# STUDIES ON THE FRUCTIFICATION OF "SHINANO WALNUT", A STRAIN OF JUGLANS REGIA L.\*

# 1. Morphology and ecology of the staminate catkin and the pollen

by

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# Introduction

So-called Shinano walnut, a strain of *Juglans regia* L., has been cultivated mainly in Nagano -pref., Japan. It is said as the hybrid between the ancient variety (*J. regia* L. var. *orientis* Kita.) and some varieties of Persian walnut (*J. regia* L.). Therefore, the essential characters resemble closely those of Persian walnut, though the both strains more or less differ from each other in detailed points.

Since ten and more years ago the author has investigated the morphology and the ecology of Shinano walnut, especially on the fluctification.

A part of the results is given in the present paper, which contains the following subjects.

- 1. Quantitative characters of staminate catkin
- 2. Development of staminate flower
- 3. Pollen shedding and relative humidity
- 4. Amount of pollens produced
- 5. Shape and size of pollen
- 6. Dispersion of pollen
- 7. Viability of pollen

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# 1. Quantitative characters of staminate catkin

The stanimate flower develops from the catkin bud which is formed on the preceding branch.

The catkin bud takes a cone shape with the diameter  $3\sim4mm$  and the length  $4\sim6mm$ . When warm weather comes in spring the catkin bud begins to grow and the spike bearing many immature flowers elongates. Generally the catkin bud points upward at first and lately pendents as the catkin before the pollen grains are shed. In some individuals, however, the catkin tekes a horizontal direction without pendency.

The length of the catkin varies according to individual tree. It is considered that the standard length in an individual will be decided inherently.

Thirty pieces of the matured catkins already finished pollen shedding were collected from each individual tree to measure an average length of the catkin. The results are shown in Table 1.

No. of	C	lass	۲	val <b>u</b> e	(le	(length)		cm	Mean	
individual	7	8	9	10	11	12	13	14	length	·
39	1	6	4	12	7	3	2	4	$10.2 \pm 0.30$	1.86

Table 1. Frequency of individual tree based on average length of catkin

According to Table 1, the length of the catkin ranges  $7\sim14$ cm, and the mean length is  $10.2 \pm 0.30$ cm. The length is as long as half of that of Japanese walnut (*J. sieboldiana* Maxim.), and is near to that (average 10.4) of Persian walnut measured by CONSTANTA (1967).

Next the number of the staminate flower grown around the axis of the catkin was measured on 10 catkins per individual tree. The results are shown in Table 2.

No. of Class value Mean σ individual 110 90 100 120 130 140 value 59 22 2 16 9 6  $112.2 \pm 1.62$ 4 12.46

 
 Table 2.
 Frequency of individual tree based on number of staminate flowers per catkin

The number of the staminate flowers per catkin varies  $90 \sim 140$  and the mean value is  $112.2 \pm 1.62$ . The number stands about half as compared with that of Japanese walnut.

Regarding the number of the staminate flower in Persian walnut IMPIUMI and RAMINA (1967) and CONSTANTA (1967) reported that it was about 130 and  $110\sim140$  (average 121.5), respectively. The number in Shinano walnut seems a little smaller than that of Persian walnut. It is considered to be originated by an inherent character of the ancient variety.

# 2. Development of staminate flower

The staminate flower seats radially around the axis of the hanging catkin and takes an oval shape in plane view. It consists of a bract, two bracteoles, four sepals and numerous stamens (MANNING, 1948). The bract is distinguishable from the other parts in structure and position, connecting with the pedicel at the basal portion. It is hardened and takes dark-brown in coloration.

Two bracteoles and four sepals form a calyx-like-circle composed of six organs (lobes) around the stamens (anthers). The bracteoles are somewhat larger than the sepals, which lie at their posterior part.

The size of the flower is commonly 5mm in length and  $3\sim$ 4mm (narrower foward basal part) in width, though it decreases toward the distal part of the catkin.

Next, the author observed the florescence of the staminate flower. The above-descrived calyx-like-circle has been packed strongly at the beginning of growth, but becomes loose gradually as the time proceeds. Then each lobe of the calyx trends toward outside, and the inner anthers come in sight. This process proceeds with the elongation of the catkin axis from the proximal part toward the distal one.

A true florescence, however, occurs when an anther bursts open and also pollens are shed. The author decided the first blooming time of the catkin and the individual tree with the dehiscence of anther.

