Pollination success and full seed percentage in European larch (*Larix decidua* MILL.)

B. SLOBODNÍK

Technical University, Faculty of Forestry, Department of Phytology, Zvolen, Slovak Republic

ABSTRACT: A relationship between the success of pollination and the percentage of full seeds of European larch (*Larix decidua* MILL.) was studied using several models of non-linear correlation analysis. Although the proportion of pollinated ovules was high in most cases (especially in the middle parts of open-pollinated conelets), the mean percentage of full seeds was extraordinarily low (after the controlled self-pollination even close to zero) and most of the calculated correlation coefficients are lower than the corresponding critical value. This fact gives an evidence for the strong effect of additional important factors causing the empty seed formation in *Larix* even after the successful pollination of ovules.

Keywords: European larch; pollen; empty seeds

It is well known that individual species of *Larix* MILL. are characterized by the extremely high percentage of produced non-vital empty seeds and therefore (despite the appreciable importance of this genus in forestry and landscape ecology) their breeding, seed management and artificial regeneration are complicated. Concurrently, the *Larix* species are characterized by large, heavy and wingless (so-called non-saccate) pollen grains whose mobility is considered relatively low in most cases (DYLIS 1948; CHALUPA 1961, 1985, and others). Besides that, the receptive period of female generative organs is relatively short (BARNER, CHRISTIANSEN 1960; VILLAR et al. 1984; SAID et al. 1991; OWENS et al. 1994) and the success of pollination is closely related to the amount of produced pollen and the existing meteorological conditions during its shedding (KRIŽO, SLOBODNÍK 1996). Concerning these weak links of the pollination process and the fact that the integument is able to transform into the normally developed testa in unfertilized larch ovules as well (unlike various species of *Picea* A. DIETR. and *Pinus* L.), the insufficient pollination could be regarded as a very important prezygotic factor markedly influencing the full seed production within this genus. According to the studied literature data, however, the chronically high percentage of produced empty seeds in *Larix* is not probably caused by a single reason but it is to be considered the result of numerous accidents and irregularities in various processes of the reproductive cycle (KOSIŃSKI 1986, 1987; SHIN, KARNOSKY 1995). In this context, the success of pollination should be studied as one of several factors affecting the quality of the produced larch seeds and the actual importance of insufficient pollination should be assessed and quantified.

MATERIAL AND METHODS

The studies on the pollination success and seed quality of European larch were carried out under the conditions of "Kmeťová" seed orchard (Forest Enterprise at Slovenská Ľupča, Banská Bystrica region, Central Slovakia) in 1998. This orchard was established 25 years ago (in 1973) and is composed of grafted plants from the elite trees of one autochthonous provenance (Šumiac, Low Tatra Mts. – the clones marked with "Š") and one provenance with uncertain origin (Motyčky – the clones marked with "M").

The occurrence and number of pollen grains attached to the stigmatic apparatus of ovules were ascertained from microscopic preparations of open-pollinated and self-pollinated female generative structures (a short time before the shedding of pollen, several branches with both male and female strobili were isolated by paper bags in order to prevent the open pollination). For the purpose of rapid quantitative evaluation of pollination success, three open-pollinated and three self-pollinated female strobili were pulled out from each of the four observed grafted plants immediately after the completion of the pollination period and carried to the laboratory. By means of a binocular magnifying glass and scalpel, individual ovuliferous scales were separated gradually from the base to the top of the female strobilus. Similarly to the method

Table 1. Percentage of pollinated ovules for the different modes of pollination, clones, female strobili and their individual parts

Mode of pollination	Clone	Base (%)	Middle (%)	Top (%)	Total (%)
Open pollination	M5	98.98	100.00	91.49	96.82
	M10	87.32	97.01	71.09	85.14
	Š13	66.66	93.60	87.61	82.62
	Š14	65.65	86.59	59.26	70.50
	Total	**79.65**	**94.30**	**77.36**	**83.77**
Self-pollination	M5	38.20	36.51	53.03	42.58
	M10	80.80	94.89	97.02	90.90
	Š13	33.77	63.50	88.90	62.06
	Š14	40.28	71.83	78.78	63.63
	Total	**48.26**	**66.68**	**79.43**	**64.79**

described by BARNER and CHRISTIANSEN (1960), the apical part of each normally developed ovule was cut off, fixed and stained with a drop of the acetocarmine solution, put between the microscopic slides and observed as a squash preparation. The pollination success was evaluated separately for four individual grafted plants (belonging to the clones marked as M5, M10, Š13 and Š14) and three different parts (lower, middle and upper third) of the open-pollinated and self-pollinated female strobili.

Similarly to the treatment of the immature female strobili at the end of the pollination period, 24 full-grown lignified cones (three open-pollinated and three self-pollinated ones from each analysed clone) were deprived of the ovuliferous scales. After the manual extraction of seeds, all the normal-looking ones (i.e. the seeds with normal size, normal shape and normally developed testa) were cut longitudinally with the scalpel. After this simple manipulation, the full seeds (i.e. the seeds with normally developed embryo and nutritive tissue) became very easily distinguishable from the empty ones (i.e. the seeds with shrivelled and degenerated inners).

A relationship between the full seed percentage and the success of pollination (expressed as the percentage of ovules pollinated by a certain minimal number of pollen grains – in particular, 1, 3, 5, 7 and 9) was studied by non-linear correlation analysis. Four mathematical functions,

applied by SORENSEN and WEBBER (1997) to model a similar relationship in the seed orchards of *Pseudotsuga menziesii* (MIRB.) FRANCO and *Tsuga heterophylla* (RAF.) SARG., were chosen for this purpose as well. The analyses were carried out separately for each of the used pollination modes (open pollination and self-pollination, respectively). 12 data points (4 clones, 3 different regions of female strobili) were analysed in each data set.

