Ecological Studies on *Guignardia laricina* (SAWADA) W. YAMAMOTO et K. ITO, the Causal Fungus of the Shoot Blight of Larch Trees, and Climatic Factors Influencing the Outbreak of the Disease.

by

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Contents

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Introduction

In Hokkaido, the northern island in Japan, larch plantations have been established for several decades, most of which were started for shelter forests for railroads or for agricultural fields. Since 1958, the project of increasing afforestation of larch has been advanced intensively, aiming at the restoration of forest denudation caused by the attack of the tropical low atmospheric pressure and typhoon No. 19 in 1954. The area of larch stands has increased progressively and up to 1963, the total area of the plantations was said to amount to about 350,000 ha.

In these plantations, such diseases as *Mycosphaerella* needle cast and *Armillaria* root rot are generally increasing with the increase of the area of the plantations. Further, the damage by the shoot blight is prevailing in many localities in Hokkaido, and it is said that the disease may be one of the important factors determining the success or failure of the project.

Infected larch trees are seldom killed by the disease. This is due to the fact that the causal fungus attacks only the current season's shoots and not the shoots grown up to the previous season. But if a larch keeps on being attacked by the disease every year, the growth of tree height is disturbed and the values for the larch stands will be lost completely.

Shoot blight disease is caused by the attack of *Guignardia laricina* (Sawada) W. Yamamoto et K. Ito*, a fungus indigenous to Japan. The spores of the causal fungus exist through the whole period of growing season of larch and the pathogenicity is very strong. According to these particular characteristics of the causal fungus, the methods of control had been quite difficult and the damage of the plantations spread rapidly, the area of which amounted to about 63,000 ha or more within 5 years.

Studies on the disease have been carried out extensively in various special fields and much information has been published. Nevertheless, the methods of control in damaged plantations are still obscure, though the controlling method in forest nurseries by using a special fungicide has become of practical use, and it seems necessary to proceed with the basic studies on the ecology of the causal fungus, the environmental conditions concerning the outbreak of the disease, and so on.

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* A disease, killing the top of shoots of larch, had already been known in Hokkaido since 1943, to which the name "Sakigare" (shoot blight) had been applied. The late Mr. K. Sawada[58] (1950) described for the first time the causal fungus, collected at many nurseries in the Tohoku district in 1949, as *Physalospora laricina* Sawada. Afterwards, Yamamoto[59] (1961) proposed to emend the scientific name of the causal fungus, based on the fact that the imperfect stage is *Macrophoma* sp., as *Guignardia laricina* (Sawada) W. Yamamoto et K. Ito. At present, it has been used for the scientific name of the causal fungus.

Since 1959 the writer has studied the disease and reported on the following aspects of the disease ; morphological, physiological and ecological studies of the causal fungus, and analysis of climatic factors influencing the outbreak of the disease and the methods of control with fungicides (YOKOTA[60)61)62)63)64)66)67)68)69)71)], 1960, 1961-a, b, c, d, 1962-a, b, 1963-a, b, 1964; YOKOTA and INOUE[65)], 1961; YOKOTA, ONO and ENDO[70)72)], 1963, 1964-a; YOKOTA, TSURUTA and SUZUKI[73)], 1964-b; ENDO and YOKOTA[10)11)], 1963-a, b). In the present paper, the writer defined the problems on the ecology of the causal fungus and the environmental factors closely related to the outbreak of the disease, and wanted to determine the considerations which would give the basis of the methods for the control of or escape from the disease.

The writer wishes to express his hearty thanks to Professor Taizo INOKUMA of Tokyo University for the kindly encouragement and criticism. He also greatly appreciates and hereby acknowledges the helpful suggestions and the revision of the manuscript by Dr. Kazuo ITO, Chief of Forest Pathology Section, Dr. Osamu CHIBA, Chief of the Laboratory of Forest Pathology, and Mr. Rokuya IMAZEKI, of the Govermnent Forest Experiment Station, and Mr. Kaoru ONO, Chief of the Laboratory of Forest Pathology, Hokkaido Branch of the Government Forest Experiment Station. He is indebted to many people concerned who readily facilitated the survy and the collection of experimental materials. Thanks are due to members of the Hokkaido Branch of the Government Forest Experiment Station, especially to Mr. Katsura INOUE, former Chief of Disaster Prevention Laboratory, Mr. Takeo TSURUTA, Mr. Takao SUZUKI of the laboratory, Mr. Masao SHIOZAKI of Soil Science Laboratory, Mr. Katsuaki ENDO, Mr. Sei-ichi MATSUZAKI and Mrs. Reiko NAKAGAWA of Laboratory of Forest Pathology for their help and cooperation throughout the studies.

Chapter 1. Distribution of the disease and present status of the damage.

1. Distribution of the disease.

Distribution of the disease in Hokkaido has been reported successively to date (YOKOTA[60)61)62)64)66)71)], 1960, 1961-a, b, d, 1962-a, 1964). The results are summarized as shown in Fig. 1. Areas of damaged plantations were rare up to 1958, though it was known that damage in small areas in larch plantations near the Japan Sea, the Uchiura Bay, and in the central part had occurred. In 1959, however, heavy damage inflicted by the disease suddenly appeared in Shiraoi, facing the Pacific Ocean, so a general survey of the damage was carried out all over Hokkaido. Since then, areas of the damaged plantations has increased year after year. Up to 1963, in the southern part of Hokkaido, almost all plantations were considered to

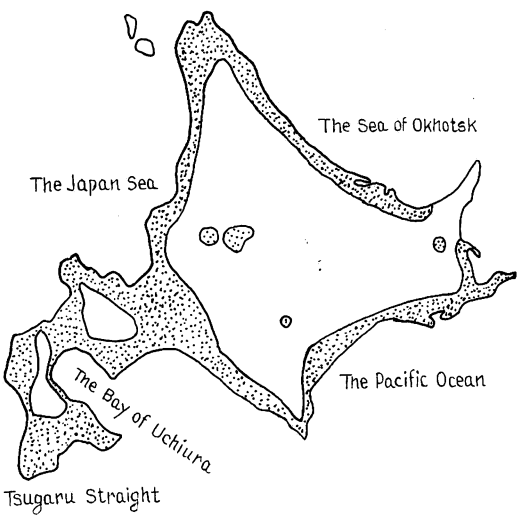


Fig. 1 Distribution of the disease in Hokkaido (dotted area).

be more or less damaged by the disease, and it was discovered that the damage was widely distributed mainly along the Japan Sea and the Pacific Ocean.

At present, the fact that the damage is spreading towards the inner part of Hokkaido (YOKOTA[66], 1962-a) and that the outbreak of the disease is found in Furano and Biei Town, at the foot of Mt. Tokachidake (OMURA[47], 1962), and in the vast plantations in the eastern part of Hokkaido (YOKOTA[71], 1964), should be borne in mind.

2. Damage in the plantations.

Since 1960, the area of damaged plantations is progressively increasing as shown in Tables 1 and 2, and their distribution is shown in Fig. 2. The increase of the area of damaged plantations is as follows: Up to Dec., 1960, 6,600 ha (YOKOTA[61], 1961-a), up to May, 1961, 13,000 ha (YOKOTA[64][66], 1961-d, 1962-a), up to Jan., 1962, 35,000 ha (YOKOTA[67], 1962-b). According to the results of the general survey carried out in 1962, total damaged areas up to Nov. 1962, amounted to 63,000 ha (YOKOTA[71], 1964), 18 percent of the total area of larch plantations in Hokkaido. Details of the damage are shown in Tables 1 and 2.

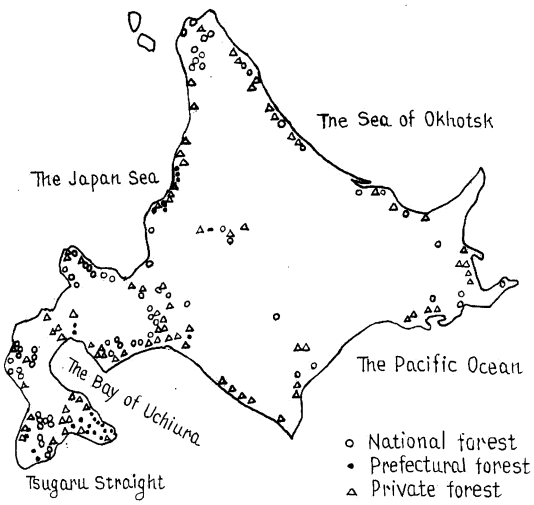


Fig. 2 Distribution of damaged larch plantations in Hokkaido.

Damaged areas in the southern part of Hokkaido, including Hakodate and Sapporo Regional Forestry Office national forests, prefectural forests and private forests belonging to Oshima, Hiyama, Iburi and Hidaka districts, have reached over 70 percent of the total of 63,000 ha. Heavily damaged plantations are situated mainly near the coast of the Tsugaru Straits, the Pacific Ocean, the Japan Sea and the

Table 1. Damaged area of larch plantations by the disease in Hokkaido, up to November, 1962. (ha)

Owner of forest	Regional forestry office	Damaged area	Area in each degree of damage		
			Heavy	Moderate	Slight
National	Sapporo	5,260	558	2,103	2,539
	Hakodate	3,227	721	1,382	1,124
	Asahigawa	590	160	50	380
	Kitami	278	22	72	184
	Obihiro	87	6	27	54
	Total	9,442	1,467	3,694	4,281
Prefectural		4,904	1,691	1,395	1,818
Private		48,866.17	15,390.23	18,419.74	15,056.20
Total		63,212.17	18,548.23	23,508.74	21,155.20

Table 2. Distribution of damaged private forest by the disease in Hokkaido, up to November 1962. (ha)

Branch office	Number of damaged plantation	Area in each degree of damage			Total
		Heavy	Moderate	Slight	
Ishikari	444	0	123.84	518.16	642.00
Sorachi	718	17.17	361.39	1,249.71	1,628.27
Kamikawa	9	45.50	42.85	124.42	212.77
Shiribeshi	374	181.58	743.92	570.20	1,495.70
Hiyama	5,857	2,740.76	2,854.27	1,001.44	6,596.47
Oshima	8,928	2,695.96	2,064.89	5,251.13	10,011.98
Iburi	9,261	2,289.74	7,713.65	3,747.06	13,750.45
Hidaka	7,992	3,624.47	2,623.24	1,185.02	7,432.73
Tokachi	817	189.73	278.44	382.29	850.46
Kushiro	318	12.32	352.86	158.22	523.40
Nemuro	88	8.84	12.69	167.99	189.52
Abashiri	121	10.27	94.49	65.55	170.31
Sôya	1,375	2,362.22	310.24	102.12	2,774.58
Rumoi	2,275	1,211.67	842.97	532.89	2,587.53
Total	38,577	15,390.23	18,419.74	15,056.20	48,866.17

Sôya Straits, where a relatively strong wind blows throughout the growing season of larch. The remainder of the damaged areas is scattered in the other parts of Hokkaido.

3. Damage in the nurseries.

In Hokkaido, many larch trees had hitherto been planted for the shelter forest or the shelter hedge in the forest nurseries, because of their fast growing character. These trees were damaged by the disease and became the source of infection to larch seedlings in the nurseries.

Relatively only a few records on the damage of the seedlings have been reported to date. It was reported that 400,000 seedlings were damaged by the disease at Shiraoi and 120,000～200,000 seedlings were damaged at Yuni nurseries in 1955 (Yokota[63], 1961-c). Uozumi[55] (1958) reported that 40,000 seedlings were found to be infected at Yuni in 1957. It seemed that the damage thus reported might be only a small part of the total damage.

Fig. 3 shows that the situation of the nurseries where the damage of the seedlings was found by the writer. As these nurseries are situated in the distributed area of the disease, it seems that the damage in the nurseries situated in the area might occur to a considerable extent. In fact, the Silvicultural Section, Hokkaido, reported that 1,160,000 seedlings, 6.3 percent of the total seedlings cultivated in 74 private nurseries were damaged by the disease in 1963 (Forestry Management Association, Hokkaido[12], 1964).

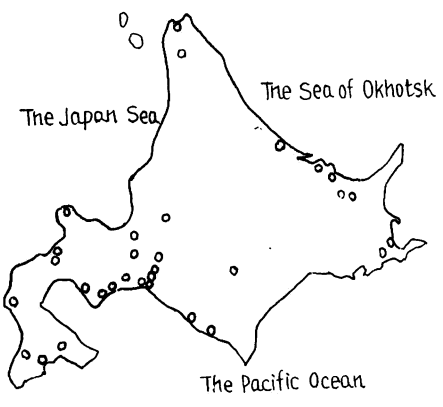


Fig. 3 Distribution of damaged nurseries in Hokkaido, recognized by the writer.

As mentioned above, the source of infection is mainly heavily infected larch shelter hedges in nurseries or shelter forests near nurseries (Plate 1, A). The writer, to determine the relationship between the damage and the distance from the heavily infected hedge, investigated the source of infection of the disease in the nursery in Tomakomai. As shown in Figs. 4 and 5, it became clear that the longer the distance from the hedge, the smaller the damage.

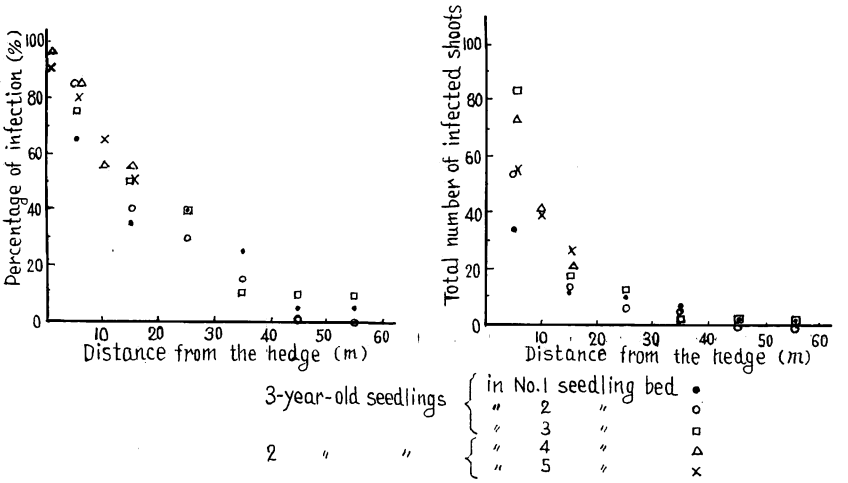


Fig. 4 The relation between percentage of infection and the distance from infected hedge.

Fig. 5 Total number of infected shoots in each distance (per 20 seedlings).

Chapter 2. Symptoms and signs of the disease, and life history of the causal fungus.

1. Symptoms.

The disease infects only the current season's shoots and leaves of adult trees and seedings, and the shoots grown till the previous year cease to be infected. If the disease continues to attack every year, many shoots grow from living parts of the shoots infected in the previous year, and they will be damaged again in the current season. Thus the growth of the tree is conspicuously retarded and many dead shoots remain on the trees, giving the tree the appearance of a broom (Plate 1, B).

The symptoms are divided into two types, dependent on the time of infection. One is the typical type hanging at the top of the infected shoots, and the other is the type remaining straight without hanging at the top.

In Hokkaido, the first symptoms appear hanging at the top of the shoots with the change of the color in the leaves from green to pale yellowish green. Then the leaves in the infected part of the shoots defoliate except at the top where dead brown leaves remain (Plate 1, C). Infected shoots soon die and dry, offering a conspicuous contrast with the healthy parts by consequent shrinkage of dead tissue (Plate 1, E). Generally, resin exudes from the diseased parts (Plate 1, D). When the disease becomes severe, the infection occurs not only at the top of the shoots and the small shoots but also at the middle parts of the shoots. Occasionally the disease attacks the leaves, on which relatively large brown spots appear, and the leaves soon defoliate.

Succulent small shoots are formed in the vigorously growing season and they are very susceptible to the disease. When a small shoot is infected, it is soon killed and the lesion sometimes progresses to the main shoot and enlarges upwards and downwards from the base of the dead small shoot (Plate 2, A). In heavily infected trees, almost all current season's shoots are infected, and healthy shoots are scarcely found (Plate 2, B).

These symptoms are seen from early July to the middle of September. Thereafter the infected shoots do not hang but remain straight. In this case, also, resin exudes frequently from an infected part of the shoot, and the dead tissue dries out and shrinks.

When infection occurs in a later season, the symptoms do not appear in the current year but appear after the buds begin to sprout in the following spring (Plate 2, C), though this type of symptom is rarely seen.

2. Signs.

From July, when the color of dead leaves remaining at the top of the curved shoots becomes purplish brown, small blackish poines appear on the under-surface of remaining leaves and the hung part of the infected shoots. These are the fruit bodies of *Macrophoma* sp. the imperfect stage of the causal fungus (Plate 2, D). Pycnidia can be found generally until late November.

Spermogonia appear from late July as small blackish points buried in the bark of the infected shoots. Often they are formed near prematured perithecia and they usually disappear by Decemeber.

In general, the perithecia of the causal fungus, *Guignardia laricina* begins to develop after November on the hung part or near the part of the shoots exuding resin, buried in the bark, solitarily or aggregately. As the perithecia begin to mature, the fungus extrudes ostiole from stromatic tissues on the bark (Plate 1, D). Sometimes the perithecia appear on the leaf scar where the black fungus tissue can be clearly seen.

3. Life history of the causal fungus.

Because the disease is propagated by spores, it is important to make clear the life history of the causal fungus in connection with its control methods. In regard to the life history of the causal fungus, some information has hitherto been reported (Uozumi[56], 1961; Sato[48], 1961; Yokota[66], 1962-a), but these reports lack the quantitative consideration of spores.

The writer collected infected shoots occasionally from various localities in Hokkaido between 1959 and 1963, and the maturity of perithecia and pycnidia was detemined. The appearance of spermogonia was also examined throughout the observation. Localities and collected date of the materials tested are shown in Fig. 6 and Table 3, respectively, where the numbers in the former apply to those in the latter.

These specimens were minutely examined and Fig. 7, regarding the time of maturity of fruit bodies of

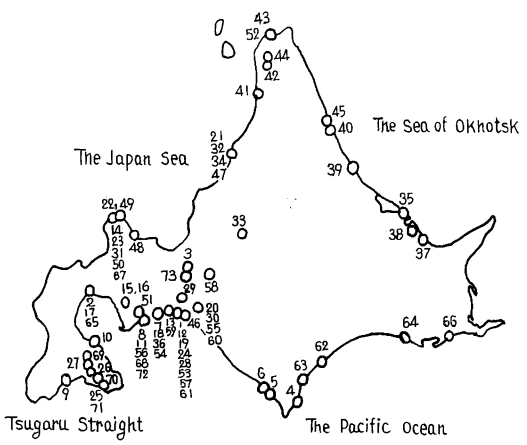


Fig. 6 Locality of collected materials for history of the fungus.

Table 3. Data of collected materials.

No.	Date	Locality	No.	Date	Locality	No.	Date	Locality
1	24/ I	Tomakomai	26	25/VII	Ônakayama	51	1/X	Noboribetsu
2	29/ II	Shizukari	27	25/VII	Fujishiro	52	4/X	Wakkanai
3	8/III	Nopporo	28	29/VII	Tomakomai	53	12/X	Tomakomai
4	9/III	Shoya	29	31/VII	Chitose	54	12/X	Shiraoi
5	10/III	Samani	30	9/VIII	Hayakita	55	24/X	Hayakita
6	10/III	Horobetsu	31	9/VIII	Nozuka	56	24/X	Kojôhama
7	26/III	Shiraoi	32	13/VIII	Senmatsu	57	26/X	Tomakomai
8	28/III	Kojôhama	33	15/VIII	Utashinai	58	27/X	Kuriyama
9	30/V	Sakkari	34	21/VIII	Senmatsu	59	11/XI	Nishikioka
10	31/V	Sahara	35	26/VIII	Tokoro	60	12/XI	Hayakita
11	8/VI	Kojôhama	36	29/VIII	Shiraoi	61	12/XI	Tomakomai
12	8/VI	Tomakomai	37	29/VIII	Hama-Koshimizu	62	18/XI	Asahihama
13	9/VI	Nishikioka	38	30/VIII	Abashiri	63	18/XI	Hiroo
14	17/VI	Nozuka	39	31/VIII	Shokotsu	64	20/XI	Kushiro
15	23/VI	Sôbetsu	40	31/VIII	Kami-Horonai	65	21/XI	Shizukari
16	29/VI	Sôbetsu	41	13/IX	Enbetsu	66	21/XI	Hamanaka
17	1/VII	Shizukari	42	14/IX	Toyotomi	67	25/XI	Nozuka
18	6/VII	Shiraoi	43	14/IX	Wakkanai	68	2/XII	Kojôhama
19	7/VII	Tomakomai	44	14/IX	Tokumitsu	69	6/XII	Ônuma
20	11/VII	Hayakita	45	15/IX	Otoshibe	70	7/XII	Yachiyama
21	19/VII	Senmatsu	46	21/IX	Numanohata	71	7/XII	Gabino
22	20/VII	Hizuka	47	25/IX	Senmatsu	72	18/XII	Kojôhama
23	20/VII	Nozuka	48	29/IX	Umekawa	73	26/XII	Hiroshima
24	21/VII	Tomakomai	49	30/IX	Hizuka			
25	24/VII	Gabino	50	1/X	Nozuka			

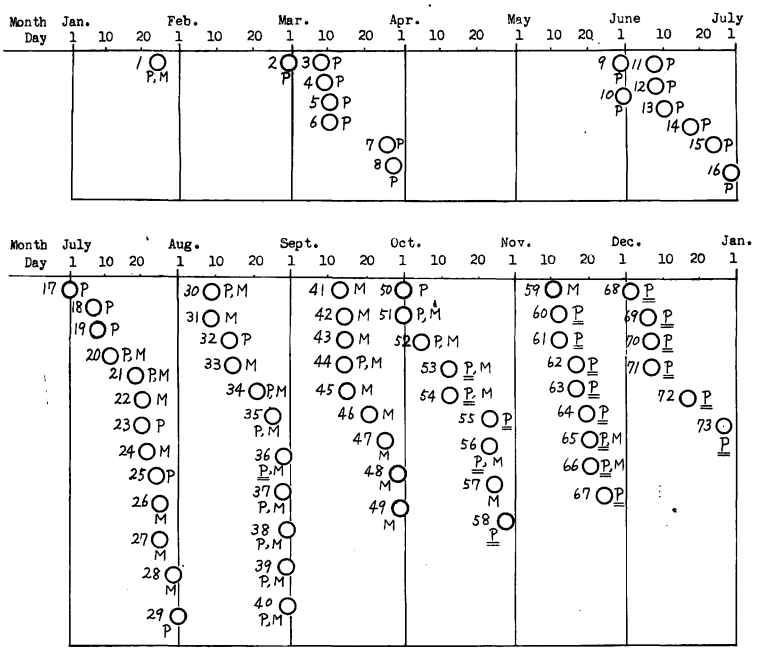


Fig. 7 Appearance of fruit bodies of *Guignardia* and *Macrophoma* in Hokkaido.

(Note)
P: *Guignardia laricina* (SAWADA) W. YAMAMOTO et K. ITO.
M: *Macrophoma* sp.
P: *Guignardia* produced on the shoots infected this year.

the causal fungus, was obtained. The circles in Fig. 7 show that fruit bodies are in mature condition, and the date as well as the locality can be found from the number on the left side of the circles by checking up with the number in Table 3.

As shown in Fig. 7, the ascigerous stage of the causal fungus exists all the year round, though perithecia on infected current season's shoots begin to appear after October. In the following spring, on over-wintered infected shoots, perithecia appear in turn and increase in number from May to June. This is closely related to the discharge of ascospores. Perithecia still remain on 2-year-old infected shoots. Pycnidia are generally found from mid-July and reach the maximum in number in August and September, and then decrease by December. They are found rarely on over-wintered infected shoots and leaves. Spermogonia are found between August and October and sometimes in winter on infected shoots.

From the above results, the diagram of the life history of the causal fungus was obtained as shown in Fig. 8, where the quantitative consideration of spores was added.

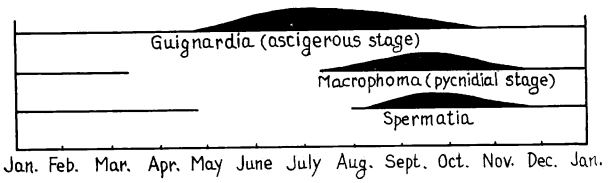


Fig. 8 Life history of *Guignardia laricina* (SAWADA) W. YAMAMOTO et K. ITO.

Chapter 3. Ecology of the causal fungus, with particular reference to the dissemination of spores.

According to the life history of the causal fungus, it is clear that ascospores exist all the year round, and pycnospores are present from middle July to November, and that the quantity of spores reaches maximum during the most vigorously growing season of larch.

Because these spores are responsible for the outbreak and propagation of the disease, it is very important to know what environmental conditions are necessary to the dissemination of spores. Of course, the pattern of dissemination will vary between ascospores and pycnospores, based on the difference in the structure of fruit bodies. Therefore, *in vitro* tests and the field observations were carried out concerning the discharge of ascospores, and *in vitro* tests were conducted to obtain the basic data on the dissemination of pycnospores, respectively.

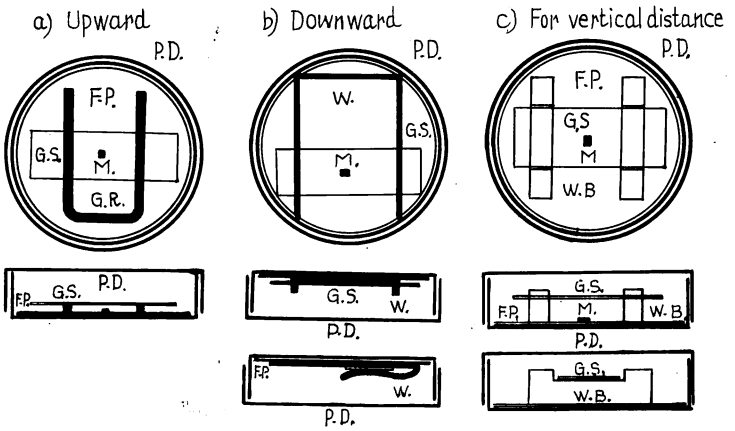
1. Discharge of ascospores.

A. Discharge of ascospores *in vitro*.

Experimental methods.

It was assured by preliminary experiments that the discharge of ascospores took place under the condition of 100 percent relative humidity or of over-saturated condition. Therefore, the apparatus shown in Fig. 9 was used for the experiments on the discharge.

In Fig. 9 a), the material M, the infected bark bearing perithecia, was put in the middle of two sheets of the filter paper, to the edge of which sterilized distilled water was supplied to keep the sheets continuously in a wet condition. U-shaped glass rod was put on the filter paper and a glass slide was set on the glass rod. Ascospores discharged from perithecia in the material were trapped on the under-surface of the glass slide. In b), the glass slide was set under the material and discharged ascospores were trapped on the upper-surface of the slide. To determine the height of expulsion of ascospores, the method shown in Fig. 9 c) was used, *i.e.*, the



P. D.: Petri dish, F. P.: Filter paper, G. S.: Glass slide, M.: Material bearing perithecia, W: Wire, W. B.: Wood block, G. R.: Glass rod.

Fig. 9　Apparatus for the test of ascospore expulsion.

distance between the material and the glass slide was adjusted by the wood blocks.

These glass slides were changed at appropriate intervals and the total number of trapped ascospores was counted under the microscope.

a)　Discharge under constant temperatures.

Materials and methods.

The effect of temperatures upon the discharge of ascospores was determined on 6 grades of constant temperatures.

A bit of bark of infected shoots, containing 1 or a few perithecia, was used as the material. Infected shoots were collected on December 26, 1961, and the test was started on December 27, 1961.

The apparatus shown in Fig. 9 b) was also used in this test. Because the dimension of perithecia is different in many cases from one another even in the same infected shoots (YOKOTA,[66] 1962-a), the number of trapped ascospores was recorded as they were. Materials were sectioned soon after the end of the experiment and the content of perithecia was observed.

Results.

The results of the experiment are shown in Table 4. As shown in Table 4, discharge was ended after about 10 days in the temperature between 20 and 30°C. This was determined by the fact that the content of perithecia was vacant. The time required until the end of the discharge became longer in the temperature at 10°C or less than at 20°C or more.

At 15°C no constant tendency of discharge was observed; No. 1 discharged abundant ascospores soon after the start of the experiment, No. 2 barely discharged after 19 days, and No. 3 was intermediate to the both. The content of the perithecium soon after the end of the test was full of asci and ascospores in every case.

b)　Discharge under the alternation of high and low temperatures.

