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Effect of Soil Submerging on the Sclerotial Survival of *Sclerotium rolfsii**

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Summary

Sclerotia of *Sclerotium rolfsii*, causal agent of southern blight disease of water chestnut (*Trapa bispinosa* Roxb.) were buried in heat-treated paddy soil at 95 °C for 30 min or autoclaved soil and submerged, subsequently incubated at 30 °C. Sclerotia were collected from the submerged soil daily and assayed for the survival of *S. rolfsii*. Viability of natural and cultured sclerotia rapidly decreased. Scanning electron microscopic observation were carried out to assess the effects of submerging on the fate of natural sclerotia buried in the soil. After being submerged for 58 hr, cortical layer of sclerotium was disrupted and contained many bacteria like organisms (BLO). On the other hand, BLO did not appear in sclerotium kept in tap water for 5 days at 30 °C. From these results, it is suggested that submerging is be an effective control measure of southern blight disease of water chestnut.

Key words: *Sclerotium rolfsii*, submerging, bacteria like organisms.

INTRODUCTION

Sclerotium rolfsii Sacc. is one of the most important pathogens of several economic crops in the tropical and subtropical regions. This fungus produces many sclerotia that can survive for long period and therefore the most potential inoculum for the fungus. Chemical control of the disease, on the other hand, has not been successfully conducted due to the long persistance of sclerotia in soil. Biological control of this pathogen has not yet been established either. However, Garren[3], Sonku et al.[8] and Tanaka et al.[9] suggested that some microorganisms contribute to the lost of sclerotial germinability of *Sclerotinia sclerotiorum* and *S. rolfsii* in field soil under submerged condition. It was hypothesized that the reduction in viability under submerged condition was associated with anaerobic bacteria.

At present, submerging methods are often practiced to control soil borne diseases of onion at Fukudomi region, Saga, Japan.

This study were carried out to evaluate the survival of *S. rolfsii* in autoclaved, heat-

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treated or unautoclaved paddy soil under submerged condition at 30 °C. Furthermore, we demonstrated the bacteria like organisms (BLO) growing in the sclerotium by scanning electron microscope (SEM) and transmission electron microscope (TEM). A preliminary investigation has been reported[9].

MATERIALS AND METHODS

Soil: Paddy soil used in this experiment was collected from cultivated rice field of Saga University, Honjou, Saga Prefecture, in spring, airdried at room temperature for 10 days, thoroughly mixed and passed through a 2 mm sieve prior to use. The physical and chemical characteristics vary with location (Table 1).

Fungus: All experiments were conducted with *S. rolfsii* sclerotia isolated from infected water chestnut, *Trapa bispinosa* Roxb.[5]

Cultured sclerotia: The fungus was cultured in potato sucrose agar (PSA) for 4 weeks at 25 °C and allowed to dry for 48 hr in a desiccator.

Natural sclerotia: The fungus infected on pumpkin fruit for 5 weeks at 30 °C was collected and allowed to dry for 48 hr in a desiccator. Ten sclerotia were placed in a wire netted bag. Then ten bags were buried in each of 200g of unsterile, autoclaved (121 °C for 30 min) or heat-treated (95 °C for 30 min) paddy soil in 550 ml glass jars (ϕ85 mm). Nylon string was attached to each bag, so that an individual bag could be removed without removing the remaining bags. The

Table 1 Characteristics of soil used submerging experiments

Characteristics		Gray lowland soil ※
Percentage	clay	37.7—44.4
	silt	42.5—50.4
	sand	7.9—21.1
pH range		4.6— 6.3
Organic Matter (%)		2.5— 4.2

※The soil samples were taken randomly from several spots in the field with a shavel to a depth of 10 cm.

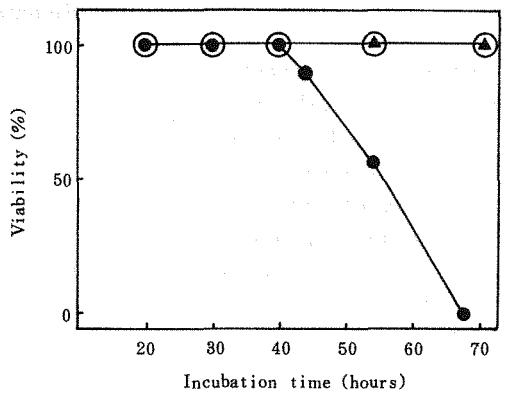

Fig. 1 Percent of viability of natural sclerotia of *Sclerotium rolfsii* buried in submerged paddy soil at 30 °C
●—● heat-treated soil (95°C,30 min), ○—○ autoclaved soil, ▲—▲ in water without soil.