RESULTS

SUCCESS OF POLLINATION

In the case of open pollination, the proportion of pollinated ovules (i.e. ovules with one pollen grain on the papillae of their pollen-collecting apparatus at least) ranged from 56.31% (strobilus No. 1 of the grafted plant belonging to Š14 clone) to 100.00% (strobilus No. 1 from M5) and a similar trend was also registered in the mean values of individual clones (Table 1). The lowest percentage of pollinated ovules (70.50%) was ascertained in the female strobili of the clone marked as Š14, whereas the M5 clone was characterized by the mean value of almost 100% (96.82% precisely). The mean values of the remaining two clones (M10 and Š13) were near the total mean for the open pollination (83.77%). In almost all the

Table 2. Analysis of variance for the proportion of pollinated ovules (transformation: $p' = \arcsin\sqrt{p}$)

Source	DF	Sum of squares	Mean square	*F* value	Pr > *F*
Mode	1	1.1139	1.1139	28.74	0.0001
Clone	3	0.8710	0.2903	7.49	0.0003
Part	2	0.7162	0.3581	9.24	0.0004
Mode × clone	3	1.9546	0.6515	16.81	0.0001
Mode × part	2	0.7918	0.3959	10.22	0.0002
Clone × part	6	0.5214	0.0869	2.24	0.0548
Mode × clone × part	6	0.0189	0.0031	0.08	0.9978
Error	48	1.8602	0.0388		
Corrected total	71	7.8478			

Table 3. Mean values and homogeneous groups at the significance levels of α = 0.05 (letters a, b and c) and α = 0.01 (letters A and B) for the proportion of pollinated ovules depending on the mode of pollination and the clone (transformation: $p' = \arcsin\sqrt{p}$). Mean values of the clones with the same letter are not significantly different

Mode	Open pollination							Self-pollination					
Clone	Mean	Duncan		Tukey				Clone	Mean	Duncan		Tukey	
M5	1.4705	a		A	a	A		M10	1.3440	a	A	a	A
						A							
M10	1.2284	b		B	b	A	B	Š14	0.9470	b	B	b	B
		b		B	b		B			b	B	b	B
Š13	1.1848	b	c	B	b		B	Š13	0.9350	b	B	b	B
			c	B	b		B				B	b	B
Š14	1.0343		c	B	b		B	M5	0.6970	c	B	b	B

observed samples, the highest rates of ovules with the attached pollen grains were ascertained in the middle parts of female strobili.

On the other hand, the proportion of pollinated ovules was not always sufficiently high in the self-pollinated female strobili, especially in M5 with the mean value of 42.58%. Unlike the previous mode of pollination, the highest ascertained values were related to the upper regions of the strobili. In total, 64.79% of ovules contained the attached pollen grains after self-pollination.

The results of analysis of variance (the arcsine-transformed values were analysed) approved the existence of significant differences at all three levels (clone, mode of pollination, part of female strobilus) as well as differences resulting from most of the possible interactions (Table 2).

The basic trends of differences between the clones and the parts of female strobili were also partly demonstrated by the results of Duncan's and Tukey's tests. In the case of open pollination, the proportions of the pollinated ovules belonging to M5 proved (with the exception of Tukey's test at the significance level of $\alpha = 0.01$) to be significantly higher than the ascertained values of the other clones. On

the other hand, the values of the clone marked as M10 were significantly the highest in the self-pollinated strobili (Table 3).

In open-pollinated ovules, the statistical significance of differences between the middle and the other parts of female strobili was confirmed by the results of both used tests at both basic significance levels. However, as Table 4 shows, no distinct trends were demonstrated in self-pollination.

Besides the determination of the proportions of pollinated ovules, the estimation of the number of pollen grains on their pollen-collecting (stigmatic) apparatus was used as an additional criterion of pollination efficiency. The corresponding histograms are shown in Figs. 1 and 2.

PERCENTAGE OF FULL SEEDS

In terms of their outward appearance, the seeds in the mature, lignified cones could be distinctly divided into the following three groups:
1. Small, flattened, rudimentary seeds that could arise as a consequence of abnormal early development or abortion of ovules at early stages of their morphogenesis.
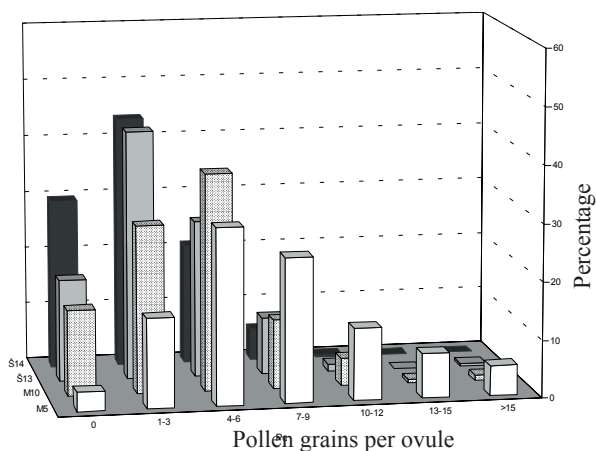

Fig. 1. The ascertained frequencies of pollen grains per pollen-collecting apparatus after open pollination
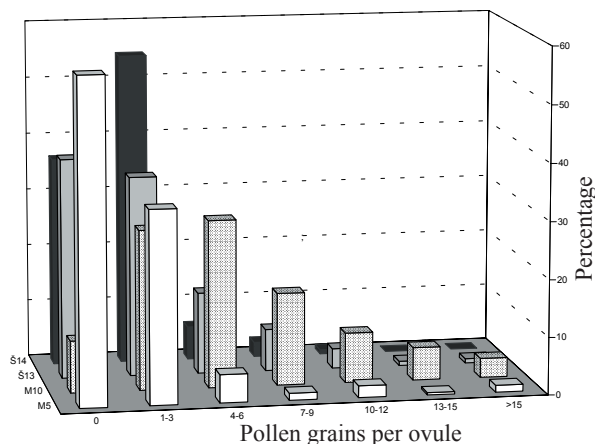

