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Temperature requirements for carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* isolates of different geographic origin[1]

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Abstract. Twenty single-ascospore isolates of *Sclerotinia sclerotiorum* from various countries including Canada, USA, Argentina, France, Taiwan, and Japan were compared for the effects of temperature during sclerotium formation on carpogenic germination. Sclerotia were harvested from cultures grown in the dark on potato dextrose agar at 10°C (cool) or 25°C (warm) for 8 weeks and tested for germination on moist sand at 20°C under light for 3 weeks. The ungerminated sclerotia were conditioned at 10°C (cool) for 4 weeks and then tested for germination again. Results showed that the ability of sclerotia formed at cool (10°C) or warm (25°C) temperatures to germinate carpogenically varied with isolates, and more specifically, with the geographic origin of the isolates. Of the sclerotia produced at 10°C, germination occurred readily in isolates from the cool climatic regions such as Hokkaido, Japan; North Dakota, USA; and Alberta, Saskatchewan, and Manitoba, Canada. However, little or no germination of sclerotia occurred in isolates from warmer climatic regions including Taiwan and California, Florida, and Hawaii, USA. Sclerotia formed at 25°C showed little or no germination unless they were conditioned and the effect of cool conditioning also varied with isolates. For the isolates from Taiwan, cool conditioning was effective in triggering carpogenic germination of sclerotia formed at 25°C but was ineffective for sclerotia formed at 10°C.

Key words: Apothecia; Carpogenic germination; Sclerotia; *Sclerotinia sclerotiorum.*

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a homothallic species and sclerotia from single-ascospore cultures are capable of producing apothecia by carpogenic germination (Henson, 1935; Keay, 1939; Huang and Kozub, 1989). Although there are numerous reports on the factors influencing carpogenic germination of sclerotia of *S. sclerotiorum,* there is a great degree of inconsistency and contradiction in the literature (Phillips, 1987). Since this pathogen is world-wide in distribution, the inconsistency in the germination behavior among the reports may be partially due to differences in the isolates used in investigations by various workers.

Temperature during sclerotium formation is one of the important factors affecting carpogenic germination of sclerotia in *S. sclerotiorum.* Purdy (1956) reported that carpogenic germination of sclerotia produced on PDA at 15 or 18°C occurred more rapidly than those produced at 4 or 27°C. Keay (1939) found that sclerotia formed at 5, 10, 15 and 20°C were capable of undergoing carpogenic germination but sclerotia formed at 25°C failed to germinate carpogenically. Harada *et al.* (1974) reported that sclerotia produced at 25°C had a longer resting period than those produced at 10 or 15°C. Huang

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and Kozub (1989) studied five isolates of *S. sclerotiorum* from different host crops in Alberta and found that sclerotia of these isolates formed on PDA cultures at 10°C for 8 weeks germinated carpogenically under light on moist sand at room temperature (22±1°C). However, sclerotia formed at 25 or 30°C required a minimum of 4 weeks conditioning under cool (10°C) moist conditions to trigger the germination. This germination technique (Huang and Kozub, 1989) was used as a standard method to compare 20 single-ascospore isolates of *S. sclerotiorum* collected from North America, South America, Europe and Asia for differences in carpogenic germination and the results are reported in this paper.

Materials and Methods

Isolates of ***S. sclerotiorum***

Twenty single-ascospore isolates of *S. sclerotiorum,* including 14 isolates from North America, one from South America, one from Europe and four from Asia, were used in this study. The hosts and countries of origin of these isolates are listed in Table 1. Sclerotia of each isolate were harvested from PDA cultures, stored in paper bags at 4°C and used as stocks.

Carpogenic Germination of Sclerotia

In each experiment, one sclerotium from each isolate was surface sterilized in 95% ethanol for 90 sec, placed on PDA, and incubated at 20°C for 5 days to establish a culture. Agar blocks containing mycelial mats from the culture were inoculated on PDA in Petri dishes, one block per dish. The cultures were placed in plastic bags, incubated in the dark at 10 or 25°C for 8 weeks, and the sclerotia produced in the dishes were harvested and tested for carpogenic germination immediately without surface sterilization (Huang and Kozub, 1989).

