ジャガイモ疫病菌遊走子のうの成熟と間接発芽速度

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者	佐藤, 章夫
巻/号	60巻1号
掲載ページ	p. 53-59
発行年月	1994年2月

日植病報 60：53-59 (1994)
Ann. Phytopath. Soc. Japan 60：53-59 (1994)

Maturation of Sporangia of *Phytophthora infestans* Affecting the Rapidity of Indirect Germination

Norio SATO*

Abstract

The rapidity of indirect germination of the sporangia of *Phytophthora infestans* was investigated. Sporangial suspensions were prepared from the infected potato tuber slices or disks cultured for various periods at different temperatures. When the suspensions were incubated at 14°C, optimum for indirect germination, sporangia from older cultures could germinate more quickly than those from younger cultures. Sporangia immediately after production could germinate only slowly, while almost all the sporangia aged by pre-incubation for 6 hr or more at 22°C could germinate quickly within 1 hr when post-incubated at 14°C. Therefore, it was considered that sporangial maturation by aging was essential to acquire the ability of indirect germination, and that matured sporangia could germinate quickly within about 1 hr at 14°C. Time required for sporangial maturation was remarkably affected by the air temperature during sporangial production and also by the incubation temperature after preparation of sporangial suspension. The shortest maturation time, about 6 hr, was found between 18 and 22°C. Temperatures below 15°C remarkably slowed maturation, and temperatures of 26°C and above inhibited it. Matured sporangia quickly lost the ability of quick germination when the suspensions were incubated at 26°C or above.

(Received May 24, 1993)

Key words: *Phytophthora infestans*, sporangial maturation, indirect germination, potato late blight.

INTRODUCTION

Phytophthora infestans (Mont.) de Bary causes the important diseases, late blight of potato and tomato. Sporangia of the fungus can germinate either indirectly releasing zoospores or directly growing germ tubes. In water direct germination of young sporangia, less than a few days old, occurs very slowly, usually takes 1-2 days, and the proportion of germination is usually low, while indirect germination occurs rapidly and some sporangia release zoospores within 1 hr[3]. Zoospores play a very important role on the disease development because they can swim in water to infection sites of plants and can germinate quickly[3,4,6,7]. Therefore, for inoculum potential, not only sporangial production but also the proportion and rapidity of indirect germination are very important factors. It has been known that temperatures of 9-13°C were the optimum for indirect germination whereas 23-24°C inhibitory to it[1,3,6], and the proportion of germination was very variable, sometimes high but sometimes low even when sporangial suspensions were incubated for 1-2 days at the optimum temperatures[1,2,3,6]. The delicate nature of indirect germination seemed to disturb further progress of physiological research. Perhaps, distilled water and the tap water used by the previous workers might not be very favorable for indirect germination, because in the tap water of Hokkaido Natl. Agric. Exp. Stn. the proportion of germination was always high, which enabled a series of detailed investigations on sporangial germina-

* Hokkaido National Agricultural Experiment Station, Hitsujigaoka-1, Toyohira-ku, Sapporo 062, Japan 北海道農業試験場

tion.

This paper reports some factors affecting the rapidity of indirect germination. Since direct germination was always less than a few percent, it was neglected and in this report hereafter indirect germination will be described simply as germination except for special notices. A part of the results was briefly referred to elsewhere before[8].

MATERIALS AND METHODS

Sporangial production. An isolate (H-0) of *P. infestans*, race 0 and Al mating type, was maintained on potato tuber slices of cultivar Irish Cobbler by weekly inoculation. To produce sporangia, two different cultures, tuber-slice and -disk cultures, were used (Fig. 1). In the tuber-slice cultures, slices of 8–10 mm thick were placed on the V-shaped glass rods on the moist newspaper sheet in a petri dish (18 cm dia.) and sprayed with a dilute sporangial suspension (about 500 sporangia/ml). The petri dishes were kept in a temperature-controlled room at about 18°C. Sporangial production was few in 5 days but abundant in 6 and 7 days (Fig. 1-A). In the tuber-disk cultures, disks were cut out from the 5-day-old tuber-slice cultures by a cork borer (20 mm dia.) and the sporulating surface tissues of about 1 mm thick were removed by a razor for making a disk of about 6–8 mm thick (Fig. 1-B and C). After washing in running tap water and gently wiping with a paper towel, four to six tuber disks were placed on the wavy folded filter papers in a 9 cm petri dish, in which a 3 cm dish containing water and a filter-paper wick was placed to keep a high moisture condition (Fig. 1-D). They were kept in electric incubators at various temperatures between 10 and 26°C (±1°C). Resporulation on the tuber disks began in about 6–8 hr and the amount of sporangia increased with time as shown in Table 1.