Fig. 2. The ascertained frequencies of pollen grains per pollen-collecting apparatus after self-pollination

Table 4. Mean values and homogeneous groups at the significance levels of α = 0.05 (letters a and b) and α = 0.01 (letters A and B) for the proportion of pollinated ovules depending on the mode of pollination and the part of female strobilus (transformation: $p' = \arcsin\sqrt{p}$). Mean values of the clones with the same letter are not significantly different

Mode	Open pollination					Self-pollination					
Part	Mean	Duncan		Tukey		Part	Mean	Duncan		Tukey	
Middle	1.4156	a	A	a	A	Top	1.1591	a	A	a	A
								a	A	a	A
Base	1.1610	b	B	b	B	Middle	1.0028	a	A B	a b	A B
		b	B	b	B				B	b	B
Top		b	B	b	B	Base		b	B	b	B
	1.1111						0.7803				

2. Seeds with the apparent symptoms of damage by insect pests (holes in seed coat, outflows of resin, etc.).

3. Normal-looking seeds with standard size and normally developed seed coat.

In the normally developed cones, the proportion of seeds of the first type was very low and therefore these rudimentary formations were not taken into consideration in the quantitative analysis. In comparison with the small underdeveloped seeds, the second category was more abundant, but the proportions of damaged seeds varied considerably among individual strobili. In order to avoid the problems in the interpretation of results, another quan-titative analysis was carried out only on relatively undamaged cones with 10% of defective seeds at most.

Although the final full seed proportions were relatively low (less than 30%) in all the investigated samples, very expressive contrasts were observed especially between two employed pollination modes. While after the open pollination the registered values ranged from 8.75% (cone No. 2 belonging to M10) to 27.28% (clone marked as M5, cone No. 3), they were close to zero in all the self-pollinated female strobili regardless of the clone. On average, in individual clones the full seed percentage ranged from 13.45% (M10) to 22.59% (M5) after the open pollination,

Table 5. Percentage of the full seeds for the different modes of pollination, clones, female strobili and their individual parts

Mode of pollination	Clone	Base (%)	Middle (%)	Top (%)	Total (%)
Open pollination	M5	22.33	23.20	22.22	22.59
	M10	14.21	22.83	3.31	13.45
	Š13	20.33	42.24	4.37	22.31
	Š14	33.03	9.96	2.25	15.08
	Total	**22.48**	**24.56**	**8.04**	**18.36**
Self-pollination	M5	5.63	2.27	0.00	2.64
	M10	0.95	5.75	3.92	3.54
	Š13	0.00	0.00	1.64	0.55
	Š14	9.32	2.42	0.00	3.91
	Total	**3.98**	**2.61**	**1.39**	**2.66**

Table 6. Analysis of variance for the full seed proportion (transformation: $p' = \arcsin\sqrt{p}$)

Source	DF	Sum of squares	Mean square	F value	Pr > F
Mode	1	1.5460	1.5460	113.27	0.0001
Clone	3	0.0690	0.0230	1.68	0.1828
Part	2	0.4461	0.2231	16.34	0.0001
Mode × clone	3	0.1901	0.0634	4.64	0.0063
Mode × part	2	0.1514	0.0757	5.55	0.0068
Clone × part	6	0.3567	0.0594	4.36	0.0014
Mode × clone × part	6	0.3357	0.0560	4.10	0.0021
Error	48	0.6552	0.0136		
Corrected total	71	3.7502			

Table 7. Mean values and homogeneous groups at the significance levels of α = 0.05 (letters a and b) and α = 0.01 (letter A) for the full seed proportion depending on the mode of pollination and the clone (transformation: $p' = \arcsin\sqrt{p}$). Mean values of the clones with the same letter are not significantly different

Mode	Open pollination						Self-pollination						
Clone	Mean	Duncan			Tukey		Clone	Mean	Duncan			Tukey	
M5	0.4887	a		A	a	A	M10	0.1525	a		A	a	A
		a		A	a	A			a		A	a	A
Š13	0.4447	a	b	A	a	A	Š14	0.1275	a	b	A	a	A
			b	A	a	A			a	b	A	a	A
M10	0.3429		b	A	a	A	M5	0.1202	a	b	A	a	A
			b	A	a	A				b	A	a	A
Š14	1.3209		b	A	a	A	Š13	0.0249		b	A	a	A

Table 8. Mean values and homogeneous groups at the significance levels of α = 0.05 (letters a and b) and α = 0.01 (letters A and B) for the full seed proportion depending on the mode of pollination and the part of the female strobilus (transformation: $p' = \arcsin\sqrt{p}$). Mean values of the clones with the same letter are not significantly different

Mode	Open pollination					Self-pollination				
Part	Mean	Duncan		Tukey		Part	Mean	Duncan		Tukey	
Middle	0.4911	a	A	a	A	Base	0.1371	a	A	a	A
		a	A	a	A			a	A	a	A
Base	0.4833	a	A	a	A	Middle	0.1222	a	A	a	A
								a	A	a	A
Top	0.2235	b	B	b	B	Top	0.0595	a	A	a	A

whereas in the cases of controlled selfing the mean proportions were between 0.55% (Š13) and 6.18% (Š14). In addition, some of the self-pollinated cones (namely, two cones of Š13 and one cone of Š14) were characterized by the absolute prevalence of seeds without a normal-looking embryo and nutritive tissue. After both employed pollination modes, the lowest mean full seed proportions were ascertained in the uppermost regions of the cones (Table 5).