Under the natural conditions, temperatures alternate continuously between high and low. Since it is experimentally difficult to make these temperature conditions, discontinuous alternation of high and low temperatures was applied for the test of ascospore discharge.

Table 4. Ascospore expulsion under the constant temperatures and the over-saturated moisture condition.

Temp. (°C)	No.	Days after the start of the test									Total	Number of perithecia	Content of perithecium*
		2	4	6	9	12	14	17	20	23			
5	1							6			6	1	—
	2					10	13	20			43	2	—, ±
	3					6					6	1	—
10	1				3		7	22			37	3	—
	2									10	10	1	+
	3		2			8	19	51	4		84	2	—
15	1	8		7	8	20	8	96			147	1	+
	2				3		9	118	12		142	1	?
	3								10	22	32	1	+
20	1	Untested											
	2			28	180	10					218	2	—
	3	5	7	52	103						167	1	—
25	1		21	110							131	1	—
	2											1	—
	3	?	29	30	27						86	1	—
30	1	36	48	28	9						121	1	—
	2	53	4		4						61	1	?
	3		8	21							29	1	—

* +, Asci and ascospores abundant,
±, 〃 〃 〃 few; —, Vacant.

Materials and methods.

For the combination of high and low temperatures, data (Meteorol. Soc., Hokkaido Branch[42], 1960) on the temperature from May to October, 1959, in Hakodate, Rumoi, Nemuro, Tomakomai, and Muroran Meteorological Stations, situated in heavily infected areas, were consulted. The data are shown in Table 5, from which the following 6 combinations were made; 5←→15°C. 0←→20°C, 10←→15°C, 5←→25°C, 15←→20°C, 10←→30°C, based on the mean of maximum and minimum temperatures, and maximum and minimum temperatures, respectively.

Materials used for the experiment were collected on December 26, 1961, the same as those used for the discharge under constant temperatures.

These materials put in the apparatus shown in Fig. 9 a) were alternated daily to be exposed to high temperatures from 9 A. M. until 5 P. M. and then to low temperatures until 9 A. M. next morning. Glass slides were exchanged at 9 A. M. every day and the number of discharged ascospores was counted under the microscope. Materials were sectioned soon after the end of the experiment and the content of perithecia was observed.

Results.

As shown in Table 6, ascospores were abundantly discharged in the alternated temperature of 15←→20°C and of 5←→25°C, where the time required until the end of discharge was

Table 5. Climatic conditions in some places where the disease prevailed in Hokkaido. (1959)

a) Mean temperature (°C)

Meteorol. sta.	May	June	July	Aug.	Sept.	Oct.
Hakodate	12.1	14.2	19.5	21.1	17.9	12.1
Rumoi	11.3	15.0	19.3	20.2	16.8	10.9
Nemuro	7.6	9.4	15.8	16.9	15.0	11.3
Tomakomai	9.7	12.4	17.7	20.1	17.4	11.7
Muroran	10.6	12.4	18.5	20.5	18.1	13.5
b) Maximum temperature (Mean) (°C)						
Hakodate	16.7	17.3	23.1	24.6	21.7	17.0
Rumoi	15.5	18.9	22.8	24.5	20.8	15.7
Nemuro	11.2	12.6	19.4	19.9	17.8	14.4
Tomakomai	13.7	14.5	20.8	23.2	20.6	16.6
Muroran	14.2	14.8	21.2	23.3	20.8	16.7
c) Minimum temperature (Mean) (°C)						
Hakodate	8.0	11.9	16.6	18.1	14.7	8.1
Rumoi	7.9	11.7	16.5	16.4	13.2	6.6
Nemuro	4.8	6.5	13.0	14.6	12.5	8.2
Tomakomai	6.1	10.3	15.5	17.6	14.4	6.7
Muroran	7.9	10.5	16.1	18.5	16.1	10.7
d) Maximum temperature (°C)						
Hakodate	23.8	24.0	27.8	27.8	25.5	21.9
Rumoi	24.8	23.3	29.4	32.9	25.2	21.3
Nemuro	19.4	22.4	25.3	26.3	22.9	20.0
Tomakomai	21.6	20.5	27.6	28.1	26.0	21.9
Muroran	21.0	24.2	27.6	28.2	24.6	21.5
e) Minimum temperature (°C)						
Hakodate	3.6	8.4	12.1	14.4	8.7	1.4
Rumoi	2.3	6.1	12.1	10.6	5.9	— 1.9
Nemuro	1.8	2.1	8.8	11.0	6.2	1.0
Tomakomai	— 0.1	7.3	12.8	14.0	8.1	— 0.8
Muroran	4.2	7.4	12.6	16.6	10.2	5.0

shorter and the number of trapped ascospores was greater than those in the other combinations of alternated temperatures (Plate 3, A). On the content of perithecia soon after the end of the experiment, asci and ascospores existed abundantly in the alternated temperature of 0←→20°C and of 10←→15°C. On the contrary, the content of perithecia in the other 4 combinations was vacant, showing the end of the discharge (Plate 3, B~H).

c) Discharge under the alternation of definite periods of wet and dry conditions.

In nature, perithecia are exposed to wet condition by the rainfall or the fog and to dry condition. The writer carried out the experiment on the effect of the alternation of definite periods

Table 6. Ascospore expulsion under the alternated temperature and the over-saturated condition.

Temp. (°C)	No.	Days after the start of the test																	Total	Number of perithecia	Content of perithecium
		2	4	7	9	11	13	15	18	21	23	25	27	29	31	34	36	38			
5←→15	1									5	49	9							63	1	Spores few
	2	2		4		5	8	10	16	12	67								124	2	Vacant
	3								3	14									17	1	Vacant
10←→15	1					4		3	9	31	23	34	71	9					184	2	Asci & ascospores abundant
	2																		0	1	
	3										7	8	11	11		39			76	2	
15←→20	1	16	1	25	365	45	457												909	2	Vacant
	2		13	25	145	29													212	1	
	3			3	80	196	7												286	2	
0←→20	1																		0	2	Asci & ascospores abundant
	2	4				4	6		34	7	23	11	54	81	13	40	3		280	3	
	3		7		8				11	12		98		130					266	3	Spores few
5←→25	1			115	86	190													391	3	Vacant
	2	18		36	50	89	294	64	16										567	4	
	3	225	16		2	101	139	32	27	18									560	3	
10←→30	1				2	13		4	1										20	2	Vacant & premature?
	2	96		12	5														113	3	Vacant
	3				11	30													41	2	

of these conditions upon the discharge of ascospores.

Materials and methods.

Materials used in the experiment were collected on April 27, 1962, at Tomakomai. Several perithecia were contained in a bit of the bark of infected shoots and they were put in the apparatus shown in Fig. 9 a).

The combinations of the alternated condition were as follows:

Treatment A: Continuously in wet condition at 25°C (for a check),

〃 B: Alternated wet and dry every day,

〃 C: Alternated wet for a day and dry for 3 days,

〃 D: Alternated wet for 2 days and dry for a day,

〃 E: Alternated wet for 2 days and dry for 3 days,

〃 F: Continuously in wet condition and left in the laboratory at 10～20°C (for a check).

Swollen materials fully absorbing water were put on two sheets of filter paper in the Petri dish after the definite period in a wet condition. They soon dried out and became about 1/2 in size. The change of these conditions was done at 9 A. M., and glass slides were exchanged every day at the same time. The number of discharged ascospores was counted under the microscope. The materials were sectioned soon after the end of the experiment and the content of perithecia was observed.

Results.

As shown in Table 7, the discharge took place after the perithecia absorbed water and never occurred in dry condition, except D-3 after 12 days and E-1 after 13 days, where discharge was observed in a dry condition. It seemed that in these exceptional cases discharge took place while the perithecia were in a wet condition soon after the exchange of the glass slide.

In regard to the relation between the interval of wet and dry conditions and the discharge of ascospores, a prominent discharge was observed in the treatment D, E, and B, and not so good in C. According to these results, it is considered that the alternation of a relatively longer period of dry condition and a shorter period of wet condition gave an undesirable condition for the discharge of ascospores. In the treatment A, the discharge ceased similarly after 10 days as in the experiment conducted under constant temperatures. In the treatment F, the discharge was conspicuously vigorous, where the temperature was changed continuously between 10 and 20°C.

The content of perithecia was vacant in the treatment A and F, showing the end of discharge, almost vacant in D, and in B, C and E abundant ascospores were observed.

d) The height of projection.

Materials and methods.

Materials used for the experiment were the same as those used for the experiment on the discharge under constant temperatures. The apparatus shown in Fig. 9 c) was used in this test. The distance between the material and the glass slides was adjusted by wood blocks.

Results.

As shown in Table 8, ascospores discharged upwards were trapped on glass slides in the distance of 10 mm from the material. On the other hand, they could not be found on glass slides in the distance of 15 mm.

B. Discharge of ascospores *in vivo*.

Table 7. Ascospore expulsion under the condition of alternating of wet and dry.

		Days after the start of the test																Total	Number of perith.	Content of perithecium
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15			
Treatment		25°C under over-saturated condition																		
A	1		14	19	13	20	47											113	3	Vacant
	2					10	63	58	3	33	32	2						201	5	
	3		14			22	4	82	121	104	58	7						412	8	
Treatment		○	×	○	×	○	×	○	×	○	×	○	×	○	×	○				
B	1		9								42		12		24		70	157	2	Asci and ascospores abundant.
	2		63		73		32		21		141		81		226		193	830	4	
	3		7		6						59		40		4			116	3	
Treatment		○	×	×	×	○	×	×	×	○	×	×	×	○	×	×				
C	1						4								13			17	5	2, Vacant; 3, Asci & ascospores abundant.
	2		5				11								19			35	5	Asci and ascospores abundant.
	3		29				24				30				6			89	9	2, Vacant; 6, Abundant; 1, Premature.
Treatment		○	○	×	○	○	×	○	○	×	○	○	×	○	○	×				
D	1			11		18	60		160	498		92	9					848	3	Vacant.
	2		7	13		10	22		203	218		214	56					743	8	7, Vacant; 1, Spores few.
	3		71	5		52	39		248	164		697	252	23	66	29		1,646	11	9, Vacant; 2, Abundant.
Treatment		○	○	×	×	×	○	○	×	×	×	○	○	×	×	×				
E	1		56					16					231	415	6			724	3	Asci and ascospores abundant.
	2		42	13				40					209	331				635	7	1, Vacant; 6, Abundant.
	3		15	20				43	39				294	243				654	5	1, Vacant; 4, Abundant.
Treatment		Over-saturated condition at room temperature (10～20°C)																		
F	1								16	209	290	149	41	27	28	6		766	7	Vacant.
	2		178	99	29		78	7	276	915	316	216						2,114	6	
	3		125	12	5	9	47	4	14	248	205	18		62				749	8	1, Vacant; 7, Spores few.

Table 8. Height of projection of ascospores.

Height (mm)	No.	Days lapsed	
		2	10
5	1	34*	121*
	2	34	199
10	1	41	76
	2	7	33
15	1	0	0
	2	0	0

* Number of ascospores trapped. (Temperature: 25°C)

Materials and methods.

To ascertain the discharge of ascospores *in vivo* and the climatic conditions during the period of observation on the discharge, two heavily infected 3-year-old larch trees were transplanted in the yard from the plantation in June 2, 1962, to facilitate observation at any time.

According to the results obtained from the experiments on the discharge of ascospores *in vitro*, it was verified that vigorous expulsion occurs only when perithecia are in a fully wet condition, and at the same time, at the tempe-

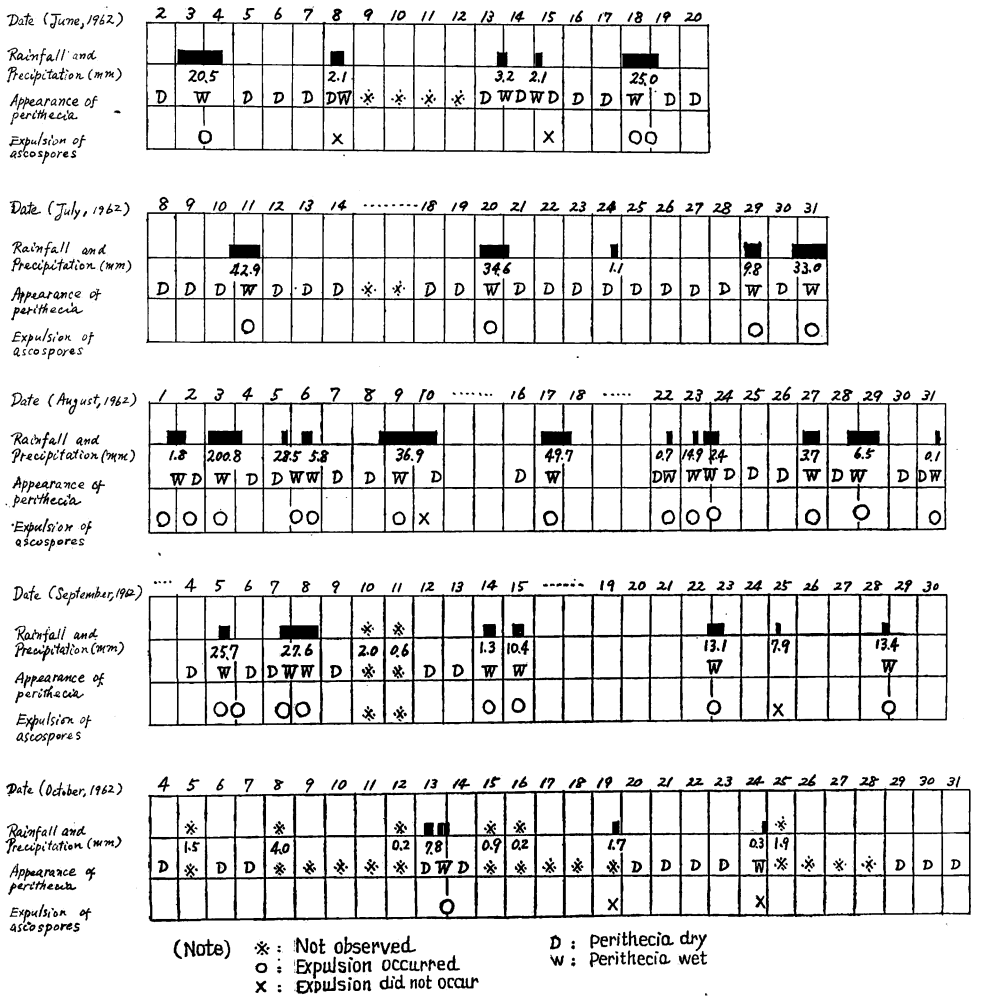


Fig. 10 The climatic condition and the ascospore expulsion from perithecia on infected shoots during the period of observation (Sapporo, Hokkaido).

rature above 15～20°C. It seemed that, under the natural conditions, these climatic conditions will be satisfied on rainy or foggy days between July and September in Hokkaido.

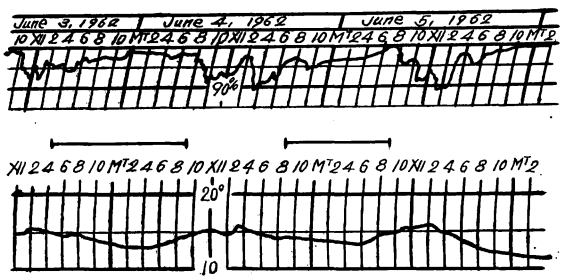
Because the ascospores are very adhesive in character, it is not necessary to spread the gelatin-jelly (KURIBAYASHI and ICHIKAWA[89]., 1941) to glass slides. When perithecia on infected shoots absorbed water and swelled in rainy weather, three glass slides were set in definite position of infected shoots of each tree bearing many perithecia (Plate 4, A, B, C). These glass slides were exchanged at suitable intervals and the number of ascospores trapped on glass slides was counted. At the same time, the germination of ascospores was observed. In regard to the climatic conditions, temperature and relative humidity were observed in the yard and the data on precipitation were obtained from the observation at Sapporo Meteorological Station. Glass slides were set at the upper part of the shoots and ascospores were trapped on the under-surface of glass slides so as to prevent their being washed down by the rain.

Observation of the discharge and climatic conditions began from early June and lasted until perithecia had ceased to discharge ascospores.

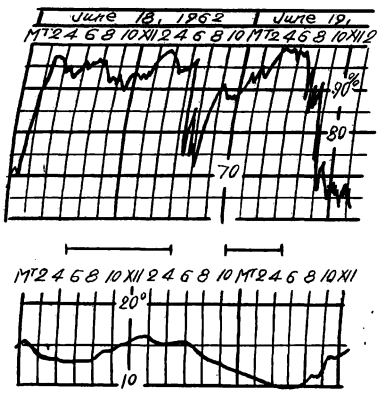
Results.

Results are shown in Tables 9, 10, and 11, and Figs. 10, 11, and 12.

Fig. 10 shows that in June no discharge of ascospores was observed in a period of least precipitation amounting to a few mm, but they were easily discharged between the middle of July



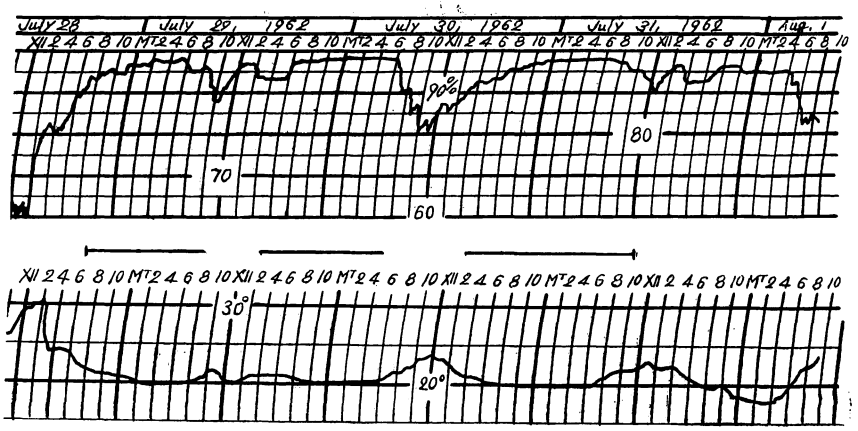
a) June 3～5, 1962.



b) June 18～19, 1962.

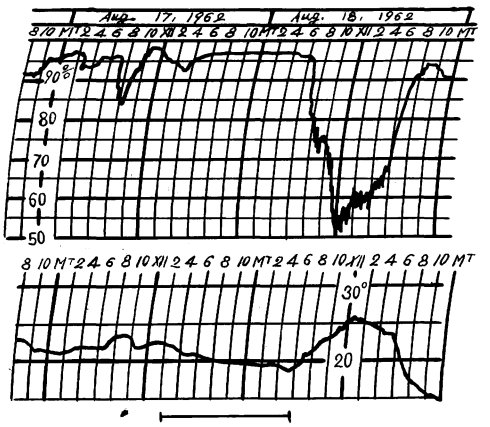
Fig. 11 Records of relative humidity and temperature during each period of observation on the expulsion of ascospores *in vivo*.

——— Period of rainfall.

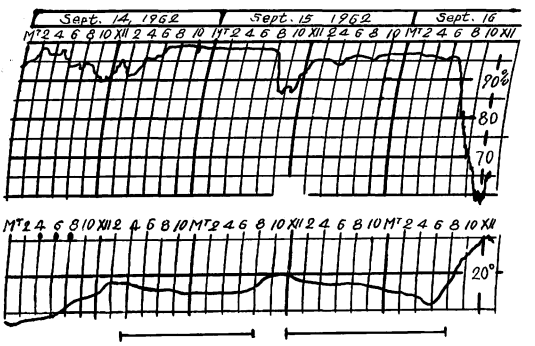


c) July 28～31, 1962.

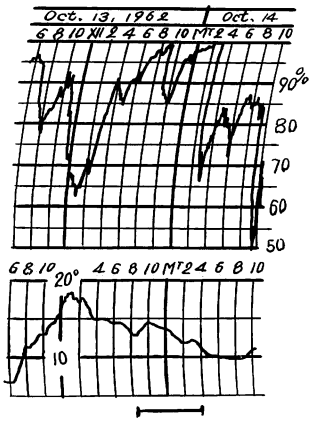
Fig. 11 (Continued)



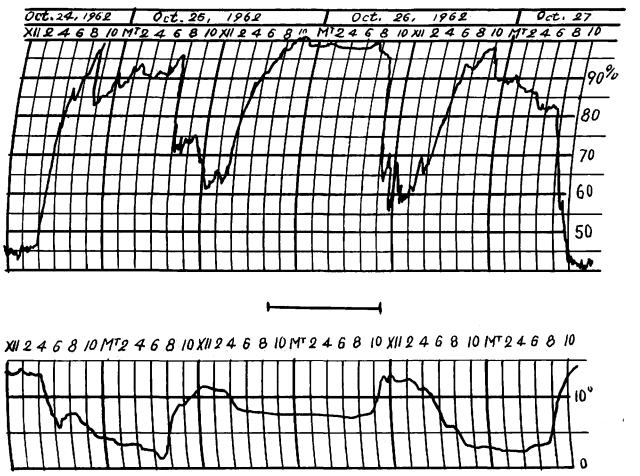
d) Aug. 17～18, 1962.



e) Sept. 14～16, 1962.



f) Oct. 13～14, 1962.



g) Oct. 24～27, 1962.

and the middle of September on rainy days. The temperature and relative humidity observed are shown in Fig. 11. In June, the temperature on rainy days is mostly below 15°C. Between early and mid-July, it increased to between 15 and 20°C and then it reached maximum of 20°C or more until the middle of August. From late August to the middle of September it began to decrease and showed between 15 and 20°C. After October, the temperature below 15°C was

Table 9. Number of trapped ascospores on glass slides and precipitation during the period of observation.

Tree number	Number of glass slide	Time of observation							
		8/VI 1600 – 15/VI 745	18/VI 1330 – 19/VI 845	11/VII 1000 – 12/VII 830	20/VII 1700 – 21/VII 800	29/VII 1300 – 29/VII 1520	30/VII 2000 – 1/VIII 820	1/VIII 1820 – 2/VIII 820	3/VIII 110 – 4/VIII 910
1	1	39	1,648	3,382	1,210	257	5,596	118	3,798
	2	41	726	3,526	1,569	223	7,753	160	2,779
	3	70	920	1,727	1,103	139	2,532	185	2,239
2	1	0	120	446	60	219	457	0	1,530
	2	513	194	119	108	78	340	103	1,933
	3	8	32	351	128	23	390	0	3,380
Total		671	3,640	9,551	4,178	939	17,068	566	15,659
Precipitation (mm)		20.5	25.0	42.9	34.6	9.8	33.0	1.8	200.8
		5/VIII 1755 – 6/VIII 1020	8/VIII 2220 – 10/VIII 830	17/VIII 900 – 18/VIII 730	22/VIII 1430 – 24/VIII 615	27/VIII 650 – 27/VIII 1800	28/VIII 1800 – 29/VIII 1200	31/VIII 1800 – 31/VIII 1830	5/IX 1230 – 6/IX 830
1	1	103	143	3,908	923	496	3	383	799
	2	42	41	4,255	2,202	4,761	53	192	7,175
	3	307	64	3,264	648	729	0	30	1,693
2	1	38	99	7,544	1,360	15,261	25	257	17,276
	2	163	47	11,208	3,393	9,158	10	1,617	18,834
	3	100	40	6,321	2,833	7,477	54	473	9,121
Total		753	434	36,500	11,359	37,882	145	2,952	54,898
Precipitation (mm)		34.3	36.9	49.7	18.0	3.7	6.5	0.1	25.7
		7/IX 1720 – 9/IX 730	14/IX 800 – 14/IX 1730	15/IX 900 – 16/IX 700	22/IX 2210 – 24/IX 700	28/IX 1930 – 28/IX 2245	13/X 1905 – 14/X 730	4/XI 1900 – 5/XI 700	
1	1	1,435	124	1,893	865	198	24	39	
	2	6,659	314	9,389	1,788	646	129	0	
	3	1,500	310	750	508	47	10	19	
2	1	10,262	956	15,945	745	2,500	442		
	2	3,983	168	9,908	1,849	274	201		
	3	4,289	466	2,862	611	219	99		
Total		28,131	2,338	40,747	6,366	3,884	905	58	
Precipitation (mm)		27.6	1.3	10.4	13.1	13.4	7.3	3.1	

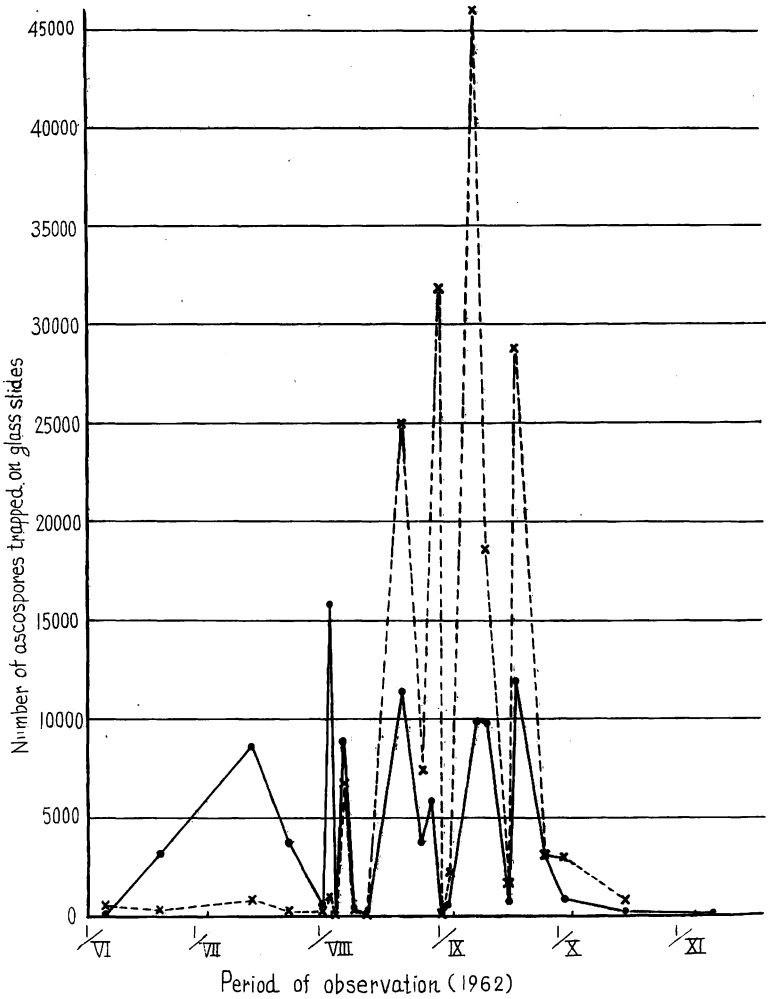


Fig. 12　Total number of ascospores trapped on glass slides during the period of observation.

observed on rainy days.

Table 9 shows the number of trapped ascospores and the precipitation in definite intervals between early June and early November. It is clear that the peak of discharge appears through August and September, *i. e.*, out of 280,000 ascospores which were trapped on 6 glass slides during the whole period of observation, 240,000 were trapped in those two months (Plate 5, D). The pattern of discharge is different in each sample tree. This is shown in Fig. 12, where the peak in the sample tree No. 1 is obscure and in the tree No. 2 the peak appeared clearly between mid-August and mid-September, depending upon the higher temperature in rainy intervals and the maturity of perithecia.

The details of Table 9 are shown in Table 10, where the range of temperature and relative humidity in observed intervals and the germination of trapped ascospores are presented. Germination of trapped ascospores was very good in almost every case. When the number of trapped ascospores was not many, it was observed that the ascospores were trapped as a unit consisting

Table 10. Ascospore expulsion and climatic conditions *in vivo* (a part).

