Studies on the Pollination Characteristics and Pollination Level of Chinese fir Seed Orchard

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(Received 24th April 2003)

Abstract

Data from three seed orchards (Chongyang, Zhangle and Laoshan) and Lintian forest were used to study pollination characteristics, pollination level, pollen and ovule production in Chinese fir. The results show that male and female cones have their own distribution patterns within crowns. Male cones are located in the middle to top and female cones the middle to bottom part of the crown. Because of lower density of trees in seed orchard than that in a stand, female cones are distributed over the entire crown in seed orchard trees. Chinese fir male and female cones appear to be very well adapted for wind as the pollination mechanism. There is no difference in the number of pollen grains produced by one pollen sac among trees in the same clone, but there are differences between clones. There are differences between both clones and years in female cone number, male cone number and their ratio. The pollen accumulation rate during pollination should be 3 to 5 pollen grains/mm² to ensure fertilization. The Chinese fir seed orchards in this study produced too much pollen and could be improved by stimulation of more female flowers in order to produce more seeds.

Key words: Cunninghamia lanceolata (Lamb.) Hook, Chinese fir; seed orchard, clones, pollen, ovules.

In the area of tree breeding and improvement, Professor CHENG YUEWU was the person who established the first seed orchard of Chinese fir in China at Yangkou, Fujian Province (Yu Xintuo, 2000) in 1970s. There are 41 out of 288 families in this seed orchard that provide a 15 to 20% improvement over unimproved material. Professor SHI JISEN initiated a Chinese fir Breeding and Improvement Program, and set up the second generation seed orchard of Chinese fir at Sanming, Fujian Province (SHI JISEN, 1994). According to information from Chinese fir Superior Trees & Seed Orchard Research Group, the wood volumes of 3 to 12 year-old trees from 337 clones are 37.8 to 81.6% greater than those of unimproved forests (SHEN XIHAN, 1990).

The purpose of setting up a seed orchard is to produce large amounts of high quality seeds. therefore increasing seed production is very important. Although there are many ways to do so, such as to increasing light intensity, fertilization, weed control and using flower stimulation techniques. An alternative method is to study the clonal flowering characteristics, which is the objective of this study.

Materials and Methods

Seed orchards and the Lintian forest used in this study

This research was carried out in three seed orchards (Chongyang, Zhangle and Laoshan) and the natural Lintian forest. The details of seed orchards and Lintian forest are shown in *Table 1*.

Sampling Methods and Data Collection

Male and female cone distribution in crowns of Chinese fir

Five trees of each clone selected at random in the seed orchard were selected for measurement. The crown was divided into upper, middle and lower regions by means of the branch number along the main trunk, selecting 4 average size branches in the east, west, north and south directions in each region of crown. After counting the female and male cones on these branches, the number of female and male cones of whole tree was estimated (ZHANG ZHUOWEN et al., 2001).

$\label{eq:main_state} Male \ and \ female \ cone \ adaptation \ to \ wind \ as \ a \ pollination \ mechanism$

During pollination some trees in seed orchard were selected and the following the characteristics of flowering of Chinese fir were observed (ZHANG ZHUOWEN et al., 2001).

Pollen production per pollen sac and per tree

Fifty microsporophylls were collected from sample male cones at random, put into 2 ml liquid of glycerol and water (1:6), the pollen sacs were opened and pollen was washed out using a tweezer and an anatomical needle. Then pollen number is counted with a Neubauer haemacytometer under a microscope.

Twenty to 50 male cones were selected from a sample of cones of each clone at random. Each morphological item is measured 20 times. From this data the pollen number produced per tree is calculated (ZHANG ZHUOWEN et al., 1990, 2001).

Pollination level estimation

Pollen collectors were set up in seed orchards and Lintian forest, using glass microscope slides coated with a thin layer of Vaseline and was changed every day at almost the same time (ZHANG ZHUOWEN et al., 1990, 2002). The accumulation rate of pollen during pollination and the maximum accumulation of

Table 1. - The details of seed orchards and the Lintian forest.

