larities that would affect seed production. It may be that female sterility is not of common occurrence in Douglas fir but the fact that it can occur makes close examination of the seed from individual trees established in seed orchards an essential part of any seed production program. On the positive side, the clones of any male and/or female sterile trees should be established as could be of considerable value in future breeding research.

Summary

In 1967, three 20 year old Douglas fir in southern Vancouver Island were control pollinated. The seeds from the conas of two of the trees were normal and yielded healthy seedlings, all the seeds from the third tree, however, were undeveloped and flat. Scions from this tree were grafted in 1968 at Cowichan Lake, located some 100 km to the northwest on Vancouver Island. In 1976, cones were collected from the two surviving ramets and all the seeds were likewise undeveloped and flat. It is considered that this tree is female sterile. The importance of female sterility in seed orchards and future breeding is discussed.

Key *words:* Ovules, clone, incompatibility, sterility, microsporogenesis, megasporogenesis, asyndetic.

Zusammenfassung

Im Jahre 1967 wurden drei 20jährige Douglasien kontrolliert bestäubt. Von zwei dieser Baume konnten normale Samen geerntet und daraus Pflanzen angezogen werden. Dagegen hatte der dritte Baum nur taube Samen. Im Jahre 1968 wurde dieser Baum abgepfropft und die Pfropflinge an anderer Stelle, d. h. einige 100 km entfernt ausgepflanzt, so daß im Jahre 1976 erneut Zapfen und Samen zur Verfügung standen. Auch diese waren unbefruchtet. Es wird daraus geschlossen, daß dieser Baum weiblich steril ist. Das Ergebnis wird im Zusammenhang mit Samenplantagen-Problemen diskutiert.

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Embryo Development and Yield of Seed in Larix

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Introduction

There are two major categories of factors influencing formation of empty seed in Larix (a) those which act between pollination and fertilization and (b) those which act after fertilization. Between pollination and fertilization, failure can occur if pollen does not reach or does not germinate on the nucellus. After fertilizstion, failure can occur if the pro-embryo fails to grow out of the archegonia or if the young embryo fails to develop at a later stage.

HAKANSSON (1960) stated that seed sterility in Larch arises from "failure of fertilization and developmental disturbances leading to embryo lethality". He described the appearance of unfertilized ovules and degenerating embryos and attributed the occurrence of all empty seed to these factors but did not quantify their proportionate contribution. In *Pinus* it has been reported that hybridity barriers result in death of hybrid embryos (HAGMAN and MIKKOLA (1963), DOGRA (1967) and KRIEBEL (1970)). It is possible that the low yield of seed per cone in hybrid crosses in Larix are a result of hybridity barriers but there is no indication of this from the literature.

As part of an investigation in the yield of secd in Larix in North-east Scotland it was decided to study the development of the female gametophyte to determine the proportionate contribution of the above factors to the production of empty seed. According to HALL and BROWN (1976) approximately one fifth to one third of the ovules remain unpollinated after controlled pollination and this must account for at least that proportion of the empty seed in the final harvest. The study reported here concerns the development in both pollinated and unpollinated ovules of known parentage. The development of hybrid embryos and intraspecific embryos was also compared.

Development of the embryo in Larix

Briefly to summarise the literature, the sequence of cvents during the development of the embryo is as follows.

The stigmatic flap collapses 7 to 10 days after pollination and the pollen is ingested into the micropyle and embedded on the micropylar side of the collapsed tissue (DOYLL and O'LEARY 1935). The pollen then swells with "the pollen tube forming a bulge of the intine up to one-fifth of the pollen diameter" (BARNIR and CHRISTIANSEN 1960). After 5 to 7 weeks the nucellus enlarges, partly filling the micropylar canal and exudes a fluid which dissolves the material holding the pollen grains on the under side of the stigmatic flap. The fluid appears to retract, bringing the pollen grains with it until they come to the top of the nucellus (BARNER and CHRISTIANSEN 1960). The pollen grains do not release the male gametes unless, and until, they come in direct contact with the nucellus.

The germinated pollen grains discharge two male gametes enclosed in a membrane which malre their way through the nucellus and penetrate the archegonia at the neck cells.

Four to six archegonia develop and are fully formed about 44 days after pollination occurs (SMOLSKA 1927).

Development of the fertilized zygote can be conventiently divided into two stages (a) Pro-embryo development from fertilization to elongation of the suspensors and (b) Early embryo and embryo enlargement and the formation of the mature embryo.

(a) Pro-embryo development During the first mitosis after fertilization the zygote nucleus becomes elongated;

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this is followed by two free nuclear divisions to form 4 free nuclei which form the pro-embryo at the base of the archegonium. These nuclei divide into lower and upper tiers with the upper tier forming the primary suspensors and the lower tier the apical initials. Both tiers divide again resulting in 4 tiers of 4 cells. These are, from the base, the relict nucleus, the rosette layer, the suspensor and the apical initials. These last 2 layers form a "polarity unit" (SCHOPF 1943).