Preparation of sporangial suspension and incubation for germination. The tap water from the well of Hokkaido Natl. Agric. Exp. Stn. was used to prepare sporangial suspensions. Sporangia

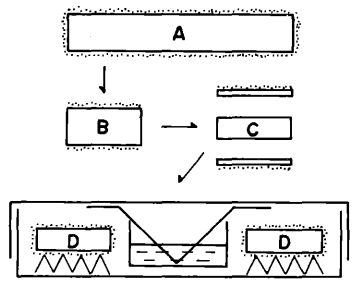

Fig. 1. Methods for obtaining the sporangia of *Phytophthora infestans*. The sporangia used for germination tests were collected either from potato tuber-slice cultures (A) or tuber-disk cultures (D).

Table 1. Effect of air temperature on sporangial production of *Phytophthora infestans* on potato tuber-disk cultures[a]

Air temp. (°C)	No. of sporangia from 12 tuber-disk cultures (×10⁴)				
	Time (hr) of culture incubation				
	12	18	24	36	48
10	0.02	0.2	1.0	2.8	7.5
12	0.1	1.1	2.9	6.4	12.2
15	0.5	4.6	13.6	26.6	57.8
18	0.9	8.8	17.2	30.8	97.6
20	0.7	11.5	19.1	51.8	93.6
22	0.9	12.8	20.3	65.8	113.8
24	0.6	7.6	15.6	36.8	35.9
26	0.2	1.7	2.9	5.2	3.7

a) Average of three experiments. The average coefficient of variation was 0.66.

were suspended by shortly dipping of the cultures in water of 22–24°C. The suspension was filtered through a gauze to remove mycelia, and after twice centrifugation (1,000 rpm, 1 min) for washing, the precipitated sporangia were resuspended in water at the concentration of 2.0×10^4 or less sporangia/ml. The suspension was dispensed into three test tubes (15 mm dia.), 2–3 ml per each, and incubated in a water bath at 14°C (±0.2°C) either immediately or after pre-incubation at different temperatures for various periods of time. The suspensions were kept undisturbed except for a brief agitation when sampling.

Determination of the proportion of germination. A small aliquot (0.2–0.3 ml) of suspension was taken from the test tubes, and added with a drop of 10% formalin to stop further germination. The proportion of the sporangia which had already released zoospores and just been releasing them was determined by observing at least 100 sporangia under a microscope, and the average percentage of three test tubes was used to indicate the proportion of germination in a suspension.

RESULTS

Relation of sporangial age to the time required for germination

Sporangia from the tuber-slice cultures 5, 6 and 7 days after inoculation were used to prepare suspensions. Either immediately after preparation or after pre-incubation for 24 hr at 22°C, the suspensions were incubated at 14°C, and the proportion of germination was determined at intervals. The results are shown in Fig. 2. In the suspensions immediately incubated at 14°C, the rapidity of germination fairly differed depending on culture period. The longer the culture period was, the more rapid the germination. The proportions of germination for the first 1 hr in the suspensions from 5-, 6- and 7-day-old cultures were 7, 19, and 34%, respectively. On the contrary, in the pre-incubated suspensions germination occurred rapidly irrespective of the culture period, and the proportion reached about 90% in the first 1