The results of analysis of variance (Table 6) demonstrated the existence of statistically significant differences at the levels of pollination mode (open, self), part of cone (basal, middle and upper third) as well as all the possible interactions. On the contrary, the differences at the level of clone proved to be statistically non-significant. However, when each of the modes of pollination was analysed separately, some statistically significant differences between the clones were demonstrated by the results of Duncan's multiple range test at the significance level of α = 0.05 (Table 7).

Regarding the differences between the regions of cones, statistical significance was demonstrated only after open pollination when the final full seed proportion was significantly lowest in the uppermost third of the strobili (at both significance levels of α = 0.05 and α = 0.01), whereas no significant differences were confirmed between the other two parts, i.e. between the basal and middle regions of cones. On the other hand, significant differences between the base, the middle and the apical part were entirely absent in the self-pollinated cones where the data on all these regions represented a single homogeneous group (Table 8).

CORRELATION ANALYSIS

Despite the large differences between the proportion of pollinated ovules and the percentage of full seeds, a certain trend is evident from the results of correlation analysis after open pollination (Fig. 3): while the full seed percentage increases relatively rapidly along with the initial increase of pollination success, it approaches subsequently an asymptote as the percentage of pollinated ovules reaches the maximal values. However, all the plotted curves are extraordinarily flat (regarding the very low mean percentage of full seeds) and, while the data points are relatively scattered, most of the calculated correlation coefficients are lower than 0.5 (the critical value for sample size $n = 12$, degrees of freedom $n - 2 = 10$ and significance level α = 0.05). In general, the lowest values of the correlation coefficient were computed if the ovules with 2 pollen grains and less were included into the calculations (the values of $r = 0.22–0.31$, image not shown).

On the contrary, this trend was absolutely indistinct after controlled self-pollination when the computed values of r ranged from 0.00 to 0.29 and the curves were extraordinarily flat (images not shown). This fact (along
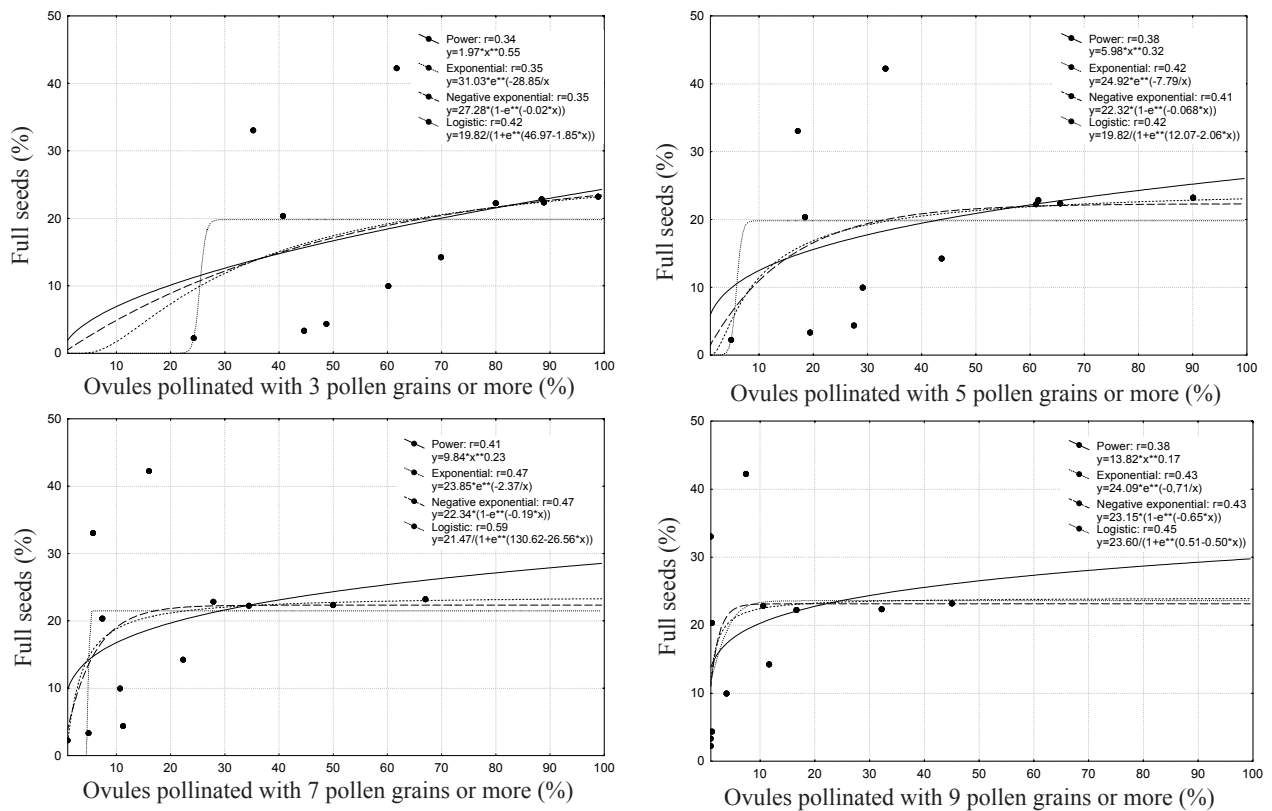
Fig. 3. The results of the non-linear correlation analysis of the relationship between the pollination success (x) and the full seed percentage (y). Used non-linear models: (1) power: $y = a \cdot x^b$, (2) exponential: $y = a \cdot e^{b/x}$, (3) negative exponential: $y = a \cdot (1 - e^{-b \cdot x})$, (4) logistic: $y = a / (1 + e^{b-c \cdot x})$

with large differences between the success of pollination and the full seed percentage, and the relatively low values of correlation coefficients after open pollination as well) suggests that the empty seed formation in *Larix* is strongly affected not only by insufficient pollination but also by additional factors, more or less independent of pollination intensity.