(b) *Early embryo and embryo enlargement* The primary suspensors elongate pushing the apical initials to the base of the archegonium and the polarity unit then progresses through the centre of the gametophyte in the corrosion cavity. The polarity unit functions as a group of 4 closely associated embryos but eventually one of them rapidly becomes dominant and the others disappear. SCHOPF (1943) terms this "delayed cleavage polyembryony", intermediate between the cleavage polyembryony of *Pinus* and the non-cleavage polyembryony in *Picea*.

When the polarity unit reaches the end of the corrosion cavity it begins to enlarge rapidly and at this stage it is termed a 'club embryo'. The development of meristematic areas follows starting with the rib meristem located about 8 to 10 cells behind the apical cells, then the generative root meristem and lastly the stele promeristem forming between the rib meristem and the apical cell. Continued growth and development is concentrated upon the establishment of the meristematic zones, the development of the cotyledons and enlargement of the embryo.

The pro-embryo stage in *Larix* is completed within a week of fertilization and the 'club embryo' stage 3 to 4 weeks after fertilization. Meristematic zones are present about 8 weeks after fertilization.

Methods

(a) *Collection and preparation of sections* Controlled crosses were made using one clone each of *L. kaempferi* and *L. decidua* as mother trees. A mixture of fresh pollen from 6 clones of *L. kaempferi* was then used to pollinate several grafts of *L. kaempferi* and *L. decidua* and a mixture of fresh *L. decidua* pollen from 6 clones was used to pollinate several grafts of *L. decidua*. The same *L. decidua* clone was used as mother tree for both the intraspecific and hybrid crosses. No hybrid crosses were done using the *L. kaempferi* clone as a mother tree as insufficient *L. decidua* pollen was available at the time. Collections were made at 5 to 10 day intervals from mid- April to mid-September from the intraspecific crosses *L. kamepferi* × *L. kaempferi* and *L. decidua* × *L. decidua* and from the hybrid cross *L. decidua* × *L. kaempferi*. In addition, several strobili were isolated but not pollinated and were then sampled at 5 to 10 day intervals.

The strobili were fixed in a mixture of ethanol, formaldehyde and propionic acid and stored until required for examination. In order to obtain sections of embryos after mid-June it was necessary to remove the seed coat to allow proper penetration of the paraffin wax. The ovules were dehydrated, embedded in paraffin and sectioned (thickness 15 microns) and mounted according to methods described by JOHANSEN (1940). The sections were then stained with safranin 0 and counterstained with fast green. For each cross approximately 700 slides were examined.

(b) *Determination of embryo abnormality leading to formation of empty seed* DOGRA (1967) lists several criteria for recognition of degenerated embryos; excessive vacuolization, erratic staining behaviour, loss of staining behaviour, loss of staining affinity, and unusually deep staining of a part or whole of the embryo. Many embryos examined had one or more of these characteristics but whether or not it was an artefact of preparation for sectioning could not be determined. Orientation of the embryos in the corrosion cavity was variable but such variation also occurs in other conifers (DOGRA 1967) and is not always associated with embryo degeneration. Since DOGRA's criteria could not be used in determining the viability of embryos directly, it was decided to use relative age as a criterion.

The most common stage of development at each sampling date was accepted as normal for this date. Usually the most advanced stage of development was also the most common. Two assumptions were made about development of the female gametophyte, first that in no strobili were all ovules abnormal and second that all ovules within the strobilus develop at the same speed. Development, although continuous, was divided for convenience of classification into seven stages:

1. pro-embryo
2. early embryo at 4—6 cell stage
3. early embryo at 8—16 cell stage
4. club embryo
5. enlarged club embryo
6. embryo at beginning of differentiation of meristematic areas
7. embryo differentiated into cotyledons and well defined meristematic zones.

examples of these stages are shown in *Figures 1, 7, 10.* At each sampling date the embryos were classified according to this scheme.

Results and Discussion

Development of unpollinated ovules

In a previous paper it was reported that, after controlled pollinations, approximately one third of *L. decidua* and one fifth of *L. kaempferi* ovules remained unpollinated (HALL and BROWN 1976). Seed from strobili which had been isolated but not pollinated, were invariably empty. When sections from such strobili were examined, it was seen that approximately one week after the normal fertilization date the archegonia of unpollinated ovules often increased in length and the egg cell remained in the same position it had occupied at the time fertilization should normally have occurred *(Fig. 9).* At this time it was noted that the egg cell often became enlarged. After 3 weeks the archegonium was filled with deeply staining bodies quite different from the homogenous appearance of fertilized archegonia *(Fig. 10).* The enlarged egg cell shown in *figure 11* had a membrane apparently retracted from the periphery of the egg cell zone. Sections taken six weeks after the normal fertilization date showed that the gametophyte had completely degenerated and separate tissues could not be distinguished. The female prothallus was so shrunken that it occupied only a small proportion of its original volume and this stage *(Fig. 13)* was seen in sections sampled as late as September. At this time (26 June) the outline of a corrosion cavity was also present *(Fig. 10)* although sections taken at later stages showed no sign of an actual cavity. This is in contrast to the observations of HAKANSSON (1960) who found no sign of a corrosion cavity in unpollinated ovules.

