日本の気象条件と好稠糸状菌胞子の自然発芽

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On the natural spore germination of some tonophilic fungi, depending upon the climate of Japan

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Abstract

Spore germinations of some obligatorily tonophilic fungi and ordinary fungi belonging to the genus *Aspergillus* were examined under experimental and natural climatic conditions. Following results were obtained.

(1) Spores were incubated under different humidities (100, 95, 90, 80, 70, and 60% RH) and different temperatures (0°～5°, 15°～18°, 24°, 28°, or 30°, and 37°C). Marked differences between the tonophilic and ordinary fungi tested were seen on the point of the germinating activities at 100% and lower than 80% RH. Actually, spores of the tonophilic fungi never germinated in the air saturated with water vapour at any temperature tested, but germinated at the lower humidities such as 80～60% RH. On the contrary, 100% RH was optimum for ordinary fungi.

(2) Spore germinations under natural conditions were observed in the rainy seasons since 1961 to 1963. From the observational results, it has been elucidated that the climatic conditions such as high temperatures and high humidities of the air caused the spore germination of the obligatorily tonophilic fungi tested. On the other hand, spores of the ordinary fungi have never germinated under the same natural conditions. Thus, the climatic conditions under which the germination of spores took place was fully accordant with the experimental conditions mentioned above.

Obligatorily tonophilic fungi, *Aspergillus vitricolae* and *Eurotium tonophilum*, which were capable of growing on glass under natural environments were isolated from lenses of optical instruments by OHTSUKI (1941, 1962). In addition to the glass moulds mentioned above, two other tonophilic fungi, *Eurotium halophilicum* (CHRISTENSEN *et al.* 1959), and *E. heterocaryoticum* (CHRISTENSEN *et al.* 1965) were isolated from wheat and rough rice, respectively. On the other hand, a new fungus identified *Eurotium halophilicum forma* A was found by the present author on an old agar-slant preserved in her laboratory (1964). These three fungi other than glass moulds, also showed high osmophily (IMAI 1962, 1964). As described in a foregoing paper (IMAI 1962), *Eurotium halophilicum* and *E. halophilicum f.* A grew better in the range of 90～80% relative humidity (RH), and also did even in those of 70～60% RH. Spores of ordinaty fungi, e.g., *Aspergillus oryzae* and *A. niger*, however, germinated best under highly moist condition, at nearly 100% of relative humidity, and did lesser at 80% RH (IMAI 1958).

As above mentioned, the spore germination of these obligatorily tonophilic fungi is characterized by its aerial germination. Spores of the fungi seemed to be capable of

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absorbing gaseous water from the air. They could not germinate in water or even in liquid media. OHTSUKI pointed out this fact and mentioned that the natural germination on the glass, therefore, should take place depending upon the atmospheric humidity (OHTSUKI 1953, 1962). In the paper, he also described that the cloudiness of optical instruments in Japan occurs most frequently during the rainy season characterized by high humidity and high temperature. In contrast to Asian countries, fungal growth occurs only exceptionally on dry materials other than foods in both Europian and American countries. The climatic condition of these countries may be responsible for the absence of fungal growth, because it is not moist in summer time and two important points i.e., high moisture and high temperature do not coexist throughout whole year.

Therefore, the author attempted to compare spore germination under natural conditions with those under experimental conditions. For the purpose of preventing the cloudiness of optical instruments and protecting the crop from damage by mould infection, too, it seems important to elucidate the correlation between experimental conditions and natural atmospheric conditions which might affect the spore germination of tonophilic fungi.

In the present paper, the results obtained from experiments on aerial spore germination and the observations regarding the spore germination under natural climatic conditions will be reported.

Materials and methods

Throughout experiments on the spore germination, following procedures were adopted: (A) A small glass tube was covered with a piece of glass, on which spores were hanged (IMAI 1958; OHTSUKI 1962). These tubes were half filled with aqueous sulfuric acid solutions of varied concentrations (WALTER 1931), by which the air inside of them might be maintained at the desired level of relative humidity. These tubes were exposed to temperatures, 0°, 15°～18°, 24°, 28°, or 30°, and 37°C, and were subjected repeatedly to microscopic observations in respect to germination of spores and their subsequent growth. (B) In order to examine the spore germination of several species simultaneously, a larger cylindrical glass container (90 mm in diameter, 80 mm in height) was used (IMAI 1962, 1967). In this case, slide glasses were smeared with malt-extract medium containing 3% agar, then kept in a calcium chloride- desiccator for four days before inoculation. By such a treatment, the agar medium became a thin film covering the slide glass. Spores were inoculated on the agar film, and laid on a glass plate supported by a glass tripod in the container mentioned above. The air inside the container was maintained at desired levels by the same method used for the case of (A).

