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Factors Affecting Seed Yield in *Larix*[1]

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落葉松의 種子結實에 影響을 미치는 要因[1]

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ABSTRACT

Various factors reducing seed yield in 4 *Larix* species throughout the whole reproductive cycle were investigated and partitionate losses attributed to them were determined. Pollen quality, lack of pollination, and degeneration of female gametophyte played minor roles in reducing seed yield. Failure of pollinated ovules to be fertilized was an important factor causing seed loss. Embryo degeneration was also a major factor causing seed loss in all 4 species. Strobili abortion, which causes loss of all potential seeds in a cone, was the most important factor in reducing seed loss in this study. Based on the results obtained from this study, hybridizations in either direction between European larch and Japanese larch are likely to resulting viable seed. However, hybridization between tamarack as a mother tree and European larch are not likely to result in viable seeds being produced.

Key words : Larix, *seed yield, reproductive cycle, hybridization.*

요 약

4가지 낙엽송(구주낙엽송, 일본낙엽송, 시베리아낙엽송, tamarack)의 종자결실에 영향을 미치는 요인과 각각의 요인들에 의한 종자손실율을 조사하였다. 화분의 질, 수분의 실패, female gametophyte의 퇴화 등은 종자결실에 큰 영향을 미치지 않았으나, 수정실패, 배의 퇴화 등은 조사된 4개의 수종에서 모두 주요한 종자손실요인으로 조사되었다. 조사된 요인들 중 종자수확에 가장 큰 영향을 미치는 것은 수분후 strobilus자체가 퇴화되는 것이었다. 조사된 4가지 교배조합중 구주낙엽송 또는 일본낙엽송을 모수로 하는 것이 종자생산측면에서는 가장 바람직한 것으로 나타났으며, tamarack을 모수로 하는 교배조합은 건전한 종자생산을 하지 못할것으로 판단된다.

INTRODUCTION

Larches(*Larix* species) are an important alternative reforestation choice because of their rapid growth rate, good pulping characteristics, and desirable ecological value. Hybrids combining the rapid growth rates of Japanese larch(*Larix leptolepis*) and/or European larch(*L. decidua*) and the cold hardiness of tamarack(*L. laricina*) and/or Siberian larch(*L. sibirica*) are particulary desirable. However, low seed yield and poor seed

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quality in these four larch species have been a problem in creating hybrid planting stock.

Seed production is affected by a variety of factors. Generally, they can be categorized into prezygotic factors and postzygotic factors. Prezygotic events including pollen - pistil interaction (Knox 1984), self - incompatibility(Cornish *et al.*, 1988), gametophytic competition(Mulcahy 1978), and selection at fertilization have been reported to reduce seed set in flowering plants.

In conifers, inviable pollen formation(Barner and Christiansen, 1959 ; Chandler and Mavrodineau, 1965 ; Eriksson 1968), pollination failure (Hall and Brown, 1977 ; Kosinski 1987a), degeneration of female gametophyte(Kosinski 1987b ; Owens *et al.*, 1990), and fertilization failure(Hall and Brown 1977 ; Kosinski 1987a ; Owens *et al.*, 1990) are known to cause seed loss.

To understand the factors affecting seed yield in conifers, all stages in the reproductive cycle must be studied to determine quantitative losses at each stage. The relative importance of the factors may vary between species and trees in different years. However, basic information on seed yield and seed quality is badly needed for larch improvement activities. Once the factors and stages at which seed loss characteristics are determined, studies of physiological and/or molecular bases for these factors will be possible.

The objective of this study was to determine at which stages of cone and seed development seed losses occur in four different larch species (*L. decidua*, *L. laricina*, *L. leptolepis*, and *L. sibirica*) growing in Michigan's Upper Peninsula and which cross combination is proper for producing healthy and abundant hybrid seeds.

MATERIALS AND METHODS

Experiments and materials described in this study were located at the Michigan Technological University Tree Improvement Arboretum located near South Range, MI. The trees used in this study were established at this site in 1968.