Unfertilized ovules were found in sections of pollinated strobili in approximately the same proportion as that recorded when ovules were treated with stained pollen (HALL and BROWN 1976).

Formation of empty seed due to failure of fertilization to occur in pollinated ovules

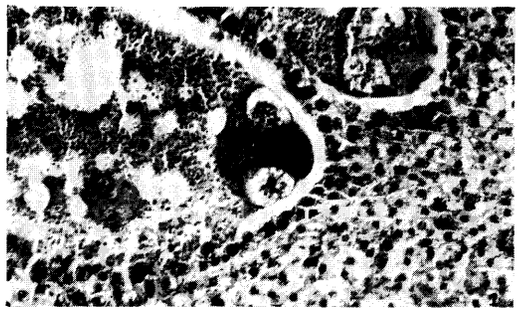
Until the middle of June it was possible to determine from sections whether or not fertilization had occurred. It was also possible to determine if the lack of fertilization was caused by failure of pollen to move from the micropyle to the nucellus or by failure of the pollen on the nucellus to germinate. The numbers and proportion of ovules which remained unfertilized after pollination are shown in *Table 1* and *Table 2* respectively. Failure of pollen to move to the nucellus occurred in *L. decidua* ovules only, and failure of the pollen on the nucellus to germinate occurred only in ovules which had been pollinated with *L. kaempferi* pollen. Hybrid ovules were much more likely to remain unfertilized than either of the intraspecific ovules.

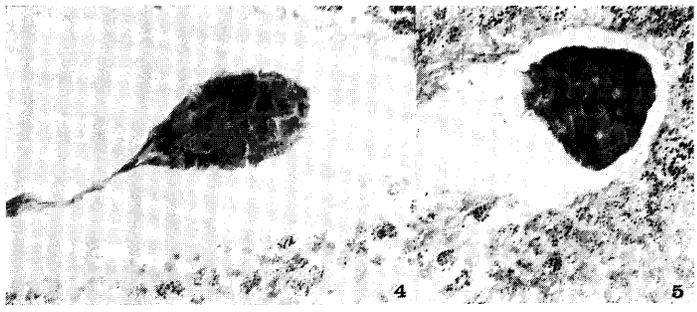
Failure of pollen to reach the nucellus occurs frequently in *Larix* as reported by DOYLE and O'LEARY (1935). Failure of pollen to germinate on the nucellus, however, has not so far been reported.

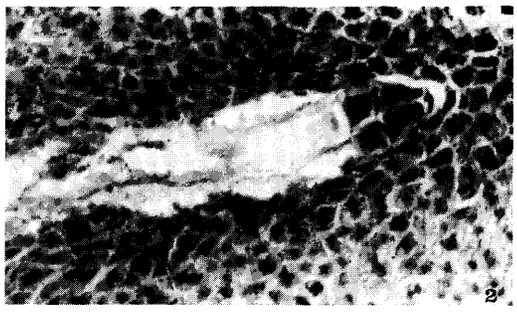
The higher percentage of pollinated (ie pollen on the micropylar side of the collapsed stigmatic flap) but unfertilized ovules in the hybrids indicate the presence of factors affecting the pollen drop mechanism. This may be due to the presence of a different stimulus or absence of a stimulus which controls the movement of pollen grains to the nucellus. It is also possible that the failure of the *L. kaempferi* pollen to germinate (ie release the gametes) on the nucellus is a result of inviable pollen rather than the presence of an inhibitory mechanism.

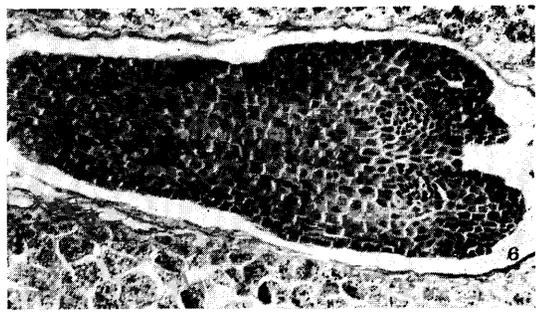
Formation of empty seed due to failure of embryos to develop to maturity

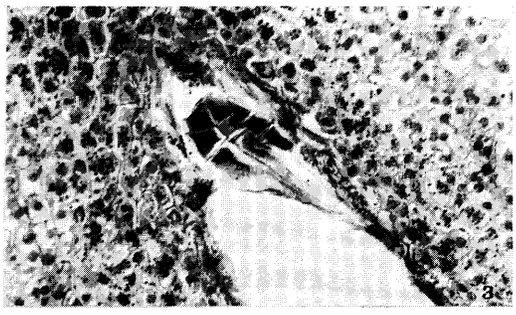
Table 1 shows the number of embryos at the different stages on each sampling date. The normal stage of each date is indicated by diagonal lines. There was considerable variation in the size of embryos at each sampling date and variation increased at later dates. Variation in embryo size is common in conifers and has been attributed to differences in date of fertilization, environmental conditions