Rate of spore germinations were expressed by days required for the first germination of spores. If necessary, the rate of germination was expressed by percentage of sprouted spores to inoculated spores, and were represented by the following marks: (–) indicates no germination, (±) and (+～–) only swollen spores and scanty germination less than 1%, respectively. (+), (++), (+++), (++++) and (+++++) indicate less than 10, 30, 50, 70% and more than 70% of germination, respectively.

Aspergillus vitricolae (OHTSUKI 1962), *Eurotium tonophilum* (OHTSUKI 1962), *E. halophilicum* (CHRISTENSEN *et al.* 1959), and *E. halophilicum* forma A (IMAI 1964) were used as tonophilic fungi. For the comparison's sake, parallel experiments were carried out with four species of ordinary fungi; *Aspergillus niger* (IMAI 1958; FUKUDA 1953), *A. oryzae* (*ibid.*), *A. restrictus* and *A. fischeri* (THOM *et al.* 1945; RAPER *et al.* 1965).

Experiments have been carried out as follows:

(I) *Aerial germination of tonophilic fungus spores under controlled conditions*: (1) *Spore germinations under varied relative humidity and constant temperature.* Spores were incubated at 28°C under constant humidity, but *Aspergillus oryzae* was exceptionally incubated at 30°C. Relative humidity was adjusted to 100, 95, 90, 80, 70, and 60%, respectively. (2) *Spore germinations under varied temperature and varied relative humidity.* Temperature of 0°~5°C and 15°~18°C were kept in a refrigerator, and in the laboratory room respectively. Thermostats were used for higher temperatures. Relative humidities ranging from 100 to 60% RH were provided as mentioned above.

(II) *Spore germination of the same fungus under natural climatic conditions*: Spores of the test organisms were inoculated both on glass covered with agar film and on glass only, and kept in different places such as on shelves, in drawers of desk, and on a floor. Neither temperature nor humidity was controlled artificially. They were kept under quite natural climatic conditions. Then, the rate of germinations was examined daily. And observations were begun in 1961, ended in 1963. Spores were inoculated on 27 May in 1961, on 7 June in 1962, and on 22 May in 1963.

Results and discussion

On the experiment (I): Data obtained from the experiment (I) are presented in Figure 1. In this experiment, the time needed for the first germination seems more important than the numerical rate of germinated spores. Therefore, days required for the first appearance of spore germination were designated as the rate of germination. The reasons are as follows: 1) Germination time may be depending on the age of each spores. 2) Even a loop of inocula may contain spores of different ages. Spores germinated gradually as we have expected. Especially in the case of tonophilic fungi, counts of germinated spores actually increased day by day.

In the course of experiments, the most interesting thing was characteristic behaviour of the spores of tonophilic fungi. They showed optimum germination at a relatively low level of air humidity, about 90~80%, when they were inoculated on chemically clean glass. On dried medium, the range of optimum RH value shifted to 95% from 90~80% and no germination of them took place at 100% RH. On the contrary, the spore germination of ordinary fungi were restricted within 100~80% RH, and the optimum humidity was 100% RH. Germinated spores of any ordinary forms never detected at the humidity less than 80% RH, even when incubated as long as 60 days. Such a marked tendency was seen throughout these experiments.