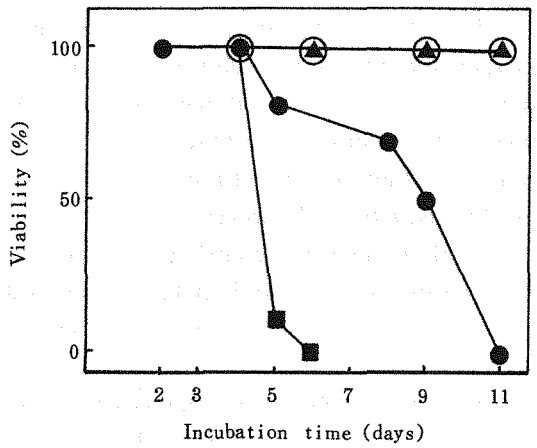

Fig. 2 Percent of viability of cultured sclerotia of *Sclerotium rolfsii* buried in submerged paddy soil at 30 °C
■—■ in soil, ●—● in heat-treated soil (95 °C, 30 min),
○—○ in autoclaved soil, ▲—▲ in water without soil.

water was added to the jars maintaining at 2 cm depth above the soil surface. Jars were then covered with glass plates to minimize evaporation of water during the experiment. Jars were placed in incubator to keep at 30 °C. Bags containing sclerotia were recovered periodically from each of the jars. Recovered sclerotia were rinsed with running tap water for 30 min, then soaked in 70 % alcohol for 2 sec and in 0.5 % NaClO for 60 sec and rinsed in sterile distilled water. Subsequently five sclerotia were placed on PSA. After incubation for 2-3 days at 25 °C, the number of germinated sclerotia was counted. The experiment was performed three times. The rest of the sclerotia were fixed with 2 % glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.0) and rinsed three times with the same buffer and post-fixed with 1 % OsO_4 for 4 hr. After dehydration in a graded alcohol series, critical -point dried, sclerotia were cut with a razor blade, ionsputter coated with gold and observed by SEM (JEM-F15). To prepare specimens for the TEM, sclerotia were fixed and dehydrated as described above. After embedding in Epon, the samples were sectioned with a glass knife. The sections were double stained with uranyl acetate and lead citrate and viewed with TEM (JEM-2,000 FX).

RESULTS

In the submerged heat-treatment (95 °C for 30 min) paddy soil, viability of natural sclerotia rapidly declined from 40 hr after incubation and completely lost for 68 hr, while under submerged autoclaved paddy soil and tap water only, the viability remained at 100 % over 10 days (Fig. 1).

The results with cultured sclerotia summarized in Fig. 2. In the submerged paddy soil, viability of the sclerotia rapidly declined at 5 days and completely lost 6 days after being buried and in the heat-treatment paddy soil, the viability declined gradually, completely lost after 11 days.

SEM observations clearly showed that the cortical layer of sclerotium contained BLO (Fig. 3-D, F, G) was disrupted (Fig. 3-C, E, G) and BLO (arrows) in sclerotia increased rapidly over a short time of submerging. On the other hand, BLO did not appear in sclerotia kept in tap water for 5 days at 30 °C (Fig. 3-A). Under TEM, BLO in the sclerotia incubated in tap water containing paddy soil appeared as very small spheres or slender rods due to cutting angles. Similarly to SEM observations, BLO did not appear in sclerotia kept in tap water for 10 days at 30 °C (Fig 4).

DISCUSSION

The results obtained from the laboratory experiments were shown in Fig. 1 and 2. This strengthen our belief that soil disinfestation by submerging in summer season could be used as a control measure for soil borne diseases. Submerging for soil borne disease control might be effective in subtropical and tropical regions with high temperature.