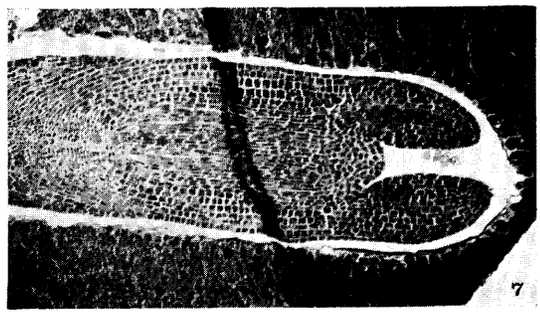




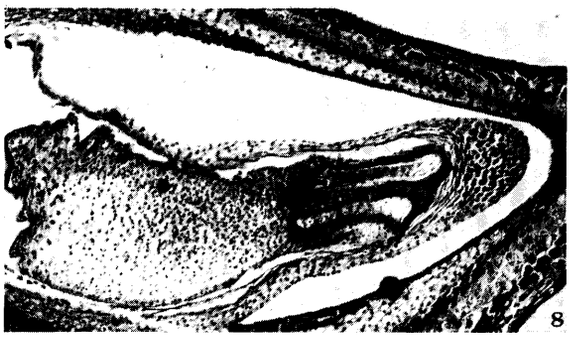


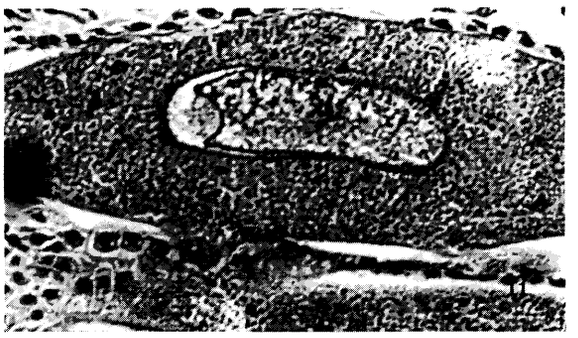


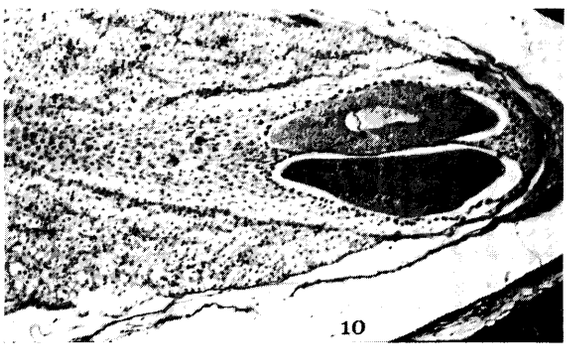




	Stage of development and Date	Magnification
Figure 1. —	Pro-embryo, 2 June	156
Figure 2. —	Early embryo at 4—6 cell stage, 9 June	168
Figure 3. —	Early embryo at 8—16 cell stage, 17 June	165
Figure 4. —	Club embryo, 26 June	120
Figure 5. —	Enlarged club embryo, 2 July	135
Figure 6. —	Embryo at beginning of differentiation of meristematic areas, 9 July	107
Figure 7. —	Embryo differentiated into cotyledons and well defined meristematic areas, 16 July	56







	Description	Date	Magnification
Figure 8.	— Unpollinated female gametophyte	2 June	54
Figure 9.	— Enlarged archegonia	11 June	114
Figure 10.	— Outline of corrosion cavity	26 June	51
Figure 11.	— Enlarged egg cell	26 June	180
Figure 12.	— Degenerated gametophyte	17 July	117
Figure 13.	— Degenerated gametophyte	17 July	108

or to the presence of dead or dying embryos (Dogra 1967).

The time of occurrence of fertilization was determined from the sections and occurred on or about 2 June in both species. In hybrid ovules, pro-embryos were present by 2 June.

In the intraspecific crosses, between *L. kaempferi* pollen and *L. kaempferi* ovules, pro-embryos were present at the same time as germinating pollen grains and male gametes were observed at the neck of the archegonia. In crosses between *L. decidua* pollen and *L. decidua* ovules, germinating pollen and male gametes at the neck of archegonia were present on 3 June but no pro-embryos were seen. Sections taken a week later showed that fertilization had been completed in ovules of all crosses and early embryos were the same size and moving through the corrosion cavity. This suggests that fertilization occurred in *L. decidua* ovules shortly after 2 June.

