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# ON THE VIABILITY OF FOREST TREE POLLEN

By

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## 林木の花粉の生存期間について

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### CONTENTS

Introduction .....	967
Materials and Methods .....	968
Experimental Results .....	969
Discussion .....	977
Conclusion .....	978
References .....	979
要 約 .....	980

### Introduction

Owing to long distance transportation of pollen or the difference of efflorescence time, the cross-fertilization of forest trees often becomes almost impossible for nearly one year. So it is important to prolong the viability of forest tree pollen for at least one year or a little longer.

PFUNDT<sup>9)</sup> investigated the effect of humidity upon the viability of pollen and optimum sucrose concentration for pollen culture with a large number of species. He reported that the optimum sucrose concentration for pollen culture varied widely from species to species and that it was from 10 to 20% for *Alnus glutinosa* and *Betula verrucosa*. He also found that over H<sub>2</sub>SO<sub>4</sub>, the pollen of *B. verrucosa* had remained alive for 53 days. NOHARA<sup>8)</sup> successfully stored willow pollen in desiccator with CaCl<sub>2</sub> in dark place for 73 days longer than in light place. DUFFIELD and SNOW<sup>7)</sup> investigated the effect of temperature and humidity upon the viability of pollen and reported that at a relative humidity of 50% and at temperature of 0° to 4°C. the pollen of *Pinus strobus* and *P. resinosa* was still

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more than 80% germinable after a 1-year-long storage with no significant differences between the pollen of the two species. DUFFIELD<sup>9</sup> emphasized the importance of extraction humidity on germinability and storage of pollen.

The present writers<sup>11</sup> have already reported that in sealed bottle with 2 g K<sub>2</sub>S or in vacuated glass tube at temperature of  $-8^{\circ}\text{C}$ . the pollen of *Salix Bakko* had been successfully stored for more than one year. This paper reports on experiments conducted for the purpose of prolonging the viability of forest tree pollens. Optimum temperature and optimum H-ion concentration for the germination of pollen are also reported in this paper.

### Materials and Methods

The pollen of four tree species was used in this study.

Branches bearing male flowers of these tree species were collected and were put in Erlenmeyer flasks filled with water in glass house. The shed pollen grains were assembled on paper spread under the flasks. The date of collecting pollen was on April 19 to 21, 1953 in the case of *Alnus tinctoria*, on May 8 in *Betula japonica*, on May 24 in *B. Maximowicziana* and on May 14 in *B. Ermani*. Each of these tree pollens was made pure by a 150 mesh sieve and was stored in the following four ways;

1. in desiccator (inside diameter; 10 cm) with CaCl<sub>2</sub> in ordinary room (normally fluctuating room temperature;  $-5^{\circ}\sim 25^{\circ}\text{C}$ ).
2. in sealed bottles (capacity; 100 cc) with 10 g adsol, with 2 g K<sub>2</sub>S or with 10 g adsol and 2 g K<sub>2</sub>S in ordinary room or in fruit storage room ( $3^{\circ}\sim 5^{\circ}\text{C}$ ).
3. in sealed bottles with various amounts of adsol, with various amounts of K<sub>2</sub>S or with various amounts of adsol and 2 g K<sub>2</sub>S in low temperature room ( $-8^{\circ}\text{C}$ ).
4. in sealed bottles without a desiccating agent or in vacuated glass tubes (inside diameter; 4 mm) in various temperature rooms.

Pollen samples were germinated on aqueous gel of 2% agar, cut into pieces about 1 mm thick and 7 mm square. The sucrose concentration of agar gel used in this study was 10% for the pollen of *B. Ermani* and 15% for the pollen of the other three tree species. Each of these sucrose concentration was found, in tests, to promote optimum germination of pollen of each tree species. The 23 agar gel pieces required for germination test of each tree species were arranged on two slide glasses and the slide glasses were placed on glass-support in a Petri dish in which the air was saturated with water vapour by the addition

of distilled water at the bottom of the dish. These dishes were placed in thermostat of 20°C. for 24 hours and the germination percentage on each agar gel piece was then estimated by counting samples of about 300 grains. The pollen grains of which pollen tube had developed normally were regarded as a germinated one.

### Experimental Results

When pollen samples of these tree species were germinated in hanging drop medium containing sucrose from 1 to 10%, the sucrose concentration of 10% was found to be the best for germination of pollen of all tree species in all cases. In the hanging drop method, however, the pollen of these tree species gathered in the center of the culture medium and showed low germination percentage. Results obtained by the use of agar gel method are shown in Table 1.