Stand	Location	Area m ²	Spacing m	Clone number	Age (years)	Height m	DBH cm	Latitude	Longitude	Elevation m
ChongyangS.O	Hillside	80,000	5×5	22 32	5 to 9 7 to 11	6.4 7.4	10.4 12.8	29°38′27″	114°8′19″	148 to 226
Laoshan S.O	In Qiandaohu	311,350	4×4	620	10 to 13	6.0	11.6	29°34'56"	118°52'24"	100 to 305
Zhangle S.O	Hillside	342,838	5×5	657	10 to 14	6.7	12.0	30°16'32"	120°12'28"	64 to 150
Lintian forest	Hillside	800,000	2.5×2.5	0	18	8.7	16.2	30°22'12"	119°47′8″	50 to 120

Silvae Genetica 53, 1 (2004)

Table 2. - Female and male cone distribution in the crown of Chinese fir.

stand	Crown	Branches on	N	lale cones per 1	tree	Female cones per tree		
	m	trunk ~	Тор	middle	bottom	Тор	middle	bottom
Lintian forest	2.4	38.4	10.2	230.1	242.9	301.1	71.0	1.3
Laoshan S.O	3.2	74.7	103.1	419.5	1,136.9	299.8	285.1	145.4
Zhangle S.O	3.4	68.2	286.5	587.4	1,033.2	242.2	189.3	54.9
ChongyangS.O (2001)	2.3	43.0	13.6	85.4	170.2	120.7	170.7	150.6
ChongyangS.O (2003)	3.1	52.0	39.4	136.5	269.7	109.1	142.6	112.8

Note: this table is based on 20 clones for 2001, and 32 clones for 2003 of Chongyang seed orchard^[4]

pollen per day for Chinese fir can be measured. From the data, the accumulation pollen indexes (Rm and Ra) can be calculated. Ra is the ratio of current accumulation rate of pollen in a seed orchard to the accumulation rate of pollen per area, Rm is the ratio of maximum accumulation pollen in a seed orchard to the maximum accumulation rate of pollen at pollination peak per day and per area. With Ra and Rm we are able to judge the pollination level in a seed orchard.

Number of Ovules per tree

Twenty to 50 female cones were selected from samples cones of each clone at random. Each morphological item was measured 20 times. The number of ovules produced per tree was then calculated (ZHANG ZHUOWEN, 2001, 1990).

Pollen sedimentation velocity in still air

A glass tube with diameter of 20 cm and length of 4 m is suspended and 100 grams pollen was shaken at the top of the tube. A stopwatch was used to record the time it takes pollen to travel from the top to the bottom of the tube, and this was repeated 5 times.

Results and Discussion

Female and male cones distribution in crowns of Chinese fir

The distribution of female and male cones in a tree crown shows that male cones are mainly in the middle to bottom region of the crown and female cones are mainly in the middle to top region of the crown. Because of the lower density of trees in seed orchard compared to a normal stand, the female cones are distributed throughout all regions of the tree crown in seed orchard. Trees in low density stands get more sunlight. The results are shown in *Table 2*.

Using the data from the Chongyang seed orchard the differences in female and male cones between clones can be seen. In the Chongyang seed orchard there are 445.8 male cones and 364.5 female cones per tree and the ratio of female cone to male cone is 0.9. There are differences in the number of male cones per tree both between clones and between years. There is a difference in the number of female cones between clones, but not between years. There is also a difference in the ratio of female to male cones between years. See *Table 3* and *Table 4*.

Pollination characteristics of Chinese fir

 $\label{eq:main_matrix} \textit{Male and female cone adaptation for wind as a pollination} \\ \textit{mechanism}$

Pollen size is small and light in weight

Chinese fir pollen is almost like a ball with an equator axes length 39.9 μm , a pole axes length 37.4 μm , a volume of 30219 μm^3 and a weight of $1.98 \times 10^{-5} mg$.

Pollen number is very large

A Chinese fir pollen sac produces 2,300 to 12,000 pollen grains, on average. A normal Chinese fir tree in a natural

stand produces 7.83×10^9 pollen grains $(1.56\times10^5 \text{ mg})$ and 39,241 ovules. By contrast a tree in a seed orchard produces on average 2.19×10^{10} pollen grains $(4.34\times10^5 \text{ mg})$ and 53,199 ovules.

The distribution of male and female cones is good for pollination

Female cones produced at the top of branches. This is good for cross pollination with little resistance of tree crown.