It has not been reported at what levels temperatures may limit growth in *Larix* but no abnormal embryos which could be directly attributed to temperatures were observed on the sections after the occurrence of the lowest recorded temperatures during the growing season. Temperatures remained above 3° C for the whole growing season *(Fig. 14)*. In addition, all ambryos were exposed to the same temperature conditions so these should have affected all embryos equally. It is possible that temperature affects ovules of the three species in different ways but it does not appear that variation in embryo size could be attributed to temperature conditions.

It was assumed, therefore, since the variation in size of

Table 1. — Stages of development in pollinated ovules of three crosses sampled at different dates.

Sampling Dates	Stages of embryo development 1	2	3	4	5	6	7	Gametophyte without embryo – Pollen immobilized in micropyle	Pollen ungerminated	Total no. of ovules examined
	L. decidua x L. decidua									
	Number of embryos in each stage									
16/7	0	0	1	0	1	0	10	0	0	12
9/7	0	0	0	0	0	11	–	0	0	11
2/7	0	0	0	4	5	–	–	0	0	9
26/6	0	1	1	10	–	–	–	0	0	12
17/6	0	6	2	–	–	–	–	1	0	8
9/6	0	10	–	–	–	–	–	1	0	10
2/6	–	–	–	–	–	–	–	–	–	–
							Total	2	0	64
	L. decidua x L. kaempferi									
16/7	0	0	0	2	2	2	0	0	0	6
9/7	0	3	1	4	3	0	–	0	0	11
2/7	0	6	2	2	0	–	–	0	0	10
26/6	0	0	0	10	–	–	–	0	1	11
17/6	1	4	5	1	–	–	–	0	0	11
9/6	0	6	–	–	–	–	–	2	2	10
2/6	2	–	–	–	–	–	–	0	3	5
							Total	2	6	64
	L. kaempferi x L. kaempferi									
16/7	0	0	1	1	1	4	6	0	0	13
9/7	0	0	2	1	5	2	–	0	0	10
2/7	0	0	2	4	5	–	–	0	0	11
26/6	0	1	3	6	–	–	–	0	0	10
17/6	1	3	4	–	–	–	–	0	0	8
9/6	1	9	–	–	–	–	–	0	3	13
2/6	11	–	–	–	–	–	–	0	0	11
							Total	0	3	76

Table 2. — Summary ovule development stages according to cross.

Cross	Total No. of embryos	Fertilized ovules: Number of embryos in different stages: Normal stage No.	%	1 Stage behind normal stage No.	%	>1 Stage behind normal stage No.	%	Unfertilized ovules: Pollen in micropyle only No.	%	Pollen on nucellus ungerminated No.	%
E x E	64	48	75.00	11	17.19	3	4.69	2	3.12	0	0.00
J x E	64	24	37.50	11	17.19	21	32.81	2	3.12	6	9.38
J x J	76	43	56.58	20	26.32	10	13.16	0	0.00	3	3.94

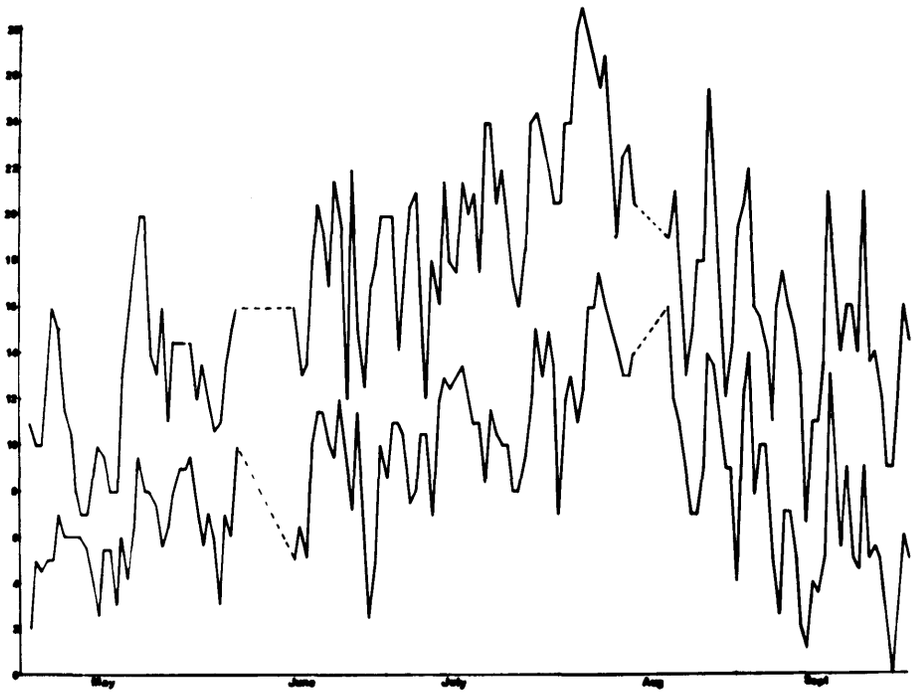


Figure 14. — Daily maximum and minimum temperatures at Newton from 1 May 1974 to 30 September 1974.

embryos could not be accounted for by differences in times of fertilization or to temperature conditions, that such variation represented dead or slowly growing embryos.