Results obtained from the experiment (2) are represented in Figure 2. Here, also

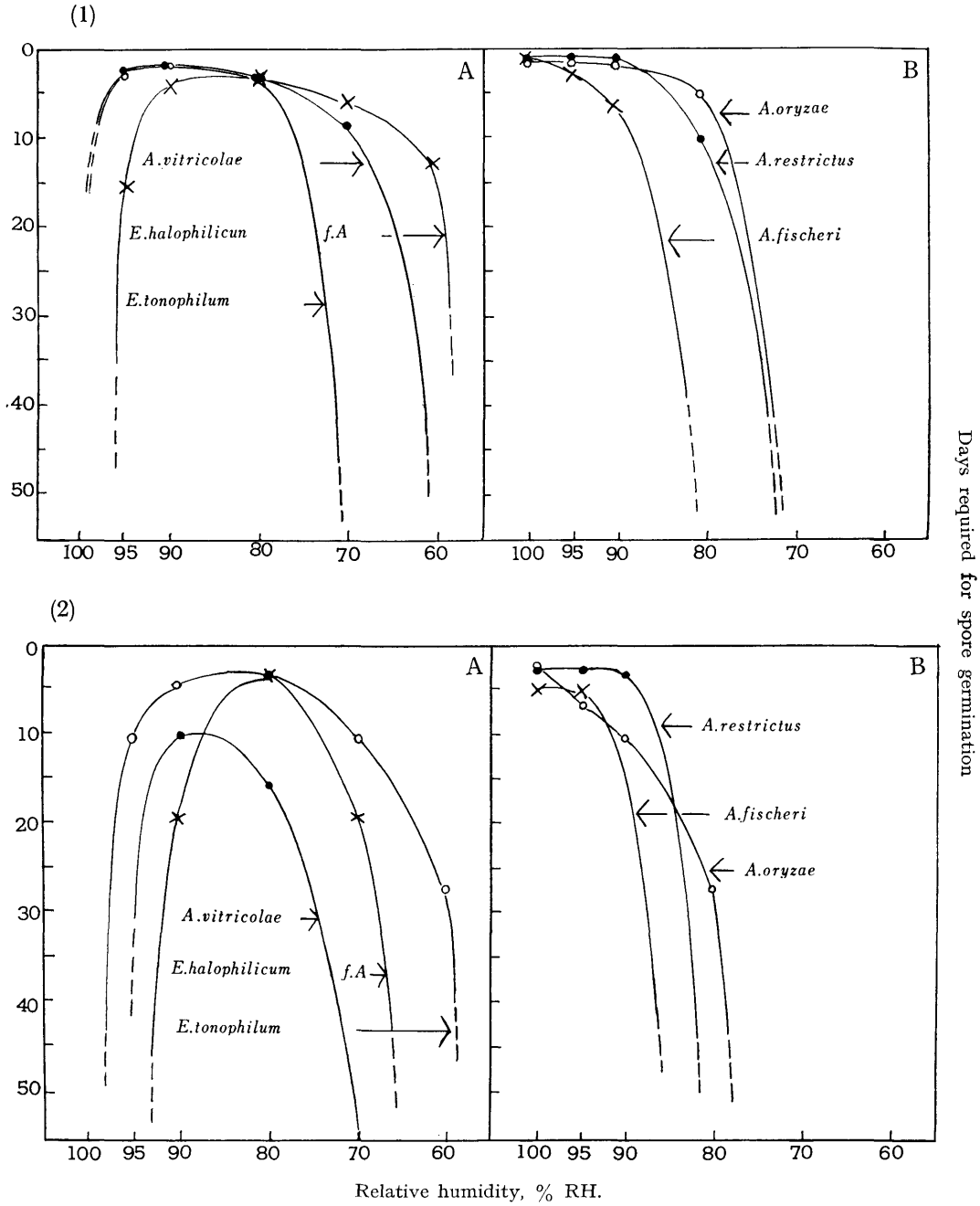

Fig. 1. Spore germination under varied relative humidities at 28° or 30°C.
(1), (2): Germination on dried medium and on chemically clean glass, respectively.
A, tonophilic moulds; B, ordinary moulds.