Table 1. Percentages of germination of pollen stored in sealed bottle with 20g adsol and 2g K<sub>2</sub>S in low temperature room in relation to sucrose concentration.

Species	Date of germination test	Days stored	Sucrose concentration (%)			
			10	15	20	25
<i>A. tinctoria</i>	June 24	64	34	37	18	2
<i>B. japonica</i>	June 11	34	15	35	27	5
<i>B. Maximowicziana</i>	June 24	31	47	59	50	20
<i>B. Ermani</i>	June 24	41	51	41	24	5

In order to test the effect of temperature on the germination of pollen, pollen samples were cultured at 20°C., 25°C. and 32°C. for 24 hours. The results are shown in Table 2.

Table 2. Percentages of germination of pollen stored in low temperature room in relation to culture temperature. (Date of germination test; March 9, 1954)

Species	Condition of storage	Days stored	Culture temperature		
			20 C.	25 C.	32°C.
<i>A. tinctoria</i>	With 20g adsol and 2g K <sub>2</sub> S Vacuum	322	24	6	9
		322	40	19	17
<i>B. japonica</i>	With 20g adsol and 2g K <sub>2</sub> S Vacuum	305	4	1	0
		305	18	8	6
<i>B. Maximowicziana</i>	With 20g adsol and 2g K <sub>2</sub> S Vacuum	289	25	18	14
		289	27	17	6
<i>B. Ermani</i>	With 20g adsol and 2g K <sub>2</sub> S Vacuum	299	12	4	2
		299	0	0	0

These results clearly indicate that optimum temperature for pollen culture was 20°C. in all tree species studied. A large number of pollen grains of all these tree species cultured at 32°C. developed much shorter and much thicker pollen tubes than pollen did when cultured at 20°C. A few pollen grains cultured at 25°C. had this abnormal pollen tube.

In order to test the effect of H-ion concentration on the germination, pollen samples were germinated on agar gel pieces of several H-ion concentrations, which were made by the use of 1/10 normal  $H_3PO_4$ , 1/50 mol.  $KH_2PO_4$  and  $Na_2HPO_4$ . The sucrose concentration of these agar gel pieces was 10% for the pollen of *B. Ermani* and 15% for the pollen of the other three tree species.

Table 3. Percentages of germination of pollen stored in low temperature room in relation to H-ion concentration. (Date of germination test; March 8 in *B. Ermani* and March 4, 1954 in the other three tree species)

Species	Condition of storage	Days stored	H-ion concentration of agar medium					
			6.2*	4.0	5.1	5.7	6.8	7.6
<i>A. tinctoria</i>	With 20g adsol and 2g $K_2S$	317	18	20	28	30	14	2
	Vacuum	317	34	40	31	37	25	6
<i>B. japonica</i>	With 20g adsol and 2g $K_2S$	300	7	7	18	12	4	0
	Vacuum	300	20	13	25	13	10	2
<i>B. Maximowicziana</i>	With 20g adsol and 2g $K_2S$	284	30	26	25	32	11	1
	Vacuum	284	19	16	24	27	12	2
Species	Condition of storage	Days stored	H-ion concentration of agar medium					
			6.2*	4.1	5.1	5.8	6.9	7.5
<i>B. Ermani</i>	With 20g adsol and 2g $K_2S$	298	9	7	6	6	2	0
	Vacuum	298	0	0	0	0	0	0

\* These agar media had not been treated with any  $H_3PO_4$ ,  $KH_2PO_4$  and  $Na_2HPO_4$ .

Table 3 indicates which H-ion concentration is favourable to promote optimum germination of pollen of these tree species. The pollen of *A. tinctoria* stored in sealed bottle with 20 g adsol and 2 g  $K_2S$  showed maximum germination percentage at pH 5.7 and in the storage under vacuum condition, the pollen of *A. tinctoria* showed two maxima germination percentages at pH 4.0 and at pH 5.7 with no significant difference between the two. The pollen of *B. japonica* whether stored in sealed bottle with 20 g adsol and 2 g  $K_2S$  or stored in vacuated glass tube showed maximum germination percentage at pH 5.1. In the case of *B. Maximowicziana*, optimum H-ion concentration for the germination of pollen was pH 5.7. The pollen of *B. Ermani* stored in sealed bottle with

20 g adsol and 2 g  $K_2S$  showed maximum germination percentage on the agar medium of pH 4.1. But this germination percentage is lower than 9% on the agar medium of pH 6.2 which had not been treated with any  $H_3PO_4$ ,  $KH_2PO_4$  and  $Na_2HPO_4$ . H-ion concentration above pH 7 exerted a harmful effect upon the germination of all the pollen studied.