Table 1 shows the variation in stage of development of embryos at each sampling date. Among the *L. decidua* × *decidua* crosses most of the embryos were at or near the normal stage and only three were more than a week behind normal development. In the hybrid embryos, which were all developing in *L. decidua* maternal tissue, there were many more embryos behind the normal stage than among embryos in the *L. decidua* × *L. decidua* cross. By the end of June it was seen that all hybrid embryos had developed more slowly than non-hybrid embryos. In the *L. kaempferi* × *L. kaempferi* crosses about half the embryos were behind the normal stage. *Table 2* shows the percentage of embryos at the normal stage and at one and two or more stages behind normal. It is apparent that the proportion of slowly growing embryos was much higher in hybrids than in the intraspecific crosses.

Both *L. decidua* and hybrid embryos showing depressed growth in *L. decidua* maternal tissue developed this condition when they were part way down the corrosion cavity. This was in contrast to the situation of *L. kaempferi* embryos where the dying embryos were found at the end of the corrosion cavity where, under normal circumstances, they would be entering their period of rapid growth which occurs during the latter part of June and early July.

These results show that failure of embryos contributes significantly to the formation of empty seed. The results do not, however, indicate the causes of embryo failure in crosses between species. Embryo failure in the hybrids may be due to a biochemical incompatibility between embryo and endosperm or it may be caused by genetic imbalance. When the seeds germinate, however and the influence of the maternal tissue is removed, the seedlings normally express heterosis. This suggests that incompatibility between embryo and endosperm may be the cause of the increased rate of embryo failure in the hybrid embryos, rather than genetic imbalance.

Hybrid inviability shown in the development of *L. kaempferi* × *decidua* embryos manifests itself as slowing of rate of growth following extension of the early embryos. KRIEBEL (1970) also showed increased embryo inviability among interspecific crosses of *Pinus* and stated that the inviable embryos had extended into the corrosion cavity before collapsing.

The data from *Table 2* were used to calculate the expected proportion of empty seeds at each stage in development and this is shown in *Table 3*. Seeds were extracted by hand to ensure accurate assessment of total seed yield and hence total potential yield given that all seeds are viable. The number of empty seeds were determined from germination and cutting tests. (HALL 1976).

The first factor considered was that of failure of pollination which reduced potential numbers of filled seed by 33.6% and 20.5% in *L. decidua* and *L. kaempferi* ovules respectively (HALL and BROWN 1976). This factor reduced the potential number of filled seeds by about 10 in *L. kaempferi* and about 16 in *L. decidua* cones. The data for failure of pollination was obtained from ovules pollinated with stained pollen and based upon 195 ovules in *L. kaempferi* and 214 in *L. decidua*. The proportion of ovules pollinated could be determined in ovules sectioned as late as mid-June but the data from stained pollen was used because it was based on many more samples than were obtained by sectioning.

In *L. decidua* ovules pollen remaining in the micropyle accounted for a reduction of less than one seed while failure of pollen to germinate on the nucellus accounted for a reduction of about 1.5 seeds in *L. kaempferi* cones and about 2.6 in the hybrid cones. Failure of pollen to germinate on the nucellus may be due to inviable pollen or an inhibition. The observed proportion of ovules with only ungerminated pollen is the best estimate of the effect on seed production of pollen inviability considering the uncertainties associated with *in vitro* tests of pollen viability.

The last factor considered was that of embryo failure. Potential yield of seed was reduced by two amounts; first the minimum amount consisting of embryos more than one week behind the normal and secondly all embryos behind the normal stage. Minimum losses ranged between about 1.4 and 9.2 seeds and maximum losses between about 6.8 and 15.5 seeds. The resulting potential yields were compared with the actual yields. The actual yields in the E × E and J × E crosses were within or close to the calculated minimum and maximum expected yields. The actual yield in the J × J cross was less than the calculated minimum expected yield.

Table 3. — Comparison of actual and expected yields of seed after losses attributed to lack of pollination, lack of fertilization and embryo failure.

Causes of reduction in potential yield	Numbers of seeds per cross E x E	E x J	J x J
Total potential yield of filled seed per cone	46.73	42.43	49.32
Loss due to failure of pollination to occur	-15.70	-14.26	-10.11
Expected yield from pollinated ovules	31.03	28.17	39.21
Loss due to pollen remaining in micropyle	- 0.97	- 0.88	0.00
Loss due to pollen remaining on micropyle not germinating	0.00	- 2.64	- 1.54
Expected yield from ovules	30.06	24.65	37.67
Loss if all embryos die which are more than one stage behind the standard	- 1.46	- 9.24	- 5.16
Expected yield if minimum no. of embryos die	28.60	15.41	32.51
Loss if all embryos die which are behind the standard	- 6.79	-14.08	-15.48
Expected yield if maximum no. of embryos die	23.27	10.57	22.19
Actual yield of full seed per cone	26.40	15.60	19.77
Loss due to non-germinability	- 3.37	- 3.29	- 3.44
Number of germinable seeds per cone	23.03	12.31	16.33

When seeds from controlled crosses in these clones were germinated it was seen that the percentage loss in seeds from the three crosses were 12.8, 21.1 and 17.4 respectively for E × E, J × E and J × J crosses (HALL 1976). Thus the yield of full seed per cone which germinated becomes 23.02, 12.31 and 16.33 for the three crosses. The numbers of full seed which did not germinated was about 3.4 in the intraspecific crosses and about 3.3 in the hybrids.

