QUANTITATIVE EVALUATION OF THE POLLINATION PROCESS OF EUROPEAN LARCH (*LARIX DECIDUA* MILL.) IN SEED ORCHARD

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ABSTRACT

The pollination of European larch (*Larix decidua* MILL.) was investigated in the seed orchard Dolné Mlynárovo (Low Tatra Mts, Central Slovakia) during years 1991-1993. The pollen in the air was captured on horizontally and vertically placed microscopical glasses in different areas of the seed orchard. After the pollination period, ovules were isolated from the female strobili in order to find out the percentage of the pollinated ovules and number of the pollen grains per collecting apparatus. Approximately 140 pollen grains per square centimeter on average were captured on the vertically placed microscopical glasses in 1991, up to 400 in 1992, and only 40 in 1993. The most rapid course of the pollination was observed in 1991. Most pollen grains (in some cases more than 2,000 per square centimeter) were intercepted on the 5th and 7th day of observation and in next days the numbers of captured pollen were significantly lower. In 1992 the pollination period was much longer and more harmonious. This fact is probably associated with stable temperatures and small amount of precipitation (unlike to 1991). When comparing the number of pollen grains on horizontally placed microscopical glasses, approximately 25 pollen grains on average were captured in 1992, 6 in 1991, and only 2 per square centimeter in 1993. Days with the maximum numbers of intercepted pollen did correspond with the maximum values obtained from the vertically placed interceptive glasses. Almost all of analyzed ovules were pollinated in 1992 and up to 27 pollen grains were found on their pollen collecting apparatus in some extreme cases. In 1991 the situation was similar to 1992 (more than 85% ovules pollinated), but in 1993 only 40-85% ovules were pollinated (depending upon the grafted plants and different parts of the crowns) and only 1-3 pollen grains were found in the majority of pollinated ovules.

KEY WORDS: larch, pollen, pollination, seed orchard.

INTRODUCTION

One of the most important factors that can limit the quality and genetic value of acquired seed is a sufficient amount of full-value pollen. The problems connected with the production and transmission of pollen grains and with the biology and ecology of pollination, come into prominence mainly in the European larch (*Larix decidua* Mill.), which is distinguished by an extremely high proportion of non-vital, so called daef seeds, and by non-saccate pollen grains with considerably decreased mobility. With regard to these factors it is inevitable to consider the phenomenon of pollination as the main one influencing high production of empty seeds within the genus *Larix* Mill. (Dyakowska 1936, Dylis 1948, Kaji 1974, Hall & Brown 1976, Pospíšil 1978, Chalupa 1960, 1985 Kosinski 1985, Križo 1990 and others). This study of these problems can considerably contribute to clearing up some significant circumstances influencing the quality and genetic value of seed produced in seed orchards of this attractive and economically important species.

The aim of the paper is to contribute to the knowledge of the time process of the release of pollen from the anther; to the laws of pollen transmission in the seed orchard Dolné

Mlynárovo (Low Tatra Mts., Central Slovakia) on the basis of many years regular investigation into the amount of pollen grains intercepted on horizontally and vertically oriented microscopic glasses regularly placed on five wind indicators. Observation of the quantity of pollen grains intercepted on the stigmatic apparatus of ovules contributes to the determination of how the above stated facts are reflected in the process of pollination of female strobili.

MATERIALS AND METHODS

The 2.5 hectare seed orchard Dolné Mlynárovo was founded in 1976 and is situated in the south-west of the Low Tatra Mountains near the village of Jasenie (region Banská Bystrica) at an altitude of 600 m above sea level.

Movement of pollen was observed during the years 1991-93. Wind indicators with interceptive microscopic glasses were set up in the shape of a cross with a longer north-south axis, so indicator No. 1 was situated in the southern part of the orchard, No. 2 in the centre, No. 3 in the north, No. 4 in the west and indicator No. 5 in the eastern part of the orchard. One microscopic glass was placed vertically and the other

Fig. 1. Quantity of pollen grains intercepted on vertically placed microscopic glasses in the year 1991.