For carpogenic germination tests, sclerotia were placed on moist, autoclaved sand in Petri dishes. Each dish was sealed with parafilm and incubated for 3 weeks at 20°C under fluorescent light (15.20 μE s^{-1} m^{-2}). The number of sclerotia that germinated and produced mature apothecia was recorded for each dish.

Table 1. *Hosts and geographic origin of isolates of* ***Sclerotinia sclerotiorum***

Isolate[a]	Host	Country of origin
Tai	Cabbage	Taiwan
Tb-1	Tobacco	Taiwan
Chr-2	Chrysanthemum	Taiwan
SS-5	Sunflower	North Dakota, USA
SS-6	Sunflower	California, USA
SS-7	Sunflower	Hawaii, USA
SS-12	Sunflower	Florida, USA
SS-8	Sunflower	France
SS-Arg	Soybean	Argentina
Sm5f-1	Bean	Hokkaido, Japan
SS-car	Carrot	Swift Current, Saskatchewan, Canada
SS-9	Sunflower	Altona, Manitoba, Canada
SS-11	Sunflower	Morden, Manitoba, Canada
physos-1	False-dragonhead (*Physostegia virginiana* L.)	Morden, Manitoba, Canada
pea-1	Field pea	Portage la Prairie, Manitoba, Canada
mun-1	Mungbean	Portage la Prairie, Manitoba, Canada
sun-87	Sunflower	Bow Island, Alberta, Canada
pea-87	Field pea	Bow Island, Alberta, Canada
bean-87	Field bean	Bow Island, Alberta, Canada
can-87	Canola	Coaldale, Alberta, Canada

[a] Tai, Tb-1, and Chr-2 from Prof. S. K. Sun; SS-5, SS-6, and SS-7 from Dr. M. Abdallah, Cargill Co.; SS-Arg and Sm5f-1 from Dr. I. Saito.

Six experiments in completely randomized designs were carried out and, except for the first experiment, there were four replicate dishes for each treatment isolate, and 20 sclerotia per replicate. The first experiment had three replicates with 15 sclerotia per replicate. Sclerotia that failed to germinate during the 3-week testing on moist sand under light were conditioned by storing the dishes at a cool (10°C) temperature for 4 weeks. They were then tested for germination again using the above method.

Statistical Analyses

Analyses of variance (Steel and Torrie, 1980) were carried out to compare isolates for carpogenic germination for each temperature at which sclerotia were formed and before and after conditioning treatment combination. For each isolate, the total percentage of sclerotia that germinated in the replicate dishes for each experiment was determined. The percentage data were transformed to logits (Bartlett, 1947) since it was evident that the isolate means and variances determined over the experiments were not independent, and then analysis of variance was carried out with sources of variation due to experiment and isolate being included in the statistical model. A cluster analysis (Scott and Knott, 1974) was carried out to separate the isolates into groups that had similar carpogenic germination within the temperature-conditioning treatments.

Canonical analyses (Seal, 1964) were carried out to compare isolates for their collective before and after

Table 2. *Comparison of 20 isolates of* ***Sclerotinia sclerotiorum*** *for carpogenic germination of sclerotia formed at 10 or 25°C before and after conditioning*

	Carpogenic germination (%)[a]			
	Sclerotia formed at 10°C		Sclerotia formed at 25°C	
Isolate	Before conditioning	After conditioning	Before conditioning	After conditioning
Tai	0.8d[b]	6.1d	0.7b	99.4a
Tb-1	0	0.5d	0	98.4a
Chr-2	0	1.7d	0	99.5a
SS-5	82.6b	97.9a	0	1.1d
SS-6	1.8d	51.2c	0.5b	98.9a
SS-7	0.6d	7.2d	12.9a	76.0b
SS-12	2.2d	64.4b	0.6b	99.7a
SS-8	0	6.0d	0	0
SS-Arg	0.5d	19.0c	0.8b	93.2b
Sm5f-1	11.0c	88.9b	0.3b	63.6b
SS-car	63.6b	99.1a	0	19.0c
SS-9	74.9b	84.5b	0	0
SS-11	28.6b	87.2b	0	0.9d
physos-1	76.6b	92.8b	0	0
pea-1	60.5b	98.3a	0	0.9d
mun-1	85.7b	98.3a	0	5.9c
sun-87	98.0a	99.3a	0	90.8b
pea-87	94.6a	99.4a	0	89.2b
bean-87	46.3c	87.7b	0	72.3b
can-87	70.7b	98.3a	0	66.2b
SE[c] (df)	0.31 (80)	0.37 (95)	0.27 (25)	0.38 (80)

[a] Germination percentages are a backtransformation of means following a $\log_{10}$ [P/(100-P)] transformation where P is the percentage germination; observed 0% and 100% germination data were replaced by 25/N and 100-25/N (N=total number of sclerotia for an isolate in an experiment), respectively, prior to transformation.