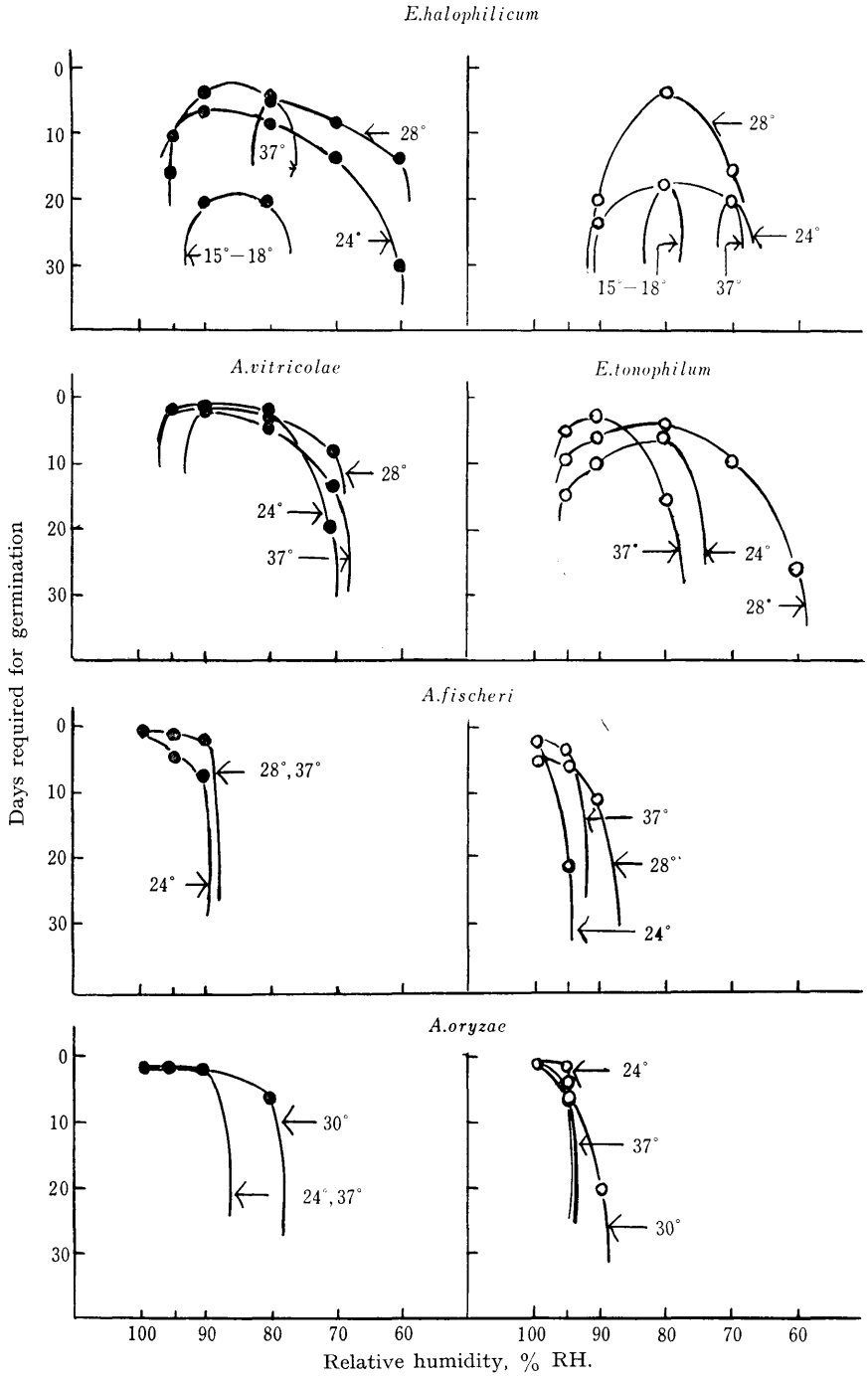

Fig. 2. Aerial spore germinations at varied temperatures and varied relative humidities.
•——•, o——o: Germination on dried medium and on chemically clean glass, respectively.

Table 1. Aerial germinations of mould spores

Observation from May 27 till July 31 in 1961

Organism / Germination / Date	*Eurotium halophilicum* form. A				Daily change in weather*	
	On Chemically clean glass	On dried medium on glass			Mean temperature	Mean relative humidity
		Agar (3%)	Malt extract agar	Sucrose agar	°C	% RH
June 21	−	−	−	−	23.8	69
〃 22	−	−	±	−	23.4	71
〃 23	±	±	±	−	24.4	73
〃 24	+～−	+～−	＋～+	+～−	24.2	82
〃 27	+～−	+～−	＋	＋	24.8	91
July 6	+～−	+～−	＋	＋	26.7	74
〃 18	+～−	+～−	＋	＋	23.3	74

Observation from May 22 till September 10 in 1963

Organism / Germination / Date	*E. halophilicum* f. A		*E. halophilicum*		*E. tonophilum*	
	On dried medium	On clean glass	On dried medium	On clean grass	On dried medium	On clean glass
June 11	−	−	−	−	−	−
〃 14	±	±	±	−	−	−
〃 17	＋～−	±～−	＋～−	−	−	−
〃 20	＋～−	＋～−	＋～−	+～−	−	−
〃 23	＋～−	＋～−	＋～−	+～−	−	−
〃 28	＋～−	＋～−	＋	+～−	−	−
July 9	＋	＋～−	＋	+～−	+～−	+～−
〃 23	＋	＋～−	＋	+～−	+～−	+～−
Sept. 10			＋	＋～−	+～−	+～−

* : Data from Monthly Report of Japan Meterological Agency.

the rate of germination is expressed as days required for the occurrence of the first germinated spores. Data regarding the spore germinations at 0°～5°C were eliminated from this figure, because there was no germination during the incubation period of 60 days.

As it is clearly shown in Figure 2, the similar tendency noticed in the previous experiment existed between the spore germination of tonophilic fungi and that of ordinary forms. Days required for the spore germination on dried medium and on clean glass (seris A and B, in Figure 2) were dependent on temperatures in certain extent, and especially conspicuous in the case of a tonophilic fungus (*Eurotium halophilicum* f. A) on clean glass. To the fungus, the optimum conditions for spore germination seem to lie within the range of 24°～28°C and 90～80% RH.

For further consideration, the results given in Figure 2 are again showed in graphs (Fig. 3). The salient characteristics in relation to temperature and humidity which can be observed on tonophilic fungi are primarily the following: The low and the high limits

as influenced by climatic conditions

Observation from June 7 till July 31 in 1962

Organism / Germination / Date	*Eurotium halophilicum* form. A.	*Eurotium halophilicum*	*Eurotium tonophilum*	Daily change in weather*	
				Mean temperature	Mean relative humidity
	On chemically clean glass			°C	% RH
July 10	−	−	−	23.5	86
〃 12	±～−	±～−	−	26.6	77
〃 17	+～−	+～−	−	25.9	75
〃 28	+～−	+～−	＋～−	25.5	88

