

黄色突然変異キュウリおよび斑入りキュウリ葉上におけるSphaerotheca fuliginea (Schlecht.) Pollacciの生育

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Development of *Sphaerotheca fuliginea* (Schlecht.) Pollacci on Yellow Mutant Cucumber and White Mottle of Variegated Cucumber

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Abstract

The relationship between development of *Sphaerotheca fuliginea* and chlorophyll in cucumber plants (*Cucumis sativus* L.) was studied. As chlorophyll mutant, yellow mutant cucumber and variegated cucumber with white mottles, which occurred by a natural mutation were used. The yellow mutant cucumber lacks chlorophyll, and white mottle parts of variegated cucumber leaves also lack chlorophyll. On normal cucumber leaves, the hyphae elongated to 1,209 μm in length 48 hr after inoculation, and 63 haustoria were formed from each conidium within 96 hr after inoculation. Conidiophores were formed 4 days after inoculation. On the white mottle parts of variegated cucumber leaves, the hyphae reached 173 μm 48 hr after inoculation, and 5 haustoria were produced from each conidium within 96 hr. The conidiophores were observed 14 days after inoculation. On cotyledons of yellow mutant cucumbers, the hyphae elongated to only 74 μm in length, and no second haustorium was formed in 72 hr after inoculation. On yellow mutant cucumber whose roots had been soaked in a 2% sucrose solution, however the hyphae elongated 1,100 μm in length in the same period, and 48 haustoria were formed 96 hr after the inoculation. The conidiophores were formed 4 days after inoculation. Chlorophyll is not necessarily requisite for the fungal growth, since *S. fuliginea* developed as readily on yellow mutant cotyledons as on green leaves, if sucrose was supplied to the plants.

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Key words: *Sphaerotheca fuliginea*, cucumber, chlorophyll mutant, yellow mutant, variegated cucumber.

INTRODUCTION

Powdery mildew of cucumber, caused by *Sphaerotheca fuliginea*, is a common trouble in the cucumber production, because its high infectivity throughout the growing stages induce severe damage on the cucumber production^{1,3,7}.

Since *S. fuliginea* is an obligate parasite, its growth is considered to be closely related to the nature of the host. However, little significant features of host plant affecting growth of *S. fuliginea* have been studied^{1,3,7}.

The authors observed that the development of *S. fuliginea* on white mottle parts of leaves of variegated cucumber (Plate I-1a) was slower than that on normal leaves. Also no conidiophore was observed on the white mottle parts even 7 days after inoculation, while conidiophores of *S. fuliginea* formed on colonies on normal leaves 4 days after inoculation.

These phenomena suggested that chlorophyll in the host plant might affect the development of *S. fuliginea*, because the cells consisting of white mottle parts of leaves lacked chlorophyll.

The authors obtained yellow mutant cucumber by natural mutation from the cultivar sagami-

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hanjiro (Plate I-1b). The yellow mutant cucumber also lacks chlorophyll⁴⁾, and dies 10 days after germination.

The development of *S. fuliginea* on yellow mutant seedlings and on white mottle parts of variegated cucumber were observed and compared to that of normal cucumber leaves, to clarify the relationship between the development of *S. fuliginea* and chlorophyll in the host plants.

MATERIALS AND METHODS

The yellow mutant cucumber, variegated cucumber with white mottles, which occurred by natural mutation of cultivar sagami-hanjiro, and normal cucumber (*Cucumis sativus* L., cultivar sagami-hanjiro) were used as host plants. Seedlings were grown in plastic pots (one seedling/pot, with 120 g of soil) in a greenhouse (at temperatures 28°C for the time of 7:00 AM to 5:00 PM and 20°C for 5:00 PM to 7:00 AM) for 6 days for the yellow mutant, and in the same conditions at 0.7 true leaf-stage for the variegated and the normal plant.