[b] Means followed by the same letter within each column indicate isolates that are homogeneous according to cluster analysis using the 5% significance level. Isolates with no germination or complete germination for all replicates were excluded from the statistical analyses.

[c] Standard error of a mean in $\log_{10}$ units.

conditioning response to carpogenic germination within each sclerotium-formation temperature (10°C or 25°C) and over temperatures. Linear combinations of the before-after conditioning germination responses (canonical variables) that best summarized the between-isolate variation were determined. The first canonical variable shows the largest differences between isolates. The means of the first and second canonical variables were plotted for each isolate and clustered to obtain approximate groupings of isolates for their joint before-after conditioning response. These analyses were carried out using the CANDISC and CLUSTER procedures of the Statistical Analysis System Institute, Inc. (SAS Institute, 1985) software.

Results

Results from six tests showed that the ability of sclerotia formed at 10°C or 25°C to germinate carpogenically differed significantly ($p<0.01$) among the 20 isolates of *S. sclerotiorum* (Table 2, Figs. 1-2). When sclerotia produced at 10°C were tested, the isolates exhibited a wide range of germination response with distinct clusters of isolates being evident (Table 2). Eight of the isolates, including Tai, Tb-1, Chr-2, SS-6, SS-7, SS-12, SS-8, and SS-Arg, had little or no germination. The increase in germination of these isolates by a 4-week conditioning of the sclerotia on moist sand at cool (10°C) temperature was not pronounced except for SS-Arg, SS-6 and SS-12 which showed increases ranging from 19 to 62% (Table 2, Fig. 1). For the remaining 12 isolates, the sclerotia formed at 10°C exhibited a low to high rate of germination without cool, moist condi-

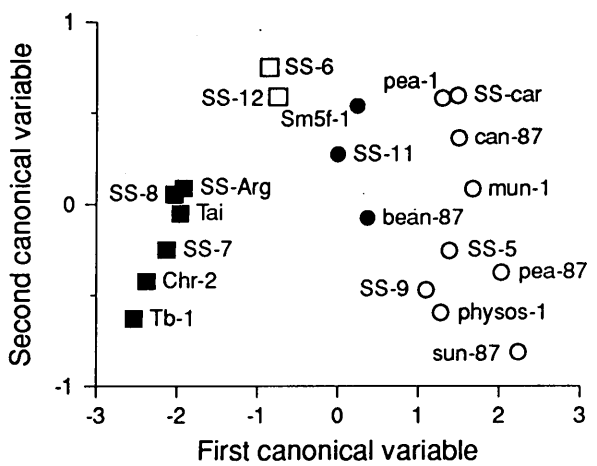

Fig. 1. Canonical analysis of the 20 isolates of *Sclerotinia sclerotiorum* for carpogenic germination of sclerotia formed at 10°C and tested before and after conditioning. Isolates with the same symbol have similar before-after conditioning response.

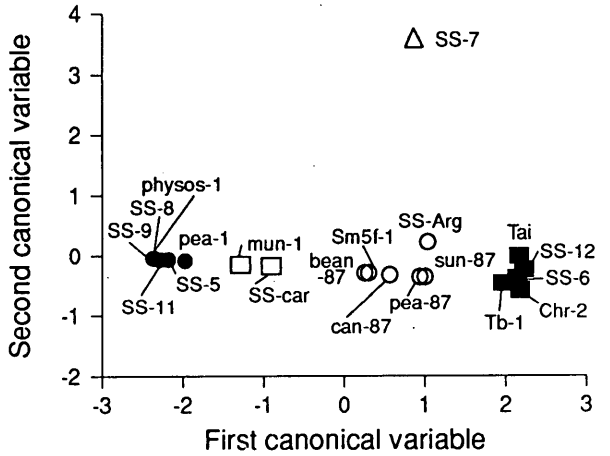

Fig. 2. Canonical analysis of the 20 isolates of *Sclerotinia sclerotiorum* for carpogenic germination of sclerotia formed at 25°C and tested before and after conditioning. Isolates with the same symbol have similar before-after conditioning response.