Tree number	Number of glass slide	Ascospores trapped	Germination of ascospores	Remarks
3/VI, 1600～4/VI, 1000				
1	1	0		Temp. range: 15.2～13.0～14.9(°C)
	2	14	+++	
	3	0		
2	1	0		Relative humid.: 96.0～99.0～91.5～93.0 (%)
	2	0		
	3	0		
4/VI, 1000～4/VI, 1500				
1	1	39	−	T. r.: 14.9～15.3～14.6～15.7
	2	27	−	
	3	52	±	
2	1	0		R. h.: 93.0～98.0～95.0
	2	513	−	
	3	8	−	
4/VI, 1500～4/VI, 1930				
1	1	0		T. r.: 15.7～14.3
	2	0		
	3	5	−	
2	1	0		R. h.: 95.0～89.5～97.2
	2	0		
	3	0		
29/VII, 1300～29/VII, 1520				
1	1	257	++	Temp. range: 19.8～21.0
	2	223	±	
	3	139	±	
2	1	219	±	Rel. humid.: 97.0～93.5
	2	78	−	
	3	23	±	
30/VII, 2000～31/VII, 845				
1	1	1,313	+++	T. r.: 19.7～21.0
	2	1,041	+++	
	3	86	+++	
2	1	62	+++	R. h.: 97.0～98.0～97.0
	2	0		
	3	46	+++	

Tree number	Number of glass slide	Ascospores trapped	Germination of ascospores	Remarks
4/VI, 1930～5/VI, 745				
1	1	0		Temp. range: 14.3～13.3～14.1
	2	0		
	3	13	−	
2	1	0		Relative humid: 97.2～94.5～100～99.7
	2	0		
	5	0		
5/VI, 745～5/VI, 1300				
1	1	0		T. r.: 14.1～15.5
	2	0		
	3	0		
2	1	0		R. h.: 99.7～91.2～92.0
	2	0		
	3	0		
5/VI, 1300～5/VI, 1500				
1	1	0		T. r.: 15.5～15.5
	2	0		
	3	0		
2	1	0		R. h.: 92.0～97.0
	2	0		
	3	0		
31/VII, 2000～1/VIII, 820				
1	1	102	+++	Temp. range: 20.0～17.6～22.2
	2	152	+++	
	3	144	+++	
2	1	0		Rel. humid.: 96.0～92.0～94.0
	2	8	+++	
	3	53	+++	
1/VIII, 1820～1/VIII, 2320				
1	1	51	+++	T. r.: 20.2～19.8
	2	62	+++	
	3	116	+++	
2	1	0		R. h.: 97.4～98.0
	2	103	+++	
	3	0		

Table 10. (Continued)

31/VII, 845～31/VII, 1700

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	4,181	++	T. r.: 21.0～22.8～21.5
	2	6,560	++	
	3	2,302	++	
2	1	395	++	R. h.: 97.0～90.0～97.0～93.0
	2	332	++	
	3	291	++	

1/VIII, 2320～2/VIII, 820

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	67	+++	T. r.: 19.8～21.2
	2	98	+++	
	3	69	+++	
2	1	0		R. h.: 98.0～87.0
	2	0		
	3	0		

17/VIII, 900～17VIII, 1800

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	3,463	+++	Temp. range: 23.2～21.0
	2	3,443	+++	
	3	3,246	+++	
2	1	6,465	+++	Rel. humid: 91.0～98.4～96.7
	2	8,341	+++	
	3	5,494	+++	

23/VIII, 1130～23/VIII, 1430

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	290	+	Temp. range: 19.4～20.4
	2	348	++	
	3	164	+	
2	1	142	++	Rel. humid.: 87.0～96.0～95.0
	2	1,205	+	
	3	110	+	

17/VIII, 1800～18/VIII, 730

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	445	+++	T. r.: 21.0～18.8～21.2
	2	812	+++	
	3	0		
2	1	1,079	+++	R. h.: 96.7～85.0
	2	2,867	+++	
	3	827	+++	

23/VIII, 1805～23/VIII, 2130

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	607	++	T. r.: 19.6～19.0
	2	1,769	++	
	3	14	+++	
2	1	1,052	++	R. h.: 95.0～99.0～98.5
	2	2,012	++	
	3	2,634	+++	

22/VIII, 1430～22/VIII, 1715

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	0		T. r.: 26.0～23.6
	2	14	−	
	3	414	±	
2	1	0		R. h.: 78.0～92.0
	2	160	±	
	3	67	±	

23/VIII, 2130～24/VIII, 615

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	26	++	T. r.: 19.0～19.4
	2	71	+++	
	3	56	++	
2	1	166	+++	R. h.: 98.5～96.7
	2	16	++	
	3	22	+++	

5/IX, 1415～5/IX, 1730

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	226	+	Temp. range: 18.3～18.2
	2	5,156	++	
	3	322	+	
2	1	13,380	++	Rel. humid.: 98.0～91.8～93.0
	2	14,120	++	
	3	3,524	+++	

7/IX, 2155～8/IX, 800

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	84	++	Temp. range: 16.0～15.0～16.0
	2	417	++	
	3	6	−	
2	1	478	+++	Rel. humid.: 96.5～97.5～88.0
	2	26	±	
	3	105	++	

Table 10. (Continued)

5/IX, 1730～6/IX, 830

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	121	+++	T. r.: 18.2～15.2～21.2
	2	37	+++	
	3	350	+++	
2	1	1,645	+++	R. h.: 93.0～98.0～75.0
	2	2,037	+++	
	3	4,433	++	

8/IX, 800～8/IX, 1240

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	0		T. r.: 16.0～19.5
	2	0		
	3	0		
2	1	31	++	R. h.: 88.0～86.5～93.5～91.0
	2	3	—	
	3	0		

7/IX, 1720～7/IX, 2155

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	33	—	T. r.: 18.0～16.0
	2	99	±	
	3	0		
2	1	1,129	++	R. h.: 94.0～96.5
	2	194	++	
	3	519	++	

8/IX, 1240～8/IX, 2200

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	1,305	++	T. r.: 19.5～21.0
	2	6,006	+++	
	3	1,494	+++	
2	1	8,577	+++	R.h.: 91.0～97.0～91.0～97.0
	2	3,675	+++	
	3	3,612	+++	

13/X, 950～13/X, 1300

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	0		Temp. range: 13.0～18.4
	2	0		
	3	0		
2	1	0		Rel. humid.: 87.0～93.0～63.0
	2	0		
	3	0		

13/X, 2210～14/X, 730

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	24	++	Temp. range: 14.4～9.8
	2	109	++	
	3	10	+++	
2	1	442	++	Rel. humid.: 87.0～100～66.0～87.0
	2	195	++	
	3	99	±	

13/X, 1530～13/X, 1615

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	0		T. r.: 15.0～15.0
	2	0		
	3	0		
2	1	0		R. h.: 87.5～91.5～89.0
	2	0		
	3	0		

24/X, 2005～25/X, 730

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	0		T. r.: 7.7～1.4～3.4
	2	0		
	3	0		
2	1	0		R. h.: 90.0～99.0～83.0～96.0
	2	0		
	3	0		

13/X, 1905～13/X, 2030

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	0		T. r.: 14.4～12.9
	2	20	—	
	3	0		
2	1	0		R. h.: 96.0～98.0
	2	9	—	
	3	0		

4/XI, 1900～5/XI, 700

Tree number	Number of glass slide	Asco-spores trapped	Germination of asco-spores	Remarks
1	1	39	—	T. r.: 15.5～9.0～9.5
	2	0		
	3	19	—	
2	1			R. h.: 80.0～88.0～51.0～67.0～56.0
	2			
	3			

(Note) +++ : Germinated more than 75%, ++ : Germinated 50～75%,
+ : 〃 25～50%, ± : 〃 less than 25%
— : Germination did not occur.

of 8 spores (Plate 4, D). Trapped ascospores are very adhesive, which could be clearly seen under the microscope (Plate 5, A).

In late October, only a few ascospores on rainy days were trapped. This may be due to the low temperature and not due to the disappearance of perithecia. As a matter of fact, the matured perithecia are still present abundantly, though the appearance of the surface of the shoots is very rough (Plate 5, B, C). Some of these perithecia easily discharged ascospores by giving them a favorable condition *in vitro*, as shown in Table 11. Therefore, if adequate water

Table 11. Expulsion of ascospores *in vitro* by using materials after the cease of expulsion *in vivo*.

No.	3 hours later		18 hours later	
	No. of spores discharged	Germination of spores	No. of spores discharged	Germination of spores
1	0		5	+++
2	84	++	65	+++
3	28	+++	69	+++
4	0		76	+++
5	0		0	
6	31	−	86	+++

The water was supplied adequately to filter papers kept at 23～25°C.

The materials were collected on October, 31, and the test was started in the afternoon.

is supplied, the temperature becomes the main factor determining the hardness or ease of discharge of ascospores. This was proved by the fact that the discharge of ascospores was observed on November 3, when the temperature during the rainfall increased above 10°C by the influence of the tropical low atmospheric pressure.

2. Dissemination of pycnospores *in vitro*.

As a result of the primary infection by ascospores, pycnidia appear on infected current season's shoots and leaves, and pycnospores continue acting as the source of secondary infection from mid-July to late October. Pycnospores are exuded from pycnidia forming small, white or pale milk-white masses. But they are not discharged, because the pattern of dissemination is different from that of ascospores. Therefore, it seems necessary to find out the climatic conditions deciding the hardness or ease of exudation of pycnospores, and the writer carried out the experiments concerning the effect of temperature and relative humidity upon the exudation of pycnospores and the mode of dispersion of spore masses.

A. Effects of relative humidity and temperature upon the exudation of pycnospores.

a) Effect of relative humidity upon the exudation.

Materials and methods.

Over-saturated aqueous solution of several kinds of salts which show given relative humidity* were prepared in the desiccator. Infected leaves bearing many pycnidia, which were collected on July 30 at Hayakita, were fixed on the end of glass slides, then they were put into desiccators of given relative humidity and kept at 25°C. Glass slides were examined at definite intervals to see whether or not the spore masses were formed.

* The following over-saturated aqueous solutions were used: 100% of relative humidity, water only; 98%, K_2SO_4; 94%, KNO_3; 92%, K_2HPO_4; 87%, KCl.

Results.

As shown in Table 12, after 20 hours, pycnospores were exuded forming small masses, in the relative humidity of 98 and 100 percent. After 40 hours, almost all pycnidia exuded pycnospores in the relative humidity of 100 percent (Plate 5, E). On the contrary, with the relative humidity below 94 percent, no exudation of pycnospores was observed even after 96 hours (Plate 5, F).

Table 12. Effect of relative humidity upon exudation of pycnospores

Relative humidity (%) / Time passed (hr)	100	98	94	92	87
20	++	++	−	−	−
40	+++	++	−	−	−
96		++	−	−	−

+++ Pycnospores exuded more abundantly.
++ Pycnospores exuded abundantly.
+ Pycnospores exuded slightly.
− Pycnospores did not exude.
Temperature at 25°C.

b) Effect of temperature upon the exudation.

Materials and methods.

Because good exudation was observed in the relative humidity of 100 percent, the same materials as before were put into the desiccator containing water, kept at various temperatures, and examined for exudation of pycnospores at definite intervals.

Results.

As shown in Table 13, exudation occurred in temperatures at 10°C or more after 20 hours, though quantitative differences were observed in each temperature. Above all, the most vigorous exudation was observed at 25°C, where almost all pycnidia exuded masses of pycnospores after 40 hours, followed by 31～32°C. In the temperatures at 20°C or less and at 36～37°C, exudation was not so goot even after 96 hours.

Table 13. Effect of temperature upon exudation of pycnospores.

Temperature (°C) / Time passed(hr)	5	10	15	20	25	31～32	36～37
20	−	±	±	+	++	+	±
40	+	+	+	+	+++	++	+
96	+	+	+	+		++	+

+++ Pycnospores exuded more abundantly.
++ Pycnospores exuded abundantly.
+ Pycnospores exuded slightly.
± Pycnospores exuded very slightly.
− Pycnospores did not exude.

B. Dissemination of pycnospores.

Because masses of exuded pycnospores stick to the surface of pycnidia and are not discharged,

it seems that certain conditions are necessary to the dissemination of pycnospores. So the writer carried out the following experiments.

Materials and methods.

Pycnospores were exuded previously by putting the infected leaves bearing many pycnidia into a moist chamber. They were put into the wind tunnel of Eiffel-type and were blown with dry and moist wind of 3 m/sec and 5 m/sec in wind velocity for 1 and 3 minutes, respectively.

Apart from above mentioned experiments, water drops of 0.1 cc were made to fall on the mass of pycnospores from the height of 10 cm to ascertain whether or not the mass could be washed down.

Table 14. Dissemination of exuded pycnospores by the wind.

Wind velocity (m/sec)	Time (min)	Dispersion
With dry wind.		
3	1	—
3	3	—
5	1	—
5	3	—
With damp wind containing sprayed water		
5	1	—
5	3	—

Results.

As shown in Table 14, no dissemination occurred by blowing of dry and moist wind, and the mass of pycnospores remained as they were before.

Table 15 shows that 60 drops of water were sufficient to wash down the mass of pycnospores. No remarkable difference was observed in the relation between the lapse of time after the exudation and the hardness or ease of washing down.

Table 15. Washing down of exuded pycnospores by water drops.

No.	Number of water drop			Condition of spore mass used
	40	60	100	
1	—	—	—	2～3 days after exudation
2	—	+		Do.
3	—	+		1 day after exudation
4	—	—	—	Do.

+ : Washed down, — : Not washed down.

3. Discussion.

As already mentiond, the discharge of ascospores of the causal fungus takes place under the condition of 100 percent of relative humidity or over-saturated condition. In regard to the mechanism of the discharge of ascospores, as Ingold[26] (1953) stated, it seems that much water permeates into the matured ascus having high osmotic pressure through its semipermeable cell wall, and the ascus bursts with the discharge of ascospores. Consequently the supply of water to perithecia is essential to the discharge of ascospores. Though some exceptions have been known that the water necessary to the discharge is stored in the stromatic tissue as in *Daldinia* or the water is absorbed from the cells of host plants as in *Epichloe* (Ingold[24)25)], 1946, 1948), in the case of the causal fungus the water is supplied by the rainfall or the fog.

Heald and Walton[18] (1914) reported that *Endothia parasitica* discharged ascospores most vigorously in the range of temperature from 20 to 27°C. Chiba and Zinno[9] (1959) ascertained that the discharge of ascospores in *Lophodermium pinastri* took place in the range of temperature from 5 to 40°C, with the optimum of 20～30°C. The writer carried out several exeriments

regarding the discharge of ascospores of the causal fungus, and the following results were obtained: Under constant temperatures, the discharge took place between 5 and 30°C, especially in the temperatures above 20°C, and the same tendencies were recognized as those obtained on *Endothia* and *Lophodermium*. But the fact that abundant ascospores were observed in the perithecium at 15°C after the end of the experiment was phenomena different from the other cases. In the experiment on the discharge of ascospores under the discontinuous change of temperatures, the best result was obtained in the alternation of 15←→20°C, though it was not ascertained whether or not the result was due to the alternation of temperature because of such slight difference as 5°C. But, considering the fact that the ascospores in perithecia soon after the end of the experiment under the constant temperature of 15°C were abundant and the time required from the beginning to the end of the discharge became shorter in the temperature at 20°C or more, it seemed that the alternation of 15←→20°C was favorable for the discharge of ascospores.

Though the contents of perithecia used in the alternation of temperature of 15←→20°C and 5←→25°C were vacant soon after the end of the experiment, greater numbers of ascospore per one perithecium were trapped than those in any other range of temperatures. From these results, it might be considered that ascospores were produced successively during the experiment in the alternation of temperature of 15←→20°C and 5←→25°C.

The fact that the alternation of temperature of the proper range is more favorable for the discharge of ascospores than under the constant temperature, may be seen by comparing the results shown in Table 6 with those in Table 4. But, if the treatment A and F in Table 7 are compared, the relation is still more clear, that is, in the treatment F the continuous change of temperature in the range of 10～20°C is given, and a 4 times greater number of trapped ascospores were obtained than the number in the constant temperature of 25°C.

The relation between the discharge of ascospores and the interval of the alternation of wet and dry conditions applied to perithecia is given in Table 7, which clearly shows that ascospores are discharged only when perithecia are in the wet condition. If the interval of the dry condition is relatively longer than that of the wet condition, as in the treatment C, a smaller number of discharged ascospores are observed. In the treatment A and F, the check of the experiment, the content of perithecia after the test was vacant. On the contrary, abundant ascospores were observed when perithecia were under the alternation of the wet and the dry conditions. These results suggest that different influences are at work on the perithecia under the continuance of the wet condition and the alternation of the wet and the dry conditions.

Sato *et al.*[49] (1963) reported that the discharge of ascospores of the fungus took place only when the perithecia were in the wet condition. Heald and Walton[18] (1914) carried out the experiment on the discharge of ascospores of *Endothia parasitica* and ascertained that the most vigorous discharge was observed in the alternation of one day's wet and one day's dry conditions, and no discharge took place in the alternation of one day's wet and 7 days' dry conditions. Further, the rainfall has an essential rôle on the maturity of perithecia together with the discharge of ascospores in *Venturia inaequalis* (Fray and Keitt[14], 1925; Howitt and Evans[20], 1926).

From the above results, it is clear that water is the substantial factor for the discharge of ascospores. If the discharge lasts for a longer period, however, the alternation of the wet and the dry conditions at proper intervals is considered conductive to the favorable condition for the

discharge rather than the continuously wet condition.

Discharged ascospores were trapped on the glass slide set 10 mm upward in distance from perithecia. Bier[4] (1940) reported that ascospores of *Hypoxylon pruinatum*, the causal fungus of poplar canker, were trapped on the glass slide set 45 mm in distance from ostioles of perithecia. These results show that ascospores as the source of infection can be disseminated at a great distance by the wind after their liberation.

According to the above-mentioned results, it seems that the discharge of ascospores of the causal fungus *in vivo* is feasible, when the water is supplied by the rain or the mist and the temperature is above 15°C. In regard to the discharge of ascospores *in vivo*, Fray and Keitt[14] (1925) reported that ascospores of *Venturia inaequalis* were discharged under the adequate supply of water by the rain, which had also the effect of promoting maturity of the perithecia. Similarly Howitt and Evans[20] (1926) stated that the rain was essential for the discharge of ascospores of the same fungus. Woodward[57] (1927) reported that in *Podosphaera leucotrica* the extent of maturity of perithecia, the supply of water and the temperature are responsible as factors deciding the hardness or ease of the discharge of ascospores. According to Bier[4] (1940), the discharge of ascospores of *Hypoxylon pruinatum* took place vigorously soon after the rainfall in July and August, and lasted about 20 or 30 hours after the heavy rain. Gruenhagen[15] (1945) said that the rainfall was the decisive factor rather than the temperature. For the discharge of ascospores in *Gibberella zeae*, the rain was the essential factor (Nishikado, Inoue and Inoue[44], 1952). Hirst and Stedman[19] (1961) carried out the studies on the frequency of airborne spores in orchards with suction traps, and it was found that most abundant airborne spores were trapped during the rain or soon after the end of rainfall, where perithecia in the lesion had been kept wetted. *In vitro* test carried out by Sato, Yokozawa and Shôji[49] (1963) showed that ascospores of the causal fungus were discharged from perithecia absorbing water.

From the results of the observation by the writer, ascospores began to be discharged least in early June. Fig. 10 shows that the discharge took place only when perithecia swelled with absorbing water supplied by the rain, and that the rain was the indispensable factor for the discharge of ascospores. The results well coincide with those mentioned above. And, the discharge in June was not easy without the lasting of rainfall in a relatively longer period. Relations between the seasonal change of the temperature during the rainfall and the discharge of ascospores *in vivo* were as follows: The temperature during the rainfall in June was below 15°C, unfavorable temperature condition on the discharge. From July to mid-September, the temperature during the rainfall was observed to exceed 15°C even at midnight, especially to reach 20°C or more from the middle of July to the middle of August. At the same time, ascospores were abundantly discharged and the discharge reached maximum between mid-July and mid-September. These results on the temperature and the discharge agree well with those *in vitro*, and with those on the time of infection in Tomakomai district carried out by Igarashi[22] (1963-b).

On the lasting period of the discharge of ascospores after the rainfall ceases, the velocity decreasing the water in the bark bearing perithecia may be the important factor. As Bier[4] (1940) mentioned, the causal fungus of poplar canker could discharge ascospores for 20 or 30 hours after the rainfall, because of the high capacity of the thick bark to hold the water. Inoue and Takasu[29][30] (1959-a, b) reported that ascospores of *Gibberella zeae* were easily discharged under a calm or a gentle wind during or soon after the rainfall. This shows that the decrease

of the water contained in the lesion by the wind becomes a factor deciding the period of the discharge of ascospores. In ascospores of the causal fungus, the discharge will cease soon after the end of the rainfall, depending upon the low capacity for a thin bark of infected shoots to keep water.

As stated by Bier[4] (1940) on the relation between the number of discharged ascospores and the precipitation, the number generally increased with the increase of the precipitation. The same tendency was also observed in the causal fungus, especially until the middle of July. From then on the number of trapped ascospores became greater in spite of less precipitation. The fact suggests that the extent of maturity of perithecia as well as higher temperature during the rainfall may be important among the factors influencing the discharge of ascospores. For instance, 38,000 ascospores were trapped with the precipitation of 3.7 mm on August 27, and 3,000 ascospores were trapped on August 31 with as little precipitation as 0.1 mm for 30 minutes. In both cases, the temperature was about 19°C. Though it is a favorable temperature for the discharge, the extent of maturity also plays its part in the discharge.

In late September, the number of discharged ascospores suddenly decreased and they were barely discharged at all after October. However, this was not due to the disappearance of the perithecia. They existed on infected shoots under the matured state. By giving these perithecia the favorable condition for the discharge *in vitro*, ascospores were easily discharged. So the difficulty of the discharge after October may be due to the low temperature during the rainfall, and if the wet period is relatively longer, it seems still capable of the discharge of ascospores. This was proved on November 3 and 4, by a passing tropical low atmospheric pressure. The temperature increased to about 10°C and the rain lasted 2 days, though the precipitation was not particularly much, and a small number of ascospores was trapped.

In regard to the discharge of ascospores and the photoperiodism, Ingold[24] (1946) and Ingold and Dring[27] (1957) reported that the discharge in *Daldinia concentrica* took place in the night and in *Sordaria fimicola* in the daytime. In the writer's fungus, the discharge took place depending upon the rain and the temperature condition without distinction of the time.

Pycnospores of the causal fungus continue to disperse from mid-July to late October. According to the *in vitro* tests, it was discovered that pycnospores were first exuded as spore masses from pycnidia at the time of dissemination, and then they were dispersed mainly with the splash of rain. In this respect, Sato, Yokozawa and Shôji[49] (1963) reported that pycnospores of the causal fungus were never discharged from pycnidia, but they would disperse mainly with the splash of rain after the exudation from pycnidia. The results coincide well with the writer's results. Pycnospores of the chestnut blight fungus, *Endothia parasitica*, exist through the year and they are washed down by the rain (Heald and Gardner[17], 1913). Thus, it follows that some supplementary actions such as the rain is necessary for the dissemination of pycnospores.

As mentioned above, ascospores of the causal fungus are discharged with almost every rainfall from early June to early October and the dissemination of pycnospores begins from the middle of July under the same climatic conditions as in the case of ascospores. Accordingly, the current season's shoots are under the conditions likely to be infected with spores of the causal fungus through the growing season of larch, whenever it rains. Especially the most vigorously growing season of larch, from late July to late August, is the most dangerous season, because the peak of the dispersion of spores coincides with the same period. Considering the fact that 280,000 ascospores were trapped on the glass slides set at 6 definite positions through the whole

period of observation, enormous spores as the source of infection will be dispersed from heavily infected larch stands during the growing season of larch.

Because the pycnospores of the causal fungus are disseminated mainly with the splash of rain, the distance of arrival must be shorter than that of ascospores. Therefore, it seems that ascospores contribute mainly to propagate the disease from one stand (tree) to the other, and pycnospores play the rôle of increasing the degree of the damage on infected trees.

4. Conclusion.

According to the results of experiments on the discharge of ascospores *in vitro*, it was found that ascospores were discharged when perithecia absorbed water and swelled (in 100 percent of relative humidity the discharge took place). Therefore, for the discharge, it is essential to keep the perithecia under saturated or over-saturated conditions, and then temperatures become the factor deciding the hardness or ease in the discharge.

When the water was supplied adequately, the discharge took place in the range from 5 to 30°C, with the optimum of above 20°C. The alternation of 2 days' wet and 1 day's dry condition had a favorable effect on the discharge, whereas the alternation of 1 day's wet and 3 days' dry condition hindered the discharge of ascospores. Comparing the treatment A, in over-saturated and constant temperature of 25°C, with the treatment F, in over-saturated and continuously alternated temperature between 10 and 20°C, better results were observed on the discharge of ascospores in the latter. Therefore, if perithecia are absorbing water, continuous alternation of temperature of a proper range must give perithecia a more favorable condition for the discharge of ascospores.

Discharged ascospores were trapped on a glass slide set 10 mm upward in distance from perithecia. This suggests that ascospores are able to travel for a longer distance by wind.

As shown in Fig. 12, the discharge of ascospores *in vivo* was the most vigorous between mid-June and mid-September, and temperatures during the time of rainfall in the period concerned were in the range from 15 to 20°C, as shown in Fig. 11. The results coincide well with those obtained from the experiments *in vitro*.

In regard to the relation between the number of discharged ascospores and the precipitation, in general, the number increased with the increase of the precipitation, and the tendency was obvious in the earlier period of observation. After late August, a great majority of ascospores were discharged without much precipitation. This suggests that the extent of maturity of perithecia is one of the factors deciding the hardness or ease of the discharge.

After late September, especially in October, the number of discharged ascospores suddenly decreased. This may be due to lower temperatures during the rainfall and not to the disappearance of perithecia on infected shoots.

As mentioned above, ascospores of the causal fungus are discharged from June to October with the rain, and the period in which discharge is most vigorous appears between August and September: 280,000 ascospores were trapped in the whole period of observation, 240,000 of which were trapped in August and September. On the other hand, pycnospores begin to disseminate from the middle of July under the same climatic conditions with those in the discharge of ascospores. Consequently, July and August, the most vigorous growing season of larch, is the most dangerous season when infection with the causal fungus is likely to occur.

Comparing the pattern of the discharge of ascospores with of the dissemination of pycnospores, it seems that ascospores serve as the main factor propagating the disease, and pycnospores play

the rôle of increasing the degree of the damage succeeding the primary infection by the attack of ascospores.

Chater 4. Longevity of spores and their germination.

In the previous chapter, it had been found that ascospores were discharged from June to October with the rain and were most abundantly discharged in August and September. Pycnospores were also disseminated after July under the same climatic conditions as those in ascospores. Consequently, the density of spores of the fungus must increase in the most vigorously growing season of larch. Therefore, it seems very important to ascertain the longevity of spores after the liberation as well as in perithecia or pycnidia.

1. Longevity of ascospores and the effects of temperature and relative humidity upon the germination.

A. Longevity of ascospores.

a) Longevity of ascospores in perithecia.

Materials and methods.

Infected shoots bearing many perithecia were used for materials. They were collected at various times and stored in a dry and cool condition.

Ascospore suspension was prepared by crushing the perithecia in sterilized distilled water, and the germination was tested at 25°C for 20 hours by VAN TIEGHEM's cell method. The spores having the germinating ability were regarded as being alive.

Results.

Table 16. Longevity of ascospores in perithecia.

Locality of collected material	Date of collection	Date of test	Spores counted	Germinated spores	% of germination
Sôbetsu	June 30, '60	June 28, '62	41	0	0
Noboribetsu	Oct. 1, '61	〃	76	31	40.8
Tomakomai	Nov. 12, '61	〃	30	6	20.0
Horobetsu	June 11, '62	〃	74	40	54.1

Ae shown in Table 16, ascospores collected 2 years before had lost the germination ability. However, 40 percent germination was observed in ascospores collected 9 months before. No germination was observed in ascospores collected 17 months before in the separate test.

b) Longevity of discharged ascospores.

Materials and methods.

Ascospores used in the experiment were trapped on glass slides on June 4, 18, and July 11, 1962 in the yard of the station. The glass slides were left in the laboratory room and at definite intervals the germination of ascospores after 48 hours was tested in 100 percent of relative humidity at 25°C. At the same time, the temperature and the relative humidity of the room were recorded.

Results.

As shown in Table 17, in 2 sets of the test in June, the maximum and minimum temperatures were about 20°C and 15°C, respectively. In the test in July, the maximum temperature was 25°C and the minimum was 20°C. The relative humidity did not show a constant tendency, but somewhat higher values were observed in July.

Table 17. Longevity of ascospores after expulsion and the condition in the period.

a) Ascospores discharged on June 4, '62.

Date	Maximum temp. (°C)	Minimum temp. (°C)	Max. rel. hum. (%)	Min. rel. hum. (%)	Date of expt. started	Result of germination experiment
June 4	18.5	16.8	92	76		(Ascospores trapped)
〃 5	19.0	16.0	84	76	June 5	+++
〃 6	20.0	15.3	83	63		
〃 7	20.8	15.0	72	60		
〃 8	18.1	15.5	80	73	June 8	−

b) Ascospores discharged on June 18, '62.

June 18	19.0	14.0	77	75		(Ascospores trapped)
〃 19	19.0	14.0	82	68	June 19	+++
〃 20	19.8	13.5	73	56	June 20	±
〃 21	21.2	14.5	*	49	June 21	±

c) Ascospores discharged on July 11, '62.

