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Germination of immature and mature sclerotia of *Sclerotinia sclerotiorum*[1]

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Abstract. Two isolates of *Sclerotinia sclerotiorum*, sun-87 and Tai, were used to study germination behavior of sclerotia that were either immature (light brown to grayish black with sacs of liquid on the surface, from 6- and 8-day-old cultures) or mature (black and dry, from 14-, 21-, and 42-day-old cultures) on potato dextrose agar at 20°C. Immature sclerotia readily germinated myceliogenically, producing hyphae, but mature sclerotia germinated carpogenically, producing apothecia directly. Germination responses of mature sclerotia from 14- to 42-day-old cultures of both isolates were similar.

Keywords: Carpogenic germination; Myceliogenic germination; Sclerotia; Sclerotial maturation; *Sclerotinia sclerotiorum*.

Introduction

The development of the sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary involves a short growth phase of 2–3 days, followed by a maturation phase (Cooke, 1970). During the growth phase, sclerotia change from white to grayish green and black (Colotelo, 1974) or from white to grayish brown, grayish black and black (Huang et al., 1990). Formation of sacs of liquid on the surface of developing sclerotia (Colotelo et al., 1971; Cooke, 1969) is characteristic of the growth phase (Cooke, 1971). Changes during sclerotial maturation include the deposition of structural and storage polysaccharides, a decrease in hydration of sclerotial tissues, and pigment formation (Cooke, 1969). A mature sclerotium is black without surface liquid sacs.

Sclerotia of *S. sclerotiorum* can germinate myceliogenically to produce hyphae or carpogenically to produce apothecia and ascospores. Myceliogenic germination of mature sclerotia is triggered by incomplete melanization, injury to the rind (Huang, 1985), or exposure to below-freezing temperatures (Huang, 1991). Black sclerotia of *S. sclerotiorum* contain both brown and black pigments (Huang, 1981, 1985). Only black sclerotia exhibit dormancy, suggesting that myceliogenic germination is inhibited by the black pigment but is unaffected by the brown pigment (Huang, 1981, 1985; Huang and Kokko, 1989; Huang and Sun, 1989; Huang et al., 1990).

Carpogenic germination of the sclerotia of *S. sclerotiorum* is affected by the temperature at which the sclerotia are formed (Harada et al., 1974; Huang and Kozub, 1989) and by the geographic origin of the isolate (Huang and Kozub, 1991). Age is also an important factor affecting carpogenic germination of the sclerotia of *S. sclerotiorum* (Phillips, 1987; Willetts and Wong, 1980), and all the published literature refers to the requirement of a rest period for germination of physiologically mature sclerotia. Carpogenic and/or myceliogenic germination of immature sclerotia has not been reported. The objective of this study was to determine the differences in myceliogenic and carpogenic germination responses of immature and mature sclerotia of *S. sclerotiorum*.

Materials and Methods

Two single-ascospore isolates of *S. sclerotiorum* were used in this study—sun-87 from sunflower in Alberta, Canada, and Tai from cabbage in Taiwan (Huang and Kozub, 1991). These isolates have different temperature requirements for carpogenesis (Huang and Kozub, 1993), so stock cultures were maintained on potato dextrose agar (PDA) for 8 weeks at 10°C for sun-87 and at 25°C for Tai. To produce sclerotia for the experiments, agar plugs (6 mm diam.) containing mycelial mats were removed from the stock cultures, transferred onto PDA in 8.5 cm petri dishes with one agar plug per dish, and incubated at 20°C under fluorescent light (15.2 μE s^{-1} m^{-2}). Immature (light brown to black with liquid sacs) and mature (black without sacs) sclerotia were harvested for use in germination tests. Some cultures were selected to study sclerotial development and maturation in situ by time-lapse photomicroscopy.

Sclerotia were tested for germination at various ages by the method of Huang and Kozub (1989): immature

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light brown to grayish brown sclerotia from 6-day-old cultures, grayish black to black (with liquid sacs on the surface) from 8-day-old cultures, and mature sclerotia (black without surface sacs) from 14-, 21-, and 42-day-old cultures. Sclerotia were surface sterilized in 70% ethanol for 90 s, placed on moist sand in petri dishes (20 sclerotia per dish) and incubated for 35 days at 20°C under light (15.2 μE s^{-1} m^{-2}). After 35 days, the sclerotia were examined for carpogenic germination, indicated by the production of apothecia. After incubation for 4 days on moist sand, samples of sclerotia from 6-, 8- (immature), and 14-day-old (mature) cultures were examined under a stereomicroscope for the development of hyphae indicating myceliogenic germination.