Pollen collection and Pollen germination test

Male - strobili - bearing branches of the 4 species were collected on April 15, 1992 and placed in 7 - up solution at room temperature until pollen dehisced. Subsamples of the pollen were placed in sterile microplates containing 100μl of pollen germination medium(Ho and Rouse, 1970) in each well. After 5 day incubation at 24℃ in the dark, germination rates were determined from five replications of 100 pollen grains from each well. Those pollen grains with a stalk cell and body cell divided from the generative cell during 5 day incubation were consisered to be viable.

Pollination and determination of pollination frequency

Branches with abundant female strobili were bagged on intact trees after removing all male strobili on April 10, 1992. On April 25, 1992 when female strobili were fully receptive, pollen grains were applied to the strobili with a fine paint brush. Crosses between *L. decidua* and *L. leptolepis*, *L. leptolepis* and *L. decidua*, *L. laricina* and *L. decidua*, *L. sibirica* and *L. decidua* were made. Two weeks after pollination, 3 cones from each cross were collected and ovules were examined by dissecting microscope to determine the presence of pollen on micropylar arms. From this stage, 3 cones from each cross were collected at weekly intervals and 6 to 7 ovules per cone were fixed for microscopic examination until seed cone development was completed. To determine the number of pollen grains in the micropylar canal, ovules were taken from cones collected 4 weeks after pollination. Ovules were fixed in FAA solution, dehydrated in an alcohol - xylene series(Sass 1958), and embedded in TissuePrep. Embedded specimens were serially sectioned longitudinally at 8μm and stained with safranin and fast green for microscopic observation. Probability of ovules being pollinated was calculated by multiplying pollen germination rate by the number of pollen in the ovule.

Abortion of female strobili

Two weeks after pollination, the number of degenerated strobili inside the pollination bags was counted and the degeneration rate was determined.

Degeneration of female gametophyte

On May 20, 1992 three cones from each cross were collected and ovules were cut in half transversely. Ovules with shrivelled megagametophyte were counted from collected cones. Ovules in basal and distal ends which are sterile were not included.

Fertilization

Frequency of fertilization for the crosses was determined for ovules collected from late May to Mid June, 1992. A total of twenty ovules from 3 cones for each cross were fixed after removing seed coats. Longitudinal serial sections were made and staining was done as described above. Ovules showing pollen tubes, egg cells with male gametes present, or vacuolated and granular - shaped egg cells were considered to have been fertilized.

Embryo degeneration

To determine the rate of embryo degeneration, a total 20 ovules from 3 cones for each cross were collected at eight week and twelve weeks after pollination. They were fixed and sectioned as described above. Presence and number of early embryo(globular stage : 6 - 8 weeks after pollination) and late embryo(torpedo stage : 8 - 10 weeks after pollination) were analyzed for the 20 ovules of each cross and the rate of embryo degeneration was determined by subtracting the percentage of ovules with an embryo at the late stage from percentage of ovules with an embryo at the early stage.

RESULTS AND DISCUSSION

Pollen viability

Dry pollen grains of larch appeared cup - shape while those in solution were spherical or elliptical. The mature pollen grains had the generative cell and the tube cell in addition to the two prothalial cells. During incubation in the medium, the cup - shaped pollen became turgid, followed by elongation of the whole pollen grain and breaKing of exine. The exine was usually broken into two halves. After 4 day incubation, it was found that the generative cell divided into the stalk cell and the body cell(Fig. 1). However, no further development was observed because of the heavy contaminations by bacteria and fungi.

Pollen tube development *in vitro* in larch has not been observed(Ho and Rouse 1970). Ekberg and Eriksson(1967) stated that there is no precise way to determine the pollen viability *in vitro* in larch. Pollen grains of shrunken or shrivelled appearance as shown in Fig. 1 are oviously not functional. On the other hand, some of the normally appearing pollen grains may be sterile.