July 11	21.0	19.3	91	81		(Ascospores trapped)
〃 12	26.0	18.7	92	74		
〃 13	26.7	22.2	77	70		
〃 14	26.7	21.9	79	72		
〃 15	25.0	21.7	76	73	July 15	3 : −, 1 : +
〃 16	24.0	21.1	85	74	July 16	4 : −

+++ Germinated more than 75%　　− Did not germinate
++ 〃 〃 50%　　* Could not observed.
+ 〃 〃 25%　　Rel. hum. 100%. 25°C. 48 hrs.
± 〃 less than 25%

Ascospores trapped on July 4 germinated with higher percentage after 24 hours, but no germination was observed after 96 hours. Those trapped on June 18 also germinated well, but only a few ascospores germinated after 48 hours or more. In ascospores trapped on July 11, the test was carried out after 96 and 120 hours. Only a few ascospores on a slide were germinated, and in 3 slides no gemination was observed after 96 hours. Germination was never observed after 120 hours.

B. Effect of temperature and relative humidity upon the germination of ascospores.

a) Effect of temperature upon the germination of ascospores.

Materials and methods.

To ascertain the effect of temperature upon the germination of ascospores, an ascospore suspension was prepared with sterilized distilled water and the germination was tested at 4～35°C by VAN TIEGHEM'S cell method. Ascospores used in the test were collected at Shiraoi, Sôbetsu, Fujishiro and Tomakomai.

Results.

Fig. 13 shows that above the half of ascospores germinated in the range from 15 to 30°C after 4 hours, with the optimum of 25°C. After 20 hours, 70 percent germination was observed even at 10°C. No germination was observed at 4 and 35～36°C after 20 hours, but when those

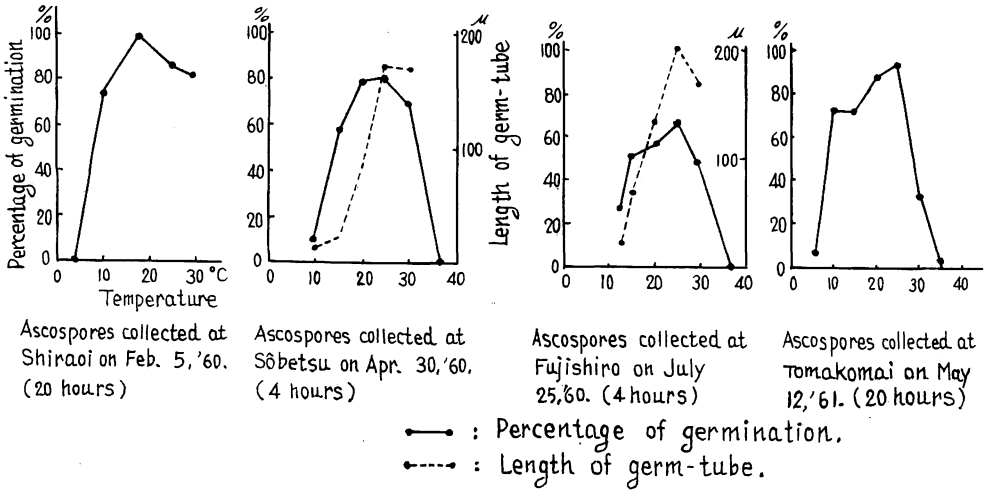


Fig. 13 Effect of temperature upon germination of ascospores.

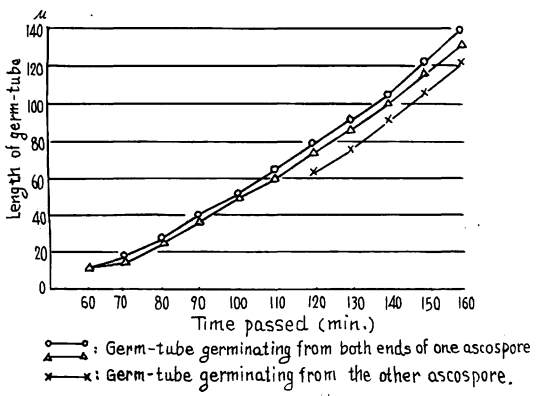


Fig. 14 Growth of germ-tubes germinating from ascospores.

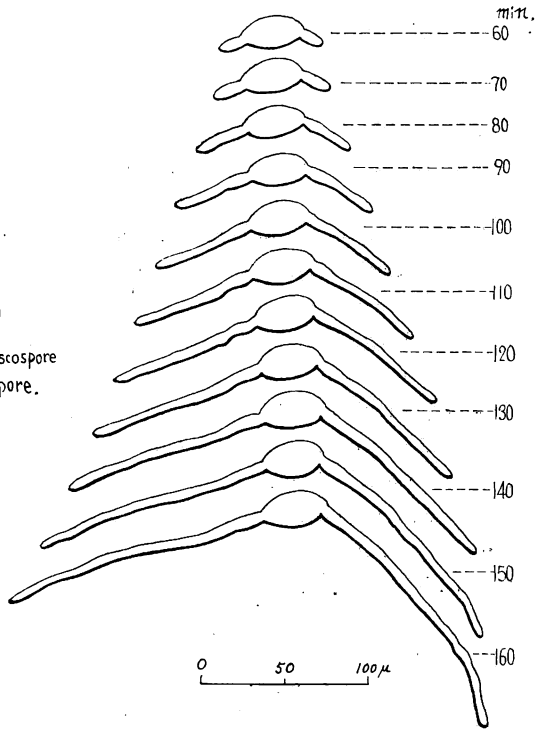


Fig. 15 Growth of germ-tubes per 10 minutes in distilled water.

materials were put at the temperature of 25°C, they germinated normally.

The growth of germ-tube was very fast. Above all, the growth at 25°C was the best, followed by the growth at 30°C.

Apart from the test, a VAN TIEGHEM's cell was fixed under the microscope and the growth of germ-tubes of definite ascospores was measured at every 10 minutes. The temperature was about 25°C. Two ascospores were observed; one germinated from both ends and another germinated from one end.

As shown in Figs. 14 and 15, the germination started within an hour and the germ-tubes grew constantly with the velocity of 13μ/10 min.

b) Effect of relative humidity upon the germination of ascospores.

Materials and methods.

To ascertain the effect of relative humidity upon the germination of ascospores, the suspension of ascospores was prepared with sterilized distilled water and then the suspension was spread on the glass slides, which were put into a desiccator. Soon after drying, glass slides were put into the desiccator of the given relative humidities (See Chapter 3, 2Aa)) and kept at 25°C. After 20 or 48 hours the germination of ascospores on the glass slide was observed. Ascospores used in the test were collected at Hayakita and Tomakomai.

Results.

Table 18. Effect of relative humidity upon germination of ascospores.

Relative humidity (%)	Over-saturated aq. solution of	Material used	Result*	Material used	Result**
100	(Water)	Hayakita,	+++	Tomakomai,	+++
98	K_2SO_4	July 9,	+++	May 12,	+++
94	KNO_3	'62	+	'61	++
92	K_2HPO_4		−		−
87	KCl		−		−

* 48 hours,　** 20 hours.

Results are shown in Table 18. It was found that in the relative humidity of 98 and 100 percent, ascospores easily germinated and in 94 percent moderately germinated, whereas no germination was observed at 92 percent or less.

2. Longevity of pycnospores and the effects of temperature and relative humidity upon the germination.

A. Longevity of pycnospores.

a) Longevity of pycnospores in pycnidia.

Materials and methods.

Infected current season's shoots bearing many pycnidia were collected at various times. They were stored in a dried and cool condition. Pycnidia were crushed in the sterilized distilled water and the suspension of pycnospores was prepared. The germination of pycnospores was tested by VAN TIEGHEM'S cell method at 25°C for 20 hours. As in the case of ascospores, pycnospores having the germinating ability were regarded as being alive.

Results.

Table 19. Longevity of pycnospores in pycnidia.

Locality of collected material	Date of collection	Date of test	Spores counted	Germinated spores	% of germination
Tomakomai	Jan. 24, '61	June 29, '62	124	0	0
Noboribetsu	Oct. 1, '61	〃	260	32	12.3
Nemuro	Nov. 21, '61	〃	94	8	8.5

As shown in Table 19, pycnospores collected in late January, 1961, had completely lost the germinating ability. On the other hand, germination was found in pycnospores collected in October, 1961, 9 months before the test.

b) Longevity of pycnospores after exudation.

Materials and methods.

Infected shoots bearing many pycnidia, collected at Chitose on August, 1962, were put into a moist chamber at 25°C and small masses of pycnospores were exuded after 20 hours from pycnidia. The suspension of pycnospores was prepared from the exuded pycnospores and spread on the glass slides, which were soon dried. The glass slides were left in the laboratory and at definite intervals the germination of pycnospores was observed by putting the glass slides into the moist chamber of 100 percent relative humidity at 25°C for 48 hours. At the same time the temperature and the relative humidity of the laboratory where the glass slides were left, were recorded.

Results.

Table 20. Longevity of pycnospores after exudation and the condition in the period.

a) Pycnospores exuded on Ang. 10, '62.

Date	Maximum temp. (°C)	Minimum temp. (°C)	Max. rel. hum. (%)	Min. rel. hum. (%)	Date of expt. started	Result of germ. expt.
Aug. 10	22.3	*	75.5	69.0	Aug. 10	+
〃 11	22.6	17.0	78.0	72.1	〃 11	+
〃 12	21.8	18.2	78.0	74.8	〃 12	−
〃 13	28.3	19.2	83.0	63.0	〃 13	−

b) Pycnospores exuded on November 24, '62.

Nov. 24	21.1	*	55.0	37.8	Nov. 24	+
〃 25	4.0	2.0	55.0	50.5	〃 25	+
〃 26	27.2	− 0.1	57.8	37.0	〃 26	±
〃 27	26.3	3.0	52.5	35.0	〃 27	−

\+ Germinated in the range of 25～50%,

± Germinated less than 25%,

− Did not germinate.

* Minimum temperature appeared before exudation. Relative humidity 100%, 25°C, 48 hrs.

Results are shown in Table 20. Germination of pycnospores was observed until 2 days after the exudation: then they completely lost the germinating ability. In the experiment started on August 10, where temperatures and relative humidites were higher than those on November 24, pycnospores maintained the ability no more than a day.

B. Effect of temperature and relative humidity upon the germination of pycnospores.

a) Effect of temperature upon the germination of pycnospores.

Materials and methods.

Pycnospores used in the test were produced on potato-sucrose agar. The method was the same as in **1** B a) in the chapter.

Results.

As shown in Fig. 16, germination of pycnospores was observed in the range from 15 to 35°C, with the optimum at 30°C, after 6 hours. Growth of germ-tubes was also the best at 30°C. Accordingly, the optimum temperature for the germination of pycnospores is about 30°C, somewhat higher than in ascospores.

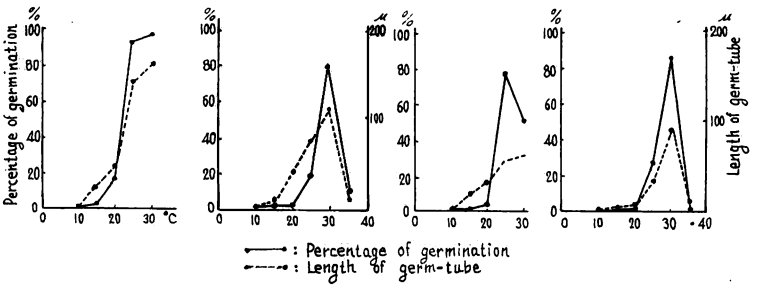


Fig. 16 Effect of temperature upon germination of pycnospores (6 hours).

b) Effect of relative humidity upon the germination of pycnospres.

Materials and methods.

Pycnospores used in the test were produced on potato-sucrose agar. The method of the test was the same as in **1** B b) in the chapter.

Results.

As shown in Table 21, pycnospores were able to germinate at 94 percent relative humidity or more after 20 hours, especially germinating easily at 98 percent or more, whereas no germination was observed at 92 percent relative humidity or less.

Table 21. Effect of relative humidity upon germination of pycnospores.

Relative humidity (%)	Over-saturated aq. solution of	Material used	Results		Material used	Result
100	(water)	Produced on	+++	+++	Produced on	+++
98	K_2SO_4	culture medi-	+	+++	culture medi-	+++
94	KNO_3	um in the	±	+	um in the	++
92	K_2HPO_4	strain	−	−	strain	−
87	KCl	KPS 43-7	−	−	SG 91 T	−

3. Discussion and conclusion.

Depending upon the difference of environmental conditions, the longevity must differ considerably between spores in perithecia or in pycnidia and those after liberation. It was ascertained that spores of the causal fungus in perithecia or in pycnidia, stored under dry and cool conditions, were able to maintain germinating ability at least 9 months. Whereas under the natural conditions spores cannot stay in perithecia or in pycnidia without dissemination, they will be disseminated by the rain, except in winter. Accrding to Bier[4] (1940), ascospores of *Hypoxylon pruinatum* maintained the germinating ability for 6 months in the laboratory room. As mentioned above, spores must maintain the germinating ability for a considerably longer period, provided they stay in perithecia or in pycnidia.

On the other hand, once spores of the causal fungus are liberated in the air, they lose the germinating ability in a shorter period. Considering the fact that the maximum period maintaining the germinating ability was about 100 hours in ascospores and about 50 hours in pycnospores *in vitro*, it seems that spores under the natural conditions must lose the ability within 2 days.

The conditions necessary for discharge of ascospores or for exudation of pycnospores of the

causal fungus are also the favorable conditions for germination of spores. For example, SATO, YOKOZAWA and SHÔJI[49](1963) reported that ascospores began to germinate within an hour and the length of germ-tubes reached 200μ in 3 hours. They were able to germinate in the range from 15 to 35°C, with the optimum at 25°C and in the range from 94 to 100 percent relative humidity. The germination of pycnospores was nearly the same as ascospores with the optimum temperature somewhat higher than in ascospores. According to UOZUMI[56] (1961), almost all ascospores germinated within 24 hours and the length of germ-tubes reached 650—1,100μ. These results accorded well with those of the writer. Further, the faster growth was observed in the extension of germ-tubes and of mycelial colonies (UOZUMI[56], 1961; YOKOTA[66], 1962-a; SATO, YOKOZAWA and SHÔJI[49], 1963). Accordingly, spores deposited on the shoots of larch are in the condition favorable for germination, and 2 days of longevity seems to be quite adequate to the propagation of the disease.

Chapter 5. Inoculation with spores, and the effect of environmental conditions upon the outbreak of the disease.

In the repeated preliminary experiments, positive results were always obtained by the inoculation with spore suspension or cultured mycelia of the causal fungus to burned wounds or incisions made on the current season's shoots of larch. Because the infection, under the natural conditions, must be done by disseminated spores and not by mycelia, the writer consistently used the spore suspension as the inoculum. Fresh wounds on the shoots to be inoculated, if necessary, were made by using a sterilized scalpel or by pulling up 3 or 4 leaves.

Potted 1-or 2-year-old larch seedlings were used as the test plants. They were brought into he laboratory room and inoculated. From then on, they were covered with polyethylene sacks to prevent the rapid drying of the inoculum. After 3 days they were put back or taken into a greenhouse. The results were observed after the lapse of definite periods.

1. Inoculation with spores.

A. Inoculation with ascospores.

Experiment-1.

Materials and methods.

The ascospore suspension was inoculated to the wounds near the top of the main and lateral shoots of the potted 1-year-old seedlings. Wounds were made with a sterilized scalpel or with a wire brush. Ascospores used in the experiments were collected in the yard of the station and at the Horobetsu nursery, respectively.

Experiment-2.

Materials and methods.

To determine whether or not the infection takes place in non-treated healthy main and succulent small shoots by inoculation with ascospores, the spore suspension was spread at the top of the main and small shoots of the potted 2-year-old seedlings. Ascospores used in the experiment were collected from infected stands at Hayakita.

Results.

Results are shown in Tables 22, 23, and 24. As can be seen in these tables, the discoloration, one of the most conspicuous symptoms of the disease, appeared after the lapse of 5 day in the case of wound inoculation, and after 2 weeks the typical symptom was observed. When ascospores were spread to the non-treated shoots, the symptom also appeared after 2 weeks, though

Table 22. Results of inoculation experiments with ascospores to the wounds made by scalpel.

Tree species	Age	No.	Period lapsed (day)			Remarks
			5	18		
				Top	Lateral*	
Larix leptolepis	1	1	Discolored	+	0/2	Typical symptom appeared after 14 days and pycnidia were abundant after 18 days.
		2		+	2/2	
L. koreana	1	1		−	1/2	
		2		+	2/2	
L. Gmelini	1	1	Discolored	+	2/2	
		2		+	1/2	
L. leptolepis (Check)	1	1		−	0/2	Healthy.
		2		−	0/2	

Inoculation: July 6.
+ : Positive result　　− : Negative result
* For example, 1/2 shows that two shoots were inoculated and 1 shoot was infected.

Table 23. Results of inoculation experiments with ascospores to the wounds made by a wire brush.

Tree species	Age	No.	28 days after inoculation		Remarks
			Top	Lateral	
L. leptolepis	1	1	+	4/4	Pycnidia appeared abundantly in diseased parts.
		2	+	4/4	
L. koreana	1	1	+	4/4	
		2	+	3/4	
L. Gmelini	1	1	+	2/4	
		2	+	2/4	
L. leptolepis (Check)	1	1	−	0/4	Healthy.
		2	−	0/4	

Inoculation: July, 24.

Table 24. Results of inoculation experiments with ascospores to non-treated shoots.

Tree species	Age	No.	Days lapsed		
			14		29
			Top	Small shoot	
L. leptolepis	2	1	−	1/8	Pycnidia appeared abundantly in diseased parts
		2	−	6/8	
		3	−	0/5	
		4	−	0/5	
Do. (Check)	2	1	−	0/ 8	Healthy
		2	−	0/10	

the percentage of infection was lower than in the case of wound inoculation.

Pycnidia were produced abundantly on the infected parts 18 days after the inoculation (Plate 6, C).

B. Inoculation with pycnospores.

Experiment-1.

Materials and methods.

A suspension of pycnospores produced on a culture medium was inoculated to the abrasion, near the top of the main and 4 lateral shoots of the potted 2-year-old seedlings. The abrasion was made with a wire brush.

Experiment-2.

Materials and methods.

(1) Pycnospore suspension was spread on the non-treated top of the main and succulent small shoots of the potted 2-year-old seedlings. They were covered with polyethylene sacks for 5 days.

(2) A suspension of pycnospores, produced on HARA-ITO's medium[16], were sprayed by using a perfume sprayer to the top of the main shoot of potted 2-year-old seedlings.

Results.

Results are shown in Tables 25, 26 and 27. From these results, it is clear that the discoloration as a symptom appeared after 4 days, and the typical symptoms were observed after 13 days when pycnospores were inoculated to wounded shoots. Spreading and spraying of pycnospore suspension to non-wounded top of shoots was also successful in causing the disease (Plate 6, A, B).

Table 25. Results of inoculation experiments with pycnospores of the wound made by wire brush.

Tree species	Age	No.	Days lapsed				Remarks
			4	9	17		
					Top	Lateral	
L. leptolepis	1	1	Faintly discolored	Clearly discolored	+	3/4	Typical symptom appeared after 13 days
		2			−	0/4	
		3			−	0/4	
		4			−	0/4	
L. Koreana	1	1		Do.	−	3/4	
		2			+	3/4	
		3			−	0/4	
		4			−	4/4	
L. Gmelini	1	1	Faintly discolored	Do.	−	0/4	
		2			−	1/4	
		3			−	2/4	
		4			+	2/4	
L. leptolepis (Check)	1	1			−	0/4	Healthy
		2			−	0/4	
		3			−	0/4	
		4			−	0/4	

Inoculation: July 7.

Table 26. Results of inoculation experiments with pycnospores.

Tree species	Age	No.	Days lapsed			
			15		19	
			Top	Small shoot	Top	Small shoot
L. leptolepis	2	1	—	1/6	—	1/6
		2	—	0/4	—	1/4
		3	—	1/4	—	2/4
		4	—	0/7	—	0/7
		5	+	2/6	+	2/6
Do. (Check)	2	1	—	0/4	—	0/4
		2	—	0/5	—	0/5

The spore suspension was sprayed on the non-wounded shoots.
Inoculation: Sept. 5.

Table 27. Results of inoculation experiments with pycnospores.

Tree species	Age	No.	Results
L. leptolepis	2	1	+
		2	—
L. koreana	2	1	—
		2	—
L. Gmelini	2	1	+
		2	—
L. leptolepis (Check)	2	1	—
		2	—

The spore suspension was sprayed on the non-wounded top of the main shoot.
Inoculation: Aug. 23.

Perithecia appeared on overwintered infected shoots which were inoculated with pycnospores in the previous year (Plate 6, D).

2. Time of inoculation influencing the infection and symptoms.

Symptoms of the disease are divided into two types, because of the difference in the degree of hardening of the tissues. The writer examined the effect of the time of inoculation upon the infection and symptoms.

Materials and methods.

(1) Wounded parts near the top of shoots of the potted 2-year-old seedlings were inoculated with a drop of pycnospore suspension on September 13. The wound was made with a wire brush.

(2) On September 25, when winter buds began to appear, non-treated or wounded parts at the top of shoots of the potted 2-year-old seedlings were inoculated with pycnospore suspension by spraying. The wound was made by pulling out 3 or 4 leaves. They were kept in a moist chamber for 3 days, and then put back to their original place.

Table 28. Relation between the time of inoculation and infection, type of symptom.

a) Inoculation: Sept. 13.

Tree species	Age	No.	Results	Symptom
L. leptolepis	2	1	1/5	Died hanging
		2	0/5	
		3	0/5	
		4	0/5	One died hanging
		5	2/5	One died straight

b) Inoculation: Sept. 25.

Tree species	Age	Treatment	No.	Results	Symptom
L. leptolepis	1	2～3 leaves at the top were pulled out.	1	0/5	Two shoots died straight
			2	2/5	
		Non-wounded	1	0/5	
			2	0/5	

Results.

Results are shown in Table 28 a) and b).

From Table 28 a) and b), it is clear that two types of symptoms resulted from the inoculation on September 13, and only wounded shoots were infected by the inoculation on September 25. The symptom in the latter case showed the type standing straight (Plate 6, E). So it seems that the time of inoculation is a factor determining the type of symptoms.

3. Effect of temperature on the infection.

To ascertain the effect of tempeature on the infection, the following experiments were carried out.

Materials and methods.

At the top of the shoots of the 10 potted 1-year-old seedlings, the suspension of pycnospores, produced on Hara-Ito's medium[16], was inoculated by spraying. Five pots were kept at 25°C for 3 days and then kept at 18°C, and the remainder was kept at 18°C soon after the inoculation. For the check, 5 pots were sprayed with distilled water and kept at 18°C.

Results.

Results are shown in Table 29.

Table 29. Effect of temperature upon infection.

Tree species	Age	Temperature after inoculation	Results	Days required to show the symptom
L. leptolepis	1	Constantly 18°C	+ 1/5	18
	1	25°C for 3 days, then 18°C	+ 4/5	13
	1	18°C (Sprayed with water)	− 0/5	

As shown in Table 29, a higher percentage of infection was observed in the pots treated at 25°C for the first 3 days. The incubation period was about 5 days shorter than those treated consistently at 18°C., and it took 18 days to show the symptom in the latter case.

4. Discussion and conclusion.

Uozumi[56] (1961) carried out inoculation experiments for the first time with the mycelium of the causal fungus to the burned wound in the shoots and ascertained the genetic relation between the perfect stage (*Guignardia laricina*) and the imperfect stage (*Macrophoma* sp.). Afterwards, the mycelia, the myeclial suspension, and spores were inoculated to fresh wounds or healthy part of the shoots of larch and a strong pathogenicity of the causal fungus was recognized (Sato, Yokozawa and Shôji[49], 1963).

From the results of the writer's inoculation experiments, it is clear that the pathogenicity of the causal fungus is very strong and only the inoculation with spores is fully sufficient to cause the infection to unwounded top of shoots, though the percentage of infection is lower

than that in wound inoculation. Positive results were obtained in the inoculation experiments with both ascospores and pycnospores to non-treated shoots, but strictly speaking, this might be true in the inoculation experiment with the spray of pycnospore suspension, because the proof that the inoculated shoots were completely non-wounded could not be given in the spread of the ascospore suspension. However, as SATO, YOKOZAWA and SHÔJI[49] (1963) reported, the pathogenicity of the causal fungus is very strong, and it seems that the causal fungus will invade from the young, succulent tissue in the top of the shoots.

As already mentioned, the symptoms of the disease are divided into two types. The time of infection may be the main factor of the phenomenon. This is clearly shown in Table 28, where typical symptoms appeared when the shoots were inoculated before the middle of September; afterwards the symptom remaining straight was observed, mainly depending upon the hardening of the tissue of the shoots.

In the time when the symptom remaining straight begins to appear, the tissue of the shoots becomes hard and winter buds are formed. Therefore, no infection is established in the non-wounded shoots by the inoculation with the spray of spore suspension, but only wounded shoots are infected. From these results, after mid-September the chance of infection will be decreased even if the discharge of spores takes place.

On the effect of temperature upon the infection or the incubation period, ABE[1)2)] (1930, 1935) ascertained that in the rice blast disease the incubation period became longer with the fall of atmospheric and soil temperature, and the number of diseased spots decreased as well with the fall of temperature. FOSTER[18] (1937) reported that, in the black rot of apples caused by *Physalospora obtusa*, the amount of leaf infection increased with the increase of the period in a moist chamber following inoculation. YAMADA and SAWAMURA[58] (1954) found that, in the canker disease of citrus caused by *Elsinoë Fawcetti*, the temperature in the incubation period was not a factor determining the length of the period, but it had a close relation to the percentage of infection, and that the temperature during the invasion of the causal fungus was closely related to the incubation period. According to KITAJIMA[36] (1958), it is said that the air temperature influenced the incubation period of black spot disease of Japanese pear caused by *Alternaria Kikuchiana*, and the lesions appeared after 24 hours at 24°C, whereas 2 or 3 days were necessary to show the symptom at 16 or 12°C.

So far as the writer's experiments are concerned, the incubation period became 5 days shorter, and a higher percentage of infection was obtained when inoculated seedlings were kept at 25°C for the first 3 days and then kept at 18°C, compared with the case under the constant temperature of 18°C.

As already mentioned, germination of pycnospores are better at 25°C than at 18°C (SATO, YOKOZAWA and SHÔJI[49], 1963; Chapter **4**, 2 B) and the same applies to the growth of the fungus colony (SATO *et al.*,[49] 1963). Therefore, this indicates that the incubation period became 5 days shorter in the seedlings kept at 25°C for 3 days soon after inoculation.

Though SATO, YOKOZAWA and SHÔJI[49] (1963) reported that the incubation period in the shoot blight disease in the Tohoku district was about 4～10 days, the writer has considered that the period in Hokkaido is about 10～14 days ascribable to the lower temperature there than in the Tohoku district.

Chapter 6. Relations between the outbreak of the disease and the effect of climatic factors, especially the wind.

From the previous chapters, the following facts have been revealed:(1) The density of airborne spores of the causal fungus reaches maximum in the vigorously growing season of larch. (2) Conditions of the temperature and relative humidity favorable for the dissemination of spores are at the same time sufficient for the germination of spores. (3) The pathogenicity of the causal fungus is very strong, and especially a higher percentage of infection was observed in wound inoculation.

On the other hand, though it has been well known experientially that the outbreak of the disease and its damage are closely related to the climatic conditions, especially the wind in the growing season of larch, and the extent of the damage is heavier in the sites blown by heavy wind during the season, few reports with detailed data regarding the effect of the wind on the outbreak of the disease have been published. So, the writer wanted to make clear the relation between the outbreak of the disease and the effect of climatic factors, especially the wind during the growing season of larch.

To accomplish this, he used two methods: (1) In the plot examination, the damage caused by the disease and the climatic factors especially the wind (mean wind velocity and blowing hours in each wind direction, etc.) in 12 (11 after 1 year) test plots established at different topographic situations (upper flat, middle slope or concave, and the bottom site) and in the automatic climatological station near the test plot No. 3, were observed. (2) In the shelter fence examination, the comparison of the damage in the inner part of the shelter fence, constructed at the upper flat, with that in the open field was carried out.

In both cases the growth of sample trees was recorded.

Based on these results, the writer wanted to make observations on the effects of the wind upon the disease.

1. General status of the examined area.

A. Situation of the examined area.

The larch plantation, planted in 1960, used for the examination is situated at Hayakita Town, neighboring Tomakomai City, Yûfutsu Gun, and belongs to Mitsubishi Mining Co., Ltd. The distance between the plantation and the coastline of the Pacific Ocean is about 16 km.