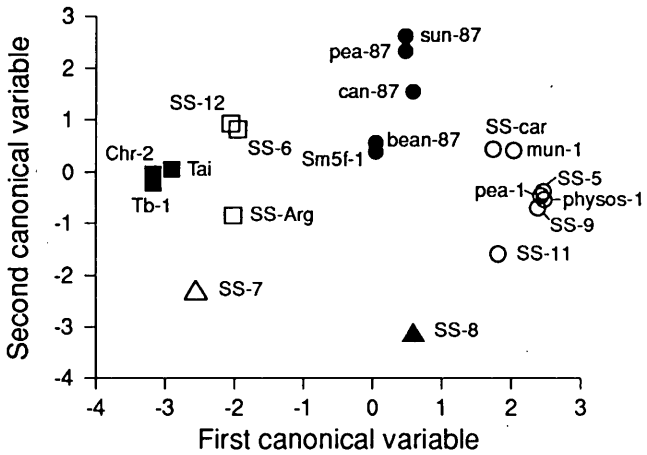

Fig. 3. Canonical analysis of the 20 isolates of *Sclerotinia sclerotiorum* for carpogenic germination of sclerotia formed at cool (10°C) or warm (25°C) temperature and tested before and after conditioning. Isolates with the same symbol have similar before-after conditioning response.