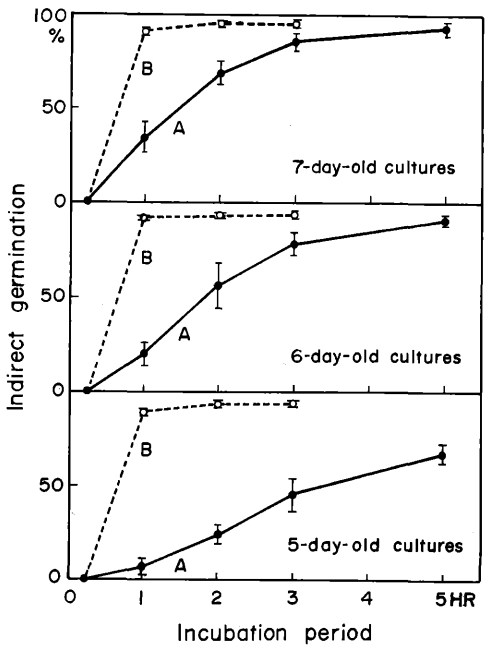

Fig. 2. Effect of the culture period to produce sporangia of *Phytophthora infestans* and the pre-incubation of sporangial suspensions for 24 hr at 22°C, on the rapidity of indirect germination. The sporangial suspensions prepared from tuber-slice cultures were incubated at 14°C either immediately (A) or after the pre-incubation (B). Each value indicates the average of five (A) and three (B) experiments, and the vertical bars show standard deviations.

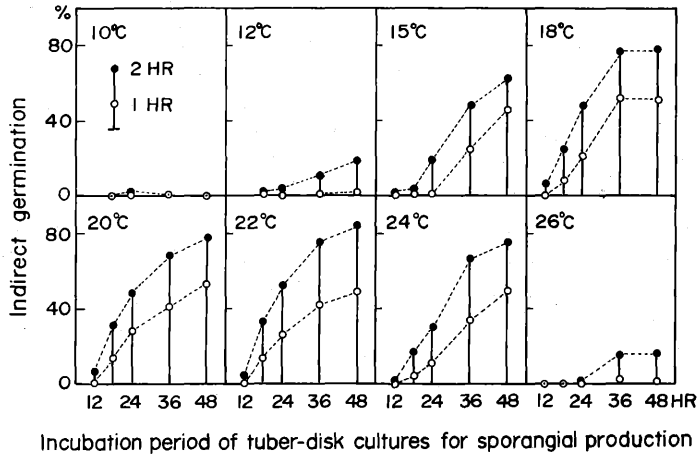

Fig. 3. Effect of the air temperature and period of incubation of tuber disk cultures of *Phytophthora infestans* to produce sporangia on the rapidity of indirect germination of the sporangia in water at 14°C. Each value indicates the average of three experiments.

hr. When the suspensions of newly produced sporangia from the infected tuber disks cultured for less than 12 hr were immediately incubated at 14°C, the sporangia germinated only slowly. Apparently sporangial age greatly affected the rapidity of germination and some period of aging appeared to be essential to acquire the ability of quick germination. Hereafter the author calls the physiological process to acquire the ability of quick germination as 'maturation'.

Effect of air temperature on sporangial maturation

Sporangia from the infected tuber disks cultured for the various periods of 12, 18, 24, 36 and 48 hr at the different air temperatures of 10, 12, 15, 18, 20, 22, 24 and 26°C, were used to prepare suspensions. The suspensions were incubated at 14°C immediately after preparation and the proportion of germination was determined after incubation for 1, 2, 3, 4, 6 and 24 hr. Irrespective of the different conditions of sporangial production, almost all sporangia germinated (av. 95.1%) within 24 hr. However, the proportions within the first few hr differed remarkably depending on the conditions of sporangial production, and so, only the proportions in the first 1 and 2 hr are shown in Fig. 3. Again, the culture period, or sporangial age, greatly affected the rapidity of germination. Newly produced sporangia from the youngest cultures at each temperature could not germinate quickly within 1 hr. As the culture period increased, the proportion of quickly germinative sporangia, or matured ones, increased. Air temperature during sporangial production also greatly affected sporangial maturation. Ten °C appeared to be almost completely inhibitory to maturation: quickly germinative sporangia were not found even in the sporangia from the oldest cultures. Twelve °C and 26°C were also strongly inhibitory to maturation. At 15°C matured sporangia began to appear from the 24-hr-old cultures. At 24°C the appearance of matured sporangia was a little earlier than at 15°C. At 18, 20 and 22°C matured sporangia began to appear from the 12-hr-old cultures, when the oldest sporangia were about 6 hr old, and the proportion increased rapidly. Therefore, it was considered that air temperatures between 18 and 22°C were the optimum for sporangial maturation, and that the shortest time required for maturation at these temperatures was about 6 hr. At those favorable temperatures for maturation including 15 and 24°C, the proportion of matured sporangia reached about 50% when the infected tuber disks were cultured for 48 hr.