### 1. On the storage of pollen of *A. tinctoria*.

How long the pollen of *A. tinctoria* has been stored successfully under various conditions in ordinary room or in fruit storage room is shown in Table 4.

Table 4. Percentages of germination of pollen of *A. tinctoria* stored under various conditions in ordinary room and in fruit storage room.

Date of germination test	Days stored	Ordinary room ( $-5^{\circ} \sim 25^{\circ}C.$ )							Fruit storage room ( $3^{\circ} \sim 5^{\circ}C.$ )				
		In unsealed bottle	In sealed bottle	In desiccator filled with $CaCl_2$	In vacuated glass tube	In sealed bottle with			In sealed bottle	In vacuated glass tube	In sealed bottle with		
						10 g adsol	10 g adsol and 2 g $K_2S$	2 g $K_2S$			10 g adsol	10 g adsol and 2 g $K_2S$	2 g $K_2S$
1953, 7, 2	72	0	0	0	25	0	2	0	21	34	10	3	0
7, 27	97	0	0	0	18	0	4	0	12	35	7	13	0
8, 24	125		0	0	6	0	0	0	0	44	0	11	0
9, 14	146				3		0		0	36	0	2	
10, 6	168				0					40		0	
11, 1	194				0					32		0	
12, 9	232									35			
1954, 2, 5	290									26			
3, 9	322									29			
3, 31	344									30			
7, 7	442									21			
8, 25	491									27			
11, 11	569									24			
12, 17	605									21			
1955, 1, 31	650									33			

Pollen stored in vacuated glass tube in ordinary room maintained its germinability for 146 days. The germination percentage of pollen stored in sealed bottle with 10 g adsol and 2 g  $K_2S$  in ordinary room was 4% after the storage of 97 days. Pollen stored under other five conditions in ordinary room lost its germinability by July 2, 1953, that is, after 72 days from the date of storage.

In fruit storage room, the pollen of *A. tinctoria* maintained its germinability longer than in ordinary room. Pollen stored in vacuated

glass tube in fruit storage room showed the germination percentage of 33% after the storage of 650 days and no significant difference is recognized among the germination percentages of each date. Pollen stored with 2 g  $K_2S$  only in fruit storage room lost its germinability after 72 days from the date of storage.

Table 5 shows the results of storing pollen in low temperature room. In low temperature room, pollen stored in sealed bottle was successfully kept for 194 days after storage. Pollen stored in sealed bottle with adsol only, regardless of the amounts of adsol, maintained its germinability for 290 days. The viability of pollen stored in sealed bottle with various amounts of adsol and 2 g  $K_2S$  is closely related to the amounts of adsol used.

Table 5. Percentages of germination of pollen of *A. tinctoria* stored under various conditions in low temperature room ( $-8^{\circ}C$ ).

Date of germination test	Days stored	In sealed bottle	In vacuated glass tube	In sealed bottle with								
				5 g adsol	10 g adsol	20 g adsol	5 g adsol and 2 g $K_2S$	10 g adsol and 2 g $K_2S$	20 g adsol and 2 g $K_2S$	1 g $K_2S$	2 g $K_2S$	4 g $K_2S$
1953, 7, 2	72	30	29	40	41	45	16	14	41	0	0	0
7, 27	97	23	44	30	36	38	25	32	38	20	3	1
8, 24	125	50	45	55	44	49	23	22	32	13	0	0
9, 14	146	27	48	42	45	42	3	10	38	2	0	0
10, 6	168	30	35	50	49	45	0	1	37	0		
11, 1	194	23	20	44	40	42	0	0	26	0		
12, 9	232*	0	38	14	16	13	0	3	28	0		
1954, 2, 5	290	0	42	3	7	1		0	23			
3, 9	322	0	40	0	0	0		0	24			
3, 31	344		22	0	0	0			30			
7, 7	442		38						25			
8, 25	491		41						14			
11, 11	569		45						11			
12, 17	605		43						2			
1955, 1, 31	650		42									

Pollen stored in sealed bottle with 20 g adsol and 2 g  $K_2S$  had been successfully stored for 605 days when pollen samples had been completely employed. But the viability of pollen stored in sealed bottle with 5 g adsol and 2 g  $K_2S$  was only 146 days shorter than in sealed bottle without a desiccating agent. Pollen stored in vacuated glass tube in low temperature room showed high germination percentages above 40% after the storage of 650 days. The use of  $K_2S$  alone

exerted harmful effects upon the viability of pollen.