Fig. 2. Quantity of pollen grains intercepted on vertically placed microscopic glasses in the year 1992.

Fig. 3. Quantity of pollen grains intercepted on vertically placed microscopic glasses in the year 1993.

horizontally on each indicator at a height of approximately 1,5 m, that is at the level of reproductive organs. The interceptive surface of the microscopic glasses was coated with white vaseline. Falling pollen grains landed on the

horizontal glasses, while pollen carried by air flow was intercepted on vertically placed glasses. The glasses were changed every 24 hours and the quantity of intercepted pollen grains was observed by luminous microscope. Measured

Fig. 4. Quantity of pollen grains intercepted on vertically placed microscopic glasses in the year 1991.

values were calculated per square centimetre. Megastrobili, necessary for observation of the movement of pollen grains to the stigmatic apparatus of ovules were gathered every year after the end of the pollen shedding period from grafted plants No. 19-14/4, 15-10/10 and 2-6/15 (the first number is the number of the clone, the second and the third state the location of the grafted plant in the seed orchard. From these grafted plants we took three megastrobili from each cardinal point of the tree top, i.e. 36 megastrobili altogether. At the same time we gradually separated ovuliferous scales with ovules from the base to the top of these megastrobiles. The apical part of each ovule was separated by means of a binocular magnifying glass and scalpel, coloured with a drop of acetocarmine, and observed under a luminous microscope.

RESULTS AND DISCUSSION

The beginning of shedding of pollen in the seed orchard Dolné Mlynárovo in the first two years was recorded approximately at the same time (in 1991 it was the 2nd April, in 1992 the 4th April), that coincides with the period observed by Kosinski in Poland (1985). Significant delay, caused by colder weather and a longer winter, was observed only in 1993, when the beginning of the pollen shedding period was not recorded until the 14th April. This fact confirms the verification of Chalupa's data (1964), according to which there can be a 6-8 day shift in strobili ripening due to different weather conditions. In our case even larger time differences were observed – in 1993 the delay was 10 days compared with 1992 and 12 days compared with 1991.

From the observed data on the quantities of pollen grains intercepted on vertical glasses we can show significant difference among these years in pollen production and in the general process of pollen shedding (Figs 1-3).

As you can see in Figure 2, the most favourable situation in the seed orchard was in 1992, when about 400 pollen grains per square centimetre on average were captured on the vertically placed microscopic glasses during 24 hours and 1,400-1,600 under extreme conditions, while in 1991 (Fig. 1) approximately 140 pollen grains were captured on the same surface and in 1993 (Fig. 3) only 10 pollen grains. According to our data, the quickest course of the shedding of pollen was

in seed orchard in 1991, which must be connected with extremely changeable weather in the blooming period. Most pollen grains (in some cases more than 2,000 per square centimetre) were intercepted on the 5th and 7th day of pollen shedding. Later in the period marked by severe cooling the quantities of intercepted pollen grains were much lower. In 1992 the highest intensity of pollen shedding was observed between the 5th and 13th day. The process of pollen shedding was thus much more fluent and balanced compared to the year 1991, which resulted from steady, dry, sunny weather without significant changes in temperature. It is low air humidity and little probability of precipitation which, according to Whitehead (1983), is one of the basic conditions of shedding of pollen grains, optimum speed of wind, a short distance between compatible plants, etc. In 1993, there was culmination during the 4th and 6th day, which is thus similar to the year 1991, however, in this case, numbers of intercepted pollen grains were considerably lower.

Significant differences among the observed years were found in the quantity of pollen grains intercepted on the horizontally placed microscopic glasses. In this case in 1992 about 25 pollen grains per square centimetre (max. 126) on average were intercepted during 24 hours, while in 1991 only 6 (max. 81) and in 1993 only 2 pollen grains (max. 9). When compared with vertically oriented interceptive glasses measured values were about 15-20 times lower (Figs 4-6). The days with maximum quantities of intercepted pollen grains are, in most cases, coincident with maximum quantities of vertically placed microscopic glasses. The exception is the year 1991 when several unimportant local maximum quantities were recorded between the 3rd and 9th day of pollen shedding.