Effect of water temperature on sporangial maturation

Newly produced sporangia from the infected tuber disks cultured for 16 hr at 24°C were used to prepare suspensions. The suspensions were pre-incubated at the temperatures of 10, 14, 18, 22, 24, and 26°C. At intervals small aliquots were taken and post-incubated for 1 hr at 14°C to determine the proportion of matured sporangia. The results are shown in Fig. 4. Water temperature also greatly affected sporangial maturation. As matured sporangia always included small proportions of a little

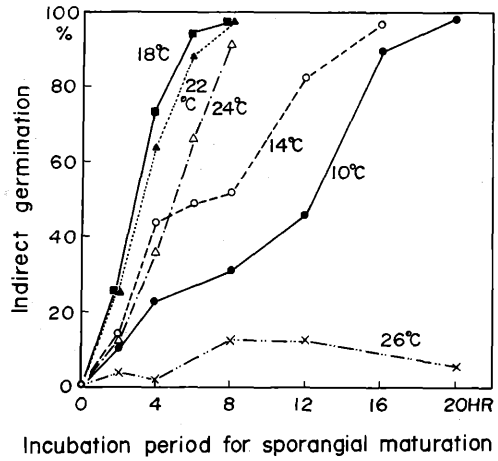

Fig. 4. Effect of water temperature on maturation of the sporangia of *Phytophthora infestans*. The suspensions of newly produced sporangia from the infected tuber disks cultured for 16 hr at 24°C were pre-incubated at the designated temperatures and then small aliquots of the suspensions were post-incubated for 1 hr at 14°C to determine the proportion of indirect germination (matured sporangia). A representative result is shown.

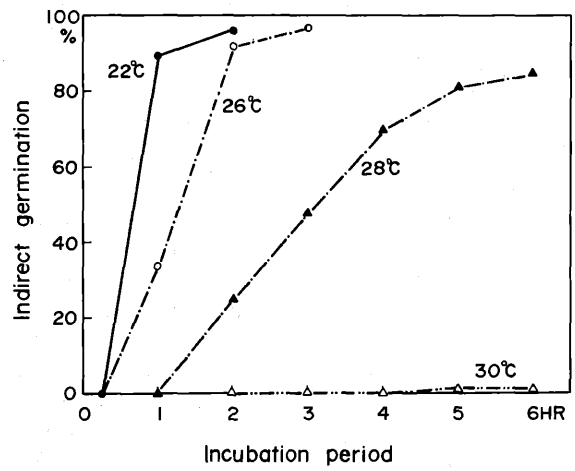

Fig. 5. Loss of the ability of quick germination of matured sporangia of *Phytophthora infestans* in water of comparatively high temperatures. The suspensions of matured sporangia pre-incubated for 2 hr at the designated temperatures were post-incubated at 14°C. A representative result is shown.

slower germinaters, the time required for attaining the level of 90% germination was presumed here to be the maturation time of the youngest sporangia in a suspension. Then the time required for maturation was about 6 hr at both 18°C and 22°C, 8 hr at 24°C, 14 hr at 14°C, and 16 hr at 10°C. Twenty-six °C was rather inhibitory to maturation. Therefore, similarly to air temperature, the water temperatures between 18 and 22°C were also considered to be the optimum for sporangial maturation. However, the water temperature of 10°C was not completely inhibitory to sporangial maturation unlike air temperature.