Asp. vitricolae		*Asp. oryzae*		*Asp. niger*		Daily change in weather*	
On dried medium	On clean glass	On dried medium	On clean glass	On dried medium	On clean glass	Mean temperature	Mean relative humidity
						°C	% RH
−	−	−	−	−	−	22.3	68
+～−	−	−	−	−	−	21.	84
+～−	−	−	−	−	−	23.2	72
+～−	−	−	−	−	−	22.9	85
＋～−	−	−	−	−	−	26.	75
＋～−	−	−	−	−	−	25.5	78
＋＋～−	+～−	−	−	−	−	31.8	80
＋＋～−	+～−	−	−	±	−	28.8	78
		−	−	±	−	21.3	87

of relative humidity for germination of tonophilic fungi are 60～70% RH, and 95% RH, respectively. Thus we find that the optimum relative humidity of the fungi is not dew point and that the optimum temperature depends upon the humidity. In marked contrast to the tonophilic fungi, higher relative humidity was desirable for ordinary fungi and the optimum RH for their germination was 100% RH, i.e., dew point.

On the experiment (II): Among the obligatorily tonophilic fungi, glass moulds were often seen on clean glass, for example, on lenses of optical instruments when kept in humid place. This is especially remarkable during the rainy season (OHTSUKI 1943, 1962). Due to the records published by Japan Meterological Agency, the favorable condition 24°～28°C, and 90～80% RH. See page 668 for spore germination of the fungi resembles the hot and humid climate of Tokyo district from late May to early August.

Therefore, the results obtained from observations were listed in Table 1 together with records of the Agency. As shown in the table, the swelling of spores was seen on 22 June