## 2. On the storage of pollen of *B. japonica*.

How long the pollen of *B. japonica* has been stored successfully under various conditions in ordinary room or in fruit storage room is shown in Table 6.

Table 6. Percentages of germination of pollen of *B. japonica* stored under various conditions in ordinary room and in fruit storage room.

Date of germination test	Days stored	Ordinary room (-5°~25°C.)							Fruit storage room (3°~5°C)				
		In unsealed bottle	In sealed bottle	In desiccator filled with CaCl <sub>2</sub>	In vacuated glass tube	In sealed bottle with			In sealed bottle	In vacuated glass tube	In sealed bottle with		
						10 g adsol	10 g adsol and 2 g K <sub>2</sub> S	2 g K <sub>2</sub> S			10 g adsol	10 g adsol and 2 g K <sub>2</sub> S	2 g K <sub>2</sub> S
1953, 6, 24	47	0	1	1	16	0	3	0	1	27	2	2	0
7, 21	74	0	0	0	20	0	6	0	1	29	1	12	1
8, 22	106		0	0	8	0	0	0	0	30	0	1	0
9, 16	131				2		0		0	17	0	0	0
10, 11	156				0					12		1	
11, 1	177				0					7		0	
12, 9	215									1		0	
1954 2, 5	273									15			
3, 9	305									13			
3, 31	327									12			
7, 7	425									12			
8, 25	474									11			
11, 11	552									15			
12, 17	588									1			
1955, 1, 31	633									4			

Pollen stored in unsealed bottle, in sealed bottle with 10 g adsol or in sealed bottle with 2 g K<sub>2</sub>S in ordinary room lost its germinability after 47 days from the date of storage. Pollen stored in vacuated bottle in ordinary room had been successfully stored for 131 days.

In fruit storage room, pollen stored in vacuated glass tube has been successfully stored for 633 days, but its germination percentage becomes lower in relation to the days of storage. Pollen stored in sealed bottle with 10 g adsol and 2 g K<sub>2</sub>S showed the germination percentage of 1% after the storage of 156 days.

Table 7 shows the results of storing pollen in low temperature room.

Regardless of the amounts of adsol, pollen stored with adsol in low temperature room maintained its germinability for 156 days after the

Table 7. Percentages of germination of pollen of *B. japonica* stored under various conditions in low temperature room (-8 C.).

Date of germination test	Days stored	In sealed bottle	In vacuated glass tube	In sealed bottle with								
				5 g adsol	10 g adsol	20 g adsol	5 g adsol and 2 g K <sub>2</sub> S	10 g adsol and 2 g K <sub>2</sub> S	20 g adsol and 2 g K <sub>2</sub> S	1 g K <sub>2</sub> S	2 g K <sub>2</sub> S	4 g K <sub>2</sub> S
1953, 6, 24	47	43	38	48	51	58	14	34	51	1	1	0
7, 21	74	43	44	44	47	50	14	31	45	1	1	0
8, 22	106	36	29	41	33	36	11	22	26	0	0	0
9, 16	131	32	21	32	30	36	3	17	20	0	0	
10, 11	156	17	15	21	15	19	4	8	18			
11, 1	177	0	10	0	0	0	2	5	11			
12, 9	215	0	10	0	0	0	2	2	4			
1954, 2, 5	273	0	13	0	0	0	0	0	6			
3, 9	305		18				0	0	4			
3, 31	327		10				0	0	2			
7, 7	425		16						5			
8, 25	474		11						0			
11, 11	552		7						0			
12, 17	588		3						0			
1955, 1, 31	633		11									

date of storage and no effect of adsol upon the viability of pollen of *B. japonica* was recognized. Pollen in vacuated glass tube in low temperature room has been successfully stored for 633 days. Pollen stored in sealed bottle with 20 g adsol and 2 g K<sub>2</sub>S maintained its germinability for more than one year. But in the case of storing pollen with 10 g adsol and 2 g K<sub>2</sub>S or with 5 g adsol and 2 g K<sub>2</sub>S, the viability of pollen was only 215 days. Regardless of the amounts of K<sub>2</sub>S, pollen stored in sealed bottle with K<sub>2</sub>S only lost its germinability much earlier than pollen stored in sealed bottle.