The germination test revealed that there was little correlation between frequency of meiotic abnormality(Shin, submitted for publication) and pollen germination rate(Table 1). For example, Siberian larch had the highest frequency of meiotic irregularity but it had a relatively high

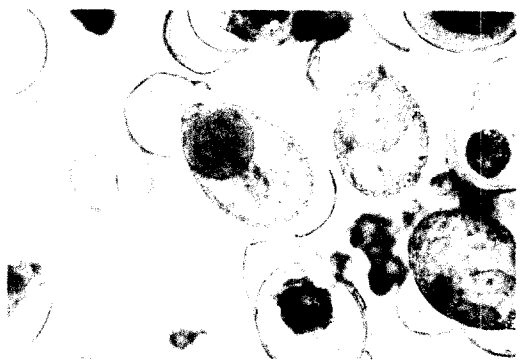

Fig. 1. Germinating pollen with broken exine and stalk cell, body cell, and body nucleus(upper) while sterile pollen shrunken with deteriorated cytoplasm (lower).

Table 1. Frequency of pollen germination of 4 larches growing in Michigan's western Upper Peninsula in 1992.

Species	Pollen germination	
	number	(%)
L.decidua	342/735*	(46.5)
L.laricina	785/998	(78.7)
L.leptolepis	528/896	(58.9)
L. sibirica	729/1032	(70.6)

* Number germinated/number observed.

pollen germination rate.

Furthermore, Japanese larch had the lowest in meoitic irregularity but had a low pollen germination rate. These results suggest that some meiotic abnormalities in PMC's may recover to form normal pollen while pollens which seemed normal may have internal deficiencies. Alternatively, factors influecing mature pollen formation from microspore may influence pollen germinability more than those up to microspore formation.

Pollination

Pollination success was observed in more than 90% of the control pollinated ovules except in tamarack in which only 75% of the ovules were pollinated(Table 2). Open - pollinated ovules showed lower frequencies compared to control pollinated ovules, ranging from 75% in European larch to only 13.2% in tamarack. Hall and Brown(1977) reported that one third of their control pollinated ovules lacked pollination. This was the major factor reducing seed yield in their study with European and Japanese larches. However, it was not the case in our study. Far lower pollination success in tamarack seemed due to its smaller cone size than others. The numbe of pollen grains per micropyle ranged from 1 to 11, and most of them were pollinated with more than 2 pollen grains(Fig. 2 ; Table 3). Thus, the probability of more than one viable pollen grain in these ovules was 1 or more than 1 depending on the pollen viability as expressed by the pollen germination rate.

Pollination success is affected greatly by the timing of the pollination. Pollen should be applied onto the strobili when they are fully receptive. Said *et al.*,(1990) reported that the length of time for ovule receptivity in larch may be less than 48hr. The high rate of pollination failure reported by Hall and Brown(1977) was probably due to application of pollens at the wrong time. Multiple pollen applications at different times during the receptive period can increase pollination success because the receptive period of individual ovules is often different(Owens *et al.*, 1981).

Abortion of female strobili

Strobili loss soon after the pollination is a common phenomenon in many conifers and usually atributed to low temperature(Colangeli *et al.*, 1990 ; Owens and Blake, 1985 ; Owens *et al.*, 1991). In Siberian larch, about 80% of the strobili aborted as compared to about 28% in tamarack(Table 4). Most of aborted strobili were found to be in contact with the pollination bags. It seemed that a touching the bags resulted in

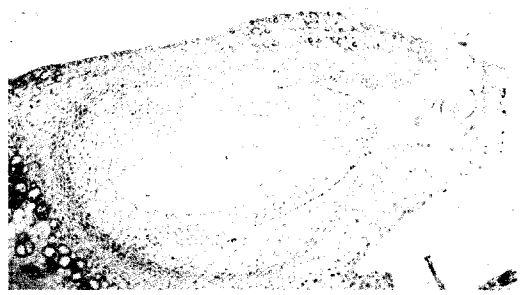

Fig. 2. Pollens engulfed by the mouth of micropyle.

Table 2. Mean number and standard deviation of ovules with pollen from control pollinated and open pollinated cones of larch in 1992*.

Species	No. Ovules examined	Open pollination(%)	Controlled pollination(%)**
L. decidua	60	45.0±12.0(75.0)	55.7±1.5(92.7)
L. laricina	20	2.7± 3.1(13.3)	15.0±1.0(75.0)
L. leptolepis	60	38.7± 9.0(64.4)	57.3±2.5(95.5)
L. sibirica	60	39.0± 9.0(65.0)	58.7±1.5(97.7)

* Calculated based on the presence of pollen on the micropylar arms of ovules from 3 cones of each crosses by examining with a dissecting microscope.