In crosses with *L. decidua* as the mother tree, the number of full seed which germinate lies within or close to the range of minimum and maximum expected yields. It is apparent that embryos which are more than a week behind the standard in development do not develop into full seed. Some of the embryos which are a week behind the standard also die.

In the cross with *L. kaempferi* as the mother tree it appears that all slowly growing embryos die, in addition some of the 'normal' ones may die as well.

Although the data are based on only one clone of each species it appears that there are differences in embryo development between species and that the slower growing embryos are better able to survive in *L. decidua*.

It is apparent that the largest single factor affecting formation of empty seeds is that of failure of pollination to occur. The next factor in importance is that of embryo failure, especially in hybrid crosses.

At this point it is appropriate, with regard to future research, to speculate upon the causes of embryo failure in both intraspecific and hybrid crosses.

In the intraspecific crosses the probability is that embryo mortality is a result of the action of homozygous recessive lethal genes such as has been reported in *Picea abies* and *Pinus sylvestris* (KOSKI 1971). In *L. kaempferi* embryos these genes appear to effect changes in the embryo after it arrives at the base of the corrosion cavity. At this point normal embryos begin to enlarge rapidly and meristematic tissues to form, so presumably many more genes are involved in development than at earlier stagse and thus there is greater possibility of lethal genes expressing themselves in the developing embryo. Alternatively in *L. decidua* where most of the embryo mortality seems to occur as the embryos are moving down the corrosion cavity, the action of lethal genes may be different. As the embryos move away from the archegonial region they cease to be influenced by that region through their suspensors and come into direct contact with the endosperm which then begins to break down and be utilized by the embryo. The lethal genes may express themselves during this movement, possibly by affecting the growth of the suspensors or also by the lack of synthesis of a substance in the endosperm which is needed by the developing embryo. At the same time the embryo is growing, though slowly, and the lethal genes could act to prevent development at an earlier stage than they do in *L. kaempferi* embryos.

The most important feature of the data from this study, however, was the increased proportion of hybrid embryos which die compared to those from intraspecific crosses. Most mortality in hybrid embryos began as they moved down the corrosion cavity and came into contact with the *L. decidua* endosperm ie at the same stage in development as in the *L. decidua* embryos. Some embryos die after they reach the base of the corrosion cavity as in *L. kaempferi*. The cause of embryo mortality in the hybrids is probably of a different nature from that in *L. decidua*. It is possible that there are lethal genes which are common to both parent species which express themselves in the developing embryo, but since these parent species are not considered to be closely related, the probability of the presence of such genes must be considered small. A common barrier to hybridization in conifers is inhibition of growth of the pollen tube or failure of the pro-embryo to be formed but neither of these occurred in hybrid embryos in larch.

If, as has already been suggested, there is an interaction between the embryo and endosperm an alternative explanation would be that the hybrid embryo, if it is to survive at this time, must be provided with the required maternal genes in an operative form. With any degree of heterozygosity in such genes there will be a proportion of hybrid embryos from which operative genes will be missing. In such cases embryo failure will be the result. The fact that *L. decidua* embryos also fail at this time indicates a fairly high degree of heterozygosity of genes involved in this part of the developmental process.

Since the reasons for failure of embryos appear to have physiological and/or genetic origins there is probably nothing which can be done to increase yield of seeds in individual clones. Rigorous selection of clones which have consistently high yields of viable seeds is probably the only practicable method for increasing yields in a seed orchard.

Summary

The development of embryos in controlled crosses of *Larix decidua* × *L. decidua*, *L. decidua* × *L. kaempferi* and *L. kaempferi* × *L. kaempferi* was studied. Fertilization of archegonia occurred on or about 2 June, early embryos were formed within the first week and embryos with distinct meristematic zones and cotyledons were formed by mid-July.

Lack of pollination was the major factor resulting in the formation of empty seed. Most empty seeds from pollinated ovules were a result of embryo failure which was much more common in the hybrid cross than in crosses between the same species. The high proportion of empty seeds in hybrid crosses was therefore explained by increased embryo inviability in these crosses. Failure of fertilization to occur was also much more common in hybrids than in intraspecific crosses.

Failure of pollen grains to move to the nucellus from the micropyle and failure of pollen on the nucellus to germinate accounted for a small proportion of the empty seeds.