The analysis of variability has not confirmed the existence of statistically significant differences in the quantity of pollen grains intercepted in various parts of the seed orchard. Extremely large amounts of intercepted pollen were recorded every year on indicator No. 3 placed in the northern, regularly poorly blooming part of the orchard. This fact gives evidence of the prevailing valley winds blowing from the end of the Jasenienska Valley, where this seed orchard is situated, towards the ridge of the Low Tatra Mts., i.e. from indicator No. 1 to indicators 2 and 3.

In 1991 most pollen grains were captured on stigmatic apparatus of megastrobili ovules coming from grafted plant

No. 19-14/4 which is situated in the south-eastern part of the seed orchard. The average quantity of pollen grains fallen on one pollinated ovule reached the values of 7.27-8.70 and the proportion of pollinated ovules ranged between 96.09 and 97.49 per cent depending on the location of megastrobili in the tree top. Considerably fewer pollen grains were present on ovules coming from the other two grafted plants where the measured average values were 4.00-5.18 (grafted plant No. 15-10/10, growing in central part of the orchard) or 3.27-4.57 (grafted plant No. 2-6/15 growing in its western part) and most ovules were pollinated by 2-4 pollen grains. The proportion of pollinated ovules in the first case was 91.36-94.98%, in the second case 84.35-88.49% (Table 1).

More pollen grains were counted also in 1992 on stigmatic apparatus of ovules isolated from megastrobili of the grafted plant N. 19-14/4, when 27 pollen grains were found on one ovule and most ovules were pollinated by 11-13 pollen grains (average 12.74-13.08 depending on the cardinal point). On the whole more than 99% of the ovules were pollinated. Unlike the year 1991 for the second best was considered the grafted plant No. 2-6/15 with ovules pollinated by 5-8 (16) pollen grains and with an average number of pollen grains on one pollinated ovule of 5.11-7.41. Relatively least favourable situation was with the grafted plant No. 15-10/10 with average values ranging from 2.85 to 4.53 where a maximum of 11 pollen grains were found on ovules, but mostly only 2-5 and 91.36-94.98% of the total amount of the ovules analysed were pollinated (Table 2).

The worst situation coming from the low pollen production occurred in the seed orchard in the year 1993. Here most pollen grains, however, were found on the ovules coming from the grafted plant No. 15-10/10 which has not been one of the best ones in the last few years. In this case 56.90-84.66% of the ovules were pollinated and there were, on average 2.23-3.70 pollen grains on one pollinated ovule. At the other two grafted plants the measured values were much lower. At the grafted plant No. 2-6/15 1.7-2.8 pollen grains on average were found on the stigmatic apparatus of ovules and at the grafted plant No. 19-14/4 there were only 1.52-2.32 pollen grains. The proportion of pollinated ovules was 48.98-69.51% in the first case, while in the second case the values were 40.48-59.18%. These values are, from the viewpoint of successfulness of pollination, very adverse, first of all when we realize according to Pospíšil (1978) that pollination by one pollen grain need not be the guarantee of successful fertilization with regard to its possible sterility.

The following analysis of variability of features investigated, confirmed statistical significance of observed differences on the level of grafted plant as well as on the level of individual years. On the contrary, the differences, coming from the position of female strobili in the crown, proved statistically unimportant.

Several authors (Kaji 1974, Hall & Brown 1976, Pospíšil 1978, Kosinski 1985, Križo 1990, Said et al. 1991 and others) dealt with quantitative analysis of pollination in the seed orchards of individual representatives of the *Larix* Mill. genus. Their data on the percentual proportion of pollinated ovules, or average quantity of pollen grains on ovules differ more or less from our values observed in central Slovakia, but as they are always connected with particular conditions of stated years (the age of the seed orchard, the total pollen production, the weather in the period of pollination), their generalization and mutual comparison are considerably limited.

