The Influence of Light and Temperature on the Sporulation, Germination, and Infection of Phytophthora colocasiae Rac.

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The Influence of Light and Temperature on the Sporulation, Germination, and Infection of *Phytophthora colocasiae* Rac.*

By Masao TAMORI **

I. Introduction

*Phytophthora colocasiae* was first described by Raciborski in Java in 1900. Since that time the fungus was reported from various places in tropical and subtropical countries. The study of the influence of environmental factors to the fungus is very few. Butler and Kulkarni (2) reported the influence of temperature to the fungus, and Uppal (11) studied the influence of oxygen to the fungus. The infection studies were done by Sawada in Formosa in 1911, by Butler & Kulkarni in 1913, and by Gomez in Philippines in 1925.

The fungus spreads with zoospores which are produced from sporangia, and are disseminated by water. The study of the influence of environmental factors on the sporulation and infection helps to know about the disease and to control it. The present paper shows the influence of light and temperature on the sporulation, sporangial germination, and infection of the fungus.

II. Materials and Methods

The fungus *Phytophthora colocasiae* was obtained from the pure culture which was isolated on 17th of April in 1963. V-8 juice agar media which consists of 100 ml. of V-8 juice, 2.0 g. of CaCO₃, 15 g. of agar, and 900 ml. of water was used through the tests. The fungus was transferred to plates, containing the V-8 juice agar media, with a cork borer (5mm. in diameter). The plates were placed into incubators which kept different temperature and light.

1. The influence of light and temperature on the sporulation

Twelve plates of th culture were prepared. Three of them were exposed to the light at 20° C, in a incubator, 3 to the dark at the same temperature, 3 to the light at 24° C., and 3 to the dark. Two hundred foot-candle of fluorescence lamps were used for the light source. Five milliliter of water was poured onto a culture. Sporangioles with sporangia on the culture were rubbed with a fine brush and the sporangia were suspended in the water. The sporangia in the suspension was counted with a Haemacytometer under a microscope.

2. The minimum time for the beginning of indirect germination

Two incubators which were kept at 16° and 24° C. were used. The 200 foot-candle of flouesence lamp was set at 24° C. The 10-day-old culture was used to the test. The culture was cut into pieces with a cork borer (13 mm. in diameter). One of the pieces was transferred into a small beaker contained 5 milliliter of sterilized water. The sporangia were removed into the water with a fine brush. The suspension contained 9,500 of sporangia. The beakers for the dark chamber in the incubator were covered with a dark bag. The indirect germination of the sporangia was examined in 5 minutes intervals.

3. The relation between concentration of sporangia and their germination

The 10-day-old culture grown at 24° C. in the dark incubator was used to this test. The sporangial suspension was prepared as follows: 1) the culture was cut into pieces at the same distances from the center of the plate with a cork borer (13 mm. in diameter), 2) one of the pieces was put into a small beaker, which contained 5 milliliter of sterilized water in it, and the sporangia were removed in the water with a fine brush. The suspension contained 9,000 of sporangia per milliliter, 3) two other pieces were put into another beaker, and the sporangia in the suspension was 30,000 per milliliter, 4) three other pieces were put into another beaker, and the sporangial suspension was prepared. The number of sporangia in

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** Department of Agriculture, University of the Ryukyus.
this beaker was 40,000 per milliliter. 5) four other pieces were put into another beaker, and the sporangial suspension was prepared. The number of sporangia in the suspension was 50,000 per milliliter. These four beakers, prepared in the above, were kept at 24° C. in a dark incubator for 10 hours, and the germination was examined.

4. The influence of light on the sporangial germination

The cultures were grown at 24° C.; three plates in the light, and three in the dark. Sporangial suspensions were prepared from these cultures. From the culture grown in the light, a piece of the culture which was cut with a cork borer (13 mm. in diameter) was obtained. It was put into a small beaker (50 ml.) containing 5 milliliter of sterilized water, and the sporangia were removed into the water with a fine brush (30,000 sporangia/ml.). The sporangial suspension was prepared in 2 beakers as the same way with the above. From the culture grown in the dark, a piece of the culture was obtained by cutting with a cork borer (20 mm. in diameter). The sporangial suspension was prepared in two beakers (each beaker contained 25,000 sporangia ml.). These 4 beakers containing sporangial suspension, two from the culture grown in the light and the other two from the culture grown in the dark, were kept at 24° C. for 10 hours. Two suspensions, one from the culture grown in the light and the other from the culture grown in the dark, were exposed to the light. The 2 other suspensions, one from the light and the other from the dark, were kept in the dark. The germinated sporangia were examined under a microscope after 10 hours incubation.