The influence of isolate and sclerotial age on myceliogenic and carpogenic germination were examined in eight experiments. There were four dishes (20 sclerotia per dish) for each isolate x sclerotial age treatment combination except when sufficient sclerotia could not be produced for some treatment combinations. The percentages of sclerotia germinating for each treatment combination were determined, and unbalanced analyses of variance (Snedecor and Cochran, 1980) were carried out using the General Linear Models (GLM) procedure of SAS (SAS Institute, Inc., 1989). Effects due to experiment, isolate, sclerotial age, and the isolate x sclerotial age interaction were included in the statistical model. A logit transformation, given by $\log_{10}$ [P/100-P] (Bartlett, 1947), was used on the percentage data (P) prior to analysis to stabilize the variance. Observations of 0% and 100% sclerotial germination were replaced by 25/N and 100–25/N (N = Number of sclerotia tested), respectively, prior to transforming the data. Although the logit-transformed data were used in the analyses, results are presented in percentages to simplify interpretation.

Results

Formation and maturation of sclerotia

Sclerotinia sclerotiorum grown on PDA at 20°C produced sclerotial initials (primordia) in 4–5 days. The initials appeared as dense white mycelial mats covered with small sacs of clear liquid (Figure 1a). The white initials changed to brown or grayish brown within 24 h of formation (6-day-old cultures, Figure 1c) and to grayish black or black within 48 h (7-day-old cultures, Figure 1d). As sclerotial pigmentation increased, sacs of liquid formed, increased in size for 24 to 48 h (Figures 1a–d), then wrinkled and collapsed. All liquid sacs disappeared 96 to 144 h after the formation of the initials (Figure 1e).

Germination of sclerotia

The germination response (myceliogenic or carpogenic) varied considerably among the eight experiments with both isolates. Nevertheless, there were statistically significant main effects and interactions among the factors.

(a) Myceliogenic germination—Almost all immature sclerotia of sun-87 and Tai isolates, harvested at the brown stages from 6-day-old cultures (Figure 2), or at the grayish black or black stages from 8-day-old cultures germinated myceliogenically within 4 days of incubation on moist sand (Table 1). Brown sclerotia germinated and developed into colonies with small daughter sclerotia (Figure 3, left dish), but in some cases, the melanization process continued in myceliogenically germinated, light brown sclerotia (Figures 2a–2c). Melanization reduced hyphal growth, preventing the myceliogenically germinated sclerotium from developing into a colony

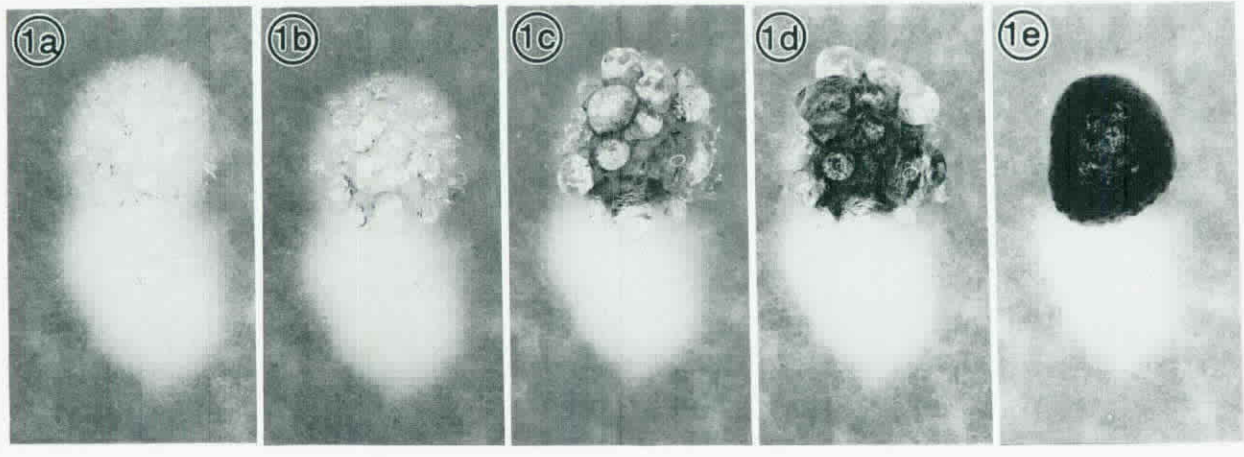

Figure 1. Time-lapse photomicrographs showing the developmental phase of a sclerotium of *Sclerotinia sclerotiorum* isolate, sun-87, on PDA culture. The time sequence was 0 hour on a 6-day-old culture (1a), 6 hour (1b), 24 hour (1c), 48 hour (1d) and 144 hour, i.e., 12-day-old culture (1e). The white sclerotial primordium (1a, 1b) turned grayish brown (1c) and then black (1d, 1e). Note sacs of liquid on the surface of the developing sclerotium (1a–1d) and no sacs on the matured sclerotium (1e). Magnification: 1a–1e, ca. x5.6.