Cotyledons of yellow mutant cucumber, mottle parts (almost white) of true leaves of variegated cucumber, and the normal true leaves were inoculated with fresh conidia of *Sphaerotheca fuliginea* (Schlecht.) Pollacci (Riken isolate) according to the methods described earlier⁵⁾. Immediately after inoculation, 5 seedlings of yellow mutant cucumber were scooped from the pot, and their roots were washed with tap water and soaked in 2% (w/v) sucrose solution. All the inoculated cucumbers, yellow mutant (5 treated and 5 non treated seedlings), variegated (3 seedlings) and normal (5 seedlings), were again placed in the greenhouse. The inoculated parts of the leaves or cotyledons were cut from the plants for observation.

A part of leaves or cotyledons were cut from those inoculated cucumbers and adhered without any other treatment on a specimen tube for observation of conidiophore formation (10 colonies for each treatment) with scanning electron microscope (MINI-SEM 101, ABT). Other parts of them were dipped in boiling water for 2 min and then soaked into aqueous ethanol (95%, v/v) for 2 hr²⁾. After being decolorized, those leaves were stained with a KHCO₃ (M/15) solution of aniline blue (0.1%, w/v) for 2 hr, and then stained again with basic fuchsin (2%, w/v) of aqueous ethanol (50%, v/v). After being rinsed with distilled water, the leaves were used for observation and photography under a fluorescent microscope (VANOX, OLIMPAS) with ultra-violet ray (exciter and absorption filters: V) and visible light²⁾.

The development of *S. fuliginea* (germination of conidia, elongation of germ tube and hyphae, formation of haustoria and conidiophore) on mottle parts of variegated leaves or yellow mutant seedlings, with or without sucrose, were observed and compared with that on normal leaves. Fifty conidia were observed for each treatment. Ten conidia were observed at the point of elongation of hyphae which was 48 hr after inoculation, and formation of haustoria, 96 hr after inoculation, on normal leaves and yellow mutant cotyledons with sucrose.

RESULTS

Development of S. fuliginea

a. Germination of conidia, elongation of germ tube and formation of first haustoria

On normal leaves, white mottle parts of variegated leaves, and yellow mutant cotyledons with or without sucrose, the conidial germination of *S. fuliginea* began 6 hr after inoculation, and reached a maximum level (60%) 12 hr after inoculation, and germ tubes elongated about 40 μ m in length. And then, the first hyphae elongated from the germ tube about 20–25 μ m long 24 hr after inoculation.

The first haustorium was formed under germ tube 12 hr after inoculation on normal leaves, white mottle parts and yellow mutant cotyledons with or without sucrose, and the percentage of the haustorial formation of germinated conidia reached about 40, 90 and 100% at 12, 24 and 30 hr after inoculation, respectively.

Table 1. Elongation of hyphae of *Sphaerothea fuliginea* on yellow mutant cotyledons and white mottle parts of variegated leaves of cucumber

Host plant	Hyphal length (μm) ^{a)}		
	18 hr ^{b)} (S.E.) ^{c)}	24 hr ^{b)} (S.E.) ^{c)}	48 hr ^{b)} (S.E.) ^{c)}
Normal cucumber leaves	60 (0.5)	135 (4.1)	1209 (74.7) ^{d)}
Variegated cucumber leaves	65 (0.5)	82 (0.6)	173 (4.4)
Yellow mutant cotyledons (no sucrose)	60 (0.4)	74 (0.4)	74 (0.4)
Yellow mutant cotyledons with sucrose	60 (0.5)	123 (4.5)	1100 (71.3) ^{d)}

a) Total length of hyphae elongated from each conidia (included germ tube). Fifty conidia for each treatment were observed.

b) Time after inoculation.

c) Standard error.

d) Ten conidia for each treatment were observed.

