ッルマメベと病菌の病斑部での蔵卵器および卵胞子形成に及ぼす水と温度の影響

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Effect of Water and Temperature on the Oogonium and Oospore Formation of *Peronospora manshurica* in Lesions on *Glycine soja*

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稲葉忠興*・日野稔彦*：ツルマメべと病菌の病斑部での蔵卵器および卵胞子形成に及ぼす水と温度の影響

Abstract

Oogonia and oospores of *Peronospora manshurica* were proved to be formed in lesions on *Glycine soja* (*G. ussuriensis*) by immersion treatment in water at 10-20 C. A few oogonia were formed by 3-day immersion at 15 and 20 C, and their number increased by 4-day immersion at 10, 15 and 20 C. The oogonium formation was most abundant at 15 C. After the formation of oogonia, oospores were more rapidly formed when temperature was higher in the range of 10-20 C. The size and shape of oospore formed by immersion treatment were identical to those hitherto reported on natural host, average diameter being 27.1 μm.

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Introduction

Oospore which is a sexual organ of Peronosporaceae and related fungi has a thick cell wall enabling it to tolerate various adverse environmental conditions. Therefore, oospore is of great importance especially in taking part in overseasoning, i.e., overwintering and aestivation[5,6]. Many researchers have attempted to clarify the disease cycle of the fungi belonging to Peronosporaceae and also clarify the conditions of oospore formation and its germination[1,2,4,9].

The factors affecting oospore formation have, however, not thoroughly been investigated and even the effect of water and moisture still remains to be elucidated, although water and moisture have been considered to be among the factors enabling the fungi to form oospores, on the basis of results obtained in field observations and laboratory tests[9].

Glycine soja (L.) Sieb. et Zucc. (*G. ussuriensis* Regel et Maack) is commonly found along roadsides and in grassland in Japan and is considered to be at the origin of cultivated soybean. The plant was reported to be infected with the fungus, *Peronospora manshurica* (Naoum.) Sydow ex Gäumann[7] and the disease on this host is easily detected even in the dry season in Japan.

The writers aimed to clarify the effect of water and temperature on oogonium and oospore formation using *P. manshurica* on *G. soja,* as it is possible to obtain material from field under dry condition.

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Materials and Methods

Materials used. The diseased leaves of *Glycine soja* (*G. ussuriensis*) were used throughout the experiment. The diseased leaves were collected from the plants naturally infected with *Peronospora manshurica* in a grassland at Sakura-mura, Niihari-gun, Ibaraki, in the period extending from August 7 to 18, 1978. The lesions used were yellowish green in color and were at the middle stage of disease development, forming conidiospores vigorously. After thorough rinsing in tap water, leaves with lesions were cut into pieces 5 × 5 mm square, each of which containing a single lesion. The leaf pieces thus obtained were again rinsed with sterilized distilled water for 10 min by stirrer. The leaf pieces thus prepared were used for various treatments in the following experiments described below.

Observation by microscope. The leaf pieces treated in the following experiments were stained by the method of Shipton and Brown[8]. Outline of the staining method was as follows : The leaf pieces were boiled in a solution containing phenol, glycerin, lactic acid, aniline blue, alcohol and distilled water. After incubation at room temperature for about 2 days, the leaf pieces were rinsed with water and placed in chloral hydrate solution for 30-50 min; and then mounted with 50 % glycerin for observations under the microscope. To observe the number of oogonia and oospores, a microscope Nikon S-Ke was used at the magnification of 200 times. The diameter of a field (× 200) was 0.91 mm.

Effect of temperature and duration of immersion in water on oogonium and oospore formation. Two hundred leaf pieces were immersed in 200 ml sterilized distilled water placed in a 500 ml beaker. The beakers were then incubated at different constant temperatures, 5,10,15,20 and 25 C. Twenty-five leaf pieces were taken out from each beaker after different incubation periods, namely 1,2,3,4,5,6,7 and 10 days after the beginning of immersion in water. The number of oogonia and oospores in 4-6 microscopic fields per leaf piece, i. e., 90-150 fields per 25 leaf pieces was counted. In the control, the number of oogonia and oospores in 150 fields per 25 leaf pieces was counted before immersion in water.