B. Topography of the examined area.

So-called "Yûfutsu waste land" spreads from the coastline, from the end of which it becomes the hill including the examined plantation. The examined area is situated at the southernmost part of the hill, where a strong sea breeze blows in summer and early autumn. The detailed topography of the examined area is shown in Fig. 17.

Though the topography of the Company's forest is, as a whole, in a hill condition, ups and downs are remarkable in the examined area. As shown in Fig. 17, the upper flat is in the central part, running from east to west, then the slope decends toward the south and north. The total area is about 4.5 ha. The altitude is between 50 and 70 m above sea-level.

The shelter fence was constructed at an upper flat, on which the larch plantation was established in 1960, and the topography around which was in a hill condition, about 300 m southward from the area of plot examination. The altitude is about 60m above the sea.

C. Soil conditions of the examined area.

The soil survey was carried out by the courtesy of Mr. M. Shiozaki on the soil in the upper

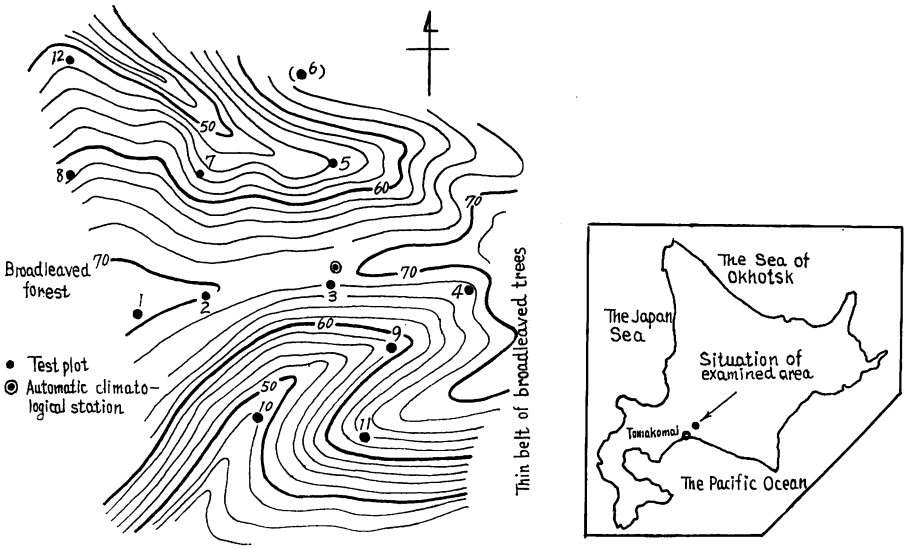
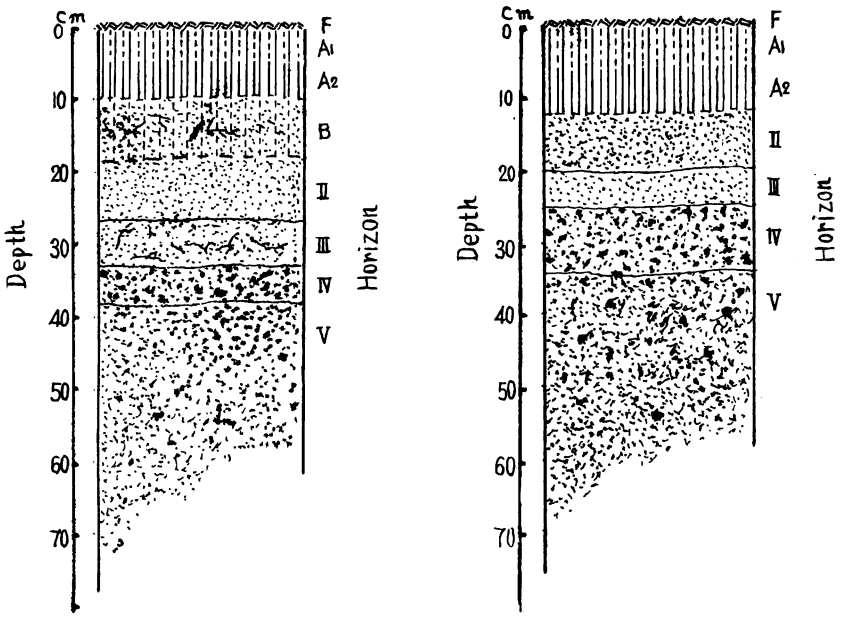


Fig. 17　Topography of surveying area, and position of test plots and automatic climatological station.



a) Upper flat　　b) Bottom site

Fig. 18　Soil profile. (By Mr. M. Shiozaki)

flat (near the plot No. 3) and the bottom site (near the plot No. 5). Results are shown in Fig. 18 and Tables 30 and 31.

From the results, it was found that no remarkable difference of the profile and the physical properties of the soil was observed between the upper flat and the bottom site. Generally it may be said that the soil condition is favorable for the larch plantation.

Table 30. Description of profile. (By Mr. M. Shiozaki)

a) Upper flat

Horizon	Depth (cm)	Color	Humus	Gravel	Texture	Structure	Compactness	Moisture condition
F	1.0							
A_1	4.0	blackish Br	very rich	—	S L	cr	loose granular	moist
A_2	5.0	〃	rich	—	S	gr	soft	〃
B	8.0	light Br	rare	pumice-stone (0.2～0.3cm)	〃		〃	〃
II	9.0	grayish Y-Br		〃	〃		〃	〃
III	6.0	blackish Br	rich	〃	〃		〃	wet
IV	4.0	grayish white		pumice-stone (0.3～0.5 cm)	〃		〃	〃
V	10(+)	〃		〃	〃		〃	〃

b) Bottom site

F	0.5							
A_1	5.0	blackish Br	very rich	—	C L	cr	loose granular	moist
A_2	6.0	〃	〃		S L	gr	soft	wet
II	8.0	grayish Y-Br	rare	pumice-stone (0.2～0.3cm)	S		〃	〃
III	5.0	blackish Br	rich	〃	〃		〃	〃
IV	9.0	grayish white		〃	〃		〃	〃
V	15(+)	〃		pumice-stone (0.3～0.5cm)	〃		〃	〃

Table 31. Physical properties of soil. (By Mr. M. Shiozaki)

Surveyed sites	Horizon	Fine soil %	Gravel %	Root %	Moisture content of fresh soil %	Air minimum %	Water holding capacity %	Total soil porosity %	Fine soil porosity %	Rough soil porosity %
Upper flat	A_1	13.8	0	1.7	40.5	25.5	18.5	84.5	32.8	61.7
	A_2	27.5	0	1.4	35.9	14.5	20.7	71.1	26.9	44.2
	B	33.1	0.9	1.7	31.6	14.0	18.7	64.3	18.6	45.7
Bottom site	A_1	11.7	0	1.1	48.6	18.4	20.2	87.2	33.7	53.5
	A_2	17.1	0	1.8	48.4	15.0	17.7	81.1	31.7	49.4
	II	30.0	0.6	3.8	32.2	16.3	17.1	65.6	20.7	44.9

2. Methods and results of the examination on climatic factors.

A. In the plot examination.

Because the intensity of the wind is remarkably different with the variation of topographic conditions, 12 (11 after the next year) test plots were established in 1961 according to the difference of the topography as shown in Fig. 17. Each test plot contained 20 numbered larch trees.

By using anemometers of Robinson's type, mean wind velocity in each test plot was odserved from simultaneous observation in 10-minute-intervals. The observation was carried out many times during the growing season of larch (from June to September, 1962). Results are shown in Table 32.

Table 32. Mean wind velocity in 10-minute-interval by simultaneous observation in each test plot throughout the growing season of larch. (1962)

Position of plots	No. of plots	Repeat of observ.	S E		S		NW	
			Mean	Ratio	Mean	Ratio	Mean	Ratio
Upper flat	1	41	4.2	0.75	1.4	0.45	1.2	0.32
	2	44	5.7	1.02	1.4	0.45	4.1	1.08
	3	44	5.6	1.00	3.1	1.00	3.8	1.00
	11	44	4.5	0.80	3.0	0.97	3.7	0.97
Middle slope or Concave	4	44	0.9	0.16	2.1	0.68	1.5	0.39
	7	43	1.0	0.18	0.8	0.26	1.3	0.34
	8	42	0.8	0.14	0.1	0.03	1.3	0.34
	12	42	1.6	0.29	1.0	0.32	1.2	0.32
Bottom site	5	43	1.3	0.23	1.3	0.42	1.4	0.37
	9	44	1.1	0.20	1.4	0.45	1.4	0.37
	10	41	2.5	0.45	1.9	0.61	1.3	0.34

As shown in Table 32, the wind was always stronger in the upper flat than in the other sites, during the time of observation. The rate of the intensities of the wind in each test plot, observed simultaneously in 10-minute-intervals, was calculated on those of the test plot No. 3, situated near the automatic climatological station. The rate of the intensity in the upper flat was several times stronger than those in the middle slope or the bottom site in every wind direction, throughout the growing season of larch.

Table 33. Comparison of wind velocity recorded by automatic climatological station with those in test plot No. 3. (1962)

Date of observation	Test plot No. 3			Auto-recording climatograph			Ratio of wind velocity
	Interval of observation	Mean wind velocity (m/sec)	Repeat of observation	Interval	Wind direction	Mean wind velocity (m/sec)	
25/VI	1530～1600	1.7	2	1530～1600	NW	17.3	*
24/IX	1200～1500	4.3	9	1200～1500	NW	19.2	≒ 1/4
2/VII	1530～1540	2.4	1	1530～1540	S E	12.0	*
9/VII	1230～1535	6.0	5	1220～1535	S E	20.6	≒ 1/3
23/VII	1230～1500	6.6	8	1230～1500	S E	20.2	≒ 1/3
13/VIII	1300～1500	5.6	6	1300～1500	S E	17.0	≒ 1/3
20/VIII	1345～1445	5.1	3	1345～1445	S E	13.3	≒ 1/3
3/IX	1320～1430	4.5	4	1320～1430	S E	13.6	≒ 1/3
16/VII	1250～1500	3.1	6	1250～1500	S	10.7	≒ 1/3

* Omitted because of the less number of repeat.

To obtain the detailed climatic factors, an automatic climatological station was set up in May, 1962, at the point marked ◎ in Fig. 17 (Plate 7, C). The records of wind directions, mean wind velocities (10-minute-interval), temperatures and precipitations were obtained during the growing season of larch in the year of 1962 and 1963.

Because the wind velocity observed in the station is the mean of the 10-minute-interval, mean wind velocities in plot No. 3 were compared with those recorded by the automatic climatological station in the same intervals with the simultaneous observation in each test plots, and Table 33 was obtained. Table 33 shows that the wind velocities in plot No. 3 were about 3 times weaker in the southern wind and about 4 times weaker in the northern wind than those in the station.

From the records of the automatic climatological station, it is clear that the prevalent winds

Table 34. Frequency of wind direction having mean wind velocity in each month during the growing season of larch (day).

a) In the year 1962.

Month	Wind direction					
	E	S E	S	W	NW	N
6		11	10	3	5	
7		24			7	
8	4	17	1		7	2
9	5	8	8	4	5	
Total	9	60	19	7	24	2

b) In the year 1963.

Month	Wind direction							
	N E	E	S E	S	S W	W	NW	N
6	1	3	4	13	4	1	2	5
7			8	14	7	2		1
8			18	8		1	6	
9		1	6	2			7	
Total	1	4	36	37	11	4	15	6

Table 35. Frequency of wind direction having the maximum wind velocity in each month during the growing season of larch (day).

a) In the year 1962.

Month	Wind direction					
	E	S E	S	W	NW	N
6		11	8	2	8	
7		27			4	
8		21	4	1	5	
9	1	8	10	6	3	2
Total	1	67	22	9	20	2

b) In the year 1963.

Month	Wind direction							
	NE	E	SE	S	SW	W	NW	N
6		2	4	12	4		2	7
7			8	15	6	1		1
8			12	14		1	4	
9			2	6			7	
Total	0	2	26	47	10	2	13	8

are southeast and south, followed by north, as shown in Tables 34, 35 and Figs. 19 and 20. These tables and figures show further that the wind in 1963 was far weaker than in 1962, especially in August and September.

From the records on the wind in the automatic climatological station, the wind runs, the mean wind velocities and the blowing hours were calculated in each month and in each wind direction. The calculation was done as follows:

$$\text{Wind run}=\frac{(\text{Total of mean wind velocities in 10-min.-interval})\times 10\times 60}{1000}\ \text{km}$$

$$\text{Mean wind velocity}=\frac{\text{Total of mean wind velocities}}{\text{Total of blowing hours}\times 6}\ \text{m/sec}$$

As the result, the wind directions and corresponding mean wind velocities in 10-minute-

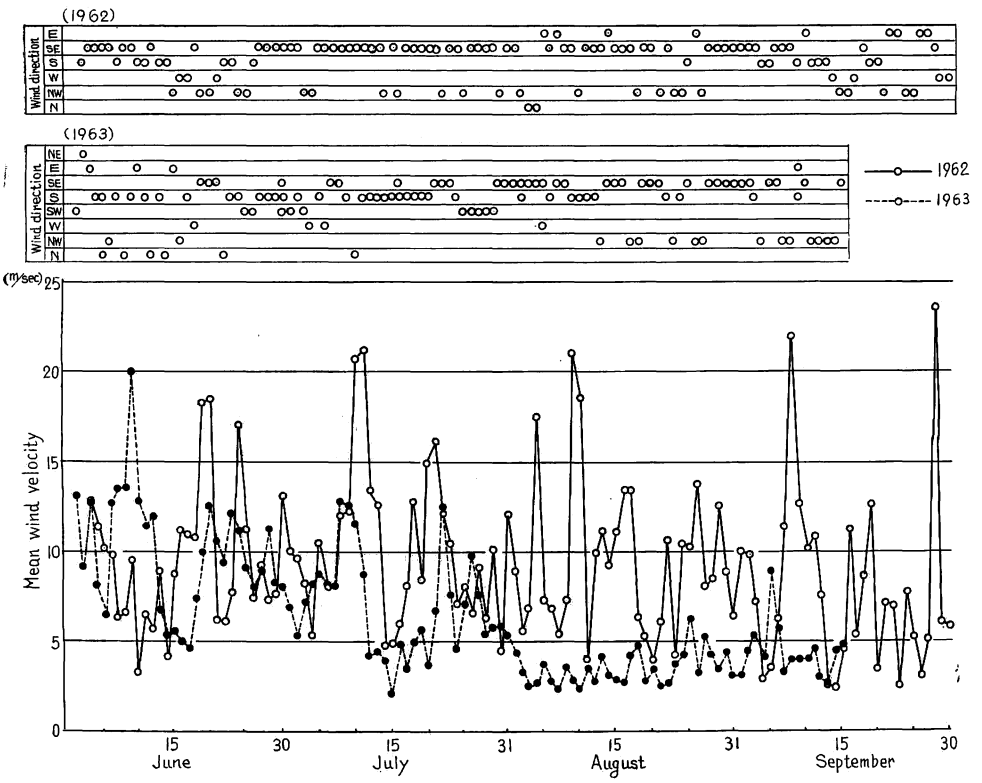


Fig. 19 Mean wind velocity and wind direction.

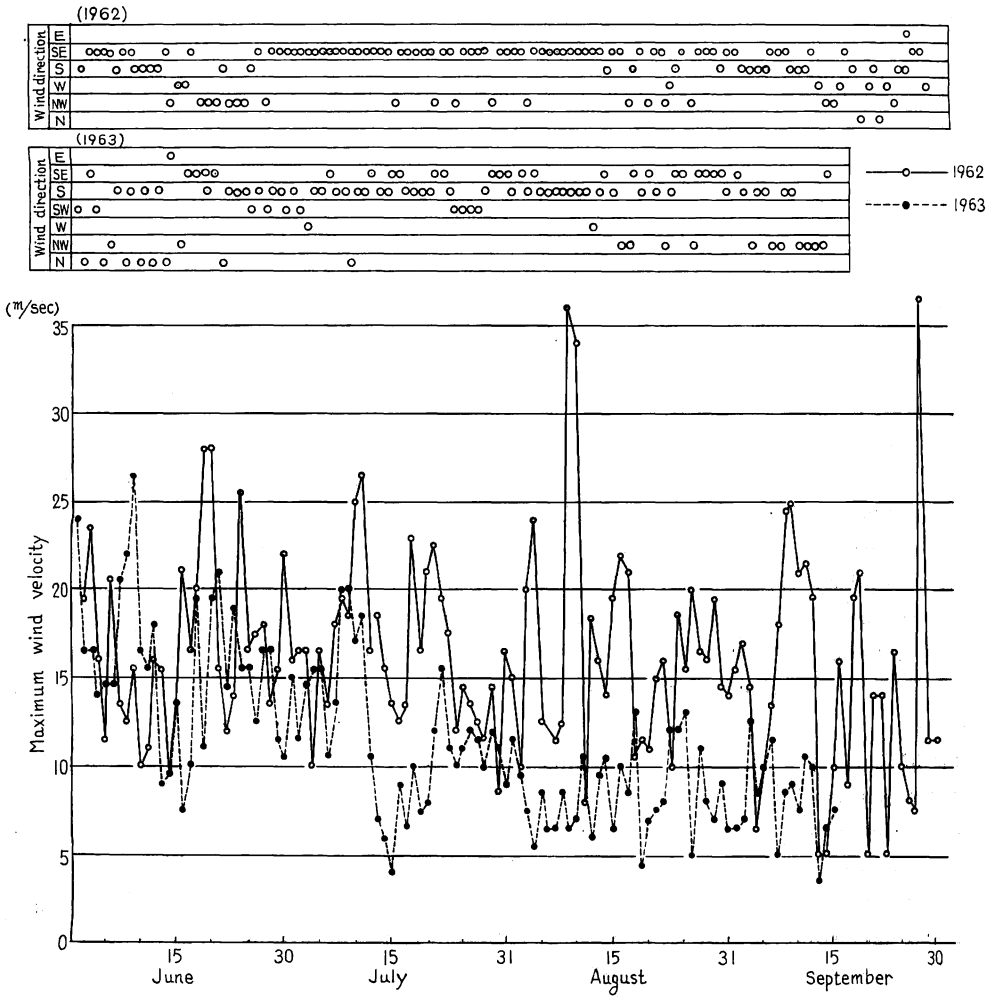


Fig. 20 Daily maximum wind velocity and wind direction.

Table 36. Sum of wind run and mean wind velocity in each wind direction obtained from the automatic climatological station during the growing season of larch (June～September).

a) In the year 1962.

Wind direction	Blowing hour	Sum of wind run (km)	Mean wind velocity (m/sec)	Wind direction	Blowing hour	Sum of wind run (km)	Mean Wind velocity (m/sec)
E	67	1,404.6	5.8				
S E	1,471	68,872.2	13.0				
S	213	8,893.2	11.6	southern	1,751	78,052.2	12.3
S W	20	286.8	4.0				
W	107	4,391.4	11.4				
N W	545	22,842.6	11.6				
N	28	514.2	5.1	northern	584	23,533.8	11.2
N E	11	177.0	4.5				

b) In the year 1963.

Wind direction	Blowing hour	Sum of wind run (km)	Mean wind velocity (m/sec)	Wind direction	Blowing hour	Sum of wind run (km)	Mean wind velocity (m/sec)
E	62	1,398.0	6.3				
S E	605	11,047.2	5.1				
S	954	26,758.2	7.8	southern	1,565	37,900.2	6.7
S W	6	94.8	4.4				
W	63	892.8	3.9				
N W	249	4,012.8	4.5				
N	29	1,112.4	10.7	northern	287	5,379.0	5.2
N E	9	253.8	7.8				

intervals and wind runs in each month were obtained as shown in Table 36.

Because of the fact that wind runs and blowing hours for the southeast, south and northwest winds are considerably great as shown in Table 36, converted values of winds in test plot No. 3 were calculated from the table as 1/3 in southern winds and 1/4 in northern winds, based on the results of Table 33. The converted results were used for the calculation of data for winds in each test plot, based on the rate of the intensity of winds in Table 32. As the result of the calculation, the monthly sum of wind run, the monthly mean wind velocity and the blowing hours in each test plot, are obtained as shown in Table 37 and Fig. 21, and Table 38, respectively.

Table 37 and Fig. 21 show that total wind run in test plot No. 2 and No. 3, situated in the upper flat, amounted to over 30,000 km, followed by over 20,000 km in No. 1 and No. 11, throughout the whole period of observation. For these high values in the upper flat, 1/3 or more smaller wind runs were calculated in the other test plots. In regard to the mean wind velocity and the blowing hours during the growing season, it was shown in Table 38 that southern wind above 3 or 4 m/sec blew for 1,700 hours in the plots situated in the upper flat in 1962. On the other hand, in the test plots situated in lower positions, the mean wind velocity was almost below 1 m/sec.

The temperatures and the precipitations were observed by the automatic climatological station for 2 years. These results are shown in Table 39 and Fig. 22.

Table 39 shows the sum of daily mean temperature (mean of temperatures every 2 hours in a day), the sum of the daily maximum and minimum temperature and their mean temperatures. In the sum of the maximum temperatures, no remarkable difference was observed between both years, whereas in the sum of the minimum temperatures there existed some differences in both years, especially in September.

In addition, in 1962 the sum of temperatures in each test plot measured between 9 and 10 A. M. every day is shown in Table 40. These values had not a great significance, but only a reference, because the measurement was made only once a day.

Table 41 shows the precipitation in each month. Values in 1963 were observed at the Tomakomai Meteorological Station, situated near the examined area, because of a malfunction in the precipitation gauge. From Table 41, it seemed that there was sufficient precipitation to the dissemination of spores of the causal fungus, though the frequncy of rainfall and the total precipitation were greater in 1962 than in 1963.

Table 37. Monthly sum of wind run in each test plot (km).

a) In the year 1962.

Test plot	June			July			August			September			Total		
	SE, S, SW	NW, N	Total	SE, S, SW	NW	Total	SE, S	NW, N	Total	SE, S, SW	NW, NE	Total	SE, S, SW	NW, N, NE	Total
1	4,771	569	5,340	6,264	374	6,638	4,909	599	5,508	3,569	341	3,910	19,513	1,883	21,396
2	6,488	1,920	8,408	8,519	1,261	9,780	6,676	2,022	8,698	4,854	1,150	6,004	26,537	6,353	32,890
3	6,361	1,778	8,139	8,352	1,168	9,520	6,545	1,872	8,417	4,759	1,065	5,824	26,017	5,883	31,900
11	5,089	1,725	6,814	6,682	1,133	7,815	5,236	1,816	7,052	3,807	1,033	4,840	20,814	5,707	26,521
4	1,018	693	1,711	1,336	456	1,792	1,047	730	1,777	761	415	1,176	4,162	2,294	6,456
7	1,145	605	1,750	1,503	397	1,900	1,178	636	1,814	857	362	1,219	4,683	2,000	6,683
8	891	605	1,496	1,169	397	1,566	916	636	1,552	666	362	1,028	3,642	2,000	5,642
12	1,845	569	2,414	2,422	374	2,796	1,898	599	2,497	1,380	341	1,721	7,545	1,883	9,428
5	1,463	658	2,121	1,921	432	2,353	1,505	693	2,198	1,095	394	1,489	5,984	2,177	8,161
9	1,272	658	1,930	1,670	432	2,102	1,309	693	2,002	952	394	1,346	5,203	2,177	7,380
10	2,862	605	3,467	3,758	397	4,155	2,945	636	3,581	2,142	362	2,504	11,707	2,000	13,707

b) In the year 1963.

Test plot	June			July			August			September			Total		
	S, SW	NE	Total	SE, S	N	Total	SE, S	NW	Total	SE, S	NW, N NE	Total	SE, S SW	NW, N NE	Total
1	2,644	17	2,661	4,621	68	4,689	1,505	165	1,670	705	180	885	9,475	430	9,905
2	3,596	58	3,654	6,284	231	6,515	2,047	556	2,603	959	608	1,567	12,886	1,453	14,339
3	3,525	54	3,579	6,161	214	6,375	2,007	515	2,522	940	563	1,503	12,633	1,346	13,979
11	2,820	52	2,872	4,929	208	5,137	1,606	500	2,106	752	546	1,298	10,107	1,306	11,413
4	564	21	585	986	83	1,069	321	201	522	150	220	370	2,021	525	2,546
7	635	18	653	1,109	73	1,182	361	175	536	169	191	360	2,274	457	2,731
8	494	18	512	863	73	936	281	175	456	132	191	323	1,770	457	2,227
12	1,022	17	1,039	1,787	68	1,855	582	165	747	273	180	453	3,664	430	4,094
5	811	20	831	1,417	79	1,496	462	191	653	216	208	424	2,906	498	3,404
9	705	20	725	1,232	79	1,311	401	191	592	188	208	396	2,526	498	3,024
10	1,586	18	1,604	2,772	73	2,845	903	175	1,078	423	191	614	5,684	457	6,141

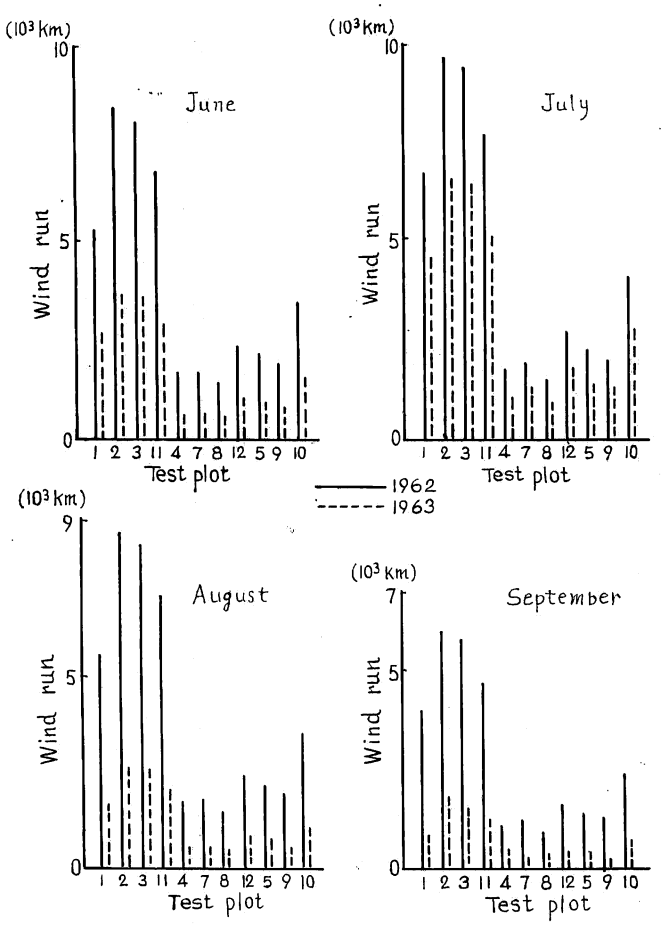


Fig. 21　Wind run in each test plot.

Table 38.　Mean wind velocity (m/sec) in each month and wind direction in each test plot and blowing hour.

a)　In the year 1962.

Test plot	June		July		August		September		Total	
	SE, S, SW	NW, N	SE, S, SW	NW	SE, S	NW, N	SE, S, SW	NW, NE	SE, S, SW	NW, N, NE
1	2.9	1.2	3.2	0.7	3.3	0.9	3.5	0.7	3.2	0.9
2	3.9	4.2	4.4	2.5	4.5	3.0	4.8	2.5	4.3	3.0
3	3.8	3.9	4.3	2.3	4.4	2.8	4.7	2.3	4.2	2.8
11	3.0	3.8	3.4	2.2	3.5	2.7	3.8	2.2	3.4	2.7
4	0.6	1.5	0.7	0.9	0.7	1.1	0.8	0.9	0.7	1.1
7	0.7	1.3	0.8	0.8	0.8	1.0	0.8	0.8	0.8	1.0
8	0.5	1.3	0 6	0.8	0.6	1.0	0.7	0.8	0.6	1.0
12	1.1	1.2	1.2	0.7	1.3	0.9	1.4	0.7	1.2	0.9
5	0.9	1.4	1.0	0.9	1.0	1.0	1.1	0.9	1.0	1.0
9	0.8	1.4	0.9	0.9	0.9	1.0	0.9	0.9	0.8	1.0
10	1.7	1.3	1.9	0.8	2.0	1.0	2.1	0.8	1.9	1.0
Blowing hour	466	128	543	141	414	185	281	130	1,704	584

b) In the year 1963.