The anther consists of two sacs like sausage, each of which attaches to one another at one side, the other side to which makes a deep groove. Lately the groove bursts open to shed the pollen. Moreover, the anthers are grobrous in the flowers of Shinano walnut, but pubescent in those of Japanese walnut. Not burst anthers take greenish milky color and burst opened anthers brown color, but become blackish before long. From the above observation, the form and development of the flower and the anther in Shinano walnut seems to have a close resemblance to that in Persian walnut.

In regard to the number of the anthers per flower, MANNING (1948) descrived that of Persian walnut was  $10\sim28$  in the studies of staminate flowers of many species belonging to Juglandaceae. The author measured the number of the anthers on the flowers sampled from the top, the middle and the base of catkin (10 pieces per tree), because the size of the flower varies according to position on the catkin.

The results are shown in Table 3.

Position of flower	Anther per flower Class value	Moon voluo	
on catkin	9 10 11 12 13 14 15 16 17 18 19 20 21 22	Weath value	0
top	2 8 17 21 7 4 59	$11.6 \pm 0.15$	1.17
middle		$15.2 \pm 0.19$	1.42
base		$18.1 \pm 0.20$	1.53

Table 3. Frequency of individual tree based on number of anther per flower.

The author has considered that the number of anther per flower is constant as a specific character of plant, while discovered an interesting fact on that of Shinano walnut as seen in Table 3. The number of the anther per flower varies  $9\sim22$  in the flowers collected from 59 trees, and the average

Individual	No. of flowers per		Number o	of anther	
tree	catkin	top	middle	base	average
No. 46	99	. 10	12	14	12
No. 30	107	10	14	16	13
No. 102a	102	11	12	16	13
No. 59	97	11	15	15	14
No.11	113	11	15	19	15
No. 18	113	10	16	18	15
No. 80	99	12	15	19	15
No.4c	112	11	17	20	16
No.5b	116	12	16	19	16
No.7b	111	13	16	19	16
No.14	107	13	16	19	16
No. 15	104	14	16	17	16
No.6b	129	13	16	21	17
No. 21	117	14	17	19	17
No. 28a	137	12	18	22	17
average	111	12	15	18	$15 \pm 0.39$

Table 4. Several examples in which number of anther differs

number at the base, the middle and the top positions is 18.1, 15.2 and 11.6, respectively.

Several examples of the number of the anther counted by the author are shown orderly in Table 4.

As seen in Table 4, there is a decreasing tendency of the number of the anthers toward the distal position of the catkin. This tendency is also found in the size of the flowers. And the average number is  $15.0 \pm 0.39$  in regard to 15 instances,

# 3. Pollen shedding and relative humidity

As above-mentioned, the pollens are shed by burst of the groove of the anther. All the anthers of a flower do not burst at the same time, and one hundred and more flowers of a catkin do not bloom at the same time. The blooming comes up usually from the basal portion toward the distal portion on a catkin. Therefore, the pollen shedding per catkin extends to several days, though the duration is influenced by the weather.

The author has examined the correlation between relative humidity and pollen shedding.

Method :

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Small rooms with the following relative humidities were made with

	time		Relative	humidity	(%)		Laborator	y (9a.m.)
	(hr)	20	50	80	100	Laboratory	Temp.(°C)	R.H.(%)
*	12	20	10	0	0	5		
(1)	24	40	50	0	0	20	16.7	57
	36	70	100	0	0	50	19.5	44
	48	100	100	0	0	100	10.0	**
	22	12	15	0	0	0		
(2)	30	40	40	0	0	0	17.6	63
	36	80	60	0	0	0	16.2	64
	42	100	100	0	0	15		
	16	20	10	0	0	0	9.1	91
(3)	30	50	40	0	0	10	14.0	
	44	90	90	0	0	50	14.3	71

Table 5. Percentage of pollen shedding under various relative humidities (Expt. 1)

\* Beginning of observation :

(1) 16 May, 1 p.m. (2) 19. May, 6 p.m. (3) 22. May. 2 p.m.

desiccators using REGNAUL's method, and laid under a constant temperature of 20°C.

Expt. 1 20, 50, 80, 100% (R. H.)

Expt. 2 50, 60, 70, 80, 100% (R. H.)