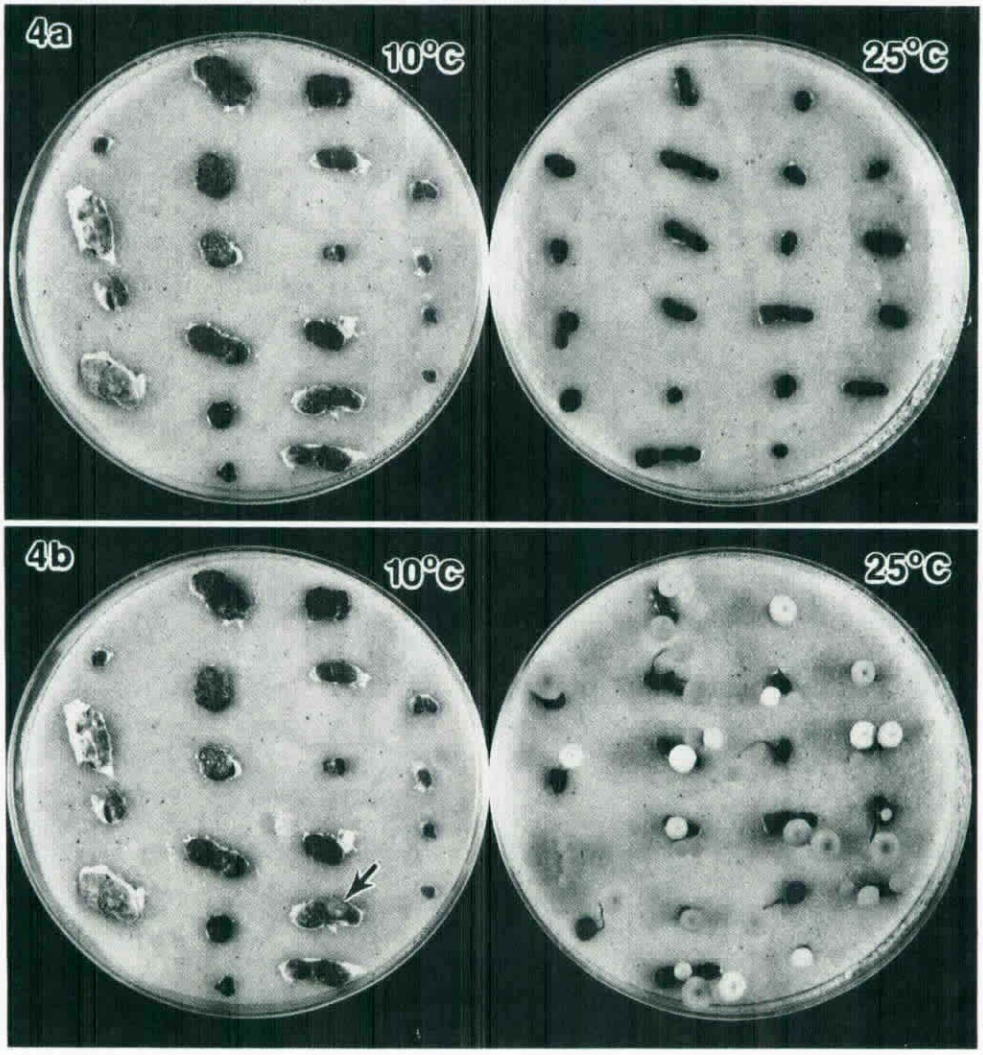

Fig. 4. An isolate of *Sclerotinia sclerotiorum*, Tai, from Taiwan showing differences in carpogenic germination of sclerotia formed at 10°C and 25°C before (Fig. 4a) and after (Fig. 4b) conditioning. Note poor germination on sclerotia formed at 10°C (Fig. 4b, arrowhead in left dish) but good germination on sclerotia formed at 25°C after conditioning (Fig. 4b, right dish). Magnification, ca. × 0.9.

tioning (range of 11 to 98%). These isolates were Sm5f-1, can-87, bean-87, sun-87, pea-87, SS-car, SS-9, pea-1, SS-11, physos-1, mun-1, and SS-5. Isolates Sm5f-1, bean-87 and SS-11 had similar before-after conditioning response patterns with mean initial germination levels of less than 50% increasing to more than 85% after the sclerotia were conditioned on cool, moist sand for 4 weeks (Table 2, Fig. 1).

Of the sclerotia formed at 25°C and tested without conditioning, there was little or no germination for any of the isolates except for the Hawaiian isolate SS-7 (Table 2). When the sclerotia were conditioned, the germination rate was moderate to high (range of 64 to 100%) for the isolates Tai, Tb-1, Chr-2, SS-6, SS-7, SS-12, SS-Arg, Sm5f-1, can-87, bean-87, sun-87, and pea-87, but was less than 20% in the isolates SS-8, SS-9, SS-5, pea-1, SS-11, SS-car, physos-1 and mun-1 (Table 2, Fig. 2). Generally, the germination response for sclerotia from each isolate was consistent between experiments with the exception of isolates SS-7, SS-