5. The influence of temperature on the infection

1) Host plant: Leaves of Taro of 3rd or 4th leaf from the top of the plant, collected from the different aged plant, were applied to the tests. The age of the plants are: 4, 10, and 16 months old. The leaves were cut into pieces. The diameter of the leaf pieces was 8 cm. Each of the pieces was put onto a wet filterate paper in a Petri dish.

2) Inoculum: Sporangial suspension was prepared from the 10-day-old culture grown at 24° C. In order to obtain zoospores, the suspension was kept at 24° C. in a incubator for 2 hours. The number of zoospores in the suspension was 2,000 per milliliter.

3) Inoculation: Inoculation tests were done at 16°, 20°, 24°, 28°, 30°, and 32° C. The each three pieces of the leaf were incubated at the each temperature. Five spots of zoo- sporangial suspension, each spots contained 0,1 ml. were inoculated to the each piece. The infection was examined by measuring the diameter of symptoms and by counting the infected spots.

III. Results

1. The influence of light and temperature on the sporulation

When the fungus was exposed to the light, it produced 230,000 of sporangia per milliliter at 20° C., and 450,000 at 24° C. When the fungus was not exposed to the light, the sporulation was 150,000 at 20° C., and 200,000 at 24° C. (Table 1).

2. The minimum time for the beginning of indirect germination

The indirect germination of the sporangia of the fungus began in 15 minutes when the sporangia were placed in the light at 24° C. while it began in 20 minutes in the dark at the same temperature. The fungus also germinated indirectly in 25 minutes in the dark, at 16° C.

3. The relation between concentration of sporangia and their germination

As the Table 2 shows total germination of the sporangia decreased as the number of the sporangia increased; eity-five percent of the

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>Temperature (° C.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In the dark</td>
<td>20</td>
</tr>
<tr>
<td>In the light</td>
<td>24</td>
</tr>
</tbody>
</table>

* Number of sporangia per milliliter. Average number of three plates.
sporangia were germinated in the suspension which 9,000 of sporangia were contained per milliliter, 77% in 30,000, 63% in 40,000, and 62% in 50,000 of sporangia. Direct germination increased as the number of sporangia increased; nine percent of the sporangia were germinated in the suspension in which 9,000 of sporangia were contained per milliliter, 11% in 30,000, 16% in 40,000, and 20% in 50,000. Indirect germination decreased as the number of sporangia increased; seventy-six percent of the sporangia were germinated in the suspension in which 9,000 of sporangia were contained per milliliter, 66% in 30,000, 47% in 40,000, and 42% in 50,000.

4. The influence of light on the sporangial germination

The total germination of sporangia obtained from the culture which was exposed to the light during the growth period was low. When the sporangial suspension was kept in the light, the total germination was 49%, and that in the dark was 70%. The direct germination of sporangia obtained from the culture which was exposed to the light was rather high. It was 13% in the light, and 17% in the dark. The indirect germination of sporangia obtained from the culture which was exposed to the light was very low. It was 36% in the light, and 53% in the dark.

5. The influence of temperature on the infection

Temperature range for the infection of the fungus lies between 20° and 30° C. Optimum temperature seems to lie between 24° and 28° C. (Tables 4, 5, & Figs 1, 2). The visible symptoms, brown spots, were appeared in 70 hours at 20° C., in 33 hours at 24° and 28° C., and in 40 hours at 30° C.

The diameter of symptom decreased as the plant becomes old. At 20° C., the diameter of the symptom was 8 mm. on the leaf which was taken from 4-months-old plant in 90 hours after inoculation, 7 mm. on the leaf of 10-months-old plant, and 6 mm. on the 16-months-old plant. At 24° C., they were: 26.5 mm. on the 4-months-old plant, 20 mm. on the 10-months-old plant, and 19 mm. on the 16-

Table 2. The relation between number of sporangia and their germination.