Three matured catkins were put in each room and observed a grade of the pollen shedding with some interval time. On the other hand, three catkins were put under a natural condition of the laboratory. This observation was repeated several times during the flowering periods.

Result and consideration :

The results of Expt. 1 are shown in Table 5.

According to Table 5, it is clear that the pollen shedding takes place when the dryness falls into less than 50%, while not under the wetness of more than 80%. The anthers of the matured catkin burst open within about two days, whereas there is little difference in the pollen shedding between 50 % and 20%.

The results of Expt.2 are indicated in Table 6. There occurs the pollen shedding in both 60% and 70%. It is recognizable that a higher critical point of the pollen shedding exists at 70% (R. H.) with the temperature of about  $20^{\circ}$ C.

The author considers conclusively that the rain and the fog during the blooming period of the staminate flowers may prevent the anthers from pollen shedding.

	Time		Relalive h	Laboratory			
	(hr)	50	60	70	80	100	temperature (°C)
*	6	50	50	50	0	0	20
(1)	24	60	60	70	0	0	17
	48	100	100	100	0	0	20
(2)	15	100	95	95	0	0	20

Table 6.Percentage of pollen-shedding under variousrelative humidities (Expt. 2)

\* Beginning of observation :

(1) 16 May, 11 a.m. (2) 18 May, 6 p.m.

#### 4. Amount of pollens produced

The blooming period of the pistillate flower, the pollen shedding of the anther, the amount of the pollens produced and the dispertion of pollen

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grains are of important factors for the pollination in fructification of walnut belonging to the anemophirous plant. In this section the amout of the pollens per anther in Shinano walnut is described.

WOOD (1934) made a study on the pollination and the blooming habits of Persian walnut in California, and described that a single catkin produces pollen grains of 1,000,000 to 4,000,000.

In Chap.1 the number of the flower per catkin and the number of the anther per flower were given. Now the author counts the number of the pollen grains per anther for calculation of the pollen grains per catkin.

In order to count the pollen grains the following method was taken. Forty-eight sections were made on a slide-glass, and there laid a single anther taken from the fixed staminate flower and broken by a needle.

The pollen grains diffused over in 60% alcohol solution on the slide were counted under a microscope. Five anthers were taken respectively from five flowers growing at the base, the middle and the top of the catkin.

The results are given in Table 7.

Flowe's portion on catkin	Base	Middle	Тор	Average
No. of pollen grains	2,172	2,532	1,680	2,130

Table 7. Number of pollen grains per anther

According to Table 7, the number of the pollen grains produced are less in the top than in the others, indicating that the anther's size in the top seems smaller than in the others.

The average number (2,130) is very large as compared with that of Persian walnut, on which IMPIUNI and RAMINA (1967) reported as 900. It may be caused by the difference in the measuring method.

Next the author calculated the number of the pollen grains produced in a single catkin with the number of the pollen grains per anther which was regarded as 2,000 and with the number of the anthers per flower which was considered as 15.

Table 8 shows three cases which differ from each other in the number of the flowers (see Table 2).

The number of the pollen grains per catkin is a little larger than that of Persian walnut, on which WOOD (1934) reported as  $1,000,000 \sim 4,000,000$  by means of the different counting method from the author's.

In another report (IMPIUMI and RAMINA, 1967), the number in Persian walnut was 1,755,000. It is very much smaller than the author's count. In

Cases	No. of flower per calkin	No. of pollen grain
А	90	2,700,000
В	110	3,300,000
С	140	4,200,000

Table 8. Number of pollen grains produced per catkin

any case, a single catkin produces extraordinarily large numbers of pollen grains.

In Shinano walnut as well as Persian walnut, the number of catkins differs according to variety and age of tree. The variety, therefore, bearing much large catkins is recognized as a nice pollinizer.

# 5. Shape and size of pollens

The fruiting of Shinano walnuts is achieved by wind-pollination, so that the shape and the size of the pollen grains take an important role for distribution. In Persian walnut the pollen size was reported as  $46\mu$  (WOOD, 1934), 40.  $2\mu$  (STACHURSKA, 1961) and  $40 \sim 50\mu$  (SCHANDERL, 1964).