### 3. On the storage of pollen of *B. Maximowicziana*.

Table 8 indicates that vacuum storage is favourable for prolonging the viability of pollen of *B. Maximowicziana*. Pollen stored in vacuated glass tube in fruit storage room has been successfully kept viability for 617 days and is still continued to store. Pollen stored in sealed bottle in fruit storage room maintained its germinability longer than pollen stored in sealed bottle with 10 g adsol.

Table 9 shows the results of storing pollen in low temperature room.

Both pollen stored in vacuated glass tube and in sealed bottle with

Table 8. Percentages of germination of pollen of *B. Maximowicziana* stored under various conditions in ordinary room and in fruit storage room.

Date of germination test	Days stored	Ordinary room (-5~25°C.)							Fruit storage room (3°~5°C.)					
		In unsealed bottle	In sealed bottle	In desiccator filled with CaCl <sub>2</sub>	In vacuated glass tube	In sealed bottle with			In sealed bottle	In vacuated glass tube	In sealed bottle with			
						10 g adsol	10 g adsol and 2 g K <sub>2</sub> S	2 g K <sub>2</sub> S			10 g adsol	10 g adsol and 2 g K <sub>2</sub> S	2 g K <sub>2</sub> S	
1953, 7, 8	45	0	0	0	31	0	9	0	37	40	44	4	0	
7, 30	67	0	0	0	22	0	1	1	39	43	38	24	0	
8, 27	95		0	0	23	0	0	0	36	47	0	5	0	
9, 21	120				20		0	0	28	40	0	1		
11, 1	161				5				0	14	0	0		
12, 9	199				0				0	3		0		
1954, 2, 5	257				0					16				
3, 9	289									2				
3, 31	311									13				
7, 7	409									15				
8, 25	458									12				
11, 11	536									16				
12, 17	572									28				
1955, 1, 31	617									6				

Table 9. Percentages of germination of pollen of *B. Maximowicziana* stored under various conditions in low temperature room (-8°C.).

Date of germination test	Days stored	In sealed bottle	In vacuated glass tube	In sealed bottle with								
				5 g adsol	10 g adsol	20 g adsol	5 g adsol and 2 g K <sub>2</sub> S	10 g adsol and 2 g K <sub>2</sub> S	20 g adsol and 2 g K <sub>2</sub> S	1 g K <sub>2</sub> S	2 g K <sub>2</sub> S	4 g K <sub>2</sub> S
1953, 7, 8	45	53	28	63	67	60	8	39	56	1	0	0
7, 30	67	50	54	60	55	48	22	50	55	11	1	0
8, 27	95	46	48	61	60	52	9	44	57	2	1	0
9, 21	120	25	51	52	57	55	14	39	58	5	1	
11, 1	161	0	5	21	26	16	0	1	25	0	0	
12, 9	199	0	15	0	0	0	1	15	39	1	0	
1954, 2, 5	257	0	19	0	0	0	0	7	31	2	0	
3, 9	289		29					6	25	0		
3, 31	311		17					16	33	0		
7, 7	409		40					17	21	0		
8, 25	458		34					14	2			
11, 11	536		45					12	0			
12, 17	572		7					9	0			
1955, 1, 31	617		10					11	0			

Table 10. Percentages of germination of pollen of *B. Ermani* stored under various conditions in ordinary room and in fruit storage room.

Date of germination test	Days stored	Ordinary room (-5°~25°C.)							Fruit storage room (3°~5°C.)				
		In unsealed bottle	In sealed bottle	In desiccator filled with CaCl <sub>2</sub>	In vacuated glass tube	In sealed bottle with			In sealed bottle	In vacuated glass tube	In sealed bottle with		
						10 g adsol	10 g adsol and 2 g K <sub>2</sub> S	2 g K <sub>2</sub> S			10 g adsol	10 g adsol and 2 g K <sub>2</sub> S	2 g K <sub>2</sub> S
1953, 7, 5	52	0	1	0	3	0	1	0	0	13	14	5	0
8, 1	79	0	0	0	3	0	1	0	0	15	11	3	0
8, 25	103		0	0	2	0	0	0	0	11	1	2	0
9, 24	133				1		0			1	0	0	
11, 1	171				1					5	0	1	
12, 9	209				0					2		0	
1954, 2, 5	267				0					0		0	
3, 9	299									0		0	
3, 31	321									2			
7, 7	419									3			
8, 25	468									0			
11, 11	546									3			
12, 17	582									1			
1955, 1, 31	627									5			

Table 11. Percentages of germination of pollen of *B. Ermani* stored under various conditions in low temperature room (-8°C.).