<table>
<thead>
<tr>
<th>Number of sporangia per milliliter</th>
<th>Germination of sporangia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
</tr>
<tr>
<td>9,000</td>
<td>9</td>
</tr>
<tr>
<td>30,000</td>
<td>11</td>
</tr>
<tr>
<td>40,000</td>
<td>16</td>
</tr>
<tr>
<td>50,000</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 3. The influence of light on the sporangial germination of P. colosiae.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
</tr>
<tr>
<td>L</td>
<td>13</td>
</tr>
<tr>
<td>L</td>
<td>17</td>
</tr>
<tr>
<td>D**</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
</tr>
</tbody>
</table>

* L = The culture was grown in the light, 1 = The sporangial suspension was exposed to the light,
** D = The culture was grown in the dark, d = The sporangial suspension was not exposed to the light.
months-old plant. At 28° C.: 28 mm. on the 4-months-old plant, 22 mm. on the 10-months-old plant, and 21 mm. on the 16-months-old plant. At 30° C.: 21 mm. on the 4-months-old plant, 19 mm. on the 10-months-old plant, and 0 mm. on the 16-months-old plant. (Table 5, & Fig. 2).

Table 4. The influence of temperature on the infection of *P. colocasiae* to the different aged Taro plant (1).*

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Age of Taro</th>
<th>4-months-old</th>
<th>10-months-old</th>
<th>16-months-old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate of infect. (%)</td>
<td>Ave. size of sympt. (mm.)</td>
<td>CK. Rate of infect. (%)</td>
<td>Rate of infect. (%)</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
<td>14.5</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>28</td>
<td>80</td>
<td>16.8</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>30</td>
<td>7</td>
<td>9</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* In 64 hours after inoculation.

Table 5. The influence of temperature on the infection of *P. colocasiae* to the different aged Taro plant (2).*

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Age of Taro</th>
<th>4-months-old</th>
<th>10-months-old</th>
<th>16-months-old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate of infect. (%)</td>
<td>Ave. size of sympt. (mm.)</td>
<td>CK. Rate of infect. (%)</td>
<td>Rate of infect. (%)</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>87</td>
<td>3</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
<td>26.5</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>28</td>
<td>83</td>
<td>22</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>30</td>
<td>7</td>
<td>21</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* In 90 hours after inoculation.

**IV. Discussion**

The sporulation tests of the fungus were done by some writers. Leonian (7) reported that the media contained dextrose was the best for sporulation. Gomez (4) found that sporangia were produced after a week in the most cases. Butler and Kulkarni (2) reported that light does not effect on sporulation when temperature conditions are suitable (25° C.).

According to the present tests, sporulation of the fungus is influenced by the light. When the sporangia were obtained from the culture which was exposed to the light at 20° C., the sporangia sporulated more than 1.5 times of the sporangia grown in the dark at the same temperature. At 24° C., the sporangia grown in the light sporulated approximately 2.5 times of the sporangia grown in the dark.
In the present tests, the indirect germination of the sporangia of the fungus was rapid in the light than in the dark at the optimum temperature of the germination. In the light, the germination began within 15 minutes at 24°C, while it began in 20 minutes in the dark at the same temperature.

The total germination of the sporangia of the fungus decreased as the number of the sporangia in the sporangial suspension increased. The direct germination increased, and the indirect germination decreased as the number increased.

The total germination of sporangia obtained from the culture which was exposed to the light during the growth period was low. The direct germination of sporangia obtained from the culture in the same condition with the above was rather high. The indirect germination was very low. The germination of sporangia obtained from the culture which was exposed to the light during the growth period and the germination period was the lowest among all the treatment.

There are many studies of infection process of genus Phytophthora using P. infestans. Crosier (3) and Roder (9) reported the germ tube of cystic spore of P. infestans invaded directly through the epidermis cell wall. Hori (5) wrote the cystic spore produced appressoria on the tip of the germ tube, and a invasion hypha was grown on the appressorium. The penetration mostly occured at the intercellular spaces of epidermis. Pristou and Gallegly (8) found the germ tube of cystic spore produced appresoria no matter the host plant was susceptible or resistance. They also reported the germ tube which germinated from a sporangium directly does not concern to the infection. Sawada (10) successfully infected Taro (Colocasia antiquorum) and "Water-potato" (C. antiquorum var.) with sporangia taken from a leaf and germinated in distilled water. Butler and Kulkarni (2) inoculated the fungus to 16 species of plants, and they found the infection on 12 species. Gomez (4) reported the all variety of
Gabis are equally susceptible. He also got infection on Youtia, Caladium spp. Katsura (6) found, on P. capsici, that there were three ways of penetration with cystic spore. They are: 1) penetration with a germ tube of miniature zoosporangium which was produced on the tip of the germ tube of cystic spore, 2) direct penetration with a germ tube of cystic spore without forming appresoria of miniature zoosporangium, 3) penetration with invasion hypha which was grown on the tip of the germ tube of cystic spore.