Table 1. Effect of maturity stage of sclerotia on myceliogenic and carpogenic germination of two *Sclerotinia sclerotiorum* isolates.

Isolate[c]	Sclerotia type	Age[d] (days)	Myceliogenic germination[a] (%)			Carpogenic germination[b] (%)		
			N[e]	Mean	SE[f]	N[e]	Mean	SE[f]
Sun-87	Immature	6	8	100	0	8	62	10
		8	8	99	1	8	84	7
	Mature	14	8	15	4	8	89	6
		21	-[g]	-	-	6	84	6
		42	-	-	-	7	77	8
Tai	Immature	6	6	100	0	6	18	9
		8	6	100	0	6	74	17
	Mature	14	6	16	6	6	72	12
		21	-	-	-	5	80	4
		42	-	-	-	5	82	9

[a]Myceliogenic germination was observed after 4 days incubation on moist sand.
[b]Carpogenic germination was observed after 35 days incubation on moist sand.
[c]Stock cultures for Sun-87 (Alberta) maintained at 10°C and Tai (Taiwan) at 25°C.
[d]Age of culture from which sclerotia were harvested.
[e]Number of experiments (80 sclerotia per experiment) used to determine mean myceliogenic or carpogenic germination of sclerotia.
[f]Approximate standard error of the mean proportion.
[g]Not determined.

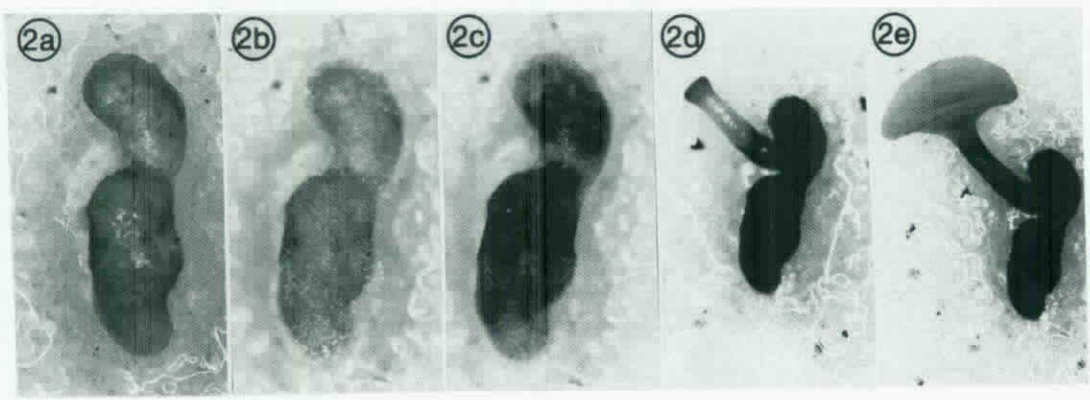

Figure 2. Time-lapse photomicrographs of a sclerotium (2a) of *Sclerotinia sclerotiorum* isolate, sun-87, on moist sand showing initial myceliogenic germination at immature stage (2b, 2c) and subsequent carpogenic germination at mature stage (2d, 2e). The light brown sclerotium was harvested from a 6-day-old PDA culture and surface sterilized in 70% ethanol for 90 sec and placed on moist sand for 0 days (2a), 1 day (2b), 2 days (2c), 30 days (2d) and 34 days (2e). Myceliogenic germination occurred during the 1-day incubation on sand, with formation of short hyphae on the surface (2b). Note patches of dark pigment on the surface of the myceliogenically germinated sclerotium (2c). Magnification: 2a–2c, ca. x8; 2d, ca. x5.3; and 2e, ca. x4.5.

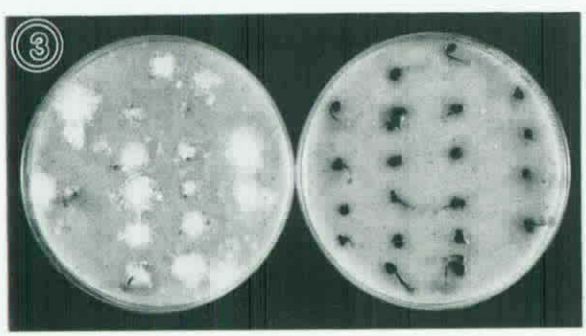

Figure 3. Germination of immature sclerotia of *Sclerotinia sclerotiorum*, isolate sun-87, on moist sand at 20°C. Light colored sclerotia (left plate) from 7-day-old PDA cultures germinated myceliogenically and developed into white mycelial colonies, but dark sclerotia (grayish black with liquid sacs) (right dish) from 8-day-old cultures germinated carpogenically to form stipes and apothecia after 35 days on moist sand. Both myceliogenic and carpogenic germination occurred on two of the light colored sclerotia (left dish). Magnification: ca. x0.5.