Table 2. Formation of haustoria of *Sphaerothea fuliginea* on yellow mutant cotyledons and white mottle parts of variegated leaves of cucumber

Host plant	Number of haustorium ^{a)}		
	48 hr ^{b)} (S.E.) ^{c)}	72 hr ^{b)} (S.E.) ^{c)}	96 hr ^{b)} (S.E.) ^{c)}
Normal cucumber leaves	5.5 (0.1)	15 (0.5)	63 (2.3) ^{d)}
Variegated cucumber leaves	1.0 (0.0)	3 (0.1)	15 (0.3)
Yellow mutant cotyledons (no sucrose)	1.0 (0.0)	1 (0.0)	1 (0.0)
Yellow mutant cotyledons with sucrose	3.8 (0.1)	11 (0.3)	48 (1.7) ^{d)}

a) Number of haustorium formed from each conidia. Fifty conidia for each treatment were observed.

b) Time after inoculation.

c) Standard error.

d) Ten conidia for each treatment were observed.

b. Elongation of hypha

On normal leaves, the hypha of *S. fuliginea* elongated from the germ tube and conidia reaching 60, 135 and 1,209 μm in length 18, 24 and 48 hr after inoculation, respectively (Table 1). The hypha on yellow mutant cotyledons soaked in 2% sucrose solution were also elongated and reached 60, 123 and 1,100 μm in length in the same period. On the other hand, the hypha of *S. fuliginea* on the white mottle parts reached 65, 82 and 173 μm in length 18, 24 and 48 hr after inoculation, respectively (Table 1). On yellow mutant cotyledons without sucrose, the hypha grew slightly, and reached 74 μm in length 48 hr after inoculation (Plate I-2, Table 1).

c. Formation of haustoria

On normal leaves and yellow mutant cotyledons with sucrose, formation of the second haustoria was observed 36 hr after inoculation. The number of haustoria of *S. fuliginea* increased with time, attaining to 5.5, 15 and 63 haustoria on each conidium on normal leaves, and 3.8, 11, and 48 on yellow mutant cotyledons with sucrose 48, 72 and 96 hr after inoculation, respectively (Plate I-3, Table 2).

On the other hand, the formation of the second haustoria on white mottle parts was observed 56 hr after inoculation, and 3 and 15 haustoria were formed on each conidium 72 and 96 hr after inoculation, respectively. The yellow mutant cotyledons without sucrose were also observed 48, 72 and 96 hr after inoculation (Plate I-2), and no second haustorium was observed within 96 hr after inoculation (Table 2).

d. Formation of conidiophore

On normal leaves, a part of colonies of *S. fuliginea* formed conidiophores 96 hr after inoculation, while others failed to do so. The conidiophores were observed in the center of a colony with more than 63 haustoria. The number of conidiophore at the center of the colony was 69.6 per mm^2 within 7 days after inoculation (Plate I-4).

On yellow mutant cotyledons with sucrose, 22.2 per mm^2 conidiophores were formed within 7 days after inoculation (Plate I-6). On white mottle parts, no conidiophore was observed in the colony 7 days

after inoculation (Plate I-5). The conidiophores were observed 14 days after inoculation.

DISCUSSION

Seeds of yellow mutant cucumber germinated similarly to that of normal cucumber, but the cotyledons (15 mm long $\times$ 8 mm width) were smaller than those (50 mm $\times$ 30 mm) of normal seedlings, and no true leaf developed from them. The yellow mutant seedlings lack chlorophyll in their cotyledons⁴⁾. The yellow mutant seedlings were unable to obtain external energy for their photosynthetic growth, and died 10 days after germination. The yellow mutant may be termed "xantha" by the classification of Gustafsson⁴⁾.

By soaking roots in a 2% sucrose solution, yellow mutant seedlings kept their activity 20 days after germination, but the cotyledons (17 mm $\times$ 10 mm) were also smaller than that of normal seedlings, and no true leaf developed.

The cells forming the white mottle parts were also unable to carry out photosynthesis. Mottled cells not surrounded by normal green cells died within 10 days after leaf development. However, the cells forming island-like white mottles maintained their activity for more than 20 days after the development of the leaves. These cells are considered to have been supplied with some nutrition from neighboring normal cells.

On these three types of host plants, i.e. green parts of normal leaves, mottle parts (almost white) of variegated leaves which were surrounded by normal green cells, and cotyledons of yellow mutant seedlings, the early stages of development of the fungus, such as germination of conidia, elongation of germ tube, formation of first haustoria and elongation of hypha (20–30 μ m long) of *S. fuliginea*, were almost the same. This means that these stages of development of the fungus mainly depend on nutrients contained in the conidia itself.