** Controlled pollinations : *L. decidua*=*L. decidua* / *L. leptolepis*, *L. laricina*=*L. laricina* / *L. decidua*, *L. leptolepis*=*L. leptolepis* / *L. decidua*, *L. sibirica*=*L. sibirica* / *L. decidua*

Table 3. Probability of ovules pollinated with at least 1 pollen grain*

Cross	No. pollen grains in an ovule	Per cent of ovules	Pollen germination rate	Probability of more than 1 viable pollen grain in the ovule**
	1	5	0.59	0.59
L. decidua ×	2	25	0.59	1.0
L. leptolepis	3	40	0.59	1.0
	4+	30	0.59	1.0
	1	25	0.47	0.47
L. larcicina ×	2	25	0.47	0.94
L. decidua	3	25	0.47	1.0
	4+	25	0.47	1.0
	1	10	0.47	0.47
L. leptolepis ×	2	25	0.47	0.94
L. decidua	3	25	0.47	1.0
	4+	40	0.47	1.0
	1	10	0.47	0.47
L. sibirica ×	2	15	0.47	0.94
L. decidua	3	35	0.47	1.0
	4+	40	0.47	1.0

* Calculated based on the number of pollen grains observed microscopically in the micropylar canal of 20 ovules(6 to 7 ovles from each of 3 cones for each cross).

** Determined by multiplying pollen germination rate by the number of pollen in the ovule.

Table 4. Degeneration of female strobili in pollination bag in the first two weeks after pollination.

Species	Total No. strobili pollinated	No. strobili aborted	Abortion rate (%)
L. decidua	171	114	66.7
L. laricina	116	32	27.6
L. leptolepis	213	126	59.2
L. sibirica	163	130	79.8

either total abortion of a strobilus or damage on the side in contact with the bag. In addition, about 20% of the strobili were aborted without being in contact with the bags was probably due to frost damage as indicated by Owins *et al.*,(1991) in Douglas - fir.

There was no external signs of abortion at the early stages. However, aborting strobili were soft, and when dissected, the insides were rotten. There may be other factors causing cone abortion. Colangeli *et al.*,(1990) suggested that an ice - nucleating bacterium *Pseudomonas* may cause cone abortions. They found significantly higher populations of the bacteria in cones when cone abortion rate was high in Douglas - fir. These ice - nucleating bacteria are known to cause ice formation at temperatures just above freezing which occurs frequently during or after pollination. The bacteria may affect cone abortion in combination with cold temperature. Strobili that had experienced low temperatures may also be more vulnerable to bacterial infection. Besides temperature and bacterial related abortions, abortion by a function of female choice(Weins *et al.*, 1987) has been suggested in *Pinus*. In *Pinus*, growth regulators produced by pollinated ovules affect nutrient transport to developing cones. If too few ovules are pollinated, the cone aborts (Sweet 1973). Sarvas(1962) estimated that when more than 20% of the ovules in a *Pinus sylvestris* cone fail to be pollinated, the cone aborted. This possibility might be excluded for our case in which more than 90% of ovules of control pollinated cones had pollen present.

Ovule development and female gametophyte degeneration

On the first collection date(May 3) ovules were no longer receptive and pollen grains were engulfed by the micropyle(Fig. 2). At this point,

Table 5. Degeneration of female gametophyte tissue before fertilization*

Species	Number of Ovules per cone	Avg. No. Aborted Ovules per cone	
		Open pollination	Controlled pollination**
	X±s.d.	X±s.d.	X±s.d.
L. decidua	81.3± 1.2	8.3±1.5	4.7±3.5
L. laricina	32.7± 3.1	1.3±1.5	1.3±0.6
L. leptolepis	87.7±12.7	4.3±2.5	6.0±2.0
L. sibirica	93.3± 4.2	5.7±2.1	4.3±2.5

* Calculated based on the number of undeveloped ovules observed before fertilization. Basal and distal sterile ovules were not included in these figures.