Loss of the ability of quick germination of matured sporangia

Sporangia from the infected tuber slices cultured for 7 days were used to prepare suspensions. After sporangial maturation was induced by incubation for 22 hr at 22°C, the suspensions were pre-incubated for 2 hr at 22, 26, 28 and 30°C. Then the suspensions were post-incubated at 14°C, and the proportion of

germination was determined at intervals. The results are shown in Fig. 5. The proportions of quickly germinative sporangia within 1 hr in the suspensions pre-incubated at 22, 26, 28 and 30°C were 89, 34, 0 and 0%, respectively. The rapidity of germination was remakably slowed down by the pre-incubation at 26°C, and greatly slowed down at 28°C, at which it took 5 hr to attain 80% of germination. All the sporangia pre-incubated at 30°C could not start germination before 4 hr. The results suggest that the ability of quick germination of matured sporangia may be lost quickly at the temperatures above 26°C.

DISCUSSION

The optimum water temperatures for indirect germination were reported as 12-13°C by Melhus[6] and Crosier[3], and 9°C by Bohnen[1]. However, the author used 14°C in the germination tests, because the temperature was best not only for the proportion but also the rapidity of germination. Distilled water has usually been used to test sporangial germination, in which nearly 100% of germination was sometimes achieved as shown by Bohnen[1], but usually it was not so favorable for germination as shown by Clark *et al.* (30-80%)[2] and Crosier (0-60%, av. 21%)[3]. Such instability of germination has strongly disturbed the progress of physiological research on germination. On the contrary, the tap water of Hokkaido Natl. Agric. Exp. Stn. was very favorable for germination (av. 95.1%), which enabled detailed investigations on sporangial germination.

The present results revealed that physiological properties of sporangia were variable by their ages and sporulating temperatures. Sporangia immediately after production didn't have the ability of quick germination, releasing zoospores within about 1 hr at 14°C, but acquired the ability by attaining maturity by aging. Air temperature during sporangial formation and water temperature after preparation of suspension showed similar effect on sporangial maturation. Temperatures between 18 and 22°C were the optimum for maturation and the time required for it was about 6 hr at these temperatures. The ability of quick germination was lost rapidly when sporangial suspensions were incubated at the comparatively high temperatures above 26°C. Such observations have not been carried out before.

Once Weille[9] also used the word maturation. He determined the total proportion of indirect and direct germinations after 2-3 days of incubation and called the both germinative sporangia as matured ones. Since indirect and direct germinations are quite different in physiological processes and should not be dealt with together, his use of the word maturation appears not to be adequate. Elsner *et al.*[5] also used the word maturation from the viewpoint of morphology. They designated those sporangia still exhibiting cytoplasmic continuity with their sporophores immature, and those ready for detachment mature. Cleavage vacuoles and well developed flagella were found in the cytoplasm of matured sporangia destined for indirect germination, while flagella resorption was found in the sporangia destined for direct germination. The relation between the morphological observations and the physiological process of maturation still remains unsolved.

Sporangial maturation will greatly affect the inoculum potential of the disease caused by *P. infestans* by affecting zoospore production. Therefore, further investigations on sporangial maturation under the field conditions will be required for the progress of the epidemiology of late blight of potato and tomato.

The author thanks Dr. D.H. Lapwood, Rothamsted Exp. Stn., England (retired), and also his colleagues, Dr. K. Takahashi and Mr. M. Kato, Hokkaido Natl. Agric. Exp. Stn., for critical reading of the manuscript.

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和 文 摘 要

佐藤章夫：ジャガイモ疫病菌遊走子のうの成熟と間接発芽速度

ジャガイモ疫病菌(*Phytophthora infestans*)遊走子のう(胞子)の懸濁液を調製して間接発芽(発芽)適温の14°Cに置いても，形成直後の若い胞子は発芽に長時間を要した。懸濁液を発芽不適温度の22°Cに6時間以上置いて胞子の齢を進めてから14°Cに置くと，ほとんどすべての胞子が1時間以内に発芽した。したがって，遊走子のうは形成直後は速やかに発芽する能力を持たず，一定の時間を経過してその能力を獲得すると考えられたので，この現象を「成熟」と称した。成熟は胞子を形成しているコロニー上でも懸濁された水中でも進み，成熟に好適な温度は18～22°Cであり，これらの温度における成熟所要時間はおよそ6時間であった。26°C以上の高温と15°C未満の低温では成熟が抑制された。また成熟胞子の持つ速やかに発芽する能力は，26°C以上の高温の水中では速やかに失われた。