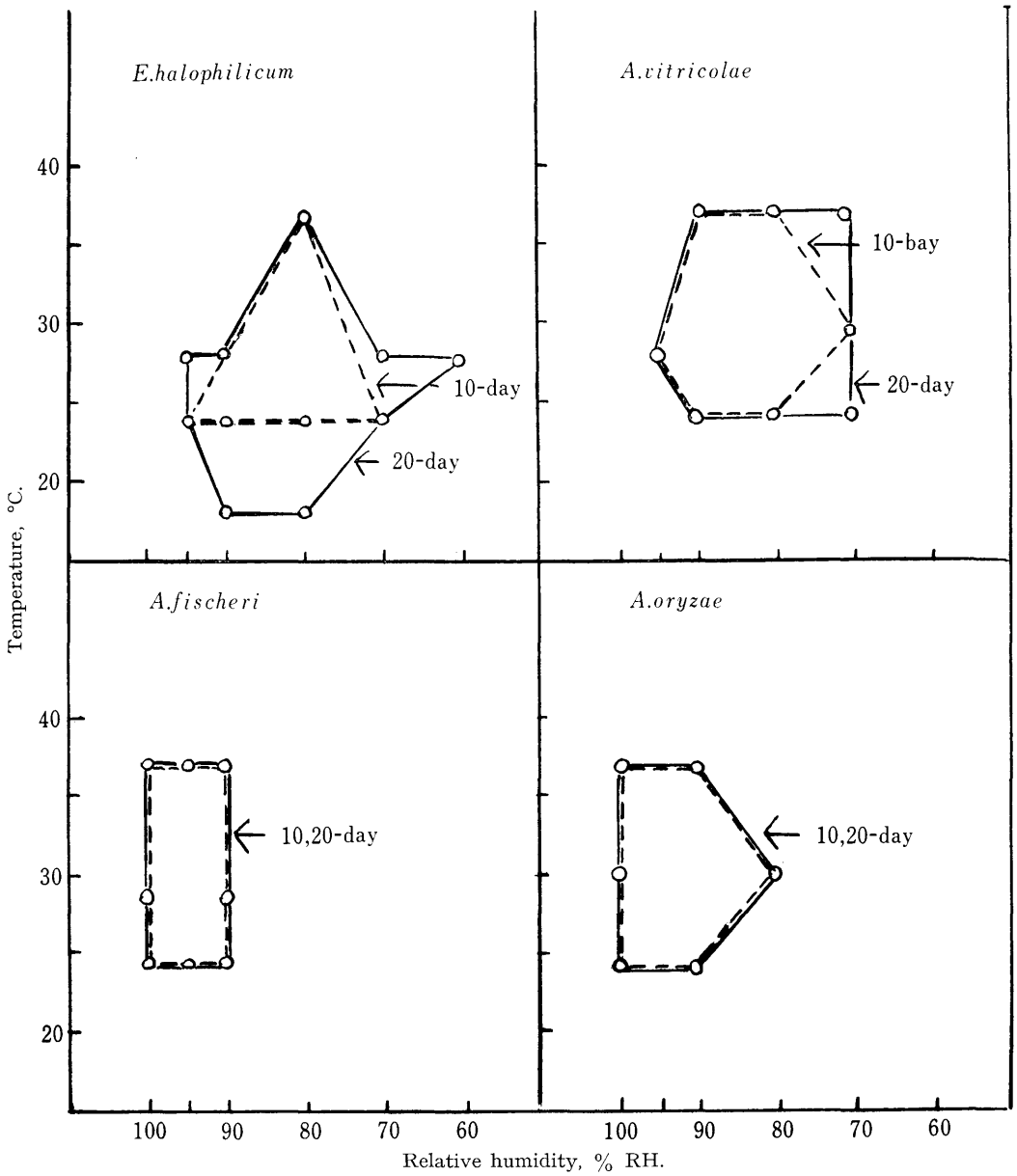

Fig. 3. Aerial spore germinations depending on relative humidities and temperatures.
———, - - - - - -: Germinations on dried media after 10 and 20 day incubations.

on dried malt-extract medium containing 3% of agar and the first germ-tubes appeared on 24 June in 1961 (*E. halophilicum f.* A), on 17 July in 1962 (*E. halophilicum* and *E. halophilicum f.* A), 14 June in 1963 (*A. vitricolae*), and on 17 June in 1963 (two strains of *E. halophilicum*), respectively. Adding this, ranges of the humidity and the temperature required for the first germination of the tested fungi were about 70~90% RH and 24°C,

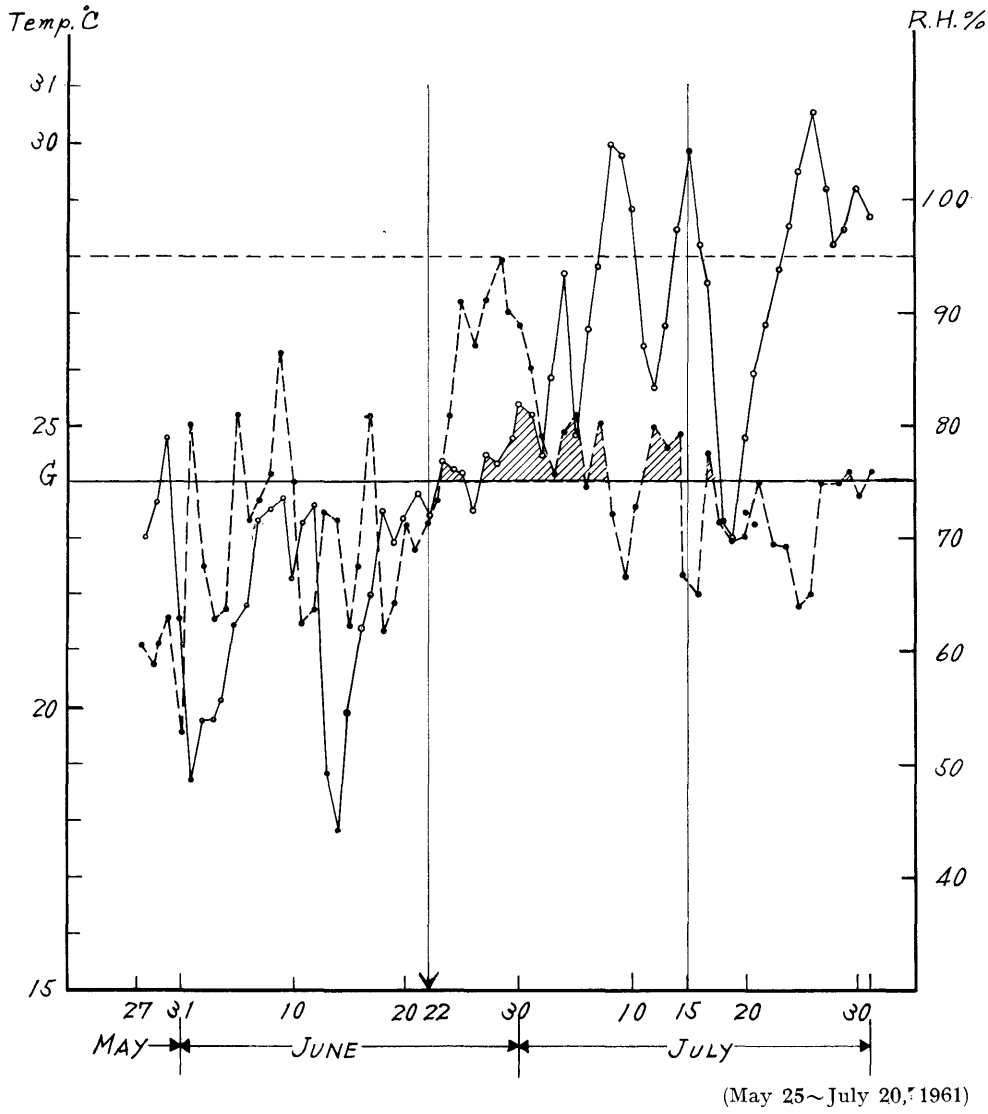

Fig. 4. Temperature and humidity of Tokyo District in 1961.
o——o: Temperature. •- - -•: Relative humidity. G: Germination line.