A marked decreased in viability of sclerotia was observed in submerged heat-treatment and non-treated paddy soil (Fig. 1, 2). Decrease of fungi in submerged soil might be

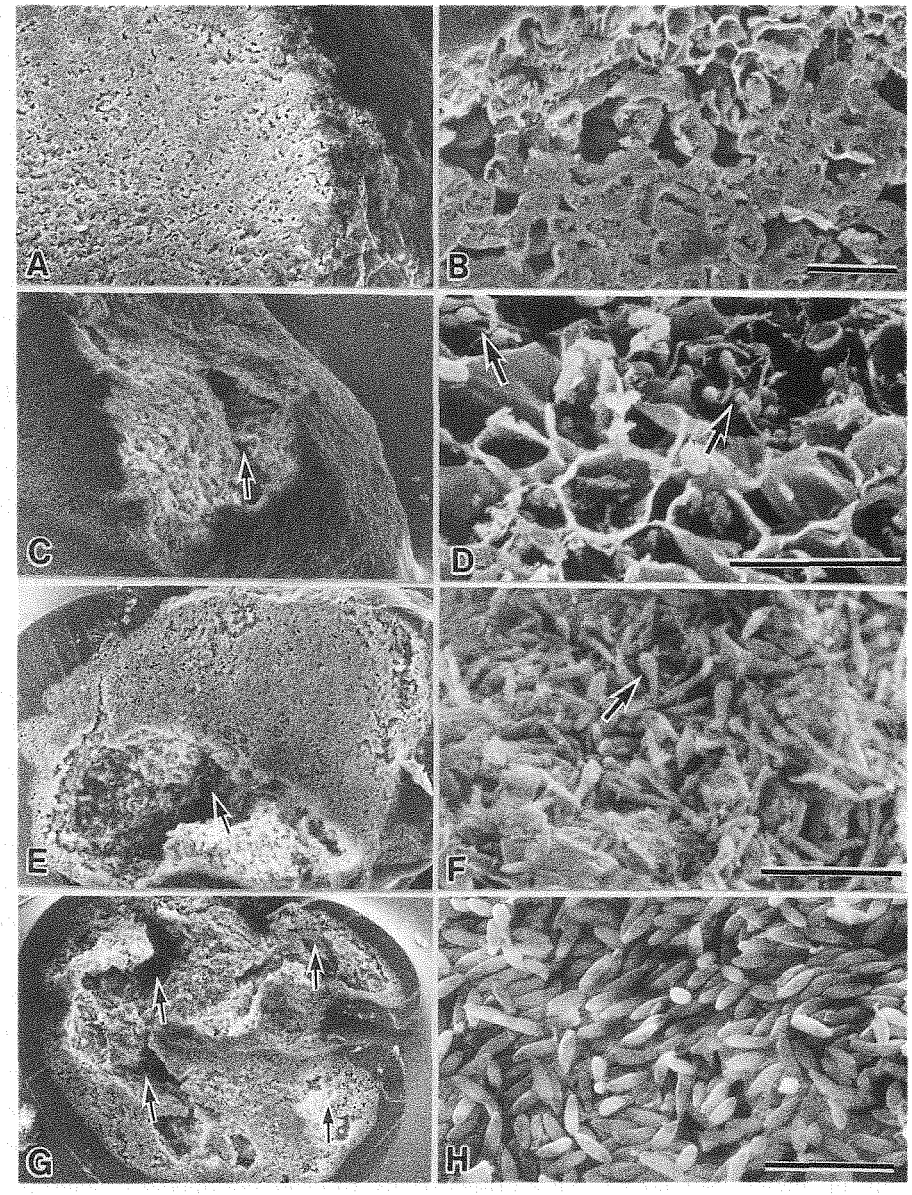

Fig. 3 Scanning electron micrographs of bacteria like organisms (arrows) in the *Sclerotium rolfsii* sclerotium.

A. Surface of sclerotium were cut with a razor blade kept in water at 30 °C for 5 days.

B. Enlarged figure of A.

C, F, G. Collapse (arrows) of sclerotium incubated in water containing paddy soil at 30 °C for 44 hours (C), 50 hours (E) and 58 hours (G).

D, F, H. Bacteria like organisms (arrows) in collapsed sclerotium incubated in water containing paddy soil at 30 °C for 44 hours (D), 50 hours (F) and 58 hours (H). Bar represents 10 μm.

attributed to a depletion of available oxygen, production of a fungicidal substance (organic acid etc.), the increase of competitive microorganisms in soil and so on. In the present study, our data indicate that submerging favor decay of *S. rolfsii*. This submerging effect is presumably due to a combination of biological and other environmental factors rather than a single factor. The same phenomenon was observed by Bamks and Edgington[1], Eastburn[2], Kodama and Fukui[4], Nakagawa et al[6] and Sonku and Kita[8].