Shape :

The pollen grains of Shinano walnut belong to a porous-foraminate type with reticulum pattern in the surface, showing a polygon in polar view, and are suboblate in equaterial view. The pores, of which margins are slightly projecting, are  $18\sim20$  in number and situated at each vertex of polygonal angles. The number is larger in the equatorial region than in the other part. It is equal to that of Persian walnut observed by SCHANDERL (1946), differing from STACHURSKA's observation ( $10\sim15$ ) (1961).

In Japanese walnut the pollen grains are smaller not only in size but also in pore number than in Shinano walnut, while resemble to each other in type. Size :

The size was measured on the pollen grains sampled from 18 trees in our farm and 15 trees in other local regions. The results are shown in Table 9 and 10.

According to Table 9, the pollen size varies from 43.8 to 48.9 $\mu$  in diameter and averages 46.5  $\pm$  0.27 $\mu$ . But, in Table 10, the pollen size is a little smaller than that in Table 9, varying from 41.6 $\mu$  to 45.8 $\mu$  (average 43.9  $\pm$ 0.29 $\mu$ ). The author presumes that the variance depends on the difference in variety of the walnut, and the pollens with a diameter of 60 $\mu$  and more can be said "Giant pollen". Be that as it may, it is recognizable that the pollen

Tree			Class n	umbers	in diame	ter ( $\mu$ )			Mean
1100.	35.5	39.0	42.5	46.0	49.5	53.0	56.5	60.0	INICALL
No. 7a	2	—	6	41	71	25	3	2	48.9
14		1	10	60	65	14			47.9
201a	—	5	30	36	56	20	2	1	47.5
24a		_	11	80	49	7	1	2	47.5
77	—		25	67	46	11		1	47.1
83		1	28	57	54	9	1		47.1
44	—	6	22	59	54	9	Resolution		40.9
71d		3	22	65	56	3		1	46.9
87		1	24	84	36	5			46.5
47		14	25	54	44	12	1		46.4
73		4	24	78	43		Antonia	1	46.4
52		6	35	67	33	8	_	1	46.1
201c		4	38	71	34	3			45.9
16b	1	3	48	63	28	6	1		45.7
75	5	14	32	50	37	10	2		45.7
41	1	12	39	60	36	2			45.4
101a		5	43	68	33	1			44.9
79	—	14	76	53	6		1	—	43.8
Total	7	93	538	1113	781	145	12	9	
Mean									$\begin{array}{r} 46.5 \\ \pm 0.27 \end{array}$
σ Anoth-			-						1.14
er. Jap- anese walnut	27	65	44	10	3	Randauun			41.0

Table9. Distributive frequency of pollen size (trees in our farm).

size of Shinano walnut is as similar as that of Persian walnut.

Further, the pollen size is larger than that of Japanese walnut, the average value of which is about  $40\mu$ .

In Japanese walnut the pollen size was reported as  $34 \sim 42\mu$  (NAKAMURA, 1943),  $30 \sim 31 \times 34 \sim 37\mu$  (IKUSE, 1956). Their size is smaller than that of

Table 10.Distributive frequency of pollen size<br/>(trees in other local regions)

Tree				Cla	ss nu:	mbers	in d	iamet	er (	μ)				Mean
1166	32.5	35.0	37.5	40.0	42.5	45.0	47.5	50,0	52.5	55.0	57.5	60.0	62.5	Wican
K–15	-	_	2	7	22	69	61	33	4	2	_	_		45.8
S-11	3	1	19	27	37	48	39	18	8	_				45.5
S- 5		1	3	26	42	72	31	16	1		2	—	1	44.9
S- 9			5	15	57	76	36	9	2			_		44.5
N-33		3	10	18	57	60	29	22	1			—		44.3
S- 7		—	9	29	43	59	47	12	1			_		44.3
S-15		3	6	17	53	74	34	13		_	_			44.3
K-17		1	8	28	52	73	21	11	4		1		1	44.1
K-15		1	1	6	49	84	46	11	2	-			—	44.0
K-11		1	3	28	54	82	29	3	—	_				43.9
K-28	-	5	12	25	49	62	37	8	2	—	—			43.8
N-17	6	7	28	29	48	54	17	1	2	2	2	1	3	43.1
K–13	2	5	24	30	52	62	23	1	1			_		42.7
N36	1	4	18	50	68	47	10	2				-		42.1
N-35	2	2	29	51	58	28	11	7			1	1		41.6
total	14	34	177	379	741	941	471	167	28	4	6	2	5	
Mean														43.9±0.29
δ														1.1
Another Japanese walnut	2	11	61	87	37	2						_	_	39.4
	•													

the author's measurement. It may be arisen from difference in species, because the scientific name used for Japanese walnut by each author is different.

