1970) but so far in few tropical ones (Posnette, 1950; Venkatesh and Sharma, 1974). The present note records the occurrence of such mutations in Bombax ceiba L. (n = 36, 46, 48) and B. insignis Schott et Endl. (n = 36), two tropical broadleaved forest tree species which constitute India's principal industrial matchwood resource and hence are included in a genetic improvement programme at this Institute (Venkatesh, 1974). As per the standard procedure adopted in such forest tree improvement work, phenotypically superior plus trees (so far of only B. ceiba) have been selected and assembled as grafts in clonal banks and seed orchards (Venkatesh and Arya, 1973).

Material

The present study is based on progenies raised from time to time at the Institute's forest genetics nursery and mostly from open pollinated seeds of individual tree or clone origin.

Observations

Initially when one-parent progenies were raised out of some B. ceiba trees chosen at random on the New Forest estate, some seedlings with yellow-green mottled cotyledons had been noticed in certain of the families. Iodine test