DISCUSSION

A prevailing majority of gymnosperms (including European larch) are wind-pollinated. Various authors (OKUBO, LEVIN 1989; DI-GIOVANNI, KEVAN 1991, and others) tried to explain the long-distance pollen transport from source to recipient using mathematical models based on the laws of atmospheric dispersal of small particles and their infiltration by plants. However, the movement of pollen grains around the receptive female strobili seems to be, above all, a matter of very fine aerodynamic principles. According to the data of POWELL and TOSH (1991), the pollen grains of *Larix laricina* are directed to the vicinity of ovules by means of broad and awned bracts. In Douglas-fir, the bract scales are apparently curved, which also funnels the pollen grains into the pollen-collecting apparatus (TAKASO, OWENS 1995). By means of the simulation of the pollination process of various *Pinaceae*

in a wind tunnel, NIKLAS and PAW U (1983) and NIKLAS (1984) demonstrated that individual structures of receptive megastrobili regulate the direction of air flow and deflect the wind-borne pollen grains towards the ovules. Nevertheless, according to the results of similar experiments carried out by ROUSSY and KEVAN (2000), the relative pollination success of *Pinus banksiana* LAMB. was not correlated with the speed of wind.

According to the embryological literature, the pollen-collecting apparatus of *Larix* and *Pseudotsuga* CARR. is of special type. It consists of two unequal lobes, the larger of which is equipped with so-called stigmatic hairs or papillae (DOYLE 1926, 1945; DOYLE, O'LEARY 1935; OWENS, MOLDER 1979; VILLAR et al. 1984; KO-RÍNEKOVÁ et al. 1988; POWELL, TOSH 1991; SAID et al. 1991; OWENS et al. 1994, and others). At the beginning of its development, the integument of various species of larch appears as a ring of the homogeneous meristematic tissue in the basal region of young ovule. Nevertheless, its further growth is rather unequal and consequently, the tip of the integument becomes bilobate. The adaxial lobe enlarges more intensively and after some time, it outgrows the tip of the nucellus. Cells of the apical region of this lobe become elongated and transform to unicellular papillae, the function of which lies in the capture of pollen grains. The abaxial part of the integument is considerably

Table 9. Comparison of the literature data on the ascertained proportions of pollinated ovules (%) and the mean numbers of adhered pollen grains in various species of *Larix*

Author(s)	Open pollination		Controlled pollination	
	(%)	Mean	(%)	Mean
BARNER, CHRISTIANSEN (1960) – various species, Denmark[a]	62	3	17	2.5
KAJI (1974) – *Larix kaempferi*, Japan (Hokkaido)[b]	67–77	3–5	–	–
HALL, BROWN (1976) – *L. decidua* and *L. kaempferi*, Scotland[c]	–	–	66.4–79.5	3.1–4.1
POSPÍŠIL (1978) – *L. decidua*, Bohemia[d]	–	0.42–6.75	–	0.9–4.42
KOSIŃSKI (1986, 1987) – *L. decidua*, Poland[e]	67–99	2.1–4.5	62–92	–
OWENS et al. (1994) – *L. occidentalis*, Canada (Victoria)[f]	–	–	–	1.0–10.1
SHIN, KARNOSKY (1995) – various species, USA (Michigan)[g]	13.3–75.0	–	75.0–97.7	–
KRIŽO, SLOBODNÍK (1996) – *L. decidua*, Slovakia[e]	40.48–99.64	1.52–13.08	–	–

[a]Random check
[b]Mean values for the different years of investigations
[c]Mean values for two clones of the different species in the same year
[d]Mean values for the different years and clones with the mean numbers of the captured pollen grains calculated for each of the ovules (pollinated as well as unpollinated)
[e]Mean values for the different years and clones with the mean numbers of the captured pollen grains calculated for pollinated ovules
[f]Mean values for the different stages of female receptivity at the time of the application of pollen
[g]Mean values for the different species and interspecific hybridizations

smaller and, with some exceptions, it does not contain any papillae. The identical development of the pollen-collecting apparatus was also described in *Pseudotsuga* (ALLEN 1963; HO 1980; OWENS et al. 1981, and others).

The quantitative analysis of the pollination success in larch (i.e. detection of the proportion of pollinated and unpollinated ovules or calculation of the mean number of adhered pollen grains to the pollen-collecting apparatus) was the topic of interest of many authors (BARNER, CHRISTIANSEN 1960; KAJI 1974; HALL, BROWN 1976; POSPÍŠIL 1978; KOSIŃSKI 1986, 1987; OWENS et al. 1994; SHIN, KARNOSKY 1995; KRIŽO, SLOBODNÍK 1996, and others). Understandably, their data are considerably varied as they are in close relationship to the meteorological conditions and many additional factors, e.g. total production of male strobili and previous accomplishment of artificial pollination (Table 9).

