# Response of Plant pathogenic mold in a Scratch model of Leaf's surface

Takatomo KIMURA, Roka OGAWA, Tomoyasu SAKATA, Ryo SANO, Kao MATSUKAWA
(Kobe High School 1st Grade)

## Purpose

■Reaction of plant pathogenic mold around the scratch of acrylic sheet

■Contribution to the development of new technology for controlling mold infestation

◇It is very important to protect crops from plant pathogenic mold.

■ Plant pathogenic mold is likely to recognize the invasion location on the leaf's surface by scratch depth.
■Scratch recognition is likely to be through substances.

## Background

(1) Invasion of the mold on the surface of non-host plants
■After germination of the mold, they invade the plant without using an appressorium around scratches.
■Mold senses the plant's condition and selects an appropriate invasion strategy.
(2) Stepping in guard cells and appressorium formation
■*Pucciniales* →It forms an appressorium on the PS plate with a step of almost the same height as the guard cells.
◇Infection behavior is based on step recognition on the leaf's surface.

A common method of invasion into plant tissues is to form appressorium.

## Methods

### (1) Isolating and culturing plant pathogenic mold

■Preparing PDA plate culture(20 petri dishes, depth 90mm Φ20mm)
*Solanum tuberosum*, glucose, and agar powder were used.

Fig.1. Plant pathogenic mold【Not identified】
*Colletotrichum orbiculare (Berkeley & Montagne) Arx*


Fig.1

■Isolating diseased areas from leaves
1.Collecting two leaves from the premises of Kobe High School.
2.Cutting out 3 to 5 mm wide strips containing the diseased areas.
3.Sterilizing surface and culturing using 70% ethanol and 1% sodium hypochlorite solution.
■Culture conditions →Culturing in a biotron at 27℃ and 75% humidity.
■Inoculating mold →Cutting and inoculating mold using the inoculation loop. Scalpel was used for the scratches. (width 40 μm± 5 μm)

Scalpel is used for scratch (width 40 μm±5 μm)

### (2) Scratch (concave) on acrylic sheet (Co)

■Scratches were made on seven acrylic sheets (30 mm × 90 mm) as shown in the table below.

| Acrylic sheet number | The alphabet indicates the type of scratch. The distance between the medium and the scratch is 8mm. | |
|---|---|---|
| | Part of the scratch1 Depth(μm) | Part of the scratch1 Depth(μm) |
| 1 | A-98.0 B-54.7 | C-128.5 D-119.9 E-43.6 |
| 2 | F-63.7 G-46.4 | H-53.8 I-39.8 |
| 3 | J-104.1 | K-94.7 |
| 4 | L-77.3 | M-64.9 |
| 5 | N-48.6 | O-50.1 |
| 6 | P-86.9 | ――― |
| 7 | Q-0.0 | Remarks: Control |

Table1

Acrylic plate (red) and culture medium (gray) used in the experiment

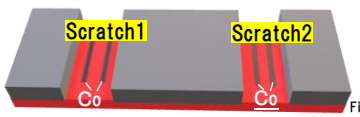

Fig.2

Measuring the width and depth of scratches using a digital microscope

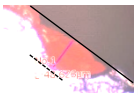
Fig.3

### (3) Culturing and observing on the acrylic sheets

■Observing the whole mold and mycelium (binocular stereomicroscope, optical microscope, digital microscope)
Object: Mycelium (Hp), Germination tube (GT), Adherent organ (Ap), Spore (C)

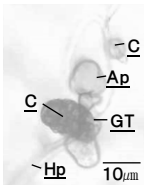

Fig.4

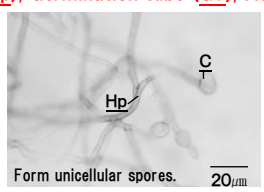

Fig.5

Fig.6

## Results

### (1) Culture and observation of mold.

Fifth mycelium Y


Fig.7
Day 0 of culture

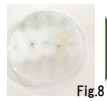
Fig.8
Day 2


Fig.9
Day 4

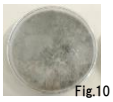
Fig.10
Day 8

Day 2→Mycelium extended in all directions.
Day 8→Overall color changes from white to black


Fig.11


Fig.12

The culture medium on acrylic plate discolored to salmon pink the 7th day of cultivate.