Oospore formation from oogonium at different temperatures. Samples used in this experiment were taken from the treated ones in the previous experiment 4,5, 6,7 and 10 days after the beginning of immersion in water at 10,15 and 20 C. Number of oogonia and oospores formed was counted and the percentage of oospore formation (number of oospores × 100 / number of oogonia and oospores) was calculated. In the samples obtained after 10 C treatment, 70-230 oogonia and oospores were observed for each duration of immersion in water, and in the samples of both 15 and 20 C treatment, 120-230 were observed.

Results

Effect of temperature and duration of immersion in water on oogonium and oospore formation

Leaf pieces immersed in water were incubated at different temperatures for different durations. At 10,15 and 20 C, a few oogonia were formed after 3-day immersion in water except for the 10 C treatment, whereas they were abundantly formed after 4-day immersion in all the treatments at 10-20 C, as shown in Table 1 and Figure 1. Among these treatments, 15 C was considered to be the optimum temperature, as oogonia appeared to be more abundant than at any other temperature. In the 10 C treatment the amount of oogonia and oospores formed was somewhat smaller than that at 15 C and 20 C even after 7-10 day immersions. Oogonia and

Table 1. Number of oogonia and oospores formed at different constant temperatures for various durations of immersion in water

Duration of immersion in water (day)	Number of oogonia and oospores formed (per 10 fields)				
	at 5	10	15	20	25 C
1	0	0.2	0	0	0.2
2	0	0	0	0	0
3	0	0.1	1.4	2.1	0
4	0.1	4.9	19.5	8.9	0.1
5	0.1	19.4	40.7	14.5	0.5
6	0.2	5.4	37.2	29.5	0.3
7	0.7	25.7	45.3	30.8	2.9
10	0.4	10.7	60.6	12.7	0.3
Not treated	0.3				

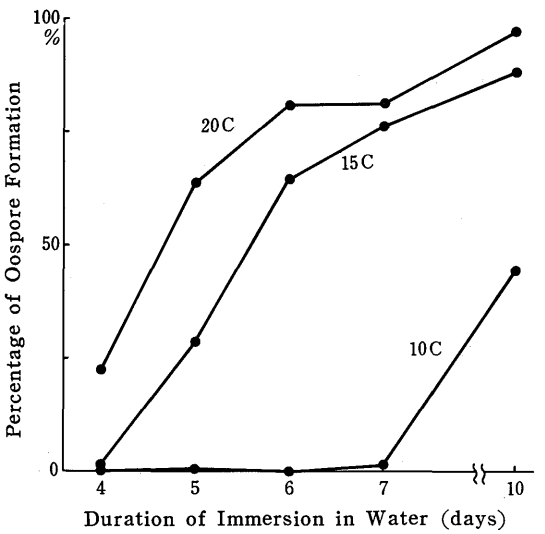

Fig. 1. Rate of oogonia and oospores formed at different constant temperatures. Percentage of oospore formation = [number of oospores × 100]/[number of oogonia and oospores].

oospores were formed in almost all the lesions used at 10-20 C after 5- to 10-day immersion, though these numbers formed in each lesion were not so uniform. At 5 and 25 C, the number of oogonia and oospores was almost the same as those of the control for all the periods of immersion in water up to 10 days. In the control, the number was 0.3 per 10 fields.

Oospore formation from oogonium at different temperatures

Samples used were taken from the previous experiment at 10,15 and 20 C after 4- to 10-day immersions in water. The percentage of oospore formation was calculated as follows: number of oospores × 100 / number of oogonia and oospores.

As shown in Figure 1, at a temperature of 10 C, oogonia were only formed after 4-day immersion in water. After 7-day immersion oospores were scarcely formed and even after 10-day immersion only 44 % of oogonia developed to oospores. At 15 C, only a few oogonia developed to oospores after 4-day immersion, whereas 64,76 and 88 % of oogonia developed to oospores after 6-, 7- and 10-day immersions, respectively. At 20 C, the percentage of oospores formed reached 22 % even after 4-day immersion, and 64, 81 and 97 % after 5-, 6- and 10-day immersions, respectively. These results suggest that oospore formation from oogonium was more rapid when temperature was higher in the range of 10-20 C and that most of oogonia formed could develop to oospores at 15-20 C.