Further development of *S. fuliginea* on mottle parts of variegated leaves was slower than that on normal leaves, though it did not stop. Further the development of *S. fuliginea* on the yellow mutant plant without sucrose was arrested completely. On the other hand, the fungus well developed on yellow mutant cucumber supplied with sucrose.

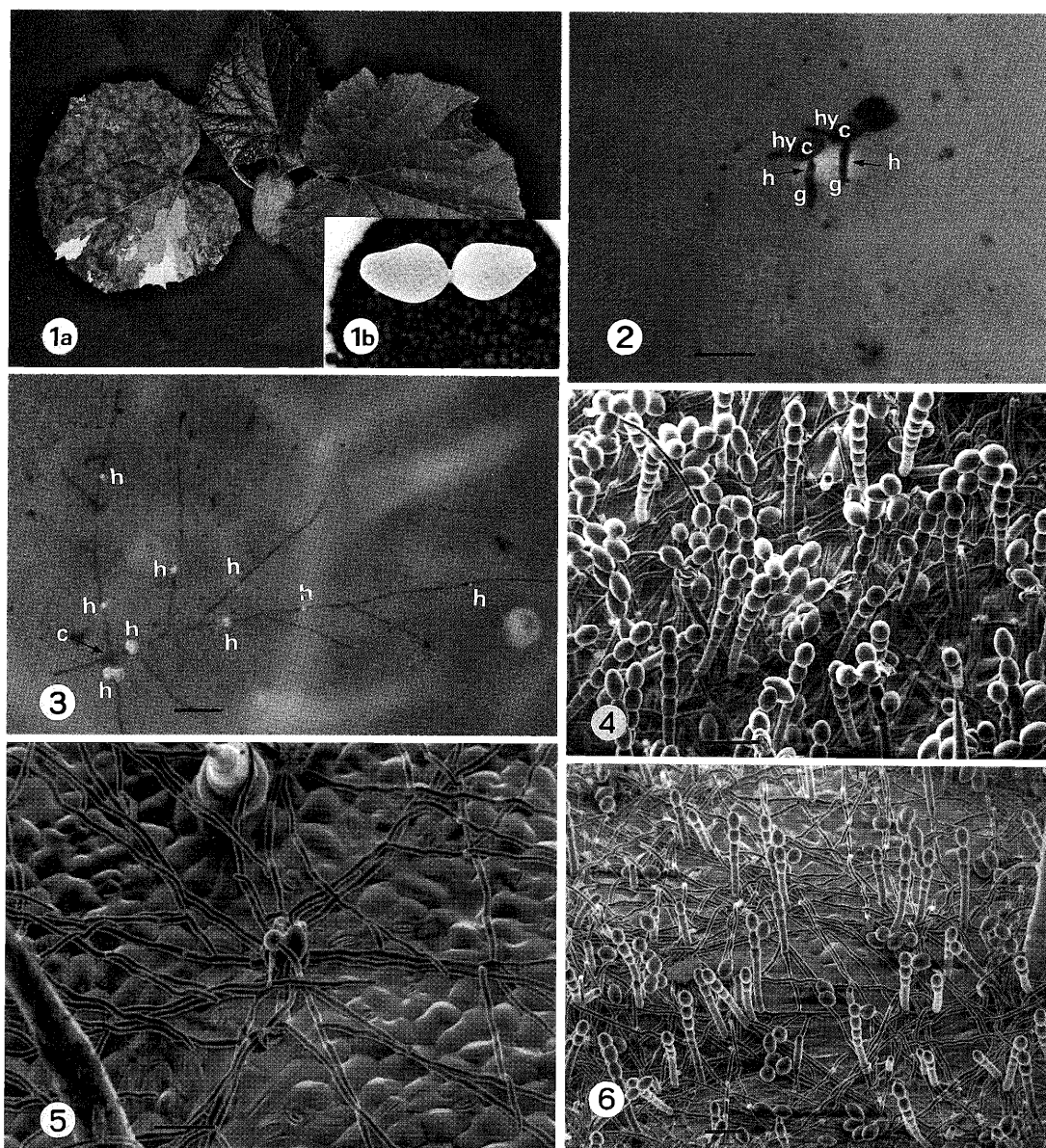
Trelease⁶⁾ reported experiments on the effect of light and carbohydrate supply upon the development of *Erysiphe graminis* on wheat. He found that when the host was able to obtain a supply of carbohydrates from a solution, light was not necessary for the development of the parasite⁶⁾.