We have created the eighth mycelium and succeeded with obtaining a pure culture.

### (2) Observation on the acrylic plate

Fig.13

Mycelium extended on the acrylic plate (after 72 hours of cultivation) (Black line→boundary between medium and acrylic surface. Red line→scratches.)
※ In this study, the mold is considered to be 'active' when it extends mycelium against the scratches, creates appressorium, and obvious collection of mycelium occurs.

■Depth of scratches and reaction of plant pathogens (after 15 days of cultivation)

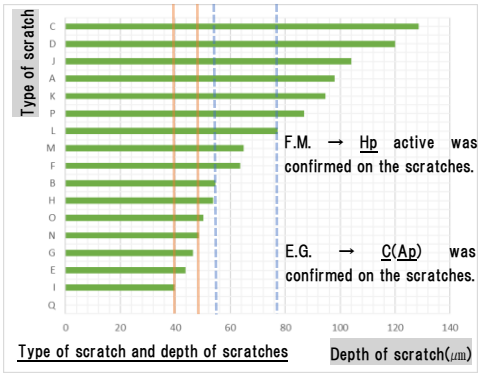

Fig.14

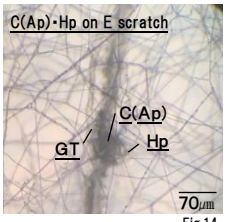

Fig.14

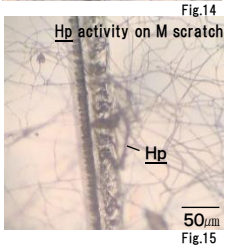

Fig.15

■Scratches in plant surface layer
Scratches of all shapes, widths, and depths are present in plant leaves, but the epidermal tissue of Camellia sinensis has a thickness of 35-50 μm.

## Discussions

The invasion site was recognized in the range larger than 39.8 μm and less than 48.6 μm, larger than 54.7 μm and less than 77.3 μm. It is because the appressorium was formed on the scratches of E and G and collective activity was formed on the scratches of F and M. Regarding E and G, it is consistent with the actual size of the scratch on the mold epidermis tissue. In addition, since it was performed on an acrylic plate, the reaction was not mediated by a substance. Considering the invasion method that does not form an appressorium, which was done in previous research, the intrusion location can be obtained by step recognition. However, for that location, it is highly probable that the information about the scratch is obtained through the substance.

【Reflection points】 We couldn't get sufficient data. We should have observed in a way where the mold can be defined as GT, Ap, or C.

【Future Prospects】 First, the mold should be identified and then, the results should be linked to the topographical characteristics of the actual leaf's surface. Through these actions, we can better understand the invention process and reaction of the plant pathogenic mold. Clarifying the invention process will lead to the development of new pesticides that intercept the invention. This pesticide also has the advantage that it does not act directly on the mold.

## References

京都大学農学研究科. 植物病原性カビの新たな侵入戦略の発見.京都大学. http://www.kyoto-u.ac.jp/static/ja/news_data/h/h1/news6/2010/100707_1.htm (参照 2020-10-05)
京都大学基礎生物学研究所.植物の新しい免疫メカニズムの発見.京都大学. http://www.kyoto-u.ac.jp/static/ja/news_data/h/h1/news6/2009/091015_1.htm (参照 2020-10-05)
Harvey C. Hoch, Richard C. Staples, Brian Whitehead, Jerry Comeau and Edward D. Wolf. Signaling for Growth Orientation and Cell Differentiation by Surface Topography in Uromyces. Science.1987, 235, 4796, 1659-1662. https://www.jstor.org/stable/1698314?seq=1 (2020-11-17).
Nick D. Read, Lesley J. Kellock, Tony J. Collins and Alan M. Gundlach. Role of topography sensing for infection-structure differentiation in cereal rust fungi. Planta.1997, 202, 2, 163-170. https://www.jstor.org/stable/23384962?seq=1 (2020-11-17).