** Controlled pollinations : *L. decidua*=*L. decidua*×*L. leptolepis*, *L. laricina*=*L. laricina*×*L. decidua*, *L. leptolepis*=*L. leptolepis*×*L. decidua*, *L. sibirica*=*L. sibirica*×*L. decidua*

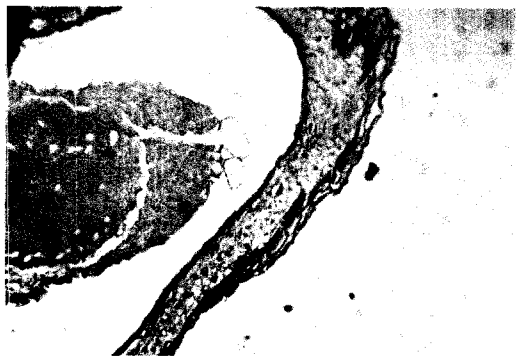

Fig. 3. Ovule showing pollen tube penetrating the nucellus.

pollen grains ingested into the micropylar canal were observed in the ovule sections of European larch and Siverian larch, but not in Japanese larch and tamarack. Ovule abortion was noticeable at the archegonial development period. Abortion frequency ranged from 4% to 6.8% depending on the species(Table 5). There seemed no significant different between control and open-pollinated cones. This frequency was similar to that reported in other conifers(Colangeli and Owens, 1990 ; Kossuth and Fechner, 1973 ; Sweet 1973). This post-pollination ovule abortion occurs in some conifers if pollen grain is not present. Thus, the presence of pollen in the ovule is essential for the continued development of the ovule. The integuments of abvorted ovules differentiated into thin seed coats and did not expand resulting in small, flat nonviable seeds.

Fertilization

By the end of May(in tamarack and Siberian larch) and the early June(in European and Japanese larches), the ovule was almost fully enlarged with the egg nucleus in the center of the egg cell. Pollen grains reached top of nucellus and ready to penetrate the tube through the nucellus for fertilization. Pollen tube penetration and the fusion of gametes were observed from the material taken on June 1 in Siberian larch, on June 6 in tamarack and European larch, and on June 14 in Japanese larch(Fig. 3). Because of difficulties related in sectioning, I was not able to observe the fusion of male and female gametes in all ovules sectioned. Thus, ovules with pollen tube penetration and fusion of gametes, ovules whose egg cell was highly vacuolated and granular-shape were considered to have been fertilized as stated in *L. occidentalis*(Owens and Modler, 1979) and Douglas-fir(Owens *et al.*, 1990). Eighty percent of the sectioned ovules showed early embryos present indicating healthy seed. This matched with the fertilization frequency ranging from 75% to 90% depending on the species (Table 6). In some ovules, fertilization was observed in more than one archegonium resulting from polyembryony. The fertilization frequency observed in this study was similar to that reported in European larch(Kosinski 1987a), but it was higher than that of European larch and Japanese larch reported by Hall and Brown(1977).

Several possible reasons for the failue of fertilization in larch have been suggested. Failure of pollen to reach the nucellus can cause fertilization failue. In addition, failure of pollen to

germinate on the nucellus can cause fertilization failure(Hall and Brown, 1977). They suggested that there may be the factors affecting pollen drop mechanism from micropylar canal to the nucellus or it may simply due to inviable pollen grains being ingested into the micropylar canal.

Seeds whose megagametophytes were degenerated at early stages before fertilization were small and flat. However, seeds with degenerated megagametophytes due to fertilization failure were round and normal in appearance so that it was not possible to distinguish them unless they were dissected.

Table 6. Fertilization frequency of *Larix* hybrids following controlled pollinations*.

Cross	Frequency of fertilization	Failure of fertilization
L. decidua × *L. leptolepis*	90	10
L. laricina × *L. decidua*	75	25
L. leptolepis × *L. decidua*	85	15
L. sibirica × *L. decidua*	80	20

* The frequency of fertilization is based on microscopic examination of thin median longitudinal sections. Ovules showing pollen tubes, egg cells with male gametes present, or vacuolated and granular-shaped egg cells were considered to have been fertilized.

Fig. 4. Four free nuclei of the pro-embryo moved to the base of the archegonium.