In the present tests, using P. colocasiae, these three types were observed. In the most cases the appresoria, grown on the germ tubes of cystic spores, were observed. When the germ tube passed through the epidermal cell, the germ tube became narrow at the cell wall of the host. Before and after passing the cell wall the germ tube was swelled. In the mesophyll the germ tube pursed a strictly intercellular course, sending houstoria into the neighbouring cells. Butler and Kulkarni (2) found that inoculation on the leaves with living active zoospores in distilled water gave in some cases visible signs of infection within 6 hours. In 24 hours, brown spots at the point of inoculation were well developed. On the second day the patches were larger and the yellow drops of liquid were oozing out on their surface. On the third day sporangia were formed. The germ tubes from sporangia which germinate directly as sporangia were also found capable of penetrating leaf, entering across or between the lower epidermal cells. Gomez (4) found the brown spots appeared at the inoculated point in 3 to 7 days under field condition. In the chamber they occurred in 36 to 48 hours. According to the present studies, brown spots as the visible symptoms were observed in 70 hours at 20° C., in 36 hours at 24° and 28° C., and in 40 hours at 30° C. Temperature range for the infection of the fungus lies between 20° and 30° C. Optimum temperature lies between 24° and 28° C. The diameter of symptom decreased as the plant becomes old.

Literature Cited

10. Sawada, K. 1911. Infection of Taro. Special Reports of the Formosan Agric. Exper. Sta. II.
光と温度が Phytophthora colcasiae  
Rac の胞子のう形成、胞子のう発芽、
および宿主への侵入と発病に与える影響（摘要）

田 盛 正 雄

1. この研究は、光と温度が、サトイモ疫病菌
Phytophthora colcasiae Rac の胞子のう形成、胞子のう発芽開始、胞子のう密度と発病関係、胞子のうの直接発芽と間接発芽、遊走子の宿主への侵入および発病に与える影響について研究した結果をまとめたものである。

2. その実験には、病原菌の菌株として、V—8 ジュース寒天を使用し、また供試植物の葉は、4カ月、10カ月、16カ月目のタロイモの頂葉から3〜4番目のものを使用した。

3. この病菌は、光をあてると胞子のう形成が促進される。10日目の培養菌で 20°C の恒温室内部に、明るいところと1mlあたり（5mlの蒸留水を菌の生育しているシャーレ1個に注いで胞子のう浮遊液をつくり、それから1mlをとり出す）150,000 個の胞子のうを形成するのに対し、光をあてると、230,000 個の胞子のうを形成した。また、24°Cでは、暗室で200,000個に対し、光をあてると450,000個の胞子のうを形成した。

4. この菌の胞子のうは、発芽最適温度で、光をあてると間接発芽が早くなる。すなわち、24°C において光をあてると15分間で遊走子を出して間接発芽をするのに対し、暗室では、それが20分間もかかった。また、16°Cにおいては、暗室で、25分間もかあって間接発芽をする。

5. この菌は、胞子のう浮遊液における胞子のうの数が増えると、その総発芽率はさまでわれる。直接発芽（胞子のうから直接に発芽管を出して発芽すること）は胞子のう数が増えると、やや増える。これに対し、間接発芽（遊走子を出して発芽する）は、胞子のう数が増えるにしたがって、いちじるしく減る。（Table 2参照）

6. 生育期間中、光をあてたこの菌の胞子のうの総発芽率は低いうち。ここでは、直接発芽率は高くなるのに対し、間接発芽率はいちじるしく低い。とくに発芽処理期間中に光をあてると、その現象ははっきりとわかった。これに対し、生育期間中暗室におくと、総発芽率は高く、直接発芽率は低くなり、間接発芽率は高くなる。（Table 3参照）

7. この菌の宿主への侵入には間接発芽によって生じた遊走子のみが関係することがわかった。

8. この病気の発病する温度の範囲は、20°C から30°Cの間にある。発病最適温度は、24°C から28°Cにある病斑は、20°Cで70時間後、24°Cと28°Cで36時間後、30°Cで40時間後に認められた。

9. 病斑の直径は、植物体の年令が長くなるにしたがって小さくなる。とくに、4カ月目の植物体からとった葉では、その直径はいちじるしく大きいのに対し、16カ月目のそれには、小さな病斑しかあらわれなかった。（Table 4, 5参照）