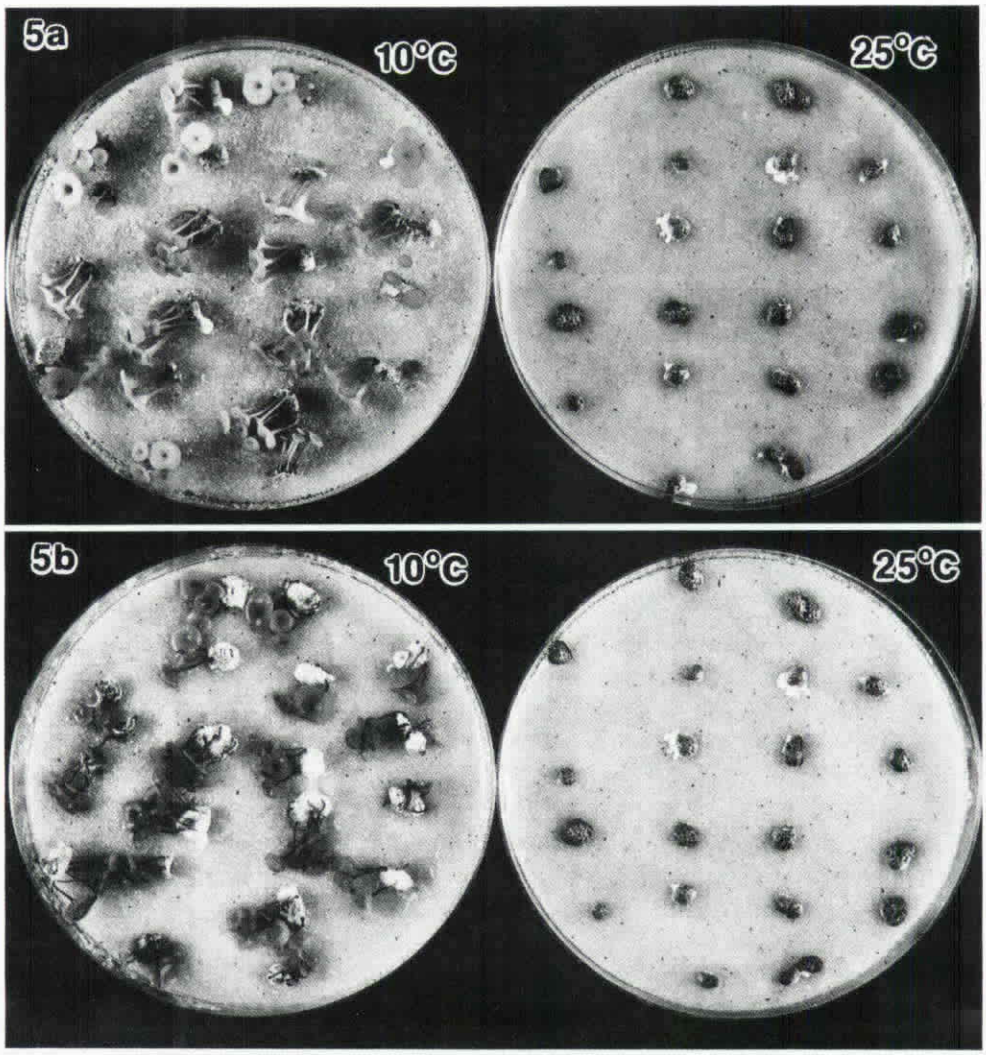

Fig. 5. An isolate of *Sclerotinia sclerotiorum*, SS-9, from Manitoba, Canada showing differences in carpogenic germination of sclerotia formed at 10°C and 25°C before (Fig. 5a) and after (Fig. 5b) conditioning. Note good germination on sclerotia formed at 10°C (Fig. 5a, left dish) but no germination on sclerotia formed at 25°C even after conditioning (Fig. 5b, right dish). Note also the old and rotted apothecia after conditioning (Fig. 5b, left dish). Magnification, ca. × 0.9.

Arg, SS-car, and mun-1, which showed some degree of inconsistency in germination between experiments.

Given the canonical and cluster analyses to examine the germination response pattern of sclerotia formed at cool or warm temperatures, with or without a cool, moist conditioning (Fig. 3), the 20 isolates of *S. sclerotiorum* were divided into the following groups.

Group A. Sclerotia formed at 10°C or 25°C had little or no germination before conditioning, but they had generally moderate or high percentage of germination after conditioning for sclerotia formed at 10 or 25°C, respectively. This group included isolates SS-6 from California and SS-12 from Florida, USA, and SS-Arg from Argentina.

Group B. Sclerotia formed at 10°C or 25°C had little or no germination before conditioning and only those formed at 25°C had high percentages of germination after conditioning. This group included isolates Tai (Fig. 4), Tb-1 and Chr-2 from Taiwan.

Group C. Sclerotia formed at 10 or 25°C had little or no germination before conditioning and their germination remained poor after conditioning. This group

included isolate SS-8 from France.

Group D. Sclerotia formed at 10°C had generally moderate to high percentages of germination before conditioning but sclerotia formed at 25°C had moderate to high percentages of germination only after conditioning. This group included isolates Sm5f-1 from Hokkaido, Japan; can-87, bean-87, sun-87, and pea-87 from Alberta, Canada.

Group E. Sclerotia formed at 10°C had generally moderate to high percentages of germination before conditioning but sclerotia formed at 25°C had generally poor germination both before and after conditioning. This group included isolates SS-9 (Fig. 5), SS-11, pea-1, physos-1 and mun-1 from Manitoba, Canada, SS-car from Saskatchewan, Canada, and SS-5 from North Dakota, USA.

Group F. Sclerotia formed at 10°C had little germination, which remained low after conditioning, while sclerotia formed at 25°C had low initial germination, which increased to a moderate level after conditioning. Isolate SS-7 from Hawaii, USA, was included in this group.