# 6. Dispersion of pollen

The flowers in the matured catkin downwardly grow overlapped each other, so the anthers in the flowers are hanging down. When the anthers

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dehisce, a majority of the pollen grains, if there is no wind, fall into a concaved part formed at the back of the flowers just below. Later, when wind arises, the pollens are blown away from the concaved part, and float into the air. Therefore, the presence of wind during the flowering period brings good effect on the dispersion of the pollens.

Then the author observed distribution density of the pollens into the air during the flowering period of the trees in our farm.

Method :

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The pollen grains floating into the air were gathered on a slide-glass placed at various distances (10, 30, 60, 100m, etc.) from the tree in open field. The stations are indicated in Fig. 1. The slide-glass was smeared by a



Fig.1 Stations where pollens were collected.

mixture of glycelin and albumin, and ten leaves of the slide-glass were placed on a table with 1m high at each station. Afterward, the slide-glass was collected with some interval time, and the number of the pollen grains per 1cm<sup>2</sup> were counted. Such an observation was repeated 4 times.

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	Station	A	В	С	D	E	F	Rei	nark.
D	vistance (m)	Under tree	10m	30m	60m	100m		Wind direction	Wind v <b>e</b> rocity (m)
SI	Time after urvey setting (hr)								
* (1)	24	50	16	14	11	4	68	EN	1.3
(2)	12 (night time)	38	20	16	8	0	27	s	0
	12 (day time)	258	126	124	46	20	57	S	0.4
	24 (total)	296	146	140	54	20	84		
(3)	12 (day time)	24	15	10	5	3	114	NW	2.0
(4)	24	9	10	10	7	1	16	E	0

 Table 11.
 Distributing density of pollen grains

(Number of pollens per 1cm<sup>2</sup> at definite position)

\* Date of the experiment.

(1) 21. May, 5 p.m. ~22. May, 5 p.m.

(2) 23. May, 6 p. m.  $\sim$ 24. May, 6 a. m and 6 a. m $\sim$ 6 p. m.

(3) 26. May,  $8 a. m. \sim 8 p. m.$ 

(4) 31. May, 6 p. m~1. June, 6 p. m.

The results obtained by the avobe method are shown in Table 11. It is considered that a decrease of the pollen number in the 4th observation is likely caused by the decline of pollen shedding in most of all trees.

Through the 1st to the 4 th observations the distribution density of the pollens is closely related to the distance; the longer the distance the smaller the density. The pollens tend to distribute to a leeward side of the tree which is shedding the pollen. And the pollen grains reach still a distance of 100m, though this examination gives no data on further more distance.

A great difference is found in the number of the pollen grains between night time and day time in the 2nd obsevation. It seems to be caused by the high wetness in night time, viz. in such a condition the anthers do not burst open and also hydrated pollen grains fall speedily.

In any case, when the pollen shedding is at the best condition, the pollens are distributed of  $4\sim20$  grains per  $1\text{cm}^2$  per day in number at a distance of 100m, and of  $11\sim54$  grains as well as at 60m. Now supposed the surfase areas of the receptive stigma is about  $25\text{mm}^2$ , each stigma in the above condition at 100m has reasonably a chance of receiving  $1\sim5$  grains in a day. At 60m distance, if so, the number of the pollens received becomes as large as 3 times.

It can be estimated that a range where the pollination is effectively achieved exists within 60m from the tree.

WOOD (1934) described that the number of the pollen grains of Persian walnut distributed per  $1\text{mm}^2$  per day under rather strong wind was 1.7 and 1 at a distance of 250 and 500 feets (75m and 150m) respectively, to the leeward side of the tree. Further, IMPIUMI and RAMINA (1967) reported that  $16\sim24$  grains per  $1\text{cm}^2$  per 15min. were found on the slides placed at the farthest station of observation (160m), which is about 20 times to that of WOOD's observation.

The above data indicate that the distributing density of pollens at a simiar distance is higher than the author's. Thus taking the above data into consideration, the most effective distance of the pollination of Shinano walnut may be ranged more than 60m.