From our experimental results, it was clarified that chlorophyll is not necessary, since *S. fuliginea* developed as readily on yellow mutant seedlings which lacked chlorophyll as on green leaves, if sucrose was supplied to the plants.

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Plate I



plants. Ann Phytopath. Soc. Japan 16: 123-126.

和 文 摘 要

有本 裕・本間保男：黄色突然変異キュウリおよび斑入りキュウリ葉上における *Sphaerotheca fuliginea* (Schlecht.) Pollacciの生育

うどんこ病菌, *Sphaerotheca fuliginea* の生育と宿主のクロロフィルとの関係について観察した。実験にはキュウリ (品種：相模半白) の自然突然変異で生じた黄色突然変異キュウリおよび本葉に白斑の入る斑入りキュウリを用いた。黄色突然変異キュウリはクロロフィルを持たない。また斑入りキュウリの白斑部細胞もクロロフィルを持たない。正常キュウリ葉上では菌糸長は接種 48 時間後平均 1209 μm になった。また、それぞれの分生孢子から形成される吸器数は接種 96 時間後には平均 63 個であった。新たな分生子柄は接種 4 日後に形成された。一方、斑入りキュウリ葉の白斑上でも *S. fuliginea* の生育は続いたが、48 時間後の菌糸長は平均 173 μm で、また 96 時間後の吸器数は平均 5 個であり、分生子柄の形成は接種 14 日後に初めて観察された。黄色突然変異子葉上では接種 48 時間後の菌糸長が平均 74 μm であり、第 2 吸器の形成も起らず、その生育は停止した。しかし、根を 2% しょ糖溶液に浸せきすると黄色突然変異子葉上での *S. fuliginea* の生育は正常キュウリでのそれと同程度まで回復した。したがって、外部から糖などを与えれば *S. fuliginea* の生育に宿主細胞中のクロロフィルは必ずしも必須ではないことが明らかとなった。

Explanation of plate

Plate I

- 1 a. Variegated cucumber with white mottles (cultivar sagami-hanjiro).
- 1 b. Yellow mutant cucumber seedling (cultivar sagami-hanjiro, 5 days after hastening of germination).
2. The first haustorium formed under germ tube in epidermal cells of cotyledon of yellow mutant cucumber without sucrose. The leaf was stained with a KHCO_3 (M/15) solution of aniline blue (0.1%, w/v) for 2 hr, and then stained again with basic fuchsine (2%, w/v) of aqueous ethanol (50%, v/v) (Photo taken by fluorescent microscope, Bar 50 μm). (72 hr after inoculation).
3. Haustoria formed under germ tube in epidermal cells of cotyledon of yellow mutant cucumber with 2% sucrose. The leaf was stained with a KHCO_3 (M/15) solution of aniline blue (0.1%, w/v) for 2 hr, and then stained again with basic fuchsine (2%, w/v) of aqueous ethanol (50%, v/v) (Photo taken by fluorescent microscope, Bar 100 μm). (72 hr after inoculation)
4. Conidiophores formed in a center of a colony on normal leaf (Photo taken by SEM, Bar 50 μm). (7 days after inoculation)
5. No conidiophore was observed in a colony on mottle parts of variegated leaf (Photo taken by SEM, Bar 50 μm). (7 days after inoculation)
6. Conidiophores formed in the center of colony on yellow mutant cotyledon treated with 2% sucrose (Photo taken by SEM, Bar 50 μm). (7 days after inoculation)

Abbreviation : c, conidium, g, germ tube, h, haustorium, hy, hyphae.