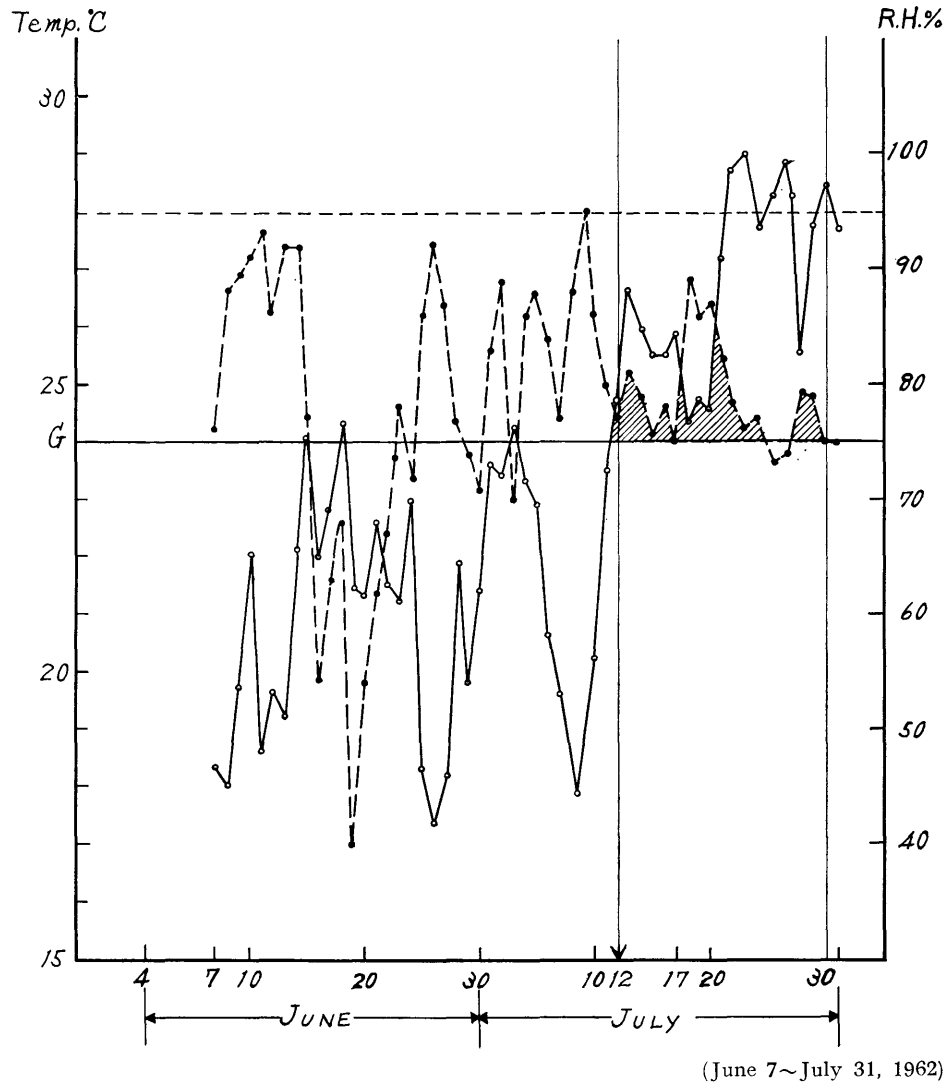

Fig. 5. Temperature and humidity of Tokyo District in 1962.
o——o: Temperature. •- - -•: Relative humidity. G: Germination line.