A special protein liquid ball is produced at the ovule aperture

The special protein liquid ball (pollen drop) at the ovule aperture is secreted by ovules. It is used to collect pollen, recognize pollen and nourish pollen during pollen germination.

Protection of pollen and ovule when raining

On the surface of a female and male cone there is a waxy covering, which can keep rain drops away.

Both the macrosporophyll and microsporophyll are able to open or close freely according to the weather.

Female cones and male cones mature at almost the same time

Although male cones are differentiated in May to June, and female cones from August to September, both female and male cones mature, pollinate and fertilize at almost the same time in March to April (YU XINTUO, 1981).

Chinese fir pollen sedimentation in still air

In the condition of still air, when pollen sac open a pollen is effected by two forces, gravity and the resistance of air. We obtain:

 $m \times d v/dt = mg - kv$

we finally get: V = mg/k. The sedimentation velocity of a pollen in still air is related to pollen mass (or weight) and air resistance is measured at V = 16.3 cm/s, k = 1.22×10^{-9} g.cm/s.

Estimation of Chinese fir pollination level

Pollen production per a pollen sac and per tree

The pollen number per sac of a tree in Laoshan seed orchard, Zhangle seed orchard, Chongyang seed orchard and Lintian forest is 3,703, 5,286, 7,653 and 4,365 grains respectively. There is no difference in pollen number per sac among trees in the same clone, but there are differences between clones, and this result is shown in *Table 5* and *Table 6*.

Chinese fir pollen production in a seed orchard

Chinese fir pollen production in a seed orchard is determined by pollen number per pollen sac, the numbers of each different clones, the density and the total area (*Table 7*).

Pollen sedimentation accumulation number during pollination

Chinese fir pollination level is determined by not only pollination characteristics, tree number, tree age, density, but also

Table 3. - Cone distribution in crowns in the Chinese fir seed orchard located at Chongyang.