According to a great deal of the compared literature data, the lack of pollination does not seem to be the most important factor affecting the empty seed production of *Larix* (after the open pollination, in most cases more than 60% of ovules contained one adhered or engulfed pollen grain at least). According to the data of KOSIŃSKI (1986, 1987), the lack of pollination reduced the potential amount of vital seeds of European larch by approximately 20% (on average) and except the insufficient production of pollen grains, it could also result from the low synchronization of male and female flowering and unfavourable meteorological conditions during the receptivity of ovules. Based on my observations,

the proportions of ovules with the total absence of pollen grains were not higher than 30% (they ranged from 3.18% to 29.50% depending on the clone) and on average, the lack of pollination caused the loss of potentially produced and normally developed seeds by approximately 16.23%. The different situation was, however, registered in the self-pollinated ovules. In this case, the decrease in the full seed production was, on average, as high as 35.21% and in individual clones it ranged between 9.10% and 57.42%. Because the branches with the strobili were just isolated to prevent the open pollination and the pollen grains from the same individuals were not injected to the isolating bags, this phenomenon was most likely to be caused by the asynchronism between the shedding of pollen grains and the receptivity of female generative organs on the same grafted plant.

Using simple mathematical models, the relationship between the number of captured pollen grains (calculated per square millimetre over the period of 24 hours in a pollen trap placed in seed orchard) and the production of full-value seeds was studied by SORENSEN and WEBBER (1997). From among the various non-linear models, the logarithmically transformed power function gave, on average, the highest adjusted coefficients of determination for all the analysed files. Despite the relatively high values of the adjusted coefficients of determination (0.46–0.82 in Douglas-fir and 0.71–0.83 in western hemlock), however, some of the demonstrated values support the assumption of the existence of several additional factors affecting the percentage of full seeds in gymnosperms.

Within the species of *Larix*, except the more or less frequent cases of insufficient pollination, a significant portion of ovules can remain unfertilized although their previous pollination was successful (HALL, BROWN 1977; KOSIŃSKI 1986, 1987; SHIN, KARNOSKY 1995). This phenomenon is usually ascribed above all to the abnormal development of female generative organs, disturbances in the pollination mechanism (in particular, restraint of the pollen displacement to the nucellus), and to the decreased viability of pollen grains. Finally, the most significant portion of the produced empty seeds of larch is caused by the abortive degeneration of developing embryos that occurs, above all, as a consequence of genetic load after the self-pollination or the consanguineous mating between neighbouring trees with high degree of relatedness (DIECKERT 1964; PARK, FOWLER 1982; KOSIŃSKI 1982, 1986, 1987; SATO 1997) or contrariwise, after the distant, interspecific hybridization (HALL, BROWN 1977; SHIN, KARNOSKY 1995). In addition, the premature abortion of female conelets (regardless of the success of pollination) can be considered another factor, significantly decreasing the potential amount of produced seeds in conifers, especially in *Pseudotsuga* and *Larix* (OWENS et al. 1991; SHIN, KARNOSKY 1995; SLOBODNÍK, GUTTENBERGER 2000).

In the case of my investigations, the increasing percentage of pollinated ovules was only poorly associated with the increase in the full seed percentage. While almost 100% effect of genetic load is assumed after the controlled self-pollination, large differences between the pollination success and full seed percentage from open pollination can be ascribed to both the effect of genetic load and the influence of the maternal genotype, as described recently by KÄRKKÄINEN et al. (1999) in *Pinus sylvestris* L. To explain the biological mechanisms affecting the quality and genetic value of produced seeds, however, these problems are to be studied in other genera of coniferous trees (including *Larix*) as well.

References

ALLEN G.S., 1963. Origin and development of the ovule in Douglas-fir. Forest Sci., *9*: 386–393.

BARNER H., CHRISTIANSEN H., 1960. The formation of pollen, the pollination mechanism, and the determination of the most favourable time for controlled pollination in *Larix*. Silvae Genet., *9*: 1–11.

CHALUPA V., 1961. Influence of the extent of forest stands on the amount of transferred pollen. Commun. Inst. For. Czechosl., *2*: 67–77.

CHALUPA V., 1985. Přenos pylu a vytváření geneticky hodnotných semen v semenných plantážích. Lesnictví, *31*: 945–958.

DIECKERT H., 1964. Einige Untersuchungen zur Selbststerilität und Inzucht bei Fichte und Lärche. Silvae Genet., *13*: 77–86.

DI-GIOVANNI F., KEVAN G., 1991. Factors affecting pollen dynamics and its importance to pollen contamination: a review. Can. J. For. Res., *21*: 1155–1170.

DOYLE J., 1926. The ovule of *Larix* and *Pseudotsuga*. Proc. Roy. Irish Acad., *37*: 170–180.

DOYLE J., 1945. Developmental lines in pollination mechanism in the *Coniferales*. Sci. Proc. Roy. Dublin Soc., *24*: 43–62.

DOYLE J., O'LEARY M., 1935. Pollination in *Tsuga*, *Cedrus*, *Pseudotsuga*, and *Larix*. Sci. Proc. Roy. Dublin Soc., *21*: 191–204.

DYLIS N.V., 1948. O samoopyleniji i raznose pyľcy u listvennic. Dokl. Akad. Nauk SSSR, *60*: 673–676.

HALL J.P., BROWN I.R., 1976. Microsporogenesis, pollination and potential yield of seed of *Larix* in NE Scotland. Silvae Genet., *25*: 132–137.

HALL J.P., BROWN I.R., 1977. Embryo development and yield of seed in *Larix*. Silvae Genet., *26*: 77–84.

HO R.H., 1980. Pollination mechanism and seed production potential in Douglas-fir. Forest Sci., *26*: 522–528.

KAJI K., 1974. On the pollination and development of ovules, and on the sterility of seeds in Japanese larch (*Larix leptolepis* GORD.). Bull. Hokkaido For. Exp. Stn., *12*: 1–12.

KÄRKKÄINEN K., SAVOLAINEN O., KOSKI V., 1999. Why do plants abort so many developing seeds: bad offspring or bad maternal genotypes? Evol. Ecol., *13*: 305–317.