Embryo development and degeneration

Embryo development in the 4 larch species was similar to that described in previous studies of *Larix*(Hall and Brown, 1977 ; Kosinski 1987b ; Owens and Molder, 1979 ; Schopf 1943). Embryo degeneration was observed most frequently in seeds of tamarack X European larch (40%), followed by Japanese larch X European larch(35%). Thus, in this study, embryo degeneration was a major factor causing decreased seed yield.

Two days after fertilization the zygote nucleus divided in the center of the archegonium to form two, then four free nuclei(Fig. 4). The four free nuclei and neocytoplasm moved to the base of the archegonium to form eight free nuclei and then 16 free nuclei of the proembryo. It appeared that the period for the development of embryos from pro- to early embryo(club shape) varied among species. In Siberian larch, it only took 10

Table 7. Frequency of embryos observed in female megagametophyte tissue at the globular stage of embryo development(early embryo=6 to 8 weeks after pollination) and at the torpedo stage of embryo development(late stage=10 to 12 weeks after pollination)*.

No. embryos in megagametophyte tissue	*L. decidua* × *L. leptolepis*		*L. laricina* × *L. decidua*		*L. leptolepis* × *L. decidua*		*L. sibirica* × *L. decidua*	
	early	late	early	late	early	late	early	late
0	20(%)	40	20	60	15	50	20	50
1	50	60	35	40	30	50	40	50
2	20	0	30	0	25	0	30	0
3	10	0	10	0	20	0	10	0
4	0	0	5	0	5	0	0	0
5	0	0	0	0	5	0	0	0
Total	100	100	100	100	100	100	100	100

* Determined by microscopically examining 20 ovules(6 to 7 ovules from each 3 cones for each cross).

days while it took 20 days in Japanese larch. Thus, mature embryo developement in Siberian larch was observed on July 5 when embryos in other species were still in the early embryo stages. Proembryo or early embryo stages were observed in more than 80% of seeds sectioned (Table 7). In most seeds, 2 or more embryos were found. This may have been due to multi - fertilization that occured in the ovules or to delayed cleavage polyembryony as described by Kosinski(1987b) in European larch. It has been known that when simple polyembryony occured by multi - fertilization, usually one distal embryo develops more rapidly into the corrosion cavity and the less vigorous proximal embyro degenerated in a different part of the corrosion cavity. In our study, it seemed that both simple polyembryony and cleavage embryony occured in ovules because one dominant embryo was observed in some seeds(Fig. 5) whereas 2 or more embryos with the same size were found in others(Fig. 6).

Polyembryony was observed most frequently in Japanese × European hybrid seeds, followed by tmarack × European larch, and Siberian larch × European larch(Table 7). The high abortion rate of early embryos in Japanese larch × European larch and tamarack × European larch seeds probably indicates that severe competition occured among embryos within the ovule. It has been suggested that competition between genetically diverse embryos occurs within ovules because of multiarchegoniate ovules in most conifers(Owens *et al.*, 1991). This was also ovserved in my study. For example, competition of early embryos developing from opposite direction as seen in Fig. 7 may cause the degeneration of both embryos later.

The developmental selection between embryos within one megagametophyte has been suggested to cause embryo abortion(Singh 1978). Embryo abortions were more frequent when early embryos were pushed into the corrosion cavity and when intimate contact with the megagametophyte was seen(Hall and Brown 1977 ; Owens *et al.*, 1991). Any gene control from the megagametophyte could play more important role in late embryo development than during early embryo development, or that the zygotic genome is not fully activated until late embryo development when

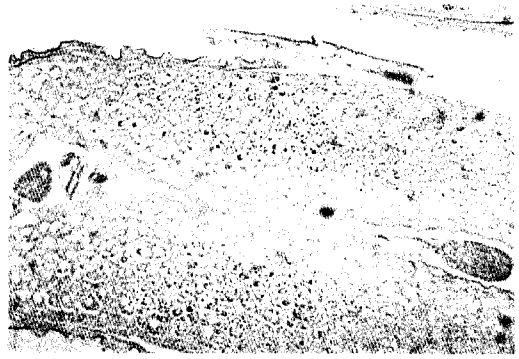

Fig. 5. One dominant distal embryo develops rapidly into the corrosion cavity while a proximal small embryo degenerates in different part of the corrosion cavity.