Test plot	June		July		August		September		Total	
	S, SW	NE	SE, S	N	SE, S	NW	SE, S	NW, N, NE	SE, S, SW	NW, N, NE
1	2.6	0.8	2.0	1.1	0.9	0.4	1.4	0.4	1.7	0.4
2	3.5	2.7	2.7	3.8	1.2	1.2	1.9	1.3	2.2	1.5
3	3.4	2.5	2.6	3.5	1.2	1.1	1.9	1.2	2.2	1.4
11	2.7	2.4	2.1	3.4	1.0	1.1	1.5	1.2	1.8	1.4
4	0.5	1.0	0.4	1.4	0.2	0.4	0.3	0.5	0.4	0.5
7	0.6	0.9	0.5	1.2	0.2	0.4	0.3	0.4	0.4	0.5
8	0.5	0.9	0.4	1.2	0.2	0.4	0.3	0.4	0.3	0.5
12	1.0	0.8	0.8	1.1	0.3	0.4	0.6	0.4	0.6	0.4
5	0.8	0.9	0.6	1.3	0.3	0.4	0.4	0.4	0.5	0.5
9	0.7	0.9	0.5	1.3	0.2	0.4	0.4	0.4	0.4	0.5
10	1.5	0.9	1.2	1.2	0.5	0.4	0.9	0.4	1.0	0.5
Blowing hour	287	6	670	17	467	133	141	131	1,565	264

Table 39. Monthly sum of temperature obtained from observation by automatic climatological station.

Year	Month	Sum of mean temperature (°C)	Mean (°C)	Sum of max. temperature (°C)	Mean (°C)	Sum of min. temperature (°C)	Mean (°C)	Total day
1962	6	433.6	14.5	549.0	18.2	328.0	10.9	30
	7	587.3	18.9	689.5	22.2	510.8	16.5	31
	8	557.0	18.0	642.6	22.9	487.1	17.4	28*
	9	507.0	16.9	610.0	20.3	407.3	13.6	30
	Total	2,084.9	17.5	2,491.1	20.9	1,733.2	14.6	119
1963	6	415.1	13.8	526.7	17.6	307.7	10.6	30
	7	481.7	17.8	569.6	21.1	382.4	14.2	27**
	8	651.7	21.0	748.2	24.1	575.9	18.6	31
	9	457.9	15.3	570.3	19.0	316.0	10.5	30
	Total	2,006.4	17.0	2,414.8	20.5	1,582.0	13.4	118

* Could not be observed 3 days.

** Could not be observed 4 days.

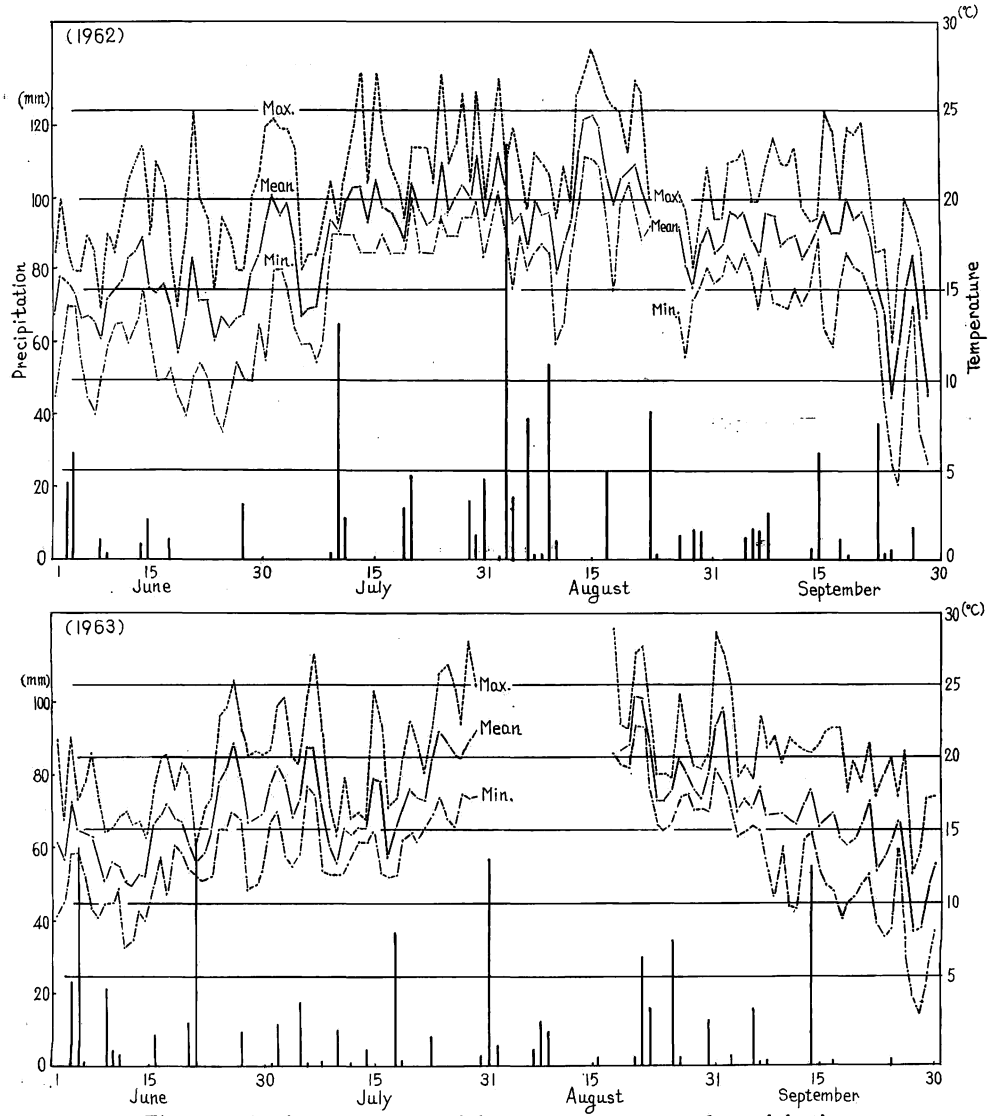


Fig. 22　Maximum, mean, minimum temperature and precipitation.

Table 40.　Sum of temperature (°C) during the growing season in each test plot. (1962)

Test plot	June		July		August		September		Total		Mean
	Days	Sum	Days	Sum	Days	Sum	Days	Sum	Days	Sum	
1	27	525.0	24	519.5	29	663.0	13	465.5	103	2,172.5	21.1
2	〃	476.0	〃	506.0	〃	657.5	〃	451.5	〃	2,091.0	20.3
3	〃	450.0	〃	492.0	〃	630.0	〃	434.0	〃	2,006.0	19.5
11	〃	469.0	〃	494.0	〃	632.0	〃	438.0	〃	2,033.0	19.7
4	〃	464.5	〃	492.0	〃	600.0	〃	408.0	〃	1,964.5	19.1
7	〃	485.5	〃	508.0	〃	639.0	〃	438.5	〃	2,071.0	20.1
8	〃	487.0	〃	505.5	〃	630.0	〃	412.0	〃	2,034.5	19.8
12	〃	492.5	〃	499.5	〃	625.0	〃	427.0	〃	2,044.0	19.8
5	〃	486.5	〃	496.0	〃	635.5	〃	436.5	〃	2,054.5	19.9
9	〃	487.0	〃	507.0	〃	635.0	〃	421.5	〃	2,050.5	19.9
10	〃	504.5	〃	510.0	〃	655.5	〃	449.0	〃	2,119.0	20.6

Table 41. Precipitation in each month.

Year	Month	Rainy days more than 1 mm	Precipitation (mm)	Year	Month	Rainy days more than 1 mm	Precipitation (mm)
1962	6	9	94.5	1963*	6	8	171.7
	7	8	162.9		7	8	176.5
	8	16	335.3		8	8	103.4
	9	12	135.0		9	9	124.2
	Total	45	727.7		Total	33	575.8

* Precipitation in 1963 was observed at the Tomakomai Meteorological Station, Tomakomai.

The relation between the precipitation and the range of temperature is shown in Fig. 22. Further by reading in detail the records of precipitation and the range of the temperature where the rainfall lasted, spore dissemination could be estimated based on the results obtained in Chapter

Table 42. Estimate of dissemination of spores during the growing season of larch based on the precipitation and temperature range (a part).

a) In the year 1962

Date	Time	Precipitation (mm)	Total precipitation (mm)	Temperature (°C) Min.	Temperature (°C) Max.	Dissemination
Ⅷ. 3	000～	113.5	131.5	17.8	25.0	++
4	700	18.0				
6	200～1300	40.5	40.5	16.5	18.8	+++
8	2300～	1.5	52.5	18.0	22.5	+++
9	900	51.0				
10	500～ 900	6.0	6.0	17.0	20.0	+
17	1100～1300	3.0	3.0	21.0	21.2	+
17	1600～2300	22.0	22.0	18.8	20.5	+++
23	1000～1400	28.0	28.0	19.0	19.0	+++
27	700～1200	5.0	5.0	20.0	20.5	+++
29	700～1500	5.5	5.5	15.0	16.1	++
30	300～ 700	2.0	2.0	15.8	18.0	+
30	1300～1500	5.5	5.5	18.2	19.0	++

b) In the year 1963

Ⅷ. 3	1200～1800	15.2	15.2	20.5	22.0	+++
13	1200～2300	1.3	1.3	21.5	28.5	+
14	2000～		40.7	20.5	22.0	+++
15	1500					
16	900～1900	1.4	1.4	21.0	25.0	±
21	600～2000	4.0	4.0	19.0	23.0	+
30	1000～2200	17.6	17.6	19.0	23.0	+++
31	000～1700	19.7	19.7	17.0	20.2	+

Dissemination of spores was estimated based on the result obtained from Chapter 3. Precipitation in 1963 was observed at Tomakomai Meteorological Station.

3. A part of the results are shown in Table 42, which gave the estimate that abundant spores sufficient for infection to larch shoots were disseminated in both years.

B. In the shelter fence examination.

To determine the effect of shelter fence upon a windbreak and the escape from the disease, a bamboo shelter fence of 4 m in height and 30 × 30 m in size was built at the upper flat in the fall of 1961 (Plate 7, A, B). At the inside of the fence, healthy larch seedlings were planted, where heavily damaged larch trees planted in 1960 had been removed.

The infected parts of shoots of the remainder (planted in 1960) were cut off in early May, 1963, before the beginning of the discharge of ascospores, thus a healthy situation was established at the inside of the fence. For the check plot, healthy seedlings were planted in the open field of 20 × 20 m on the same upper flat. The distance from the fence to the check plot was about 50 m.

Nine anemometers of Robinson's type were set up at a little above the tree height at the inside of the fence and the middle part of the check plot. The wind velocity was recorded from simultaneous observations in 10-minute-intervals several times during the growing season of larch in 1963. The results are shown in Table 43 and Fig. 23.

Table 43. Mean wind velocity in 10-minute-interval at 9 points in the inner part of the shelter fence and in the open field. (1963)

	Points in the shelter fence									Open field
	1	2	3	4	5	6	7	8	9	
Mean wind velocity	0.4	2.1	2.3	1.6	1.0	1.2	1.5	1.4	1.8	4.8
Ratio	0.1	0.4	0.5	0.3	0.2	0.3	0.3	0.3	0.4	1.0

Observed on May 7; wind direction, SW; Interval of observation, 1000～1450; Repeat of observation 15.

	Points in the shelter fence									Open field
	1	2	3	4	5	6	7	8	9	
Mean wind velocity	2.1	2.2	1.8	1.0	1.3	2.4	2.4	2.5	1.8	3.9
Ratio	0.5	0.6	0.5	0.3	0.3	0.6	0.6	0.6	0.5	1.0

Observed on Sept. 12; Wind direction, NW; Interval of observation, 1300～1420; Repeat of observation, 3.

It is clear that there exists a conspicuous difference in the wind velocities between the inside of the fence and in the check plot, showing almost a half or less velocities at the inside of the fence than in the check plot. On the distribution of wind velocities in the inner part of the fence, somewhat weaker wind velocities were observed at leeward points.

The temperature at the middle of the inside of the fence and the check plot was measured daily between 9 and 10 A. M. in 1962. The thermometer was set at 50 cm above the ground. The results are shown in Table 44.

There existed a monthly difference of 20°C or more, showing the condition for the growth of larch was more favorable at the inside of the fence than in the open field.

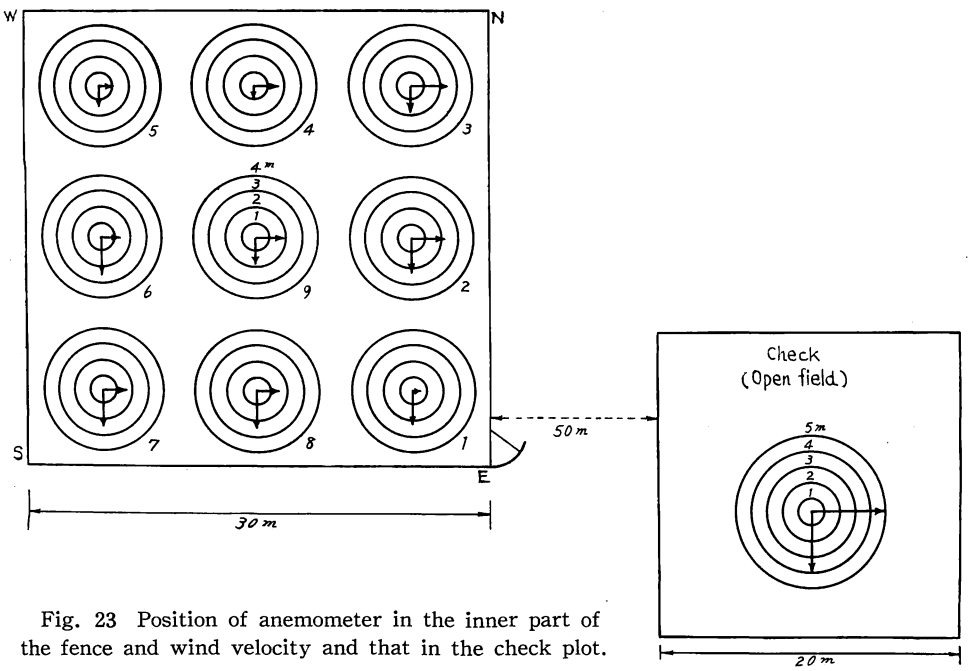


Fig. 23 Position of anemometer in the inner part of the fence and wind velocity and that in the check plot.

Table 44. Sum of temperature (°C) in the inner part of the shelter fence and in the open field. (1963)

Plot	June		July		August		September		Total		Mean
	Days	Sum	Days	Sum	Days	Sum	Days	Sum	Days	Sum	
Shelter	26	487.5	24	518.0	29	646.5	23	451.0	102	2,103.0	20.6
Open	26	464.5	24	497.5	29	622.0	23	428.5	102	2,012.5	19.7

3. Outbreak of the disease and growth of larch.

On the outbreak of the disease and the progress of the damage, the 20 numbered larch trees in each test plot were examined in October. In 1961 the number of infected in the top, lateral and succulent small shoots were recorded. In 1962 and 1963, because of the increase of the number of current season's shoots, the top shoot and the upper 5 lateral shoots of each numbered sample tree were examined according to the method by IGARASHI[21] (1963-a) whether or not they were infected.

This method for the examination of the damage is based on the consideration that whether the upper part of the tree is healthy or not is of most importance in the practical point of view. At the same time, to show the damage as a whole, indices* according to ITO's sugges-

*. The indices are given to each tree by the following basis:
0 : Healthy……No infected shoot is found.
1 : Slightly damaged……A few infected shoots are found.
3 : Moderately damaged……Many infected shoots are found.
5 : Heavily damaged……The damage is severe, rusulting in a malformation of the crown.
Mean of degree of damage=(0×a+1×b+3×c+5×d)/N,
where, N=a+b+c+d=Total number of examined trees.

tion[34] (1961-c) were given to each test tree.

A. In the plot examination.

The degree of the damage in each test plot shown by indices is given in Table 45.

Table 45. Relation between the transition of degree of damage and the position of test plots. (1961～1963)

Position	Test plot	Degree of damage												Mean of degree of damage		
		0			1			3			5					
		'61	'62	'63	'61	'62	'63	'61	'62	'63	'61	'62	'63	1961	1962	1963
Upper flat	1	1			6	3	3	13	12	11		5	5	2.3	3.2	3.1
	2				3		1	15	2	12	2	17	6	2.9	4.8	3.5
	3				2			13	2	7	5	18	10	3.3	4.8	4.2
	11		1	1	9	1	4	7	5	7	4	11	4	2.5	3.9	2.8
Middle slope or concave	4	4	2	1	15	13	13	1	5	6				0.9	1.4	1.6
	7	6	3		12	6	14	2	9	4		2	2	0.9	2.2	1.8
	8	5	3		13	6	10	2	11	7				1.0	2.0	1.6
	12	3	2		13	12	19	4	6	1				1.3	1.5	1.1
Bottom site	5	6	1	2	14	16	15		3	3				0.7	1.3	1.2
	9	3		1	13	4	8	4	12	8		4	3	1.3	3.0	2.4
	10	9	2	3	10	8	10	1	9	5			2	0.7	1.8	1.8

As shown in Table 45, in the test plots situated on the upper flat in the fall of 1961, 2 years after the planting, scarcely any healthy trees were found, and a half or more were moderately or heavily damaged. However, in the test plots situated lower than the middle slope, there were no heavily damaged trees, almost all trees were either slighly damaged or healthy. In 1962, the damage in the test plots situated in the upper flat became very severe, especially

Table 46. Damages in each test plot. (Planted on May, 1960)

a) Damages at the time of establishment. (Oct. 24, 1961)

Position of test plot	No. of test plot	Healthy tree	Top shoot			Lateral shoot		
			Top	Middle	Small shoot	Top	Middle	Small shoot
Upper flat	1	1	13	2	71	99	6	8
	2	0	17	4	108	210	13	68
	3	0	17	11	142	245	16	95
	6	0	8	24	66	219	4	32
	11	0	11	9	117	153	10	140
Middle slope or concave	4	4	1	9	32	34	12	9
	7	6	2	4	22	40	0	7
	8	5	2	6	17	27	11	27
	12	3	4	3	27	45	6	18
Bottom site	5	6	0	0	24	20	4	11
	9	3	4	5	75	61	15	75
	10	9	1	2	27	27	3	20

One plot contains 20 larch trees.

The column of top and small shoot shows the number of infected shoots, and that of middle shows the number of infected parts.

Table 46. (Continued)

b) Percentage of infection in each test plot. (%) (1962)

No. of test plot	Position of shoots							
	Top shoot				Lateral shoot			
	Top, Middle	%	Small shoot	%	Top, Middle	%	Small shoot	%
1	14/20	70.0	34/43	79.1	89/100	89.0	11/16	68.8
2	23/19	121.1	72/72	100.0	92/95	96.8	61/63	96.8
3	23/20	115.0	84/84	100.0	102/100	102.0	57/57	100.0
11	16/18	88.9	67/72	93.1	71/90	78.9	42/44	95.5
4	6/20	30.0	91/211	43.1	31/100	31.0	76/193	39.4
7	9/20	45.0	46/143	32.2	52/100	52.0	52/103	50.5
8	12/20	60.0	85/222	38.3	34/100	34.0	58/158	36.7
12	5/20	25.0	44/201	21.9	25/100	25.0	34/122	27.9
5	2/20	10.0	74/276	26.8	23/100	23.0	153/439	34.9
9	9/20	45.0	144/222	64.9	56/100	56.0	200/283	70.7
10	9/20	45.0	60/184	32.6	44/100	44.0	65/148	43.9

c) Ditto. (1963)

1	11/19	57.9	49/78	62.8	82/95	86.3	24/40	60.0
2	16/19	84.2	72/101	71.3	61/95	64.2	121/171	70.8
3	15/17	88.2	81/97	83.5	78/85	91.8	35/44	79.5
11	12/16	75.0	38/79	48.1	47/80	58.8	12/26	46.2
4	3/20	15.0	120/286	42.0	31/100	31.0	82/206	39.8
7	9/20	45.0	69/102	67.6	30/100	30.0	43/105	41.0
8	6/20	30.0	81/192	42.2	32/100	32.0	48/101	47.5
12	6/20	30.0	69/291	21.3	8/100	8.0	56/243	23.0
5	1/20	5.0	55/294	18.7	7/100	7.0	67/425	15.8
9	7/20	35.0	119/247	48.2	34/100	34.0	228/318	71.7
10	3/20	15.0	37/199	18.6	24/100	24.0	71/187	38.0

in plot No. 2 and plot No. 3, where 90 % of the sample trees were heavily damaged. In the test plots situated lower than the middle slope, the progress of the damage was slow, though the damage advanced to some extent more than it did in the previous year. The extent of the damage in 1963 was less than in 1962. This trend is prominent in the plots in the upper flat, where the mean wind velocity during the growing season in 1963 was less than in the previous year.

These changes of the damage in each year are easily found in the changes of the mean degree of damage (the mean of given indices) in the Table.

Table 46 shows the damages in each test plot quantitatively. From these results, it is clearly shown that the degree of the damage is conspicuously different between the test plots situated in the upper flat and lower than the middle slope. In 1962 the damage in the upper flat was severe, and almost all shoots examined were killed. However, the damage in the plots situated lower than the middle slope was slight, compared with those in the upper flat. Generally, the damage decreased in 1963, compared with that in the previous year, especially in the upper

flat.

The growth of larch trees was measured for the height and the increase in the current season.

General situations on the growth of larch trees in each test plot are shown in Table 47.

Table 47. Growth of larch trees in each test plot.

Position of test plot	No. of plot	Mean tree height (cm)			Mean rate of growth (%)	
		1961	1962	1963	1962	1963
Upper flat	1	108	114	132	5.6	15.8
	2	115	108	123	— 6.1	13.9
	3	108	90	112	—14.6	24.4
	11	99	98	128	— 1.0	30.6
Middle slope or concave	4	139	187	262	34.5	40.1
	7	136	182	232	33.8	27.5
	8	127	174	240	37.0	37.9
	12	111	174	234	56.8	34.5
Bottom site	5	143	208	280	45.5	34.1
	9	139	186	228	33.8	22.6
	10	142	189	247	33.1	30.7

In the fall of 1962, larch trees in the test plots situated in upper flat were heavily damaged by the disease, and the mean tree height decreased as compared with that of the previous year. In the other test plots larch trees grew normally. In 1963, the means of the tree height in the lower sites became 2 or more times taller than those in the upper flat, where the mean of the tree height increased a little over that in the previous year. The relation between the degree of the damage and the growth of trees is clearly summarized in Fig. 24.

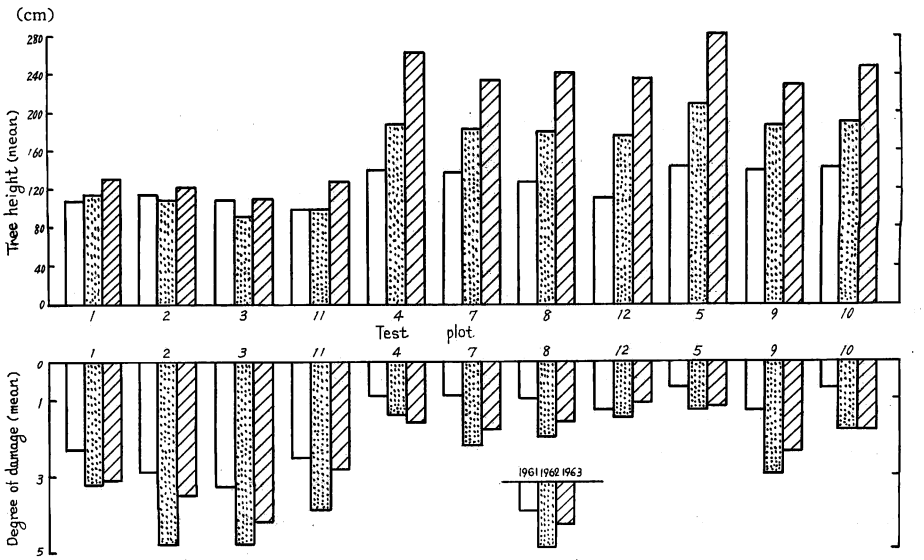


Fig. 24 Transition of the damage and the tree height (planted in 1960).

B. In the shelter fence examination.

The degree of the damage in 1963 is shown in Table 48 with the indices. Table 48 shows

Table 48. Degree of damage in the shelter fence and in open field. (1963)

Degree of damage	0	1	3	5	Mean	Planted on
In the shelter fence	74	67	8	1	0.6	1963, V
In the open field	24	42	32	2	1.5	1963, V
In the shelter fence	7	103	20	0	1.3	1960, V

that 95 percent of larch trees at the inside of the fence were healthy or slightly damaged, whereas 66 percent of trees in the open field was healthy or slightly damaged, after the lapse of one growing season (Plate 7, D, E). This is clear in the mean of degree of damage, showing 0.6 for the trees at the inside of the fence and 1.5 for the trees in the open field. Eighty five percent of larch trees at the inside of the fence planted in 1960 were healthy or slightly damaged, and moderately damaged trees were only 15 percent.

The number of infected shoots and parts, and the percentage of infection at the inside of the fence and in the open field are given in Table 49 and 50.

Table 49. Number of infected parts in the shelter fence and in the open field. (1963)

Position of trees examined	Position of shoots						Planted on
	Top shoot			Lateral shoot			
	Top	Middle	Small shoot	Top	Middle	Small shoot	
In the shelter fence	19/150	12/150	8/71	82/750	17/750	0/35	1963, V
In the open field	37/100	8/100	20/50	143/500	14/500	3/9	1963, V
In the shelter fence	13/130	6/130	373/1887	98/650	23/650	516/2424	1960, V

Top and small shoot: Number of infected shoots/Total number of shoots.
Middle: Number of infected parts/Total number of shoots.

Table 50. Percentage of infection in the shelter fence and in the open field. (1963)

Position of trees examined	Position of shoots								Planted on
	Top shoot				Lateral shoot				
	Top, Middle	%	Small shoot	%	Top, Middle	%	Small shoot	%	
In the shelter fence	31/150	20.7	8/71	11.3	99/750	13.2	0/35	0	1963, V
In the open field	45/100	45.0	20/50	40.0	157/500	31.4	3/9	33.3	1963, V
In the shelter fence	19/130	14.6	373/1887	19.8	121/650	18.6	516/2424	21.3	1960, V

These tables show clearly that the number of infected shoots and the percentage of infection were greater in the open field than in the inner part of the fence. The extent of the damage at the inside of the fence was even, and no correlation of the damage between the trees in different position at the inside of the fence was observed.

Records on the growth of larch trees at the inside of the fence and in the open field are given in Table 51 (Plate 7, D, E). For the check of the trees planted in 1960, the larch trees

Table 51. Growth of larch in the shelter fence and in the open field.

Position of trees examined	Total			Mean			Planted on
	Tree number	Tree height	Sum of current growth	Tree height	Current growth	Rate of growth (%)	
		(cm)	(cm)	(cm)	(cm)		
In the shelter fence	150	12,455	3,790	83.0	25.3	25.3/57.7 (43.8)	1963, V
In the open field	100	6,580	1,600	65.8	16.0	16.0/49.8 (32.1)	1963, V
In the shelter fence	130	30,295	10,390	233.0	79.9	79.9/153.1 (52.2)	1960, V
In the open field	50	8,170	2,355	163.4	47.1	47.1/116.3 (40.5)	1960, V

of healthy top shoots were selected in the same upper flat where no effect of windbreak of the fence was expected, and their tree height and the increase of the height in the current season were measured. As can be clearly recognized in Table 51, after the lapse of only 1 growing season, there existed the differences of 15 cm in the mean of tree height and of 10 cm in the mean of increase in the current season between those in the inner part of the fence and those in the open field. For the trees planted in 1960, the growth at the inside of the fence was very vigorous and the mean of the tree height was 70 cm taller than the mean in the open field, showing the equivalent growth to that in the lower sites where a normal growth was obtained.

4. Discussion.

On the relation between the outbreak of the disease and the climatic factors, it has been known that especially the wind becomes the main inducing factor in the following diseases: the shoot blight disease of Japanese cedar caused by *Guignardia cryptomeriae* (Kobayashi[37], 1957), the bud-blight of alder (*Alnus tinctoria*) caused by *Guignardia alnigena* (Nishikado, Watanabe and Inoue[45], 1959), the canker disease of Japanese pear caused by *Phomopsis Fukushii* (Takahashi and Nakayama[54], 1962), and so on, and it is said that the sea breeze is one of the inducing factors for the headblight (scab) of wheat and barley caused by *Gibberella zeae* (Ishii and Kawajiri[31], 1960).