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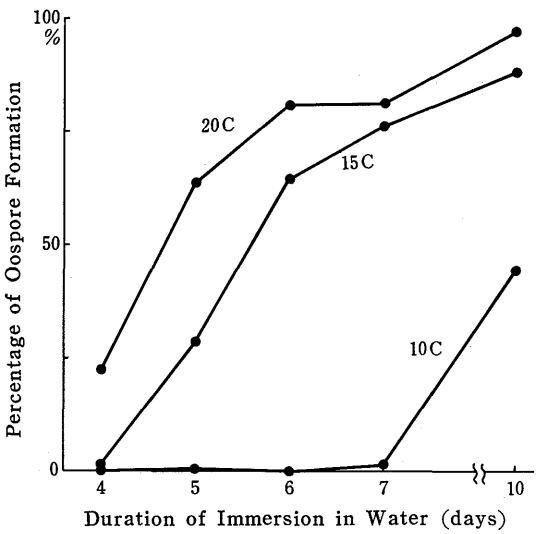
Not treated	0.3				



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Oospore formation from oogonium at different temperatures

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Oogonium and oospore formed

Various steps of oospore formation were observed in the samples immersed in water at 10, 15 and 20 C, as shown in the photographs of Figure 2. Oogonium thus formed was almost round in shape and filled with well-stained cytoplasm. Its surface was smooth and antheridium was laterally attached (Fig. 2 A and B). Thin-walled oosphere was differentiated (Fig. 2 C). The wall of oosphere became thick when the oospore and periplasm gradually became lightly stained (Fig. 2 D and E). Finally thick-walled oospore was formed with lightly stained periplasm (Fig. 2 F). Oospore was round in shape and its surface was smooth without protuberance. As a result of measurement of 200 oogonia and oospores each at the magnification of × 400, the size of oogonium was found to be 33.9 × 39.1 μm on the average, within a range of 22.7-45.3 × 28.0-57.3 μm. Oospore was 27.1 μm in diameter on the average, ranging from 20.0 to 36.4 μm.

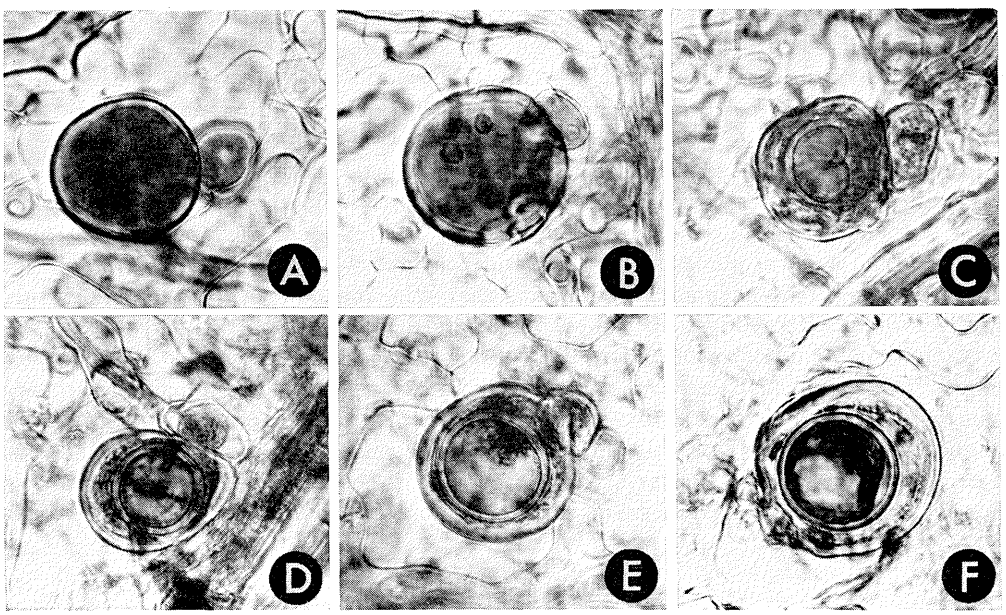

Fig. 2. Oogonium and oospore formation.
A & B : oogonium with antheridium, C : oogonium with round oosphere inside, D, E & F : oospore formed.