Date of germination test	Days stored	In sealed bottle	In vacuated glass tube	In sealed bottle with								
				5 g adsol	10 g adsol	20 g adsol	5 g adsol and 2 g K <sub>2</sub> S	10 g adsol and 2 g K <sub>2</sub> S	20 g adsol and 2 g K <sub>2</sub> S	1 g K <sub>2</sub> S	2 g K <sub>2</sub> S	4 g K <sub>2</sub> S
1953, 7, 5	52	57	10	58	66	61	6	35	50	0	0	0
8, 1	79	39	19	45	50	49	6	27	44	0	1	0
8, 25	103	0	12	40	40	39	2	19	42	0	0	0
9, 24	133	0	8	27	40	32	0	9	31		0	
11, 1	171	0	1	18	31	21	0	0	20		0	
12, 9	209		1	3	4	6	0	2	8			
1954, 2, 5	267		6	1	3	1		0	8			
3, 9	299		0	0	0	0		0	12			
3, 31	321		2	0	0	0		0	8			
7, 7	419		4	0	0	0			3			
8, 25	468		5						7			
11, 11	546		7						2			
12, 17	582		1						2			
1955, 1, 31	627		2						1			

10 g adsol and 2 g  $K_2S$  have successfully kept viability for 617 days. In the pollen of *B. Maximowicziana*, storage with 20 g adsol and 2 g  $K_2S$  is less favourable for prolonging the viability of pollen than storage with 10 g adsol and 2 g  $K_2S$ . Regardless of the amounts of adsol, the viability of pollen stored in sealed bottle with adsol was 161 days. Pollen stored in sealed bottle with 1 g  $K_2S$  maintained its germinability for 256 days, though its germination percentage was always very low.

#### 4. On the storage of pollen of *B. Ermani*.

Pollen stored in vacuated glass tube in fruit storage room maintained its germinability for 627 days, but its germination percentage was very low at all times. Even if the pollen of *B. Ermani* had been stored in fruit storage room, pollen in sealed bottle lost its germinability after 52 days from the date of storage.

Table 11 indicates that storage in sealed bottle with 20 g adsol and 2 g  $K_2S$  is more favourable for prolonging the viability of pollen than storage in vacuated glass tube, though pollen under both conditions has been successfully stored for 627 days. The use of  $K_2S$  alone, exerted harmful effects upon the viability of pollen of *B. Ermani*. Regardless of the amounts of adsol, the viability of pollen stored in sealed bottle with adsol only was 267 days. This is more than three times as long as that of pollen stored in sealed bottle without a desiccating agent.

### Discussion

All the pollens of these tree species cultured by hanging drop method showed bad germination percentage in all cases. By hanging drop method, all the pollens of these tree species gathered in the center of the convex surface of culture medium because of their high specific gravity. This condition seems to exert harmful effects upon the germination of pollen.

As shown in Table 2, all the pollens cultured at high temperature showed bad germination percentage. The pollen cultured at high temperature developed much shorter and much thicker pollen tube than pollen did when cultured at low temperature. Even if the pollen which developed this abnormal pollen tube is regarded as a germinated one, the germination percentage of pollen cultured at 25°C. or at 32°C. is lower than that of pollen cultured at 20°C. These results clearly indicate that high temperature is unfavourable for the germination of pollen of these four tree species.

As shown in Table 3, optimum H-ion concentration for the germination of pollen was pH 5.7 in *A. tinctoria* and *B. Maximowicziana* and more

to the acid side in *B. japonica* and *B. Ermani* than in the former two species. The differences of optimum H-ion concentration for germination of pollen among these tree species are closely related to the differences of optimum H-ion concentration for growth of these trees.

The results of storage experiment indicate that low temperature of  $-8^{\circ}\text{C}$ . is the most favourable for prolonging the viability of pollen of these four tree species studied. All the pollens of these tree species stored in vacuated glass tube in low temperature room and in fruit storage room have been successfully stored for more than 600 days and are still continued to store. It is important that in fruit storage room ( $3^{\circ}\sim 5^{\circ}\text{C}$ .) pollen has maintained its germinability for long time, because such temperature as was kept in the fruit storage room can be easily obtained artificially.