KORÍNEKOVÁ M., KRIŽO M., BIES R., 1988. The development of integument and pollen-collecting apparatus of European larch (*Larix decidua* MILL.). Biológia (Bratislava), *43*: 393–399.

KOSIŃSKI G., 1982. Genetic load in empty seeds of European larch. Arboretum Kórnickie, *26*: 231–236.

KOSIŃSKI G., 1986. Przyczyny powstawania pustych nasion u modrzewia europejskiego (*Larix decidua* MILL.). Arboretum Kórnickie, *31*: 107–182.

KOSIŃSKI G., 1987. Empty seed production in European larch (*Larix decidua*). For. Ecol. Mgmt, *19*: 241–246.

KRIŽO M., SLOBODNÍK B., 1996. Quantitative evaluation of the pollination process of European larch (*Larix decidua* MILL.) in seed orchard. Acta Soc. Bot. Pol., *65*: 117–121.

NIKLAS K.J., 1984. The motion of windborne pollen grains around conifer ovulate cones: implications on wind pollination. Amer. J. Bot., *71*: 356–374.

NIKLAS K.J., PAW U K.T., 1983. Conifer ovulate cone morphology: implications on pollen impaction patterns. Amer. J. Bot., *70*: 568–577.

OKUBO A., LEVIN S.A., 1989. A theoretical framework for data analysis of wind dispersal of seeds and pollen. Ecology, *70*: 329–338.

OWENS J.N., MOLDER M., 1979. Sexual reproduction of *Larix occidentalis*. Can. J. Bot., *57*: 2673–2690.

OWENS J.N., COLANGELI A.M., MORRIS S.J., 1991. Factors affecting seed set in Douglas-fir (*Pseudotsuga menziesii*). Can. J. Bot., *69*: 229–238.

OWENS J.N., MORRIS S.J., CATALANO G.L., 1994. How the pollination mechanism and prezygotic and postzygotic events affect seed production in *Larix occidentalis*. Can. J. For. Res., *24*: 917–927.

OWENS J.N., SIMPSON S.J., MOLDER M., 1981. The pollination mechanism and the optimal time of pollination in Douglasfir (*Pseudotsuga menziesii*). Can. J. For. Res., *11*: 36–50.

PARK Y.S., FOWLER D.P., 1982. Effects of inbreeding and genetic variances in a natural population of tamarack (*Larix laricina* (DU ROI) K. KOCH) in eastern Canada. Silvae Genet., *31*: 21–26.

POSPÍŠIL J., 1978. Výzkum náletu pylu na samičí orgány modřínu evropského (*Larix decidua* MILL.). Lesnictví, *24*: 581–599.

POWELL G.R., TOSH K.J., 1991. The pollination mechanism and development after bud burst of cones of *Larix laricina*. Can. J. Bot., *69*: 1179–1187.

ROUSSY A.M., KEVAN P.G., 2000. How accessible are receptive megastrobili to pollen? The example of Jack pine (*Pinus banksiana*). Amer. J. Bot., *87*: 215–220.

SAID C., VILLAR M., ZANDONELLA P., 1991. Ovule receptivity and pollen viability in Japanese larch (*Larix leptolepis* GORD.). Silvae Genet., *40*: 1–6.

SATO T., 1997. Mode of fertilization and its individual variation in *Larix gmelinii* var. *japonica*. Silvae Genet., *46*: 146–151.

SHIN D., KARNOSKY D.F., 1995. Factors affecting seed yield in *Larix*. Jour. Korean For. Soc., *84*: 207–217.

SLOBODNÍK B., GUTTENBERGER H., 2000. Ovule, megaspores, and female gametophyte formation in *Larix decidua* MILL. (*Pinaceae*). Acta Biol. Cracov, Ser. Bot., *42*(2): 93–100.

SORENSEN F., WEBBER J.E., 1997. On the relationship between pollen capture and seed set in conifers. Can. J. For. Res., *27*: 63–68.

TAKASO T., OWENS J.N., 1995. Ovulate cone morphology and pollination in *Pseudotsuga* and *Cedrus*. Int. J. Plant Sci., *156*: 630–639.

VILLAR M., KNOX R.B., DUMAS C., 1984. Effective pollination period and nature of pollen-collecting apparatus in the gymnosperm, *Larix leptolepis*. Ann. Bot., *53*: 279–284.

Úspešnosť opelenia a percentuálny podiel plných semien pri smrekovci opadavom (*Larix decidua* MILL.)

B. SLOBODNÍK

Technická univerzita, Lesnícka fakulta, Katedra fytológie, Zvolen, Slovenská republika

ABSTRAKT: Vzťah medzi úspešnosťou opelenia a percentuálnym podielom plných semien smrekovca opadavého (*Larix decidua* MILL.) sa skúmal za použitia niekoľkých modelov nelineárnej korelačnej analýzy. Napriek tomu, že vo väčšine prípadov bol podiel opelených vajíčok vysoký (obzvlášť v prostrednej tretine šištíc po voľnom opelení), priemerný percentuálny podiel plných semien bol mimoriadne nízky (po predchádzajúcom kontrolovanom samoopelení dokonca blízky nule) a väčšina vypočítaných korelačných koeficientov je nižších ako zodpovedajúca kritická hodnota. Táto skutočnosť svedčí o významnom pôsobení ďalších dôležitých faktorov, spôsobujúcich vznik prázdnych semien smrekovca aj vtedy, keď predchádzajúce opelenie vajíčok bolo úspešné.