Clone	Tree height m	DBH Cm	Branch number on Trunk	Mal	e cones / b	ranch	Male cones per tree	femal	e cones	/ branch	Female cones per tree	Cone Ratio
				Тор	mid	mid		Тор	mid	mid		F:M
Xianning7415	8.0	13.6	47.4	1.3.0	6.8	29.6	595.7	7.6	12.86	11.7	507.5	0.9
Xianning 7405	7.6	14.8	50.6	2.0	7.0	18.8	468.9	5.2	5.8	1.2	205.1	0.4
Xianning 7416	7.4	11.2	49.0	2.8	9.2	19.0	506.3	15.2	16.4	19.2	829.7	1.6
Xianning 7431	7.4	12.4	69.6	1.4	4.8	10.0	375.8	11.2	13.8	14.2	909.4	2.4
Xianning 7430	8.8	12.8	48.2	0.2	1.8	6.8	140.7	5.6	7.0	16.8	472.4	3.4
Xianning 7408	8.6	15.6	72.4	4.0	11.2	26.8	1013.6	7.2	10.2	10.4	670.9	0.7
Xianning 7420	8.4	14.0	78.4	3.6	18.2	16.2	993.1	6.4	9.0	2.4	465.2	0.5
Xianning 7434	8.2	13.6	65.0	2.6	1.7	28.8	716.7	9.0	15.4	9.6	736.7	1.0
Xianning 7407	7.6	7.6	39.0	0.8	16.0	21.0	491.4	12.4	20.2	22	709.8	1.4
Xianning 7444	7.0	13.2	51.6	1.4	5.8	16.2	402.5	8.6	12.4	6.8	478.2	1.2
Fulinsuo7305	5.2	9.2	42.2	1.2	3.8	6.6	163.2	4.0	4.6	0.9	133.6	0.8
Fulinsuo 7316	6.2	13.6	42.8	1.4	6.0	10.4	253.9	5.0	8.6	3.8	248.2	1.0
Fulinsuo 6421	8.6	16.4	59.2	17.2	9.8	25.6	1038.0	2.4	10.6	5.2	359.1	0.3
Jinpinhui16	7.6	13.6	50.6	1.4	6.0	10.6	303.6	5.8	5.0	4.2	253.0	0.8
Xiang 03	7.6	14.0	48.1	6.4	10.6	9.6	426.5	12.2	14.4	8.0	554.8	1.3
Xiang 08	6.4	11.2	54.6	2.4	3.2	3.4	163.8	3.8	4.8	3.8	225.7	1.4
Yunyou 5	8.8	15.6	70.2	0.6	12.6	13.4	622.4	8.6	7.2	3.2	444.6	0.7
Fujian 431	6.4	12.0	50.4	3.6	13.6	29.6	786.2	4.8	7.8	9.4	369.6	0.5
Fujian 43	7.6	13.2	56.0	1.4	7.0	10.0	343.5	3.8	4.2	4.2	227.7	0.7
Fujian 20	7.0	12.4	40.4	0.4	3.0	16.6	269.3	5.0	9.6	2.6	231.6	0.9
Fujian 13	8.0	14.8	47.2	0.6	5.0	14.0	308.4	4.2	7.8	2.4	226.6	0.7
Fujian 35	7.0	12.8	40.4	1.6	13.4	18.0	444.4	5.8	8.4	3.6	239.7	0.5
Fujian 25	8.0	11.2	59.6	3.2	24.2	22.0	981.4	2.2	3.8	7.6	270.2	0.3
Fujian 4	7.2	14.8	45.0	2.8	7.2	19.0	435.0	8.4	8.6	4.6	324.0	0.7
Fujian 33	7.6	15.2	52.4	0.8	10.2	19.4	531.0	9.0	9.0	8.2	457.6	0.9
Fujian 2	7.6	11.6	32.0	0.0	5.2	13.2	196.3	7.6	7.8	10.6	277.3	1.4
Fujian 3	7.8	12.4	39.0	1.0	1.1	8.2	133.9	3.6	1.26	1.5	82.7	0.6
Fujian 9	6.2	12.0	34.4	1.4	1.36	6.8	109.6	3.0	3.6	2.4	103.2	0.9
Fujian 21	7.4	10.4	30.0	0.8	2.6	12.8	162.0	3.0	2.8	0.6	63.6	0.4
Fujian 27	7.2	12.8	37.4	0.8	9.6	9.8	251.8	7.2	5.0	1.1	165.8	0.7
Fujian 36	5.6	8.8	48.0	0.2	3.2	12.0	246.4	1.96	6.8	7.4	258.6	1.1
Fujian 39	6.2	12.0	42.4	3.2	10.6	13.6	387.3	4.8	4.4	2.2	161.1	0.4
average	7.4	12.8	52.0	2.3	7.9	15.6	445.7*	6.4	8.4	6.6	364.5*	0.9

* Note: two figures are not quite equal to sum of their cones of level of crown because of decimal point, these are errors of statistics.

Table 4. - Analysis of Variance for female and male cones both between clones and between years.

Variance	df		Male cones			Female cones		Female co	Female cone:male cone Ratio		
origin		SS	MS	F	SS	MS	F	SS	MS	F	
Clones	19	1206641	63507.43	2.29*	2503155	131745	7.52**	154.43	8.13	1.85	
Years	1	587970.4	587970.4	21.18**	2721.345	2721.345	0.16	23.61	23.61	5.38**	
Error	19	527497.2	27763.01		333004.3	17526.54		83.32	4.39		
Total	39	2322109			2838881			261.36			

 $F_{0.05} = 2.16825, F_{0.01} = 4.380752$

 $Table \ 5.$ – Pollen and ovule characteristics of Chinese fir trees among clones in seed orchard located at Chongyang.