# 7. Viability of pollen

The fruiting of walnut is achieved by fertilization after the pollens reach the stigma of pistillate flower by wind. The viability of the pollen must be kept until the pollens germinate at the surface of stigma after being shed from the anther. In this case, the viability, though variable naturally according to pollen, will be effected by environmental factors.

Practically the viability of the pollens is examined by fruiting based on pollination, but the author has carried out the experiment using the germinating test on artificial media. The medium contains some amount of sucrose in general. The author's media compose of the following contents.

1. Water 100cc, agar 1g, sucrose 10g.

2. Water 100cc, agar 1g, glucose 10g.

The samely matured catkins just before burst open were collected from the material trees in our farm and in local regions, and their pollens shed in the next day were used for the test. The results obtained are shown in Table 12 and 13.

The germintion percentage of the fresh pollens shed in next day is shown in Table 12. The difference in the germination percentage is difficult to detect between media and between thermal conditions. The percentage averages 45  $\sim 80\%$  (total average 63.3%) in six varieties. This value is large compared with that of Persian walnut, on which WOOD (1943) described it as  $0 \sim 80\%$ (averge 23%) in 18 varieties.

As the pollen age seems reasonably to bring reduction of the viability, the author carried out the following examinations, tried to find the best

Temp.		20	°C	25	A		
Culture Medium		Sucrose Gluco		Sucrose	Glucose	Average	
	OM-33	81.2	76.2	82.7	84.7	81.2	
	OM-55	52.1	61.9	61.4	62.6	59.5	
Verieta	OM–97	58.2	57.8	66.2	69.6	63.4	
variety	YK- 3	58.3	51.3	51.7	63.8	56.3	
	YK-15	39.3	8		50.2	44.8	
	Concord		82.4		66.7	74.6	

Table 12. Germinate percentage fresh pollens

Remarks : OM-33, 55, 97, ..... trees in our farm

YK-3,15, Concord.....trees in local region.

preservation method for maintenance of the pollen viability essential to an artificial pollination.

Preservation method :

- A. Dry-box, temperature : 20~22°C, relative humidity : about 55%, confined in a small dry-box in our laboratory.
- B. Wet-box, temperature : 22~23°C, relative humidity : about 70%, confined in a small wet-box in our laboratory.
- C. Ice-box, temperature : 10~12°C, relative humidity : about 70%, confined in a small ice-box in our laboratory.
- D. Refrigerator, temperature : 5°C, reative humidity : about 80%.

A part of the pollens which were used for the previous examination was subjected to the respective regime after enclosed in a paraffine paper. The germination percentage of the pollens were observed with some interval of time. The results are denoted in Table. 13.

The maintenance duration of the pollen viability was 2 days in Dry-box with exception of 2 varieties,  $5\sim 6$  days in Wet-box, 12 days in Ice-box, and 19 days in Refrigerator except in YK-15 variety, in which it was 26 days. It is recognizable that the pollen viability is able to continue only for 2 days under high temperature  $(20\sim 22^{\circ}C)$  with low humidity (55%), while about 20 days under low temperature  $(5^{\circ}C)$  with high humidity (80%).

Namely, based on thermal condition, the duration is longer under low temperature than under high temperature, and observing from the relative humidity, it is more lengthened under high humidity than under low humidity.

The author's results are coincided with those in Persian walnut, on which WOOD (1943) and ŠČEPOTJEV and BOUISONKO (1949) reported the pollen viability was a few days under average field conditions and about 3 days under

Preserved state		Dry	•box	Wet	Wet-box		box	Refrigerator	
Variety		OM 33	OM 55	OM 33	OM 55	OM 33	OM 55	OM 33	OM 55
Days	2	1.3	0	52.4	37.4	66.8	57.0	47.3	49.9
after	5	0	0	6.6	19.2	40.6	32.9	57.3	52.2
biginning	12		<u> </u>	0	0	7.0	16.9	23.4	28.1
of	19					0	0	6.8	24.9
test	26						÷	0	0
Variety	7	YK 3	YK 15	ҮК 3	YK 15	YK 3	YK 15	YK 3	YK 15
Days	2	0.3	0	20.0	41.6	51.2	43.2	60.3	42.7
after	6	0	0	2.4	9.4	33.3	38.3	45.4	47.4
biginning	12			0	0	6.9	37.0	15.5	53.8
of	19					0	0	2,3	6.8
test	26							0	0.6

Table 13. Germinate percentage of preserved pollens

Remarks : See Table 12.

room conditions, respectively. Further ŠČEPOTJEV and POBEGAIRO (1954) described that Ice-box method was better for preservation of the pollen grains of Ukraina black walnut.