Though it is well known experimentally that larch trees planted on the site blown by strong wind are liable to be infected by the shoot blight disease and that the damage becomes heavier in a shorter period, only a few reports of the analytical studies with detailed data on environmental factors concerning the outbreak of the disease have been made. Nakagawa, Kataoka and Koseki[43] (1960), Okamoto and Nakagawa[46] (1962), Sato, Yokozawa and Shôji[49] (1963), Yokota and Inoue[65] (1961), Yokota[67] (1962-b) reported in connection with the wind during the growing season of larch that there existed a marked difference of the damage by the disease depending upon the difference of the topography and the direction of the slope.

In the flat plantation, least damage was found on the leeward side of the shelter belt (Aoki[3] *et al.*, 1962; Kato and Ono[35], 1962). Sato, Yokozawa and Shôji[49] (1963) reported that in larch plantations neighboring the broad-leaved forest mixed with Japanese red pine trees, the damage and the spread of the disease were fairly slight compared with those in the large pure forest, and in the isolated larch stands in a tall broad-leaved forest the occurrence of the disease was also slight. According to Matsui[41] (1963) the damage caused by the disease was severe in the sites blown by heavy wind and of poor soil conditions in the southern parts of Hokkaido, especially on the sites near the seashore.

These reports show that the wind is the most important factor for the outbreak of the disease, though they have none of the quantitative data on the wind.

In the present examination, the writer carried out 2 analytical methods to clarify the effects of wind as one of the inducing factors of the disease: One is the plot examination, in which the writer wanted to consider the relationship between the outbreak and the progress of the disease and the wind, observed quantitatively, during the growing season of larch. Another is the shelter fence examination, in which the decrease of the damage in the site, where the wind during the growing season was weakened as far as possible by the shelter fence, was compared with the damage in the open field blown by strong wind.

The results of the present examination show that the heavy wind during the growing season of larch may be the most important inducing factor for the disease.

According to Ito's opinion (1961-a, b, c)[32)33)34)], the typhoon, the rain, the fog, the water contained in larch trees and so on, may be inducing factors, and the wound in the shoots is certainly a good entrance for the invasion of the causal fungus. In these conditions, two different actions are considered; the one is the factors giving larch trees unfavorable conditions, and the other is the factors giving the causal fungus favorable conditions. It seems that the strong wind during the growing season belongs to the former, and such climatic conditions as typhoon and so on belong to the latter.

Considering the effects of the strong wind during the growing season upon the current season's shoots of larch, numerous wounds will be made mechanically by rocking, which frequently cause disorders in the root. As stated in Chapter **5**, 1 and in the report by Sato, Yokozawa and Shôji[49)] (1963), it has been known that the wounds in the shoots provide more favorable condition for the infection with the causal fungus. Accordingly it may be easily understood that the wounds made by a heavy wind make infection easier.

For the effects of wind as the cause of a physiological disturbance in larch trees, much is unknown. It is known, however, that weakening of vitality in larch trees followed by the unbalance of the water relation brought about by the increase of transpiration, will take place in the site blown by heavy wind. Satoo (1949, 1952, 1955)[50)51)52)] carried out the experiments on the effect of wind upon the transpiration and absorption of water and reported the following results: when water-cultured seedlings of Japanese cedar were exposed to artificial wind, the lag of the change of absorption behind transpiration was observed. This may probably be due to the fact that the rate of transpiration responds immediately to change in environmental conditions, but absorption does not increase until the effect of saturation deficit caused in the leaves by transpiration is transmitted to the root, and subsequent deficit of water may disturb the physiological action and further influences in the growth of trees. In winter, the evaporation from wet blotting papers and the traspiration from the leaves of *Chamaecyparis obtusa* were higher on the windward side of the windbreak. Water content was always lower on the windward side. The results showed that the wind reduced the water content of needles, though the influence on transpiration was not so obvious. In the experiment on the influence of wind on the transpiration with 4 coniferous tree species, transpiration increased with increasing wind velocity, but the increase was not proportional to the wind velocity.

From Satoo's[50)51)52)] above-mentioned results, it seems that water deficient conditions in larch trees may be induced by the increase of the transpiration in the site where heavy wind blows during the growing season.

According to Kozlowski[39)] (1962), it is said that tree growth is reduced by water deficits indirectly through interference with physiological processes such as photosynthesis, nitrogen metabolism, salt absorption, and translocation, and directly by the effects of reduced cell turgor

on cell enlargement and other processes more directly involved in growth.

Recently, Bier[5)6)7)] (1959-a, b, c), Broomberg[8)] (1962) proved experimentally that, in some dieback diseases caused by facultative parasites, water contents in the bark expressed by "relative turgidity" had a close relation with the development and the progress of diseases, and when the relative turgidity became a definite level or more, the development of the disease was apparently hindered or the progress of the disease was stopped. Kobayashi[37)] (1957) reporting on the shoot blight disease of Japanese cedar caused by *Guignardia cryptomeriae* explains that the disease was liable to develop in the weakened condition from the drying as well as in the condition blown by the wind.

In the writer's results in the plot examination, the wind in each test plot was estimated quantitatively between June and September, and it was found that the strong wind blew in the upper flat and much less in the middle or lower sites regardless of wind directions. In 1962 the wind was strong and 3—4 m/sec or more of mean wind velocity and 1,700 hours of blowing hour through the whole period of observation were observed in the test plots situated in the upper flat, whereas the mean wind velocity in almost all plots situated in the middle slope or bottom sites was less than 1 m/sec. Thus, it seemed that by the blowing of strong wind physiological processes of the larch trees in the upper flat were adversely affected, and many young shoots were injured, whereas no such interference for larch trees in the middle or bottom sites resulted from wind as weak as 1 m/sec or less of mean wind velocity.

In 1963, the wind was weaker than in the previous year, especially in August and September. It was estimated that the mean wind velocity was 1/2 or less and the wind run was 1/3 for the previous year; therefore, it seemed that physiological interference for larch trees, if any, was slight.

In the shelter fence examination, it was found that the wind at the inside of the shelter fence was considerably weak, where only 1/2 or less of wind velocity was observed compared with that in the open field (check plot). So the difference in the effects of the wind during the growing season upon larch trees between the inner part of the fence and the open field may be considered the same as that between the upper flat and the middle or lower sites in the plot examination.

So we may ask: how did spores as the source of infection disseminate? As already mentioned, spores of the causal fungus are disseminated mainly with rain throughout the growing season of larch, and the climatic factors necessary to the dissemination of spores are discussed in detail in Chapter 3. Therefore, by analysing the records on the precipitation, and the time and the range of temperature in the rainfall, observed by the automatic climatological station, as shown in Table 42, it was estimated that abundant spores sufficient for the infection to larch shoots were disseminated in each test plot in 1962 and 1963.

It seems that climatic and soil conditions other than wind observed in the examined area were not markedly different in the plot and shelter fence examination, except that in the latter where a somewhat higher temperature was observed at the inside of the fence than in the open field. Then how is the difference of the strength of wind to be correlated with the difference of the development and the progress of the disease between the upper flat and the middle or lower sites?

The progress of the damage in the upper flat was rapid in 1962, indices of which in plot No. 2 and plot No. 3 increased to 4.8 from 2.9 and 3.3 in the previous year, with the strong wind of 4 m/sec of mean wind velocity throughout the period of observation. On the other

hand in 1963 the wind was far weaker than in the previous year and the degree of the damage decreased from 4.8 to 3.5 and 4.2. In the middle or lower sites, the degree of the damage was nearly the same in both years, though the indices in 1962 gave somewhat higher values than the previous year. These results show that, so long as the intensity of wind is in a definite level or less, no influence of the wind on the development and progress of the disease can be expected. In the examined area, belonging to a heavily damaged area, it seems that the density of airborne spores is considerably high, and the dissemination of spores sufficient for the infection to larch shoots occurs with every rainfall during the growing season. And larch trees in the upper flats are rocked by the wind above a definite level, resulting in numerous wounds in the shoots favorable for the infection and physiological interference, and the progress of the disease is very fast. On the contrary, it seemed that larch trees in the middle and lower sites with slight wind grew normally and no considerable damage appeared.

The same explanation will apply to the results in the shelter fence examination.

According to INOUE's report[28] (1962) on the damage by prevalent wind during the growing season of larch (growth interference by a malformation of crown) for larch trees, damage resulted by the wind of 3 m/sec for 1,700 hours in the blowing hour and of 4 m/sec for 840 hours, and no damage is to be expected by the wind less than 2 m/sec of mean wind velocity, regardless of the blowing heur.

Based on INOUE's results[28], larch trees in the upper flat should be damaged by a strong wind more than 3 or 4 m/sec of 1,700 hours in the blowing hour in 1962, whereas no damage by prevalent wind should appear in 1963, because the wind was quantitatively about 1/2 that of the previous year.

Larch trees in the upper flat were affected physiologically and numerous wounds were made on the shoots by the heavy wind. Consequently, the damage by the disease was conspicuous, and almost all current season's shoots were killed, resulting in interference with the growth. In 1962, there appeared the decrease of the mean tree height in plot No. 2 and plot No. 3, where the most severe damage by the disease was observed. On the contrary, in the middle and lower sites, where the wind as an inducing factor of the disease was slight, larch trees grew normally, though the development of the disease was observed, and the mean tree height reached 2 times or more taller than that in the upper flat after the lapse of 4 growing seasons.

Growth of larch trees planted in 1960 at the inside of the shelter fence was also good, and the mean tree height was almost similar to that in the lower site having favorable growing conditions in the plot examination. This shows that even in an upper flat a good growth of larch can be expected, if the windbreak during the growing season is sufficient.

Based on these considerations, if the prevalent wind has the mean velocity of 3 m/sec or more throughout the growing season of larch, it will act as the most important factor for inducing the disease.

IZUKA[23] (1950) reported that wind velocities at 20 cm above the ground were observed 50—70% at the distance of 10 h (h was the tree height of a shelter belt) and 50% at 5 h to the original wind velocity. These results conducted by IZUKA[23] (1950) and KATO and ONO[35] (1962) suggest that, in the sites where the outbreak of the disease is forecast, the establishment of the shelter belt with the consideration of the strength and the direction of the prevalent wind during the growing season of larch may be the most effective method to lessen the damage or to escape from the disease, and further to give larch trees favorable conditions for growth. Concerning the tree species, the width and the interval of shelter belt, much remains to be

considered. And yet, if the mean wind velocity of the prevalent wind exceeds 3 m/sec or more, the afforestation of larch trees should be avoided.

5. Conclusion.

To obtain the quantitative data on the wind as an inducing factor of the disease, the writer carried out the plot examination and the shelter fence examination and it was found that, if the prevalent wind during the growing season of larch had a definite level or more, the wind would become the most important factor to induce the disease.

In the plot examination, the strength of the wind differed conspicuously with the difference of topographic situations: Test plot situated in the upper flat was blown by the southern prevalent wind of 3~4 m/sec for 1,700 hours in 1962 throughout the growing season of larch. On the contrary, the wind blown in those situated in the middle or lower sites was slight, being 1 m/sec or less. In 1963 the prevalent wind was weaker than in the previous year, being 2 m/sec or less even in the upper flat.

The outbreak and the progress of the disease in 1962 were conspicuous in the upper flat, where almost all test trees were damaged severely, and consequently the mean of tree height decreased compared with that of the previous year. On the contrary, the damage in the plots situated in the middle or lower sites was slight and the growth of trees was normal. In 1963, though the damage of larch trees was less than in the previous year and a slight increase in the mean tree height was observed even in the upper flat, the mean tree height in the middle or lower sites was far greater, and there existed a marked difference in the degree of damage and the tree height between the upper flat and the middle or lower sites.

In the shelter fence examination, the intensity of the wind was considerably weak and only a half or less of that in the open (check) field was observed in the shelter fence. The damage by the disease was slight at the inside of the fence, showing that the effect of the shelter fence in preventing the disease was great. On the growth of larch trees, a marked difference was observed between those on the inside of the fence and those in the open field after the lapse of only 1 growing season. Especially noteworthy is the growth of larch trees planted in 1960 which became equivalent to that in the lower sites in the plot examination.

These results must be attributed mainly to whether or not the site is blown by the strong prevalent wind, as an inducing factor of the disease during the growing season of larch, because environmental conditions other than wind are nearly the same in the examined area. And, it seems that the prevalent wind of 3 m/sec or more in the mean wind velocity throughout the growing season should act heavily as the inducing factor of the disease. Therefore, when the development of the disease is forecast, the establishment of the shelter fence may be the most effective method for the control of the disease.

Summary

In the present paper, ecological characteristics of the shoot blight fungus, *Guignardia laricina* (Sawada) W. Yamamoto et K. Ito, and climatic factors closely related to the outbreak of the disease were studied.

1. In Hokkaido, the disease is widely distributed, especially in the southern part including Oshima, Hiyama, Iburi, and Hidaka districts, which suffer severe damage. Damaged larch plantations are mainly situated near the seashore, though the disease is spreading to inner parts or eastern parts in Hokkaido. It is estimated that the total areas of damaged plantations amount-

ed to 63,000 ha up to November, 1962, which was equivalent to 18% of the total area of larch plantations in Hokkaido. Larch seedlings in nurseries are also suffering from the disease, and it seems that considerable damage has resulted in the nurseries situated in the area where the disease is prevailing. The source of infection in nurseries is in many cases the hedge or shelter forest of heavily infected larch trees.

2. The disease affects the seedlings as well as the adult trees. It infects only the current season's shoots and not the shoots grown before the previous year.

Infected shoots are soon killed. There are two types of symptom, one of which hangs at the top of infected shoots and the other stands straight.

The purplish discoloration in the early stage of the disease is characteristic. Frequently resin exudes from a part or parts of infected shoots.

Fruit bodies of *Macrophoma* sp, the pycnidial stage of the causal fungus, begin to appear as small black points from mid-July on the curved part or on the undersurface of dead leaves remaining at the top of dead shoots.

After late October, fruit bodies of *Guignardia laricina*, the ascigerous stage of the causal fungus are formed on the dead shoots. There exists spermogonia from August to October.

Pycnidia exist between mid-July and late November, and perithecia begin to appear from the middle of October on the shoots infected in the current season, the number of the latter increasing after the following spring and are found by the end of the year. So, it can be concluded that spores of the causal fungus exist throughout the year, if the density of spores is not considered.

3. Discharge of ascospores is greatly influenced by water and temperature. Water is the indispensable factor and temperature becomes the factor deciding the hardness or ease in the discharge. Under natural conditions, water is supplied by rain or fog. When perithecia fully absorb water, no discharge of ascospores takes place in the temperature at 10°C or less, but it becomes vigorous at 15°C or more. Consequently, the discharge of ascospores takes place from early June until October. The season in which ascospores are vigorously discharged, is between mid-July and mid-September, during which occurs the most vigorously growing season of larch trees. Discharged ascospores are very adhesive in character and they show a high percentage of germination.

When pycnidia absorb water and are in a suitable temperature, pycnospores are exuded as a small mass of spores, and then they are disseminated mainly with the splash of rain. Considering the pattern of dispersion between ascospores and pycnospores, it seems that the distance of dispersion in the former will be far longer than in the latter.

4. The longevity of spores, that is, the period maintaining the germinating ability differs conspicuously in cases in which they are in perithecia or pycnidia or not. Spores in perithecia or pycnidia are able to maintain the germinating ability at least for 9 months, whereas they lose the ability soon after dispersion. Experimentally they lost the ability within 2 days after the discharge or exudation.

Germination of spores easily takes place. In the optimal temperature they begin to germinate in distilled water within 1 hour.

5. Ascospores and pycnospores show very high pathogenicity. When the wounded shoots were inoculated with them, higher percentage of infection was observed. Even non-treated shoots were infected by spraying with pycnospore suspension.

When shoots are inoculated in early season, the hanging symptom appears; by the inoculation

after late September, the standing straight symptom appears.

Incubation period and percentage of infection are influenced by the temperature soon after the inoculation. In an optimal temperature incubation period becomes shorter and the rate of infection becomes higher.

6. To make clear the relation between the outbreak and progress of the disease and climatic factors, especially the wind, examinations were carried out in two ways. (1) In the plot examination, observations on the outbreak of the disease and the climatic factors in each topographic situation (upper flat, middle or concave or lower sites). (2) In the shelter fence examination, observations on the outbreak of the disease and wind between the inside of the shelter fence and the open field established in the upper flat.

In the plot examination, it was found that the wind was considerably different quantitatively with the situation of test plots. In the upper flat the prevalent wind (southern wind) of about 4 m/sec in the mean wind velocity blew throughout the growing season for 1,700 hours in 1962. At the same time the progress of the damage was very fast and 90 percent or more of the test trees was heavily damaged. Consequently the mean tree height in plot No. 2 and plot No. 3 decreased as compared with the previous year. On the other hand, mean wind velocity in the middle or lower sites was 1 m/sec or less, the damage was slight and the growth of trees was normal.

The wind in 1963 was about a half that of the previous year and only 2 m/sec in the mean wind velocity was observed even in the upper flat. So, the wind in lower sites was negligibly small. The outbreak of the disease was lighter than in the previous year, and the number of heavily infected trees decreased even in the upper flat.

In the shelter fence examination, the wind at the inside of the fence was about a half or less than in the open field. Larch trees at the inside of the fence were healthy or only slightly diseased, whereas those in the open field were moderately diseased after the lapse of only 1 growing season. The growth was also better at the inside of the fence than in the open field.

These results show that, among the factors influencing the outbreak and the progress of the disease, the wind during the growing season must be regarded as the most important factor. Further, based on the results that, if the prevalent wind of 3 m/sec in the mean wind velocity during the growing season blows for 1,700 hours or more, disturbance of growth in larch trees will result (Inoue[28], 1962), the afforestation of larch in the site with strong prevalent wind during the growing season should be avoided; alternatively it will be effective to utilize the shelter belt as a means of escaping the disease.

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Explanation of plates.

Plate 1.

A. Heavily infected larch in the shelter hedge of a nursery. This is the source of infection for larch seedlings.

B. A larch tree deformed like a broom, caused by repeated attacks of the disease (8-year-old larch tree).

C. Current season's shoots heavily infected by the disease. Dead leaves remain at the top of the shoots.

D. Resin (R) exudes from infected parts, around which stromatic tissues (P) containing perithecia are seen (enlarged).

E. Infected part dried up and is apparently differentiated from healthy part (enlarged).

Plate 2.

A. Small shoots are easily infected and occasionally the disease spreads vertically to the main shoot from infected small shoots.

B. Almost all the current season's shoots are damaged when the tree is attacked severely by the disease.

C. A symptom appearing in the following spring on the shoot which was infected late in the previous year.

D. The typical symptom of the disease. Numerous pycnidia are produced on the undersurface of dead leaves or curved shoots as black, small points (enlarged).

Plate 3.

A. Germinating ascospores discharged 2 days after the start of the experiment under

alternated temperature of 5 and 25°C, in the over-saturated condition (×150).

B～H. Sections of perithecia soon after the end of the experiment on the discharge of ascospores under alternated high and low temperatures (ater 40 days, × 150).

B. A few ascospores present in a perithecium, 5～15°C (1).

C. Ascospores abundantly present, 10～15°C (3).

D. The content of perithecia is vacant, 15～20°C (1).

E. Ascospores present in a perithecium, 0～20°C (1).

F. The content of a perithecium is vacant, 5～25°C (2).

G. The content of a perithecium is vacant, 10～30°C (2).

H. The perithecium is still immature (?), 10～30°C (1).

Plate 4.

A. Perithecial stromata under the dry condition. Discharge of ascospores does not occur in this situation (enlarged.).

B. Perithecial stromata swollen by absorbing water. In this condition, ascospores are easily discharged from perithecia. The material is the same as A (enlarged).

C. A glass slide spore trap set on an infected shoot bearing perithecia. It was always set on a definite place of the shoot in the period of observation.

D. Ascospores were trapped as the multiples of 8 spores on a glass slide set on July 11 (× 150).

Plate 5.

A. Ascospores are very adhesive in character (×600).

B. The appearance of the surface of shoot on which the spore trap was set. The surface was very rough and deteriorated in some places (October 31, enlarged).

C. Many perithecia still present in the bark of the infected shoot used for the experiment (white dots are the content of perithecia) (October 31, enlarged).

D. A part of a glass slide set during 12 and 18 o'clock, August 27, 1962, on which ascospores were trapped (×60).

E. The small white mass of pycnospores exuded from pycnidia in a moist chamber (relative humidity 100 percent, 25°C, after 40 hours) (enlarged).

F. The mass of pycnospores does not appear in the relative humidity of 92 percent or less (enlarged).

Plate 6.

A. The typical symptom appeared 15 days after the inoculation with pycnospores without making any wounds.

B. Ditto. Resin (R) exuded from an infected part.

C. Pycnidia appeared on the infected part 18 days after the inoculaton with ascospores to the incision (×150).

D. Perithecia appeared on the infected part which was inoculated with pycnospores and overwintered (×150).

E. Infected shoot did not hang but remained straight when inoculation was done after late September.

Plate 7.

A. Complete view of the bamboo shelter fence.

B. Inner part of the fence.

C. Part of the apparatus of automatic climatological station.

D. Larch tree in the inner part of the fence, planted in 1963.
Growth is very vigorous and infection is rare.

E. Larch tree in the open field (check plot), planted in 1963.
Growth is poor and many shoots are infected.

カラマツ先枯病の発生に関与する病原菌の生態ならびに気象因子に関する研究

摘　　要

横　田　俊　一(1)

1954 年に北海道をおそった熱帯性低気圧および 15 号台風による風倒跡地の造林計画，すなわち林力増強計画が 1958 年から開始され，カラマツが造林樹種として大面積にわたって造林されるようになり，1963 年までに北海道におけるカラマツ既造林面積は約 35 万 ha と推定される急増ぶりを示すにいたった。

この間，病害としては落葉病あるいはナラタケ病などが発生して毎年被害を生じ，さらに数年前からは先枯病が各地に発生して大面積にわたって被害をおよぼし，なお増加の一途をたどっている。東北地方においても同様に大きな被害を生じており，まさにカラマツ造林の成否を左右する重大な段階にたちいたっている。

本病は東北地方および北海道にはかなり以前からあったようではあるが，正式に記載されたのは 1950 年に故沢田兼吉氏によってなされたのが最初であり，病原菌は *Physalospora laricina* Sawada と命名された。その後故山本和太郎氏は不完全時代を属徴に加味することにより，*Guignardia laricina* (Sawada) W. Yamamoto et K. Ito とすることを提案し，今日ではこれが本菌の学名として用いられている。

ごくわずかの年月のあいだに，このように急激に被害が蔓延したのは，本病の特殊な性格に原因がもとめられる。すなわち，本病に罹病したカラマツは，これによって直接枯死することはほとんどないこと，本病は北海道においては海岸近い風当たりのよいところに発生していたが，潮風害と考えられ病気という認識がほとんどもたれなかったこと，本菌は本邦特産で諸外国には発生がないため，防除に関連した一連の研究がまったくなかったこと，さらに本菌の生態的特徴，すなわち胞子飛散の時期がカラマツの全成長期間にわたっており，病原性がきわめて強いことなどによるものと考えられる。

一方，本病に関する研究は，ここ 3～4 年来各分野において強力に進められ，とくに苗畑における防除法はほぼ実用の域に達しているが，造林地における防除法はいまだ研究途上にあり，今後さらに本病原菌の生態あるいは本病の発生を左右する諸条件などに関する基礎的な研究の積み上げが必要であると考えられる。

この意味において，本論文は造林地における本病の被害を軽減し，あるいは回避する方法を明らかにするために必要な基礎資料の積み上げを目的とし，とくに病原菌の諸性質のうちでも伝染源としての胞子の生態的な特徴，および被害の発生を左右すると考えられる環境条件を中心として考察を加えたものである。

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第1章　北海道における本病の分布ならびに被害の状況

1. **北海道における本病の分布**は，とくに津軽海峡，太平洋および日本海沿岸地方に広く分布している。しかも，最近ではしだいに内陸に侵入しつつあることが指摘される（札幌，苫小牧間の石狩低地帯，美瑛，富良野周辺，道東地方の一部）（第1図）。

2. **造林地における被害**は，1960 年第 1 回調査以来，調査のたびに増加の一途をたどり，1962 年 11 月現在では被害総面積は 63,000 ha に達した（第2図，第1，2表）（その後の調査によると 1964 年度の被害総面積は約 72,000 ha に達したことが明らかとなった)。この被害面積はカラマツ全造林面積の約 18% に相当する。地域的には胆振，日高支庁管内約 40%，渡島，檜山支庁管内約 30%，計 70% を占め，残り 30% が他の地域に分布している。

3. **苗畑における被害**の統計はきわめてすくないが，本病の被害発生地域にある苗畑では，ほとんど発病がみとめられるようである。そして罹病苗木が本病の急速な蔓延の重要な原因となっている場合がおおい。1963 年の北海道庁の調査によると，民間 74 苗畑で 116 万本の被害が生じたという。苗畑における伝染源はおおくの場合，防風垣あるいは隣接防風林の罹病したカラマツである（第4，5表)。

第2章　病徴，標徴ならびに病原菌の生活史

1. 病　　徴

本病の発生は当年生枝に限り，前年までに罹病しなかった枝は，も早罹病することはない。毎年激害をうけると樹高成長はまったく停止し，枯死した枝が多数残存するために，箒状を呈するようになる (Plate 1, B)。

病徴は感染時期の遅速によって，先端が下垂する典型的な先枯れ型 (Plate 1, C) と，先端は下垂せずに直立したままで枯死する型とに分けられる。

北海道では7月上旬ごろから病徴が現われはじめ，新梢先端が生気を失なって下垂し，罹病枝の色はきわめて特徴ある淡紫色となり，葉は先端部だけ残ってあとは落葉してしまう。おおくの場合，罹病枝の一部または数か所から樹脂を漏出する (Plate 1, C)。

本病は葉をおかすこともあり，この場合には針葉に赤褐色の大型の斑点が生じ，被害葉は早期に落葉してしまう。

カラマツの成長が旺盛な夏季には二次成長した小枝が形成されるが，これがきわめて罹病しやすく，しばしば病斑は二次枝から主軸に移行する (Plate 2, A)。激害木では当年生枝のほとんどすべてが罹病して，健全枝が見当たらないことも珍らしくない (Plate 2, B)。

上述のような病徴は7月から9月半ばごろまでにみられ，それ以後は下垂型の病徴はみられず，罹病枝は直立したままで枯死するが，この場合にも樹脂が漏出する。

感染時期がおそいと，翌春芽をふき，成長が開始されてから枝の一部が健全部よりも細まり，これから上部が枯死する場合もある (Plate 2, C)。

2. 標　　徴

下垂した罹病枝先端の枯れ葉の基部に近い部分や彎曲部に，7月下旬ごろから，黒色，半球形の微細な小点が多数現われる。これが本菌の *Macrophoma* sp. の柄子殻である (Plate 2, D)。

Spermogonia は7月下旬ごろから罹病枝の樹皮に埋まって，黒色小点として形成されるが，未熟な子のう殻の内壁に形成されることもある。おおくは 12 月すぎには空となるが，ときには翌春にも見出される場合もある。

子のう殻は主として彎曲した罹病枝先端部や，樹脂が漏出している部分に近いところに，樹皮に埋まり，孤生または数個，ときには 10 個以上も並んで形成され，熟すと樹皮を破り，または樹皮の縦溝に沿って現われる（Plate 1, D)。

3. 病原菌の生活史

本菌の生活史を明らかにするために，道内各地から時期別に採集した罹病枝上に形成されている *Guignardia laricina* の子のう殻および *Macrophoma* sp. の柄子殻の成熟期ならびにこの間随時 spermogonia の出現を調査した。この結果子実体の成熟時期が明らかとなり（第7図），なお子実体の量的関係を加味して，第8図にみられるごとき本菌の生活史がえられた。

これによると，子のう胞子時代は1年中存在するが，とくに量的に多くなるのは罹病の翌年5～6月ごろからで，10 月に入ると今年罹病した罹病枝上にも形成されるようになる。柄胞子時代は7月中旬ごろから 11 月下旬ごろまで普通にみられる。Speramatia 時代は主に 8～10 月の間存在する。したがって本菌の胞子はカラマツの成長時期を通じて存在することが明らかとなった。

第3章　病原菌の生態とくに胞子の分散

前章において，本病病原菌の胞子はカラマツの全成長期間を通じて存在することが明らかとなったので，これらの胞子の飛散がどのような条件のもとでおこなわれるかということは，きわめて重要な問題であると考えられる。これに関して本章においては，子のう胞子については室内実験と野外観察を，柄胞子については室内実験をおこなった。