Potassium sulphide is a deliquescent substance producing hydrogen sulphide which operates as a sterilizer by absorbing moisture in the air. Because of this operation as a sterilizer and other physiological operations such as control of respiration, which have been not yet determined, the existance of hydrogen sulphide more than optimum amounts seems to exert harmful effects upon the viability of all the pollens studied. Among the pollen of these four tree species, that of *B. Maximowicziana* was the most resistant to hydrogen sulphide. In the cases of storing pollen in sealed bottle with various amounts of adsol and constant amounts of  $\text{K}_2\text{S}$ , the larger the amounts of adsol were, the less hydrogen sulphide seemed to be produced. The optimum amounts of hydrogen sulphide for prolonging the viability of pollen are considered to vary from species to species and to be maintained when pollen is stored in sealed bottle with 10g adsol and 2g  $\text{K}_2\text{S}$  in low temperature room in *B. Maximowicziana* and with 20g adsol and 2g  $\text{K}_2\text{S}$  in the other three species.

### Conclusion

The principal conclusions which can be drawn from these studies are as follows.

1. Optimum sucrose concentration for the germination of pollen was 10% in *B. Ermani* and 15% in the other three tree species.
2. Optimum temperature for the germination of pollen was  $20^{\circ}\text{C}$ . in all the tree species studied.
3. Optimum H-ion concentration for the germination of pollen was pH 5.7 in *A. tinctoria* and *B. Maximowicziana* but more to the acid side in *B. japonica* and *B. Ermani* than in the former two species.
4. A low temperature of  $-8^{\circ}\text{C}$ . was found to be the most favourable

- for prolonging the viability of all the tree pollens studied.
5. The viability of all the tree pollens stored in vacuated glass tube was prolonged.
  6. The effects of adsol upon the viability of pollen can be recognized in the pollen of three of the tree species studied. The exception is *B. japonica*.
  7. The pollen of *B. Maximowicziana* stored in sealed bottle with 10 g adsol and 2 g  $K_2S$  and the pollen of the other three tree species stored in sealed bottle with 20 g adsol and 2 g  $K_2S$  have been successfully stored for more than 600 days. In the experiments of storing pollen with various amounts of adsol and constant amounts of  $K_2S$ , the viability of pollen varies widely with the amounts of adsol.
  8. The use of potassium sulphide alone is harmful to the viability of all the tree pollens studied.

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## 要 約

## 林木の花粉の生存期間について

本研究にはヤマハンノキ、シラカンバ、ウダイカンバ、エゾノダケカンバの4樹種の花粉を用いた。雄花をつけた枝を採集し、これを水に挿して培養し、花粉を採集した。この花粉を真空状態の硝子管及び異なる量、異なる種類の薬品を入れた硝子瓶に貯藏し、低温温室、果実貯藏室、室内の3箇所にて貯藏した。

花粉の培養は寒天培養法によつて行つた。

実験結果は概要次の如くである。

1. 花粉の発芽に最も適当な蔗糖濃度はエゾノダケカンバの花粉では10%、他の3樹種の花粉では15%である。
2. 花粉の発芽に最も適当な培養温度はすべての花粉とも20°C.である。
3. 花粉の発芽に最も適当な水素イオン濃度はヤマハンノキ、ウダイカンバの花粉ではpH5.7、シラカンバ、エゾノダケカンバの花粉では前2樹種の花粉より多少酸性のほうが適當である。
4. -8°C.の低温は花粉の生命を長びかすうえに有効である。
5. 真空貯藏は花粉の生命を長びかすうえに最も有効な方法である。
6. シラカンバ以外の他の3樹種の花粉ではアドソールの花粉の生命維持に及ぼす有効な影響を認めることが出来る。
7. アドソール10gと硫化加里2gと共に貯藏したウダイカンバの花粉は長期間その生命を維持している。他の3樹種の花粉ではアドソール20gと硫化加里2gと共に貯藏するほうが有効である。アドソールと硫化加里を同時に使用する場合は、アドソールの量の多少によつて花粉の生命維持期間が著しく違つてくる。
8. 硫化加里の単用は本研究に使用した花粉の生命にたいして、極めて有害な作用を及ぼす。

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