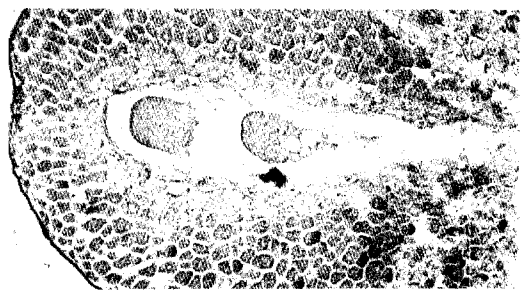

Fig. 6. Two embryos with similar size developing in corrosion cavity.

Fig. 7. Two embryos with similar size developing from opposite directions in corrosion cavity which may cause degeneration of both embryos.

losses caused by lethal genes occur. However, nothing is known about gene control of conifer embryogenesis at the present.

Self - inviability caused by selfing in conifer is commonly observed during early embryo development as a result of the accumulation of deleterious recessive genes. Embryo abortions in self - pollinated seeds vary among species and have been reported as 12% in Douglas - fir(Orr - Ewing 1957), 25% in *Pinus sylvestris*(Sarvas 1962), and 30% to 70% in European larch(Kosinski 1987a). However, it was indicated that not all conifer embryo abortion resulted from self - inviability. For example, embryo abortion of Douglas fir and *Thuja plicata* under outcrossing condition was about 20%(Owens *et al.*, 1990 ; Owens *et al.*, 1991). It was also reported that embryo abortion of European larch and Japanese larch that were outcrossed was 15% and 31%, respectively(Hall and Brown, 1977).

Previous studies on hybrid embryo development in *Larix* indicated that there is some degree of hybridity barriers(Hall and Brown, 1977). A common hybridization barrier in conifers is inhibition of growth of the pollen tube or failure of the proembryo to be formed. However, the relatively high fertilization frequency and the normal proembryo formation observed in this study indictes that they were not a problem in this study. As stated above, interaction between the embryo and its megagemetophyte was a probable cause of hybrid embryo abortions in thes study.

CONCLUSION

As summarized in Table 8, various factors throughout the reproductive cycle affect seed yield in *Larix*. Pollen quality, lack of pollination, and degeneration of female gametophyte played minor roles in reducing seed yield. Failure of pollinated ovules to be fertilized was an important factor causing seed loss. Embryo degeneration was also a major factor causing seed loss in all 4 species. Strobili abortion, which causes loss of all potential seeds in a cone, was the most important factor in reducing seed loss in this study. Finding ways to minimize strobili loss after pollination could have a significant effect in improving seed yields. Detailed studies on embryo development to determine the effects of embryo competition and interaction between the embryo and gametophyte, which may cause hybrid embryo degeneration, are needed. Based on the results obtained from this study, hybridizations in either direction between European larch and Japanese larch are likely to resulting viable seed. However, hybridization between tamarack as a mother tree and European larch are not likely to result in viables seed being produced in the western Upper Peninsula of Michigan.

Table 8. Comparison of partitionate losses attributed to various factors and actual loss of seeds determined by underdeveloped and empty seeds at harvest time.

Factors	Crosses*			
	E × J	T × E	J × E	S × E
Pollen quality	2.0(%)	14.8	6.8	6.2
Lack of pollination	7.3	25.0	4.5	2.3
Degeneration of female gametophyte tissue	5.8	4.0	6.8	4.6
Lack of fertilization	10.0	25.0	15.0	20.0
Embryo degeneration	20.0	40.0	35.0	30.0
Cumulative loss	45.1	108.1	68.1	63.1
Actual loss**	38.6	92.0	41.4	61.6

* E × J=*L. decidua* × *L. leptolepis*, T × E=*L. laricina* × *L. decidua*, J × E=*L. leptolepis* × *L. decidua*, S × E=*L. sibirica* × *L. decidua*.

** Loss determined by sorting seeds from 10 cones of each cross at harvest time.

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