Discussion

Our study confirms previous reports that carpogenic germination of sclerotia of *S. sclerotiorum* is affected by the temperature at which sclerotia are produced (Keay, 1939; Purdy, 1956; Harada *et al.*, 1974; Huang and Kozub, 1989). The ability of sclerotia formed at cool (10°C) or warm (25°C) temperatures to germinate carpogenically varied with isolates and, more specifically, with the geographic origin of the isolates. For example, of the sclerotia produced at 10°C, germination occurred readily in isolates from the cool climatic regions including northern Japan such as Hokkaido, northern USA such as North Dakota, and southern parts of Alberta, Saskatchewan, and Manitoba, Canada. However, little or no germination of sclerotia occurred in the isolates from warmer climatic regions including Taiwan and southern parts of USA such as California, Florida and Hawaii. Moreover, for the isolates from warm climatic regions, a treatment of cool, moist conditioning is more effective in triggering carpogenic germination of sclerotia formed at a warm (25°C) temperature than those formed at a cool (10°C) temperature. These differences in germination behavior among isolates suggest that temperature is an important natural selection force in *S. sclerotiorum* and that the pathogen is capable of causing epidemics on various hosts in different climatic regions.

Effects of conditioning of sclerotia formed at 25°C also varied with isolates. A period of cool conditioning is very effective for triggering carpogenic germination of sclerotia in isolates from warmer regions such as Taiwan, California, Florida and Hawaii. However, there was a difference in response to cool conditioning of sclerotia among the isolates from the North American prairies. Conditioning of sclerotia formed at 25°C was effective for the four isolates from Alberta and, to a lesser extent, for the isolate from Saskatchewan, but it was ineffective for the isolates from Manitoba and North Dakota, despite the similarity in winter conditions in the prairies. The reason for such a difference, among isolates from areas of similar climatic conditions remains unknown.

There was no interaction between the host from which isolates were obtained and the amount of carpogenic germination, before or after conditioning. It seems climatic conditions of the area of origin, rather than original host, determine the ability of an isolate of *S. sclerotiorium* to germinate carpogenically and cause a disease outbreak.

This study further demonstrates the usefulness of the technique developed for testing carpogenic germination of sclerotia of *S. sclerotiorum* (Huang and Kozub, 1989). Since formation of apothecia and production of ascospores are important in the development of sclerotinia head rot of sunflower (Huang, 1983) and safflower (Muendel *et al.*, 1985), sclerotinia stem rot of rapeseed (Gugel and Morrall, 1986) and white mold of bean (Huang and Kemp, 1989), this germination technique may be used as a standard method to compare more isolates of *S. sclerotiorum* from tropical, subtropical and temperate zones. The information from such studies may lead to a better understanding of the differentiation and adaptation of *S. sclerotiorum* as well as the epidemiology of the disease in different geographic regions.

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不同地域之菌核病菌菌株對菌核子囊盤發芽溫度的需求

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以採自加拿大、美國、阿根廷、法國、台灣、日本各國的菌核病菌(*Sclerotinia sclerotiorum*)之 20 個單孢菌株比較其菌核形成之溫度對其菌核發芽產生子囊盤的影響。將每一供試菌株移植於馬鈴薯洋菜培養基平板，經低溫(10°C)或高溫(25°C)不照光培養 8 週，隨後將所產生的菌核直接移到含有濕沙的培養皿內，於 20°C下光照 3 週後隨即測定其發芽產生子囊盤之數目；至於未發芽的菌核再給予 4 週的黑暗低溫(10°C)處理之後再移放於光照處 3 週以決定低溫處理對子囊盤發芽數目的效應。試驗結果顯示菌核發芽產生子囊盤的能力非但與菌核形成之溫度有關而且亦隨菌株而異。來自冷涼氣候地區的菌株如日本北海道、美國 North Dakota、及加拿大 Alberta, Saskatchewan 和 Manitoba 等地區的菌株，在低溫培養所產生的菌核容易發芽，而來自溫暖地區的菌株如台灣、美國 California, Florida 和 Hawaii 等地區的菌核却不容易發芽。又在高溫(25°C)下所產生的菌核，大多數都必須經過低溫處理始能發芽。這種低溫處理效應亦隨菌株而異。例如取自台灣的菌株，低溫處理可促進高溫(25°C)培養所產生的菌核之發芽，但對低溫(10°C)培養所產生的菌核卻無顯著的促進作用。