(Figure 2c). Further incubation of these dark-colored immature sclerotia led to carpogenic germination with the formation of stipes and apothecia (Figures 2d–2e; Figure 3, right dish). Apothecia were also occasionally produced on brown sclerotia that had developed into white colonies (Figure 3, left dish). Less than 16% of mature sclerotia (black and dry) of both isolates, sun-87 and Tai, germinated myceliogenically (Table 1), and the hyphae were sparse and slow growing.

(b) Carpogenic germination—There were significant effects associated with isolate ($P<0.05$), age of sclerotia ($P<0.01$), and an isolate x age interaction ($P<0.05$). For both the sun-87 and Tai isolates, the percentage of sclerotia with carpogenic germination was low in the immature, brown sclerotia from 6-day-old cultures compared to that in immature, grayish black or black sclerotia from 8-day-old cultures or the mature, black sclerotia from 14-, 21-, and 42-day-old cultures (Table 1). There was no significant difference ($P>0.05$) between isolates in carpogenic germination of immature sclerotia from 8-day-old cultures or mature sclerotia from 14-, 21-, and 42-day-old cultures, but significantly more sun-87 sclerotia from 6-day-old cultures germinated carpogenically than did those from Tai (62 ± 10 vs. 18 ± 9, respectively, $P<0.01$).

Discussion

This study shows that sclerotial maturation determines the germination behavior, myceliogenic germination in the growth and development phase (immature stage), or carpogenic germination in the mature stage. Huang (1985) reported that myceliogenic germination of sclerotia of *S. sclerotiorum* was triggered by incomplete melanization of the rind or by injury to the melanized rind. This study shows that the extent of hyphal growth in a myceliogenically germinated sclerotium varies with the degree of pigmentation, with vigorous growth in most brown sclerotia but limited growth in grayish black or black sclerotia. The inhibition of myceliogenic germination and establishment of dormancy in sclerotia of *S. sclerotiorum* by the melanization of the rind is thus confirmed.

Huang and Kozub (1993) reported that the optimum inoculum temperature for producing daughter sclerotia that subsequently germinate carpogenically is 10°C for the sun-87 isolate and 25–30°C for the Tai isolate. Since these temperatures were used in this study, most of the matured sclerotia became dormant and then germinated carpogenically when they were incubated on moist sand at 20°C. These results suggest that carpogenic germination of sclerotia of *S. sclerotiorum* is predetermined by conditions such as temperature during sclerotium formation (Huang and Kozub, 1991) or during inoculum production (Huang and Kozub, 1993). Carpogenic germination rates were similar for sclerotia of different ages, except for immature sclerotia from 6-day-old cultures, which had a lower level of germination than those from 8-day-old or from mature sclerotia from 14- to 42-day-old cultures. This finding extends a previous report (Huang and Kozub, 1993) that above a threshold age, frequency of carpogenic germination of mature sclerotia is not affected by the age of the culture from which they are produced.

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菌核病菌菌核成熟度對發芽習性之影響

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本研究係用加拿大向日葵菌核病 (*Sclerotinia sclerotiorum*) 菌株 "Sun-87" (寒帶菌株) 與台灣白菜菌核病菌株 "Tai" (亞熱帶菌株) 來探討菌核成熟度對發芽習性之影響。此二菌株在馬鈴薯平板培養基，20°C 環境下培養 6 至 8 天，所形成的菌核多未成熟而呈淺褐至黑褐色，表面具有露滴，而在此一環境下培養 14 至 42 天的菌核多已成熟而呈黑色，表面乾燥無露滴，將未成熟與成熟菌核置放於濕沙上並保持在 20°C 有光照環境下，所有褐色至黑褐色未成熟菌核均可於 1 至 3 天內以菌絲方式萌發 (myceliogenic germination) 產生菌絲或形成菌落，而所有黑色成熟菌核則無法以菌絲方式萌發，但可於 35 天後，以子囊盤方式萌發 (carpogenic germination) 產生子囊孢子，此結果顯示菌核之菌絲方式萌發是受菌核外皮層 (rind) 細胞壁色素 (melanin) 累積程度所控制。

關鍵詞：大粒菌核病菌；菌核；菌核成熟度；菌核之萌發；子囊盤。