Embryo failure occurred in *L. decidua* ovules when embryos were growing through the corrosion cavity and in *L. kaempferi* when embryos were at the end of the corrosion cavity and beginning their period of rapid growth and enlargement.

Key words: Meiosis, embryo development, seed yield, *Larix decidua*, *Larix kaempferi*, *Larix eurolepis*.

Zusammenfassung

Bei Artkreuzungen zwischen *Larix decidua* und *L. kaempferi* sowie bei Kreuzungen innerhalb der Arten wurde die Entwicklung der Embryos beobachtet. Die Befruchtung der Archegonien erfolgte um den 2. Juni. Neben einigen Embryos, die sich in der ersten Woche bildeten, zeigten sich deutliche Meristemzonen und Kotelydonen erst Mitte Juli. Leere Samen sind in erster Linie auf das Nichtvorhandensein von Pollen zurückzuführen. Samenanlagen, die bestäubt waren, gelangten nicht zur Entwicklung, weil der Embryo versagte. Dies wurde insbesondere bei den Artkreuzungen beobachtet. Nur bei einem geringen Teil der leeren Samen konnte als Ursache die ausbleibende Pollenbeförderung von der Mikropyle zum Nucellus oder die ausbleibende Pollenkeimung auf dem Nucellus erkannt werden. Auch während der Embryonalentwicklung im Endosperm wurden in verschiedenen Entwicklungsphasen Ausfälle beobachtet.

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Increased Flowering of Sitka spruce (Picea sitchensis (Bong.) Carr.) in a Polythene House

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Introduction

Sitka spruce *(Picea sitchensis* (Bong.) Carr.) is the economically most important forest tree species in Great Britain, comprising more than 40% of the total number of plants produced by the Forestry Commission in the last decade. There is considerable scope for improving the productivity of Sitka spruce by breeding, but the absence of methods for controlling flowering has been a fundamental obstacle to progress. Flowering does not occur readily in Sitka spruce and in nature there is little before trees are 25—30 years old. In the related species Norway spruce *(Picea abies)* plentiful flowering often follows high temperatures in the previous summer (Tiren 1935, Eklund 1957) and similar findings were reported for *Fagus sylvatica* (Holmsgaard and Olsen 1960), *Larix leptolepis* (Yanagihara *et al.* 1960) and *Pinus ponderosa* (Daubenmire 1960). However, very few studies have been made to assess the effect on flowering of artificially altering the environment around the tree.

Experiments have been carried out at the Forestry Commission Northern Research Station to determine the effect of growth in a polythene tunnel house on the flowering of 4-years-old grafted plants of Sitka spruce.

Materials and Methods

Grafted plants of Sitka spruce *(Picea sitchensis* (Bong.) Carr.) comprising young seedling rootstocks and scions from the tops of mature trees are known to be capable of producing strobili from about 8 years after grafting and were consequently chosen for use in this study. Scions from 19 clones were grafted on to rootstocks of 3-years-old seedlings in 1970 and transferred to 33-cm-diameter polythene buckets in 1972. Twelve uniform plants, about 0.6 m in height, were selected from each clone in the spring of 1973; nine of these plants were transferred to a polythene-skinned house while three remained outside as controls. The polythene skin, made from a mixture of polyethylene and ethyl vinyl acetate, covers a framework 3.1 × 15 × 10 m.

The buckets were weighed each week and sufficient water was supplied to keep the mean weight of those in the polythene house approximately equal to the mean weight of those containing the control plants. The polythene house was ventilated by opening the doors at both ends during the day. Air temperatures were recorded at seven different heights between 0.1 m and 1.07 m above ground level inside the polythene house and at two heights outside the polythene house; overall mean temperatures are presented in *Table 1.*

Table 1. — Mean daily maximum and minimum air temperatures (°C) in the polythene house (PH) and among the control plants outside (CON) in the spring and summer of 1976.

	May		June		July		August	
	PH	CON	PH	CON	PH	CON	PH	CON
Min	7.9	6.1	11.6	9.7	13.1	10.0	11.0	9.3
Max	34.5	16.8	39.3	22.3	39.3	23.1	38.2	23.7

Results and Discussion

Grafted plants were retained in the polythene house for periods during three growth cycles. Following an initial treatment for 3.5 months in the spring and summer of 1973 a striking increase in both the proportion of plants flowering and the numbers of male and female strobili per plant was observed in 1974 *(Table 2).* The same group of plants was returned to the polythene house in April 1974 and remained there for a further 12 months. This second and longer treatment led to a still greater increase in flowering in 1975, and a response also occurred following the third year of treatment *(Table 2).* It should be noted, however, that individual plants within the group did not flower strongly in two successive years.

Buds of Sitka spruce are initiated in April and become distinguishable as reproductive or vegetative by September in Scotland (Tompsett unpubl.); the processes leading to flower differentiation must therefore take place within the intervening period of four months. Detailed observations made during these months in 1976 indicate that there are large differences in the mean daily temperature maxima between positions inside and outside the polythene house *(Table 1),* confirming preliminary observations made

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