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Effect of Relative Humidity on Myceliogenic Germination of Sclerotia of *Sclerotinia minor*

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ABSTRACT

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A study was conducted to determine the effect of relative humidity (RH) on myceliogenic germination of sclerotia of *Sclerotinia minor* in the absence of exogenous nutrients. Results showed that fresh or air-dried sclerotia collected from cultures grown at room temperature (20±2℃) on potato dextrose agar for 3 wk were capable of undergoing myceliogenic germination when the sclerotia were placed in uncovered Petri dishes and incubated at 25℃ for 3 wk at 95% RH or higher. The germination rate for fresh and air-dried sclerotia was higher than 86.7% for the treatment of 100% RH but was lower than 54.2% for the treatment at 95% RH. In contrast, the germination rate was 0.8% and 0% for the treatments of 90% RH and 85% RH, respectively. There was no significant (P>0.05) difference in the germination rate between fresh and air-dried sclerotia tested at each RH. The possibility of infection of host plants by germinating sclerotia of *S. minor* in the absence of exogenous nutrients is discussed.

Key words: *Sclerotinia minor*, sclerotia, myceliogenic germination, relative humidity

Sclerotinia minor Jagger is a soilborne pathogen which occurs in 94 species of higher plants [9]. This pathogen overwinters in the soil by black sclerotia which serve as the primary source of inoculum in the field. Hyphae of *S. minor* produced from myceliogenic germination of sclerotia can infect plant tissues and cause diseases such as sunflower wilt [3,5] and lettuce drop [1,8,10,14].

There are two types of myceliogenic germination in sclerotia of *S. minor*, eruptive type and non-eruptive type or hyphal type. The eruptive germination occurs by the formation of closely packed hyphae causing bulging and rupture of the rind [1,14]. The non-eruptive or hyphal germination is the formation of germinative hyphae, which are initiated in the outer regions of the sclerotium, passing through degenerating outer medullary and cortical hyphae to emerge individually through the rind and aggregate outside the sclerotium to form a mycelium [2]. Wymore and Lorbeer [14] reported that hyphae produced from either eruptive or noneruptive myceliogenic germination were capable of causing infection of lettuce tissues without prior colonization of a food base.

Freshly produced sclerotia of *S. minor* were dormant before undergoing myceliogenic germination [1]. Previous reports indicate that some physical, physiological and nutrional factors are important in affecting myceliogenic germination of *S. minor*. Wymore and Lorbeer [14] reported that cold treatment of hydrated sclerotia of *S. minor* at 3℃ for 1 day stimulated rapid myceliogenic germination. Paterson and Grogan [11] found that a dry treatment of sclerotia for 24 hrs is required for eruptive germination of sclerotia. They also reported that eruptive germination was high in sclerotia produced on autoclaved potato and carrot but was low or no germination in sclerotia produced on oats and sorgham. Hau *et al.* [6] reported an increase of eruptive germination of sclerotia of *S. minor* by the treatment of volatile substances from dried and remoistened peanut leaves. Burgess and Hepworth [3] found that sterile root sap and sterile root exudates from sunflower plants stimulated germination of sclerotia of *S. minor*. In addition, they also reported that surface sterilized field sclerotia of *S. minor* germinated well on water agar, but unsterilized sclerotia failed to germinate, even after drying and re-wetting, possibly due to microbial colonization of the sclerotial rind.

Huang *et al.* [7] reported that sclerotial dryness and relative humidity are important factors affecting myceliogenic germination of sclerotia of Sclerotinia sclerotiorum (Lib.) de Bary. The objective of this study was to determine effects of sclerotial dryness and relative humidity (RH) on myceliogenic germination of *S. minor* in the absence of exogenous nutrients.

Sclerotinia minor isolate DAOM 191806, collected from a lettuce (*Lactuca sativa* L.) in a supermarket in Manitoba in

1984 was used in this study. Sclerotia were harvested from cultures grown on potato dextrose agar (PDA) at room temperature (20±2℃) for 3 wk and they were used in the germination tests, as fresh or after air-drying for 3 wk at room temperature. The RH in the laboratory during the air-drying period varied from 18.6 to 43.0%, averaging 27.9% (measured by the Humidity and Temperature Indicator HMI 31, VAISALA, Finland). The sclerotia, fresh and air-dried, were surface sterilized in 70% ethanol for 90 sec., transferred to the bottom of Petri dishes (88 mm diameter) without cover, 15 sclerotia/dish, placed in chambers at 85%, 90%, 95% or 100% RH, incubated at 25℃ for 3 wk, and examined for myceliogenic germination and hyphal growth using a stereomicroscope. The chambers with 85%, 90% and 95% RH were set up by adding 200 ml KOH solutions at the concentration of 2.8, 1.8, and 0.8 mol, respectively [13]. For the chamber of 100% RH, sterile water was added at 200 ml/chamber. The experiment was repeated once, with four replicates (Petri dishes) per treatment in each experiment. Data were analyzed by analysis of variance and treatments in each experiment were compared by Duncan's Multiple Range Test at 5% level [12].