clone	Pollen a sac	А	В	С	D	E	F	G	H×10 ⁵
Xianing7420	11,353.1	43.5	20.5	575.2	1.74×10 ¹⁰	40.2	399.3	48,155.6	3.62
Fujian 35	12,605.1	40.8	24.4	269.1*	1.01×10 ¹⁰	30.1	441.9 [*]	39,907.2	2.53
Fujian 27	5,432.5	42.4	21.1	269.1*	3.92×10 ⁹	36.9	441.9 *	48,989.1	8.00
Fujian 33	3,161.9	44.0	28.2	202.7	2.38×10 ⁹	38.1	210.9	24,108.2	9.89
Fujian 13	2,695.0	46.3	27.3	174.2	1.78×10 ⁹	37.8	222.3	25,180.0	7.07
Fulisuo 7316	2,355.5	41.6	21.7	152.5	9.72×10 ⁸	34.6	215.0	22,321.2	4.35
Xianing 7405	3,034.6	43.7	17.5	188.1	1.31×10 ⁹	30.5	390.3	35,654.8	3.66
Fujian 25	5,708.4	38.9	14.4	148.2	1.42×10^{9}	35.4	117.3	12,454.1	1.14
Xiang03	6,472.3	40.1	24.1	269.1 *	5.05×10 ⁹	36.3	441.9 [*]	48,127.3	1.05
Fujian07	9,719.1	39.2	19.3	92.5	2.03×10 ⁹	40.7	54.1	6,603.2	3.08
Xianing 7430	11,459.2	53.3	14.8	56.7	1.54×10^{9}	33.6	685.2	68,968.4	2.23
Xianing 7434	8,488.3	41.9	18.7	521.0	1.04×10^{10}	32.1	379.3	36,464.9	2.84
Fujian43	5,517.4	44.3	24.2	378.5	6.71×10 ⁹	43.0	271.2	34,983.5	1.92
Yunyou5	14,493.7	34.4	14.6	269.1*	5.86×10 ⁹	30.6	441.9 [*]	40,570.1	1.45
Fujian442	12,965.8	43.7	22.3	269.1*	1.02×10^{10}	31.4	441.9 [*]	41,564.5	2.46
Xianing 7416	10,122.3	46.8	24.4	90.5	3.14×10 ⁹	29.8	1149.3	102,575.9	3.06
Jinpinghui 16	3,798.5	44.8	19.6	205.7	2.06×10 ⁹	29.4	386.0	33,987.3	6.06
Xianing 7431	15,257.7	53.0	22.0	326.8	1.74×10 ¹⁰	34.3	1129.9	116,100.3	1.50
Xiang 04	4,201.7	42.4	19.5	269.1*	2.81×10 ⁹	33.7	441.9 *	44,680.1	6.28
Xianing 7444	3,055.8	43.8	20.4	393.8	3.23×10 ⁹	36.9	628.1	69,432.0	4.65
Fulisuo7305	4,880.8	47.7	20.1	178.3	2.50×10 ⁹	36.4	270.0	29,444.6	8.50
Fujian 431	11,310.6	44.7	25.3	351.3	1.35×10 ¹⁰	37.6	533.7	60,196.9	2.24
Fujian 6421	8,170.0	51.8	19.7	269.1*	6.73×10 ⁹	35.1	441.9 [*]	46,483.3	1.45
Fujian 04	6,684.5	44.8	20.9	274.4	5.15×10 ⁹	39.9	384.1	46,028.4	1.12
Fujian 20	4,010.7	40.8	19.2	269.1	2.54×10 ⁹	33.7	441.9*	44,680.1	5.68
Fujian7408	11,862.3	44.4	17.7	333.1	9.28×10 ⁹	32.9	461.6	45,555.0	2.04
average	7,653.3	44.0	20.8	269.1*	5.66×10 ⁹	35.1	441.9 [*]	46,483.3	1.22

** Note: The basic number for female cone and male cone is 3.

* Note: they are all averages in the table.

A the number of microsporophyll per microstrobilus C the number of male cones per tree

C the number of male cones per tree E the number of macrosporophyll per female cone

G the number of ovules per tree

B the number of microstrobili per male cone

D the number of pollens per tree

F the number of female cones per tree

H the ratio of pollen to ovule

pollen number per unit area and wind velocity. We can use the indexes of accumulation pollen rate during pollination per area or maximum pollen rate per day and per area at pollination peak to assess the pollination level. It was observed that the area of protein liquid ball (pollen drop) at ovule aperture is

Table 6. – Analysis of Variance for pollen number per sac both among trees in the same clone and between clones of Chinese fir in seed orchard located at Chongyang.