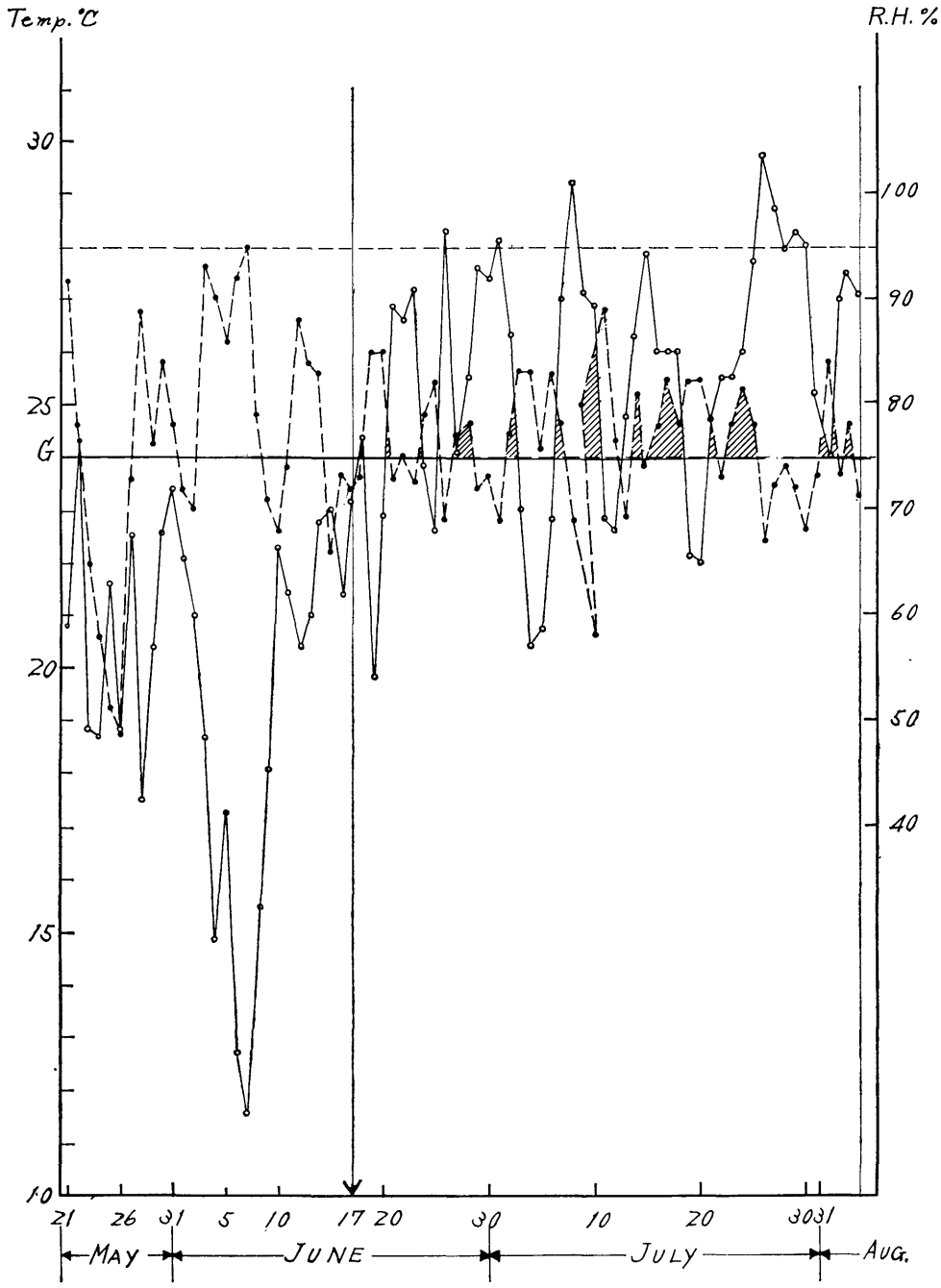

Fig. 6. Temperature and humidity of Tokyo District in 1963.
o——o: Temperature. •- - -•: Relative humidity. G: Germination line.

respectively. In order to indicate the result clearly, Figures 4, 5 and 6 are shown. At the temperature 24°C, where a line, "Germination line", is drawn, spore germinations of tonophilic fungi should be expected within 10-day incubation under appropriate humidity (see Fig. 3). Actually, spore germinations of the tonophilic fungi occurred within 10 days when both temperature- and humidity-curves rise over the germination line. Consequently, it was elucidated that there is a close relationship between germinations listed in Table 1 and the result obtained from experiment (I). From these results it may be conceivable, too, why ordinary forms could not germinate under natural climatic conditions in these three years.

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和　文　摘　要

日本の気象条件と好稠糸状菌胞子の自然発芽

今井百里江子

数種の絶対好稠糸状菌胞子と *Aspergillus* 属糸状菌胞子の実験条件下および気象条件下の気中発芽を試みた。次のような実験および観察結果が得られた。

(1) 異なる湿度 (100, 95, 90, 80, 70, 60% RH) および温度 (0～15°, 15～18°, 24°, 28～30°, 37°C) の下で胞子を培養したところ，100% RH および 80% RH 以下の湿度における好稠糸状菌胞子の発芽能と通常糸状菌のそれとの間に著しい差がみとめられた。即ち，前者は温度如何に拘らず，水蒸気飽和気中では全く発芽しなかったが，後者は水蒸気飽和 (100% RH) を最適湿度とした。

(2) 東京地方の気象条件下における胞子の自然発芽を 1961 年から 1963 年にわたり，主として梅雨期に観察した。その結果，高温多湿の気象条件は，絶対好稠糸状菌胞子の発芽をもたらしたが通常糸状菌胞子の発芽はこの気象条件下ではみとめられなかった。このように，胞子の発芽をもたらす気象条件は，上述の実験条件と全く一致する事が明らかにされた。