CONCLUSION

The results of this three-year investigation of pollen production and transmission in the seed orchard of the European larch (*Larix decidua* Mill.) in Dolné Mlynárovo show considerable variability of measured values between individual observed years. The year 1992 was marked by the highest pollen production, that was reflected in the highest number of pollen grains intercepted in the microscopic glasses as well in the highest number of pollen grains intercepted in stigmatic apparatus of ovules, while the year 1993 showed the lowest pollen production. The most stable process of the pollen shedding was recorded also in the year 1992 with steady sunny weather during the whole observed period, and the greatest differences in the amount of pollen grains intercepted during individual days were recorded in 1991 with considerable changes in temperature and humidity. The extremely large numbers of pollen grains intercepted in the northern part of the orchard and the rather small numbers of pollen intercepted in its southern part are likely due to the activity of valley winds blowing from the end of the valley to the ridge that are typical for this region. In this connection it is an advantage that this seed orchard is oriented with its longer axis in direction of prevailing winds which enable it to use released pollen more economically.

The observation of the quantity of released pollen (similar to phenological observation and quantitative evaluation of blooming) is in seed orchards significant, first of all as a source of actual information about the pollination process that plays an important role in the production of vital and genetically valuable seeds. If the amount of released pollen is small, the probability of pollination of neighbouring clones by genetically valuable pollen is decreasing and on the contrary, the probability of self-pollination, or contamination by pollen from the surrounding plants is increasing. Therefore, in these cases it is inevitable to come to supplementary mass pollination. On the other hand, when sufficiently high production of pollen grains in the seed plantation occurs, the danger of contamination by pollen of little genetic value is particularly in the case of European larch very small (Chalupa 1985) and supplementary mass pollination is thus useless. The reasoning for this measure can then be stated on the basis of total number of established male strobili, or also on the basis of the quantity of pollen grains present in the space of the seed orchard during the first days of pollen shedding.

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ILOŚCIOWA OCENA ZAPYLANIA U *LARIX DECIDUA* W ARBORETUM DOLNE MLYNANY

STRESZCZENIE

Zapylenie modrzewia *Larix decidua* Mill. było badane w arboretum Dolne Młynarowo w Niskich Tatrach w Słowacji w latach 1991-93. Pyłek był chwytny na poziomo i pionowo ułożone szkiełka mikroskopowe w różnych miejscach w arboretum. Po czasie pylenia były izolowane zalążki z szyszek żeńskich i liczono pyłek w mikropyle. Średnio 140 ziaren pyłku znajdowano na pionowo ułożonych szkiełkach: 140 na cm² w r. 1991, 400 w r. 1992 i tylko 40 w r. 1993. Wypylanie było najszybsze w r. 1991. Większość ziaren pyłku (w niektórych przypadkach ponad 2000/cm²) osadzało się w 5-7 dniach, w następnych wyraźnie mniej. W r. 1992 czas wypylania był dłuższy, bardziej wyrównany. Było to związane ze stałymi temperaturami i małą ilością opadów (inaczej jak w r. 1991). Na szkiełkach ułożonych poziomo osadzało się średnio 25 ziaren pyłku na cm² w r. 1992, 6 w r. 1991 i tylko 2 w 1993. Z podobną dynamiką osadzania w kolejnych dniach jak przy szkiełkach pionowych. Prawie wszystkie badane zalążki w r. 1992 były zapylane i do 27 ziaren znajdowało się na mikropyle. W r. 1991 podobnie (ponad 85% zalążków była zapylona) lecz w r. 1993 tylko 40-85% zalążków była zapylona (zależnie od położenia roślin i miejsc szyszek w koronie) i tylko 1-3 ziaren pyłku znajdowano na mikropyle zapylonych zalążków.

SŁOWA KLUCZOWE: modrzew, pyłek, zapylenie, arboretum.