Variance origin	SS	df	MS	F	F crit
Between clones	110,555.2	24	4,606.5	6.1**	1.6
Among the same clone	4,151.0	7	593.0	0.8	2.1
Error Total	126,374.3 241,080.6	168 199	752.2		

about 0.5 to 1.1 mm². If there are 3 to 5 pollen grains at the ovule aperture, it is enough for fertilization. So a reasonable rate for accumulation pollen during pollination should be 4 pollen grains/mm², and the rate of maximum accumulation pollen at pollination peak should be 3 pollen grains/d.mm². Therefore we can judge the pollination level of a Chinese fir seed orchard with these two indexes. As a results the pollination level of the three Chinese fir seed orchards in this study are too high. The Ra is 4 to 56 so in these seed orchards we need to stimulate more female cones in order to produce more seeds.

Ovule number and the ratio of ovule to pollen

The purpose of establishing a seed orchard is to produce seeds with high genetic quality. Seed production in a seed orchard is determined by the number of ovules, and seed quali-

Stand	Pollen/	Male-cones/ tree	Pollen per tree		All	stand
	sac	-	number	Weight (mg)	number	Weight (mg)
Laoshan S.O	3703.3	1659.5	3.51×10 ¹⁰	6.97×10 ⁵	6.55×10^{14}	1.30×10 ¹⁰
Zhangle S.O	5286.4	1907.2	3.74×10 ¹⁰	7.43×10^{5}	5.13×10 ¹⁴	1.02×10^{10}
ChongyangS.O	7653.3	269.1(2001.3)	5.66×10^{9}	1.12×10^{5}	1.81×10^{13}	3.59×10^{8}
		445.7(2003.3)	9.37×10^{9}	1.86×10^{5}	3.00×10^{13}	5.95×10 ⁸
Lintian forest	4365.2	483.2	7.83×10 ⁹	1.55×10^{5}	1.00×10^{15}	1.99×10 ¹⁰

Table 7. – Pollen production of Chinese fir*.

* note: 656.07mg/cm^3 , 5.0396×10^4 grains per mg

Table 8. - Accumulation pollen number during pollination (grain/mm²).

	Pol	m ² .	R		
Stand	Pollen accumulation	Per day	Max / Per day	Ra	Rm
	During pollination		(in peak pollination)		
Laoshan S.O	223.33	6.98	53.50	55.83	17.83
Zhangle S.O	53.74	3.61	4.04	13.43	1.35
ChongyangS.O	16.28	1.33	10.77	4.07	3.59
Lintian forest	54.44	2.85	8.95	13.61	2.98

Table 9. - Ovule production of Chinese fir*

Stand	Number of female	Number of	Seed number	Seed weight
	Cones Per tree	Ovules per tree	all stand	all stand (kg)*
Laoshan S.O	730.4	76,821	1.49×10 ⁹	10,968
Zhangle S.O	486.4	51,156	7.02×10^{8}	5,147
ChongyangS.O	441.9 (2001)	46,483	1.49×10^{8}	1,091
	364.5 (2003)	38,336	1.23×10^{8}	900
Lintian forest	373.1	39,240	5.02×10 ⁹	36,854

* note: 1. The average WKS is 7.4 gram, and there are some of ovules fail to develop into good seeds.

2. we use the average macrosporophyll number (35.1) per female cone, which can produce ovules, to estimate the number of ovules of a tree and all stand.

ty is determined by many factors, one of which is the degree of the self fertilization. In general, the higher the self fertilization proportion in a seed orchard, the more the selfed seed the seed orchard will produce, but the lower the seed genetic quality. We should try to control or avoid self fertilization in a seed orchard.

The ratio of ovule to pollen is shown in *Table 3* and *Table 5*. The ovule production in seed orchards or a stand is shown in *Table 9*. With the index of WKS (WKS is the air dried weight of 1000 mature seeds), we can forecast seed production for a seed orchard by means of ovules produced in this seed orchard discounting for failure of ovules during development.

Conclusion

From this study we can conclude that the male and the female cones have their own distribution regions in Chinese fir crown. The age, density of a stand, clone, climatic factors can effect male and female cones production, their ratio, and their distribution in crown. Both the Chinese fir male and female cones appear to be very well adapted for wind as the pollination mechanism in many ways. When a pollen leaves the pollen sac, it undergoes a slow, even sedimentation rate with the wind and topographical factors affecting this movement. With the methods used in this study we can obtain the important numbers of pollen and ovules produced in a seed orchard. We are able to judge the pollination level in a seed orchard by means of the pollen accumulation rate during pollination per area and the maximum accumulation pollen rate per day and per area at pollination peak.

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