Fresh and air-dried sclertoia of *S. minor* germinated myceliogenically when incubated at 95% RH or 100% RH in the absence of exogenous nutrients (Fig. 1). At 100% RH the germination rate was 93.3 and 96.7% for fresh and air-dried sclerotia, respectively, whereas the germination rate was lower than 54.2% for both sclerotial samples incubated at

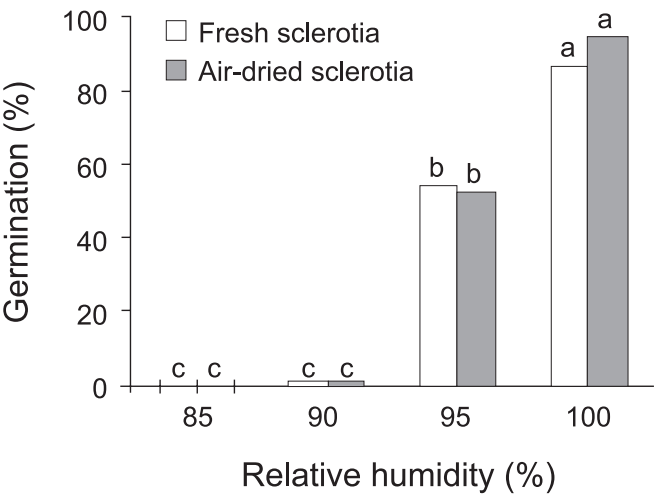

Fig. 1. Effect of relative humidity on myceliogenic germination of sclerotia of *Sclerotinia minor* in the absence of exogenous nutrients. Sclerotia were collected from 3-wk-old PDA cultures at RT (20±2℃) and tested for germination, as fresh or after air-drying for 3 wk at RT, by incubation at 25 C under 85%, 90%, 95% or 100% RH. The RH in the laboratory during the air-drying period varied from 18.6 to 43.0%. Means followed by the same letter are not significantly different (Duncan's Multiple Range Test, P=0.05); based on average of two experiments.