The pollen grains of Shinano walnut, therefore, are assumed to have the same or higher viability of Persian walnut. In practice, the fruiting cannot be achieved, if the pollens are unable to reach the stigma of the pistillate flower within  $2\sim3$  days after being shed.

A preservation method with low temperature and high humidity seems to be better for the maintenance of the pollen viability.

GRIGGS *et al.* (1971), however, stated that through the results of the pollination test on Persian walnut, laboratory test for the pollen viability was unreliable for indicating the ability of the walnut pollens to effect fertilization in the orchard.

The author feels the necessity to reconfirm the pollen viability by the pollination test.

# Summary

The author studied on the fructification of so-called Shinano walnut, which has been cultivated mainly in Japan. It is said as the hybrid between the ancient variety (*J. regia* L. var. *orientis* Kita.) and some varieties of Persian walnut (*J. regia* L.).

In the present paper, morphology and ecology of the staminate flower and the pollen grains of Shinano walnut were dealt with.

The results are described as follow :

1. The length of the catkin varies  $7\sim 14$ cm (average 10.2  $\pm$  0.30cm). It is near to that of Persian walnut and as long as half of that of Japanese walnut (J. *sieboldiana* Maxim.).

The number of the staminate flower grown around the axis of the catkin varies  $90 \sim 140$  (average 112.2  $\pm$  1.62), and seems a little smaller than that of Persian walnut, standing at about half value as compared with that of Japanese walnut.

2. The structure and the growth of the flower and the anther are investigated in detail.

The number of the anther per flower varies  $9\sim22$  (average 15.0  $\pm$  0.39) in regard to 15 instances, and it decreases toward the flower at the distal positiont of the catkin.

3. The author has discovered that the pollen shedding takes place when the dryness falls into less than about 70% (R. H.), while not under the wetness of more than 80% (R. H.).

4. The pollen number per anther varies according to the flower position (base, midde, top) of catkin. It is averaged as 2,130, by which the number of the pollen grains produced in a single catkin can be calculated as 2,700,  $000 \sim 4,200,000$ .

5. The pollen grains belong to a porous -formaminate type with reticulum pattern in the surface, showing a polygon in polar view, and are suboblate in equaterial view. The pores are  $18 \sim 20$  in number.

The pollen size varies 41.6  $\sim$  48.9 $\mu$  (average 43.9  $\pm$  0.29  $\sim$  46.5  $\pm$  0.27 $\mu$ ). It is as similar as that of Persian walnut but larger than that of Japanese walnut (39.4  $\sim$  41.0).

6. The pollen dispersion is brought by wind. The distribution number of the pollens is  $11 \sim 54 \ (1 \text{cm}^2/\text{day})$  at a distance of 60m from the shedding tree, while  $4 \sim 20$  at 100m. The longer the distance extends the smaller the pollen number becomes.

It can be estimated that a range where the pollination is effectively

achieved exists within 0  $\sim$  60m, though there is a possibility of pollination from 60 to 100m.

7. The viability of the pollens was examined by the germinating test on artificial media. The germination percentage of the fresh pollens is  $45 \sim$ 80% (average 63.3%) in six varieties.

It is recognized that the pollen viablity is able to continue only for 2 days under high temperature  $(20 \sim 22^{\circ}C)$  with low humidity (about 55% R. H.), while about for 20 days under low temperature (5°C) with high humidity (about 80% R. H.).

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- 1. Hanging catkin
- 2. Young catkin (left) and matured catkin (right)
- 3. Cross ection of young catkin
- 4. Longitudinal section of young catkin
- 5. Downwardly overapped flower
- 6. Calyx-like-circle of flower
- 7. Dorsal and ventral view of flower

Plate I.



- 1. Front and side view of anther
- 2. 3. Greenish milky color anther (left) and brown color anther (right)
- 4.
- Beginning of burst of anther groove Anther finished pollen shedding Polar view of pollen of Shinano walnut 5.
- Equatorial view of pollen of Shinano walnut 6.
- 7. 8.
- Polar view of pollen of Japanese walnut Equatorial view of pollen of Japanese walnut