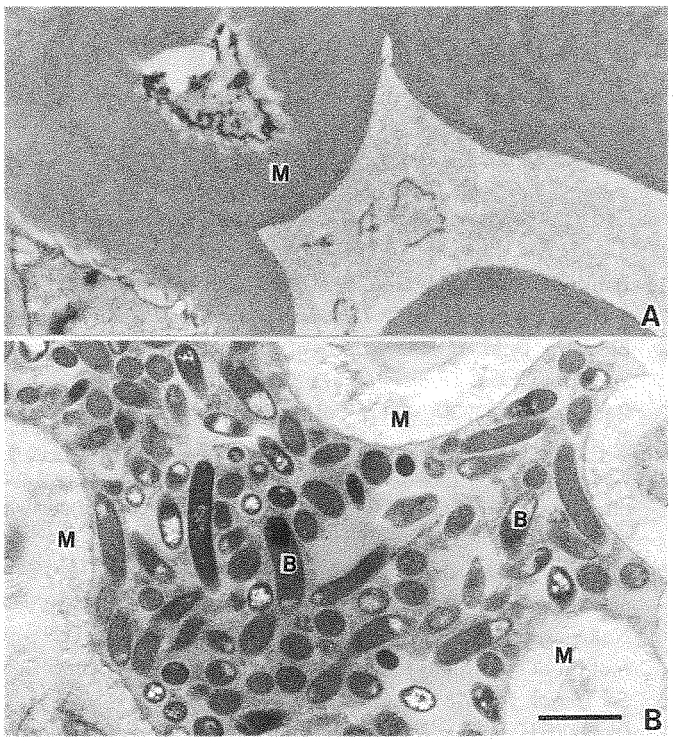

Fig. 4 Transmission electron micrographs of bacteria like organisms in the cultured sclerotium of *Sclerotium rolfsii*.
A. Mycelium of *S. rolfsii* sclerotium kept in tap water at 30 °C for 10 days.
B. Bacteria like organisms in cultured sclerotium incubated in tap water containing paddy soil at 30 °C for 5 days.
Bar represents 2 μm.
Abbreviation: B, bacteria like organisms, M, mycelium

Leakage of nutrients has been reported to occur in *S. cepivorum* when sclerotia were dried for short periods of time and then remoistened in soil[1]. This leakage of nutrients promotes rapid colonization by other microorganisms and subsequent decay within 2 to 3 weeks[2]. Leakage of nutrients may occur during submerging and this may promote invasion by BLO that cause decay.

Electron microscope (SEM and TEM) observation clearly show that contained many BLO in the sclerotia (Fig. 3, 4). This finding, further, substantiates that the BLO is responsible for the death of *S. rolfsii* under submerged condition.

Okazaki[7] reported that death of *Fusarium oxysporum* was related to a volatile fungicidal substances evolved from soil. However, we concluded that death of sclerotia of *S. rolfsii* in submerged soil is due to BLO.

Practically, flooding or submerging may be effective to control. *S. rolfsii* in the flat field and seedling bed which can be easily covered with water. To develop more effective biological control method against sclerotia diseases, further research is needed to clarify the interaction between the sclerotia and microorganism under submerged field conditions.

The method is safe and nonchemical, does not produce phytotoxic residues, is relatively inexpensive and is simple to use.

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土壌湛水処理による白絹病菌核の生存におよぼす影響

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摘　　要

ヒシの病原菌である白絹病菌の自然および培養菌核を95°C，30分間熱処理あるいは蒸気滅菌した土壌に埋設し，湛水した．その結果菌核は時間とともに急激に死滅した．さらに土壌に埋設された自然菌核を湛水処理後に走査型電子顕微鏡で観察すると，湛水処理58時間目には菌核の皮層部の菌糸細胞は崩壊し，その部分には多くの細菌様微生物（BLO）が観察された．一方，対照として水に沈めただけの菌核の中にはBLOは観察されなかった．以上のことから，土壌の湛水処理はヒシ白絹病の生物的防除法として効果的であることが示唆された．