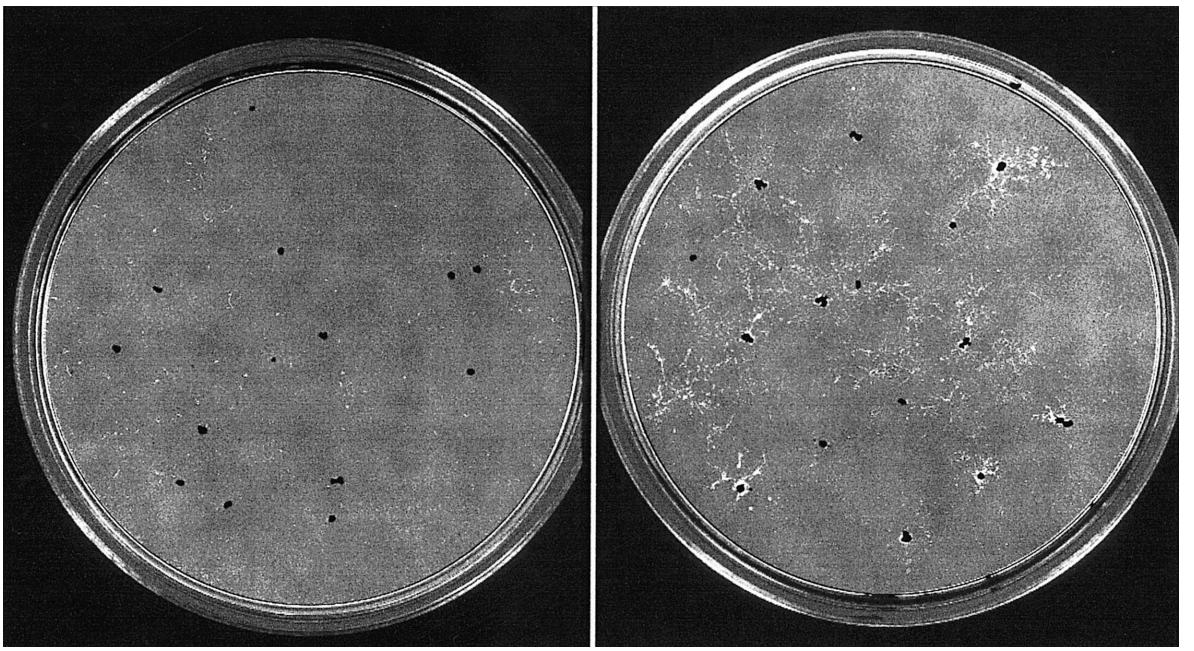

Fig. 2. Myceliogenic germination of sclerotia of *S. minor* incubated at 85% RH (left plate) and 100% RH (right plate) for 3 wk. Sclerotia were collected from 3-wk-old PDA cultures and air- dried for 3 wk at RT (20±2℃) before testing for germination. Note none of the 15 sclerotia germinated in the treatment of 85% RH (left plate) but numerous sclerotia germinated with white mycelial mates surrounding each sclerotium in the treatment of 100% RH (right plate). Ca. x 0.9.

95% RH. At 90% RH the germination rate was very low (0.8%) in both fresh and air-dried sclerotia, whereas none of the sclerotia germinated at 85% RH. The germinated sclerotia under the treatment of 100% RH showed vigorous growth of hyphae, resulting in the formation of mycelial mates and the development of white colonies (Fig. 2).

This study concludes that relative humidity is an important factor affecting myceliogenic germination of sclerotia of *S. minor* as the sclerotia germinated readily in the absence of exogenous nutrients under high RH (> 95%). Wymore and Lorbeer [14] reported that air-drying of sclerotia of *S. minor* at room temperature stimulated myceliogenic germination but this phenomenon was not observed in the present study as the sclerotia, either freshly harvested from PDA cultures or air-dried at room temperature for 3 wk, germinated readily under 100%RH (Fig. 1).

The vigorous hyphal growth for sclerotia incubated at 100%RH (Fig. 2) suggests that the energy required for the growth of hyphae and the development of colony is originated from the degradation of sclerotial tissues. Previous reports indicated that hyphae produced from myceligenically germinated sclerotia of *S. minor* were capable of causing infection of lettuce tissues without prior colonization of a food base [1,14]. The fact that sclerotia of *S. minor* geminate readily under high humidity such as 100%RH (Fig. 1) suggests that, besides nutritional factors such as root excudates [3,4] and volatile substance from host tissues [6], other physical factors such as the combination of high RH and optimum temperature can also trigger myceliogenic germination of sclerotia of *S. minor*. The evidence from this study further suggests that under the conditions of high humidity and favorable temperature, infection of host plants by *S. minor* may occur in the field if the germinated sclerotia are located in proximity of host tissues.

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摘 要

黃鴻章[1,2]、張治[1]. 2003. 相對溼度 (RH) 對小粒菌核病菌 (*Sclerotinia minor*) 菌核發芽之影響. 植病會刊 12:65-68. ([1.] 加拿大農部 Lethbridge 研究中心；[2.] 聯絡作者：電子郵件 huangh@agr.gc.ca，傳眞：+0021-403-382-3156)

本文旨在探討無外在營養物質存在的環境下，相關溼度 (RH) 對小粒菌核病菌 (*Sclerotinia minor*) 之菌核發芽能力的影響。將採自馬鈴薯平板培養基培養 3 週所形成的新鮮菌核或風乾過 3 週的菌核，放置於不加蓋的培養皿中，然後將這些培養皿放於乾燥器中，並將乾燥器的相對溼度調節至 100%、95%、90% 及 85%。在定溫 (25℃) 保存 3 週後，將這些菌核取出，在解剖顯微鏡下檢查菌核發芽及菌落形成的情形。兩次試驗結果顯示，在飽和相對溼度 (100%RH) 下，新鮮及風乾菌核發芽率均高於 86.7%；但在 95%RH，發芽率則低於 54.2%。至於在 90%RH 或 85%RH 環境下，菌核發芽率幾近於零。此外，在 100%RH 下，發芽的菌核均會形成白色菌落。因此，推測小粒菌核病菌的菌核，在高濕、適溫環境下，可以不必借助外在營養物質，即可發芽並爲害寄主作物。

關鍵詞：小粒菌核病菌、菌核、菌核性發芽、相對溼度