Discussion

In the present paper, materials used consisted of leaves of *Glycine soja* (*G. ussuriensis*) naturally infected with *Peronospora manshurica.* Oogonia were first formed after 4-day immersion in water at 10 C (Table 1 and Fig. 1). At 15 and 20 C, they were first observed after 3-day immersion and were abundantly formed after 4-day immersion, especially at 15 C. These results indicate that oogonia of the fungus are well formed when lesions are immersed in water at 10-20 C for 4 days, and that the optimum temperature would be 15 C from the view point of the amount formed.

Oospore formation from oogonium was very slow at 10 C, taking more than 6 days, whereas only 2-3 days at 15 C and 1-2 days at 20 C (Fig. 1) were required. Oospore formation from oogonium appeared to be rapid at higher temperature in the range of

10-20 C. Percentage of oospore formation (= number of oospores × 100 / number of oogonia and oospores) was 97 % at 20 C and 88 % at 15 C after 10-day immersion in water. This indicates that most of oogonia formed by immersion treatment are considered to develop to oospores under suitable conditions, though thorough study on the oospore formation from oogonium should be carried out in the future.

The size and shape of oospore formed by immersion in water (Fig. 2) were identical with those found on soybean[3] and on *G. soja*[7], being 27.1 μm in diameter on the average.

Materials used were collected in the field from August 7 to 18, 1978. According to the meteorological observations during the period extending from July 16 to August 25, rainfall occurred only 3 times, amounting to 1.0 mm on July 21, 7.0 mm on August 1, and 2.5 mm on August 17, respectively. As for the air temperature during the same period, minimum temperatures averaged for 5-day intervals were 21.8 C (lowest) and 25.9 C (highest). Average and maximum temperatures ranged from 26.0 to 28.7 C, and from 30.2 to 33.3 C, respectively. Under such conditions, neither oogonium nor oospore could be formed according to the results obtained in the present experiments. In fact, oogonia and oospores were practically not identified in any of the lesions used, and their maximum number in a lesion was only 4 when observed throughout the experiments.

In the present experiment, the yellowish green lesions at the middle stage of disease development were sampled repeatedly from the plants which were growing under such natural conditions. By the immersion treatment in water at 10-20 C for more than 4 days, numerous oogonia and oospores were formed in almost all the lesions used. This phenomenon indicates that rain and dew as well as temperature greatly influence the fungus in the oogonium and oospore formation.

Conditions of oospore formation in soybean downy mildew fungus, *Peronospora manshurica*, on soybean have not been clarified yet, though germination has been reported[1,2]. The writers also succeeded in obtaining oogonia and oospores of the fungus using the lesions present on cultivated soybean by the same method as that described in the present paper, though the results have not been published yet. This method is also applicable to downy mildew on soybean.

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和 文 摘 要

ツルマメべと病菌の病斑部での蔵卵器および卵胞子形成に及ぼす水と温度の影響

稲葉忠興・日野稔彦

ツルマメ (*Glycine soja* (*G. ussuriensis*)) べと病 (*Peronospora manshurica*) の病斑部を15Cと20Cの条件下で水に3日間浸漬することによって，蔵卵器は僅かに形成され，4日間浸漬では多数形成された。10Cでは蔵卵器の形成がやや遅れたが，4日間浸漬で形成された。蔵卵器が最も多く形成されたのは15Cの条件下であった。5Cと25Cでは10日間浸漬しても，ほとんど形成されなかった。10C—20Cの範囲では，蔵卵器から卵胞子が形成される速さは20Cで速く，10Cで遅かった。水に浸漬することによって形成された卵胞子の大きさは直径27.1μmであり，既往の報告と差がなかった。