Kľúčové slová: smrekovec opadavý; peľ; prázdne semená

Je známe, že jednotlivé druhy rodu *Larix* MILL. sa vyznačujú mimoriadne vysokým podielom nevitálnych prázdnych semien, komplikujúcim ich umelú obnovu a realizáciu šľachtiteľských programov. Predstavitelia rodu *Larix* sú zároveň charakteristickí svojimi veľkými a ťažkými peľovými zrnami, ktoré neobsahujú vzdušné vaky, a pravdepodobnosť ich prenosu na dlhšie vzdialenosti sa preto vo väčšine prípadov považuje za pomerne nízku (DYLIS 1948; CHALUPA 1961, 1985 a i.). Tento fakt (spoločne so skutočnosťou, že osemenie sa pri smrekovci normálne vyvíja aj pri neoplodnených vajíčkach) sa v lesníckych kruhoch dlho považoval za hlavnú príčinu tvorby prázdnych – tzv. hluchých – semien v rámci uvedeného rodu. Podľa väčšiny novších údajov z odbornej literatúry však chronicky nízky percentuálny podiel vyprodukovaných životaschopných semien smrekovca nie je výsledkom pôsobenia jedného faktora, ale sa hodnotí ako dôsledok početných porúch a anomálií v rozličných fázach reprodukčného cyklu (KOSIŃSKI 1986, 1987; SHIN, KARNOSKY 1995). Vzhľadom na tieto skutočnosti by sa teda úspešnosť opelenia mala sledovať a kvantifikovať ako jeden z viacerých faktorov ovplyvňujúcich kvalitu vyprodukovaného smrekovcového osiva.

Úspešnosť opelenia a kvalita semien smrekovca opadavého sa sledovala v semennom sade Kmeťová (OLZ Slovenská Ľupča, stredné Slovensko) v roku 1998 na

dvoch klonoch pochádzajúcich z výberových stromov autochtónnej proveniencie (Šumiac, Nízke Tatry, označenie „Š") a na dvoch klonoch z výberových stromov neurčitého pôvodu (Motyčky, označenie „M"). Prítomnosť peľových zŕn na stigmatickom aparáte vajíčok sa zisťovala z roztlakových mikropreparátov apikálnych častí vajíčok, získaných v jarnom období z dvanástich voľne opelených samičích šištíc (po troch z každého klonu) a rovnakého počtu samičích šištíc (megastrobilov), ktoré boli niekoľko týždňov predtým vystavené kontrolovanému samoopeleniu v izolátoroch. Podobným spôsobom sa počas jesene zozbieralo 24 normálne vyvinutých zdrevnatených šišiek (12 voľne opelených a 12 samoopelených, po troch z každého klonu) a ručne sa z nich vylúštili všetky normálne vyvinuté a nepoškodené semená, ktorých životaschopnosť sa posudzovala skúškou rezom. Percentuálne podiely opelených vajíčok a plných semien sa porovnávali prostredníctvom nelineárnych modelov, prevzatých z práce autorov SORENSENA a WEBBERA (1997).

Po voľnom opelení sa priemerný podiel opelených vajíčok (t.j. vajíčok s najmenej jedným peľovým zrnom na papilách stigmatického aparátu) pri jednotlivých klonoch pohyboval od 70,50 % (klon Š14) do 96,82 % (klon M5), celkový priemer predstavoval hodnotu až 83,77 % a štatisticky významne najväčšie percento opelených vajíčok sa nachádzalo v strednej tretine samičích šištíc. Naopak o niečo nižšia celková úspešnosť opelenia sa zistila v prípade kontrolovaného samoopelenia, po ktorom sa priemerné hodnoty za jednotlivé klony pohybovali

v rozpätí od 42,58 % (M5) do 90,90 % (M10) a celkový priemer dosiahol iba 64,79 % opelených vajíčok.

Napriek relatívne vysokej úspešnosti opelenia (a to aj s prihliadnutím na fakt, že opelenie jedným či dvoma peľovými zrnami ešte nemusí byť zárukou úspešného oplodnenia vzhľadom na ich možnú nulovú klíčivosť) boli zistené percentuálne podiely plných semien aj pri voľne opelených šiškách prekvapujúco nízke – 13,45–22,59 % pri jednotlivých klonoch (najvyššie pri M5, najnižšie pri šiškách z klonu M10) a celkový priemer dosiahol hodnotu 18,36 %. Napriek istému pozorovanému trendu (podiel plných semien sa s rastúcou úspešnosťou opelenia spočiatku prudko zvyšuje, neskôr sa blíži k asymptote) je väčšina vypočítaných korelačných koeficientov nižšia ako 0,5, čo je tabuľková kritická hodnota, zodpovedajúca rozsahu výberového súboru $n = 12$, počtu stupňov voľnosti $n – 2 = 10$ a hladine významnosti $α = 0,05$. Zatiaľ čo pri voľne opelených šiškách sa vypočítané korelačné koeficienty pohybovali aspoň v rozpätí od 0,3 do 0,6, takmer nijaká závislosť podielu plných semien od úspešnosti opelenia sa nezistila pri šiškách, ktoré boli v jarnom období vystavené kontrolovanému samoopeleniu. V tomto prípade bol zistený percentuálny podiel plných semien takmer nulový (pri celkovom priemere 2,66 % sa pohyboval od 0,55 do 3,91 % v závislosti od klonu), čo spolu s ďalšími uvedenými skutočnosťami umocňuje predpoklad, že tvorba prázdnych semien smrekovca opadavého zďaleka nie je spôsobená iba nedostatočným opelením, ale v prevažnej miere skôr inými prezygotickými, resp. postzygotickými faktormi.

Corresponding author:

Ing. BRANKO SLOBODNÍK, Ph.D., Technická univerzita, Lesnícka fakulta, Katedra fytológie, T. G. Masaryka 24, 960 53 Zvolen, Slovenská republika
tel.: + 421 45 520 62 31, fax: + 421 45 533 26 54, e–mail: slobod@vsld.tuzvo.sk