1. 子のう胞子の放出に関する室内実験

子のう胞子の放出は，まず子のう殻に水が与えられることが必要で（Plate 4, A, B)，この条件がみたされると，温度が放出の難易を決定する条件となる。

子のう殻が吸水し，温度が一定の場合は，とくに 15～20°C 以上の場合にきわめて放出が良好となる（第4表)。また，高温と低温を不連続的にくり返した場合は，15←→20°C 区がもっとも放出良好であった（第6表)。吸水期間が短く，乾燥期間が長いと放出しにくくなる傾向がみられ，2日吸水 1 日乾燥のくり返し区がもっとも放出良好であった（第7表)。放出された子のう胞子の到達高としては，すくなくとも 10mm は到達しうることが知られた（第8表)。したがって，放出後は風によって遠方まで運ばれるものと考えられる。

2. 野外における子のう胞子の放出

を明らかにするために，カラマツ激害木2本を構内に移植して，随時観察できるようにした。これらの罹病枝の一定場所に降雨のたびごとにスライドグラスをとりつけ，捕えられた子のう胞子の数を算えた（Plate 4, C)。なお観察時の気温は構内で，雨量は札幌管区気象台の観測結果をもちいた。

野外での子のう胞子の放出は，すくなくとも6月上旬から開始されたが，降雨時の気温が昼夜をとわず 15°C 以上に上昇する7月中旬から9月中旬までの間に，とくにさかんに放出され，10 月に入ると降雨時の気温の低下のために，放出されにくくなる（第 9 表，第 10, 11, 12 図)。放出された子のう胞子の

発芽はきわめて良好である（第 10 表）。10 月中旬以降は放出がみられなくなったが，罹病枝上にはまだ子のう殻は多数存在しており（Plate 5, C），実験的には，容易に子のう胞子を放出することがたしかめられた（第 11 表）。このように，野外観察の結果は室内実験の結果ときわめてよく一致していた。

3. 柄胞子の飛散についての実験

柄胞子は，飛散にあたっては，まず柄子殻が吸水して柄胞子塊として一旦殻外に排出される。この場合，排出は関係湿度 98% 以上でおこりうるが，とくに関係湿度 100% あるいは過飽和状態，温度 25°C 前後の場合にもっとも良好である（第 12, 13表，Plate 5, E）。柄胞子塊は粘着性に富み，単に風だけでは飛散しないが，水滴によって流亡する（第 14, 15 表）ことから，野外においては，雨によっていったん柄胞子塊として殻外に排出されたのち，雨しぶきとともに飛散するものと考えられる。したがって，子のう胞子の方が，はるかに到達距離は長いものと考えられる。

上述のごとき，実験ならびに観察の結果にもとづくと，カラマツはその全成長期間にわたって，降雨のたびごとに感染の危険にさらされているということができる。

第 4 章　病原菌の胞子の生存期間ならびに発芽と温・湿度との関係

前章において，病原菌の胞子はカラマツの成長期間中，降雨のたびに飛散することが明らかとなった。したがって，本病伝播の役割りを果たす胞子の生存期間ならびに発芽に必要な条件を明らかにしておくことが大切であると考えられるので，この点について実験をおこなった。

1. 胞子の生存期間

子のう胞子も柄胞子も，それぞれ子のう殻および柄子殻中において，風乾状態において飛散しないでいる場合には，すくなくとも 9 か月間は発芽能力を維持することが明らかとなった（第 16, 19 表）。しかし，いったん殻外に飛散したのちは発芽能力の持続期間はきわめて短く，事実上 1～2 日後には発芽不能になることが確かめられた（第 17, 20 表）。

2. 胞子の発芽と温・湿度との関係

子のう胞子の発芽は 10～30°C の範囲で容易で，とくに 25°C 付近で発芽率も発芽管の伸びも最大となった（第 13 図）。柄胞子の発芽は，子のう胞子の場合よりも多少高目（15～35°C）で，最適温度は 30°C 付近とみられた（第 16 図）。なお子のう胞子は約 25°C の蒸溜水中で 1 時間以外で発芽を開始し，10 分間に約 13μ の割り合いで発芽管が伸長していくことが観察された（第 14, 15 図）。

胞子の発芽は 94% でも可能であるが，とくに 98% 以上では容易に発芽しうることが知られた（第 18, 21 表）。

これらの結果から明らかなように，飛散後の生存期間は短いが，胞子飛散に必要な条件は，同時に発芽にとっても好適な条件であり，1～2 日の生存期間は，本病の伝播にとっては十分な期間とみてよいものと考えられる。

第 5 章　胞子の病原性および発病と環境条件との関係

自然状態における本病の伝播は胞子によっておこなわれるものと考えられるので，1～2 年生の鉢植えのカラマツをもちいて接種試験をおこなった。

1. 胞子の病原性

子のう胞子および柄胞子を，新梢の生傷の部分に接種するときは，きわめて高い発病率を示し（第 22, 23, 25 表），またとくに生傷をつけないで柄胞子浮遊液を噴霧した場合にも，発病率は低いが発病がみとめられた（第 27 表）。したがって，本菌の胞子はきわめて強い病原性をもっていることが確かめられた。なお子のう胞子を接種した場合の病患部には柄子殻が形成され，柄胞子を接種した場合の病患部には翌春になって子のう殻が形成されたことが確かめられた（Plate 6, C, D)。

2. 接種時期と発病および病徴との関係

本病の病徴は新梢先端が下垂するものと，下垂せずに直立したままで枯死する2型があることはすでにのべた。これは感染時期が早いかおそいかによってきまるものと考えられるので，時期を変えて接種試験をおこなった。

8月までに接種された場合には，先端が下垂する病徴を示すが，9月13日に接種した場合には2とおりの病徴が現われ，9月 25 日接種の場合には直立して枯死する病徴だけが現われた（第 28 表，Plate 6, E)。したがって，感染時期がおそくなると新梢の組織が硬化することによって，先端が下垂する病徴は現われなくなることが知られる。このように，2とおりの病徴が移り変わる時期は，ほぼ9月中～下旬とみてよいであろう。

3. 感染におよぼす温度の影響

接種後の温度が潜伏期間および発病率にどのように影響をおよぼすかを明らかにするために，接種後3日間 25°C に保ったのち 18°C に移す区と，接種直後から 18°C に保った区とを比較した。前者は後者にくらべて潜伏期間は約5日短く，発病率ははるかに高い値をしめした（第 29 表)。これらの結果から，本病の潜伏期間は最盛期においては 10 日ないし 14 日くらいとみてよいであろう。またカラマツ成長期間における高温，多湿は本病の発生には有利な条件であると考えられる。

第6章　本病の発生と風を主とした気象因子との関係

本病の発生はとくに風当たりのよい場所においていちじるしいことから，風が本病の重要な誘因となっていることは，経験的にはよく知られるようになってきた。しかし，風を数量的にとり扱って本病との関係を明らかにした報告は，ほとんどみられない。そこで本章では本病の発生，蔓延と関係深いと考えられる気象因子のうち，とくにカラマツの成長期間の風を数量的にとり扱い，誘因としての重要性を明らかにしようとこころみた。

このため，苫小牧近くの勇払郡早来町所在のカラマツ造林地激害林分において，プロット試験と防風柵試験をおこなった。

プロット試験は第 17 図にしめすように，地形別にカラマツ 20 本からなる調査区を設定して，風を主とする気象因子と本病の発生状況ならびにカラマツの成長状況を調査し，防風柵試験地では別の尾根の平坦部の激害林分に固定防風柵を設置して，風の影響をできるだけとり除いた場所と風当たりのよい場所（対照区）での風の当たりかたと発病ならびに成長状況の相違を調査した。

1. プロット試験

プロット試験では，10 分間の同時観測による各調査区での風の当たりかたを測定し，これと同時刻の総合気候計（Plate 7, C）の風向，風速の記録から，各調査区におけるカラマツの成長期間を通じての風程，平均風速および吹送時間などを推定したほか，気温，雨量についても記録をとり，また，各調査区に

温度計を設置して，一定時刻の気温（午前 9～10 時）を測定した。土壌調査の結果によると，尾根筋と沢筋とで，土壌の含水率，理学性などの差はみられず，カラマツ造林地としては好適な土壌条件であることが知られた（第 18 図，第 30，31 表）。

総合気候計の記録によると，常風は南寄りの風で，1962 年はカラマツの全成長期間にわたって強い風が吹いたのに対し，1963 年は前年の 1/2 以下しか風が吹かなかった（第 19，20 図，第 34，35，36 表）。これらの記録から，各調査区での風の当たりかたを推定すると，1962 年には尾根筋の調査区では南寄りの常風だけで平均風速 3 m/sec 以上で，4 m/sec 以上の区もみられ，吹送時間は 1,700 時間をこえていた。これに対して中腹以下の調査区では，ほとんどが 1 m/sec 程度で，わずかな地形の差によって，いちじるしいちがいが生ずることが知られた。1963 年は尾根筋でも平均 2 m/sec をやや上回るくらいで，中腹以下ではわずかしか風が当たらなかったことが推定された（第 37，38 表，第 21 図）。

各調査区における本病の発生は，とくに尾根筋にいちじるしく，1961 年秋の試験地設定当初にくらべて 1962 年には，ほとんどの調査木が激害木に移行し，これにともなって平均樹高は前年よりも低下した。中腹以下の各区では被害程度は多少進んだが成長は良好であった。1963 年は尾根筋でも被害はやや減少し，平均樹高もわずかながら増加した。中腹以下の各地では，尾根筋ほど顕著ではないが被害は減少し，成長は良好であった（第 45，46，47 表，第 24 図）。

2. 防風柵試験

防風柵（高さ 4 m，一辺 30m の正方形）（Plate 7，A，B）の内部では，対照区（皆伐区）にくらべて風当たりはいちじるしく弱く，対照区の 10～50% 程度であった。

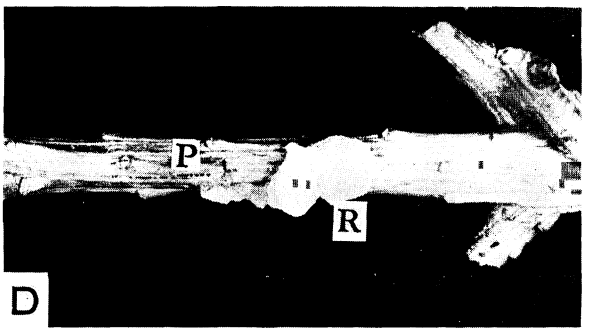
本病の発生は植栽 1 成長期間後で，すでにいちじるしい相違が生じ，柵内の平均被害度が 0.6 であったのに対して，対照区では 1.5，また平均樹高は柵内の 83 cm に対して対照区では 66 cm であった（第 23 図，第43，48，49，50，51 表，Plate 7，D，E）。

本地域においては，降水量および気温の記録から，感染の機会は各プロットにおいて十分であったと推定され（第 39，40，41，42 表，第 22 図），風以外の環境条件もほぼ同一であるが，地形の相違により，あるいは防風柵の有無によって，本病の発生およびこれにともなう樹高成長の相違が急速に現われたことは，成長期間の強い常風によって，本菌の侵入に好適な傷が新梢に多数作られ，さらに蒸散と水分吸収の不均衡にともなうカラマツの生理機能の低下が大きな原因となっているものと推定される。

以上のような結果から，カラマツの成長期間の強い常風は，本病の誘因としてきわめて重要なことが理解される。かつ，カラマツの常風害の発生と常風の平均風速および吹送時間に関する井上（1962）の報告を参照すると，カラマツの成長期間の常風の平均風速が 3 m/sec 以上で吹送時間 1,800 時間の場合は，風が本病の発生，蔓延の最大の誘因となるものと考えられる。

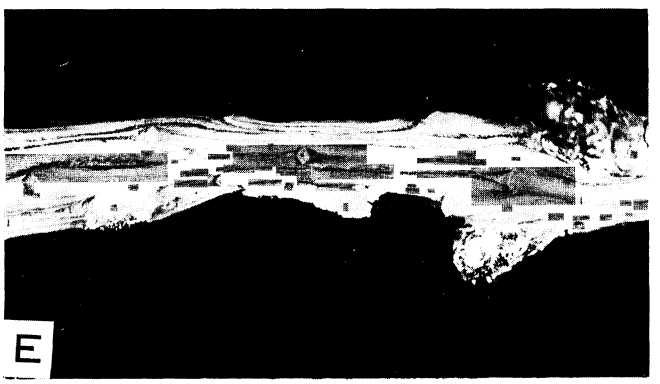

A


B



D


C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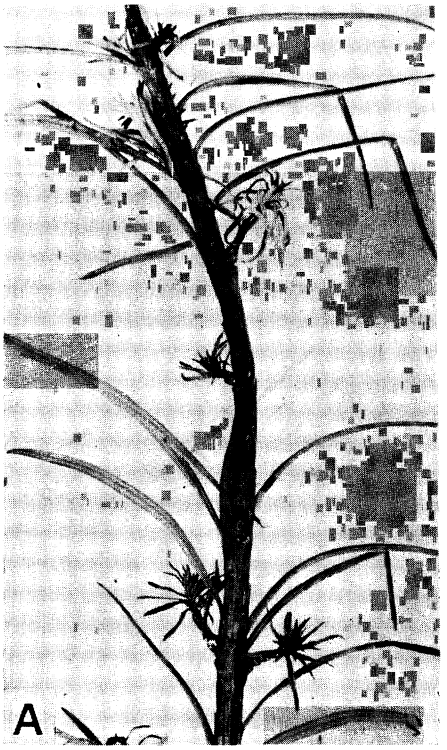
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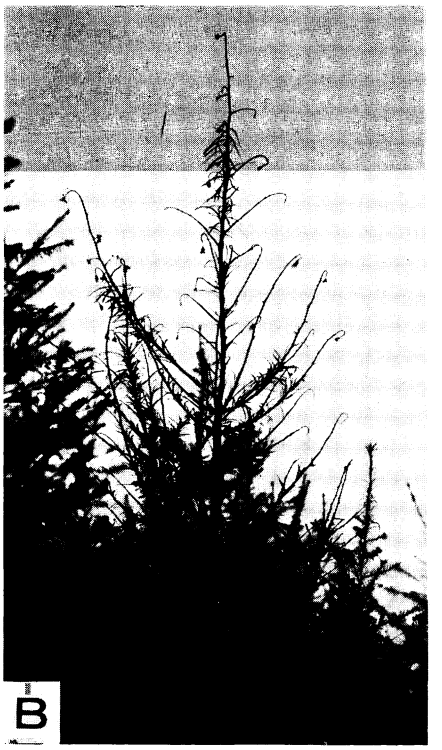
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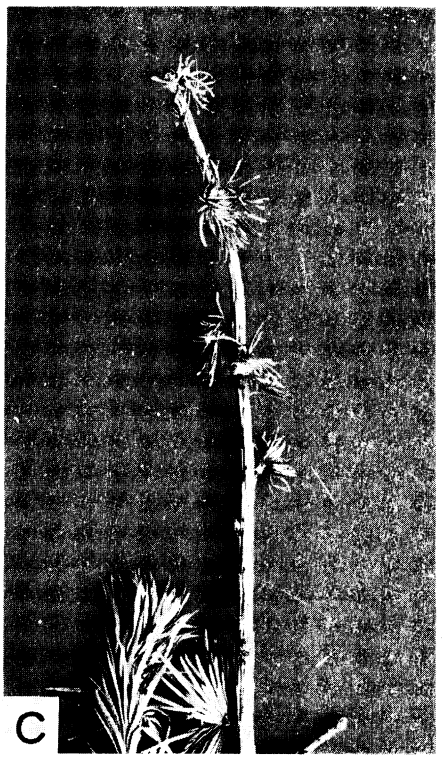
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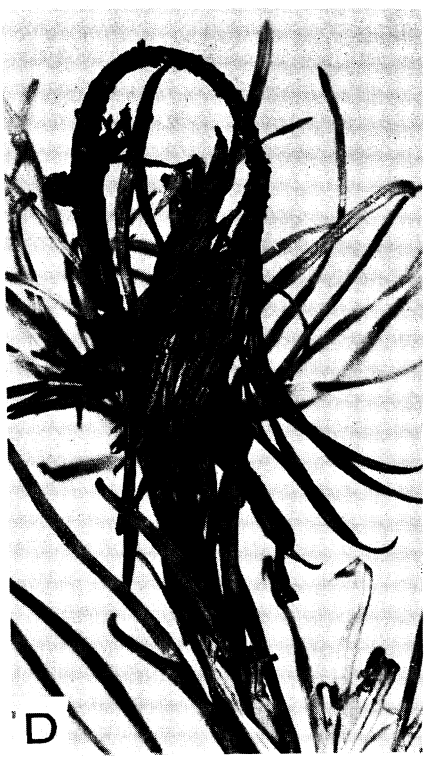
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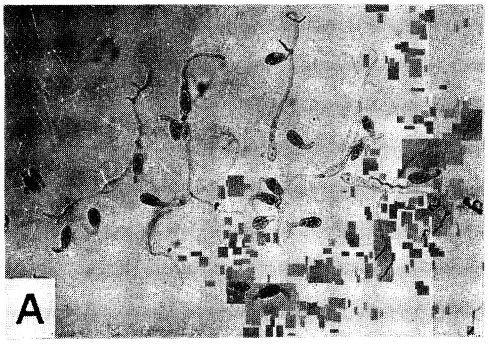
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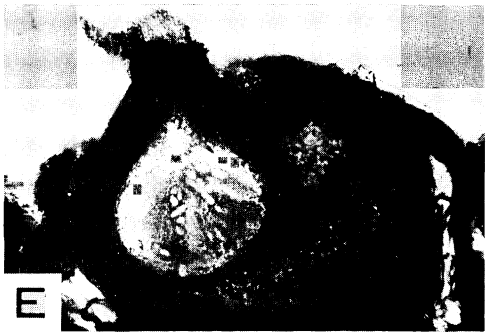
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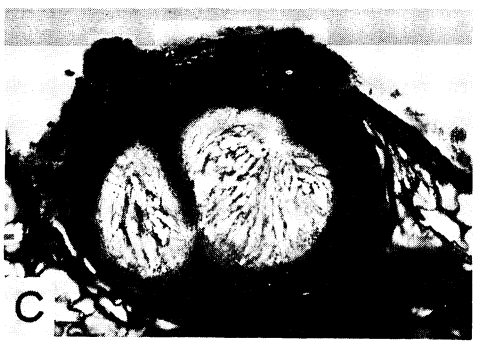
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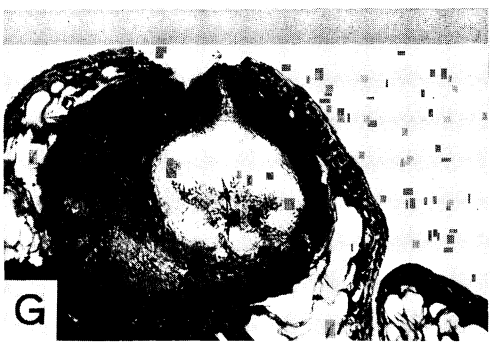
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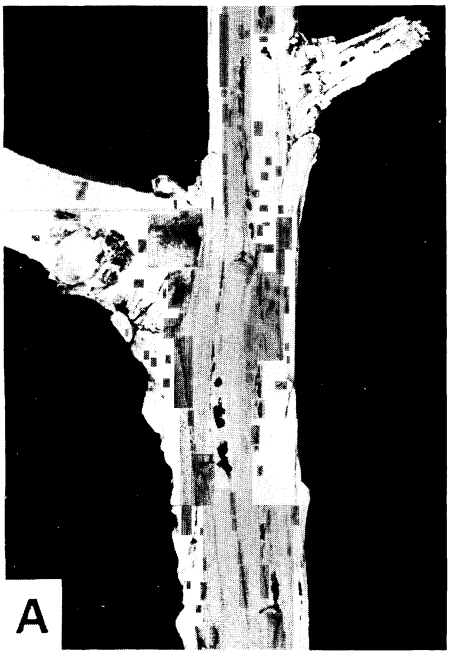
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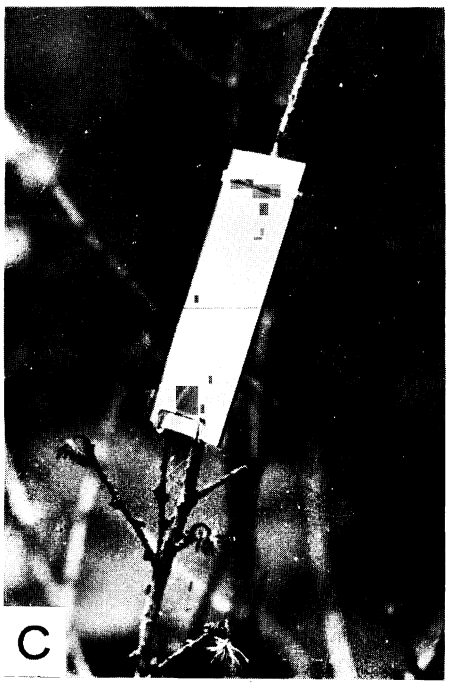
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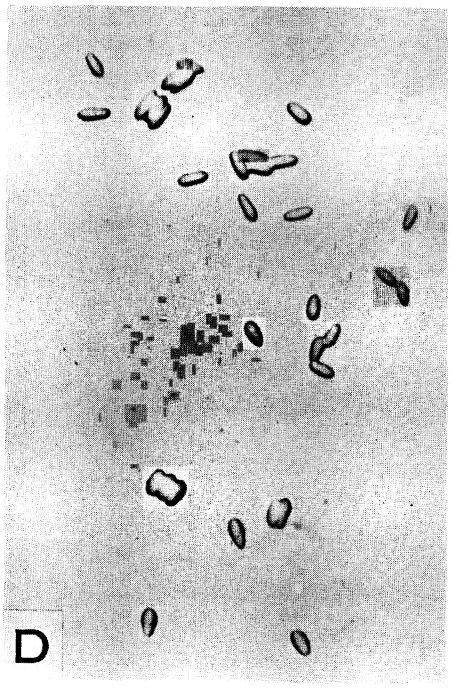
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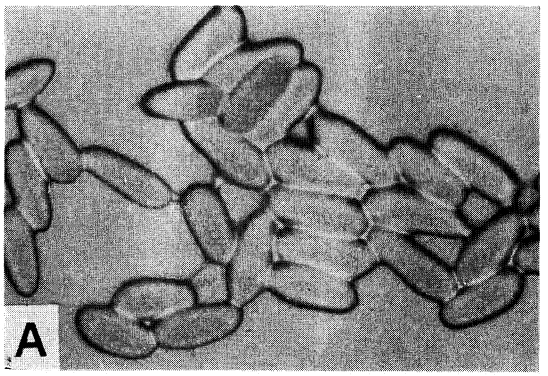
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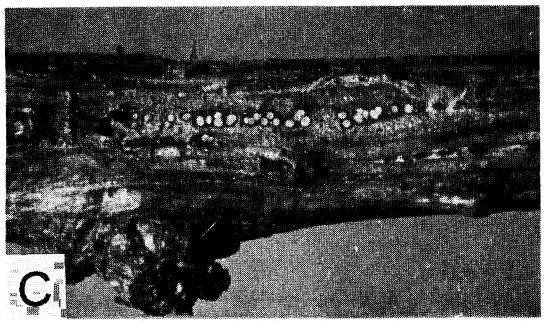
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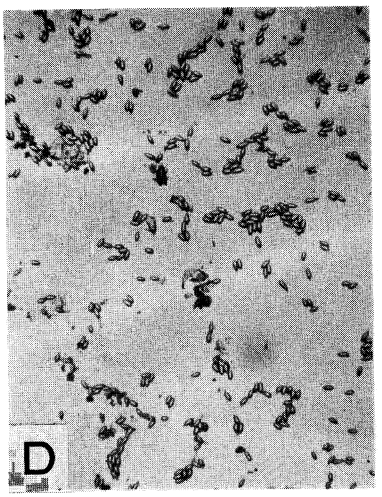
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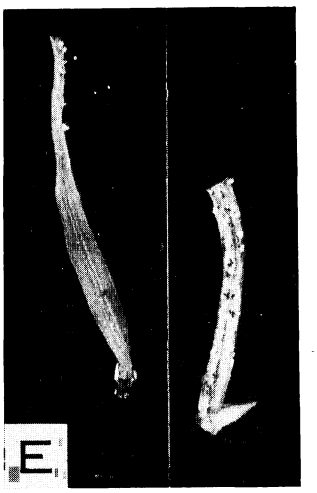
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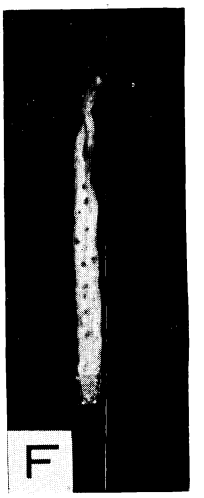
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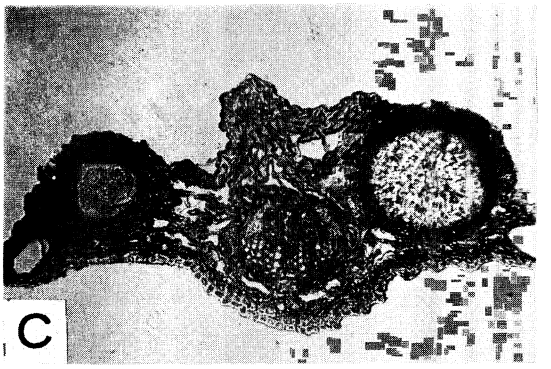
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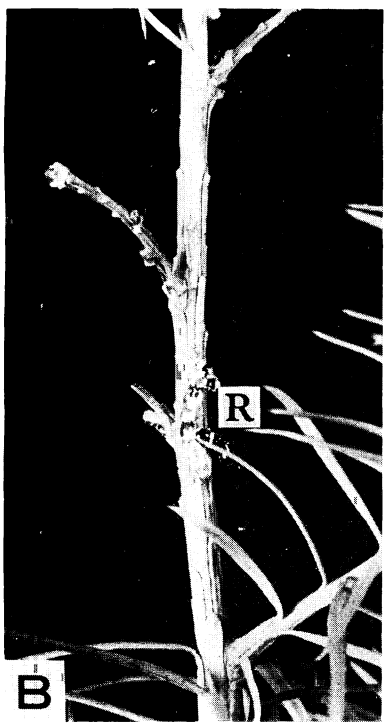
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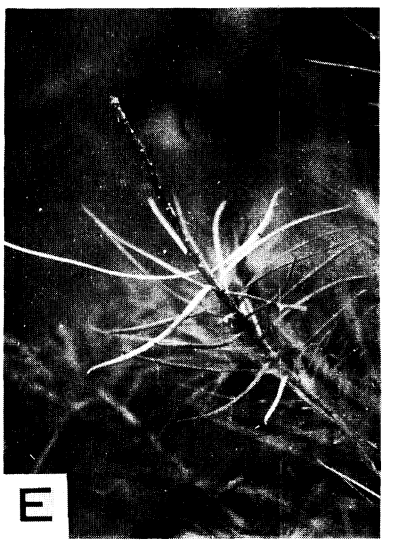
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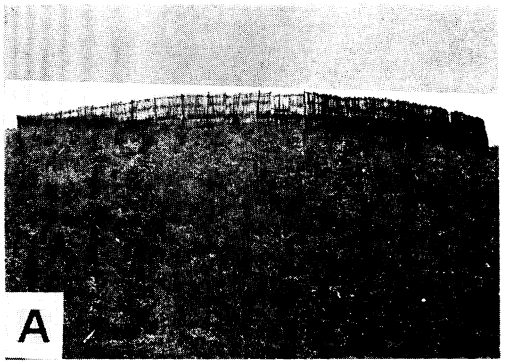
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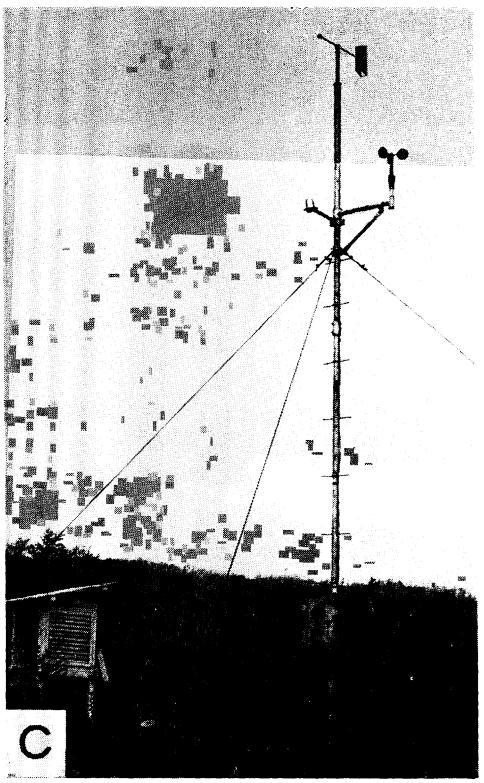
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