Effect of Cold Treatment and Drying on Mycelial Germination by Sclerotia of *Sclerotinia minor*

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Accepted for publication 7 November 1986.

ABSTRACT

Wymore, L. A., and Lorbeer, J. W. 1987. Effect of cold treatment and drying on mycelial germination by sclerotia of *Sclerotinia minor*. Phytopathology 77:851-856.

Mycelial germination by sclerotia of *Sclerotinia minor* was of two morphological types: eruptive and noneruptive. Cold treatment of hydrated sclerotia at 3 C for as little as 1 day stimulated rapid mycelial germination, whereas cold treatment of hydrated sclerotia at −12 C also stimulated rapid mycelial germination but reduced the proportion of sclerotia that underwent eruptive mycelial germination and increased the proportion that underwent noneruptive mycelial germination. Cold treatment stimulated mycelial germination of field-collected sclerotia as well as those produced in the laboratory. Air-drying (aging) of sclerotia at room temperature stimulated both eruptive mycelial germination and noneruptive mycelial germination but was not as effective as cold treatment. Fluctuating moisture conditions increased the proportion of sclerotia undergoing noneruptive mycelial germination. Hyphae produced during either type of mycelial germination were capable of causing infection of lettuce tissues without prior colonization of a food base.

Additional key words: *Lactuca sativa*, lettuce.

Sclerotinia minor Jagger is the principal causal agent of lettuce drop, a destructive disease of lettuce (*Lactuca sativa* L.) grown on organic soil in New York. *S. minor* produces sclerotia that serve as survival propagules in soil and as primary inoculum for infection of lettuce. Sclerotia may undergo carpogenic germination to produce apothecia, but this is apparently relatively rare in nature (2,10,20). Infection of lettuce plants by *S. minor* occurs primarily by myceliogenic germination of sclerotia and the production of infective hyphae (2,20).

Adams and Tate (5) described two types of myceliogenic germination. Hyphal germination was said to occur when ". . . individual hyphae . . . emerged through the rind of the sclerotia." Mycelial germination was said to occur when ". . . dense mycelium erupt[ed] from the sclerotium." Hyphal germination occurred most commonly on agar media, whereas mycelial germination usually occurred on soil or filter paper. Adams and Tate also demonstrated that mycelial germination resulted in infection without prior colonization of an organic substrate as a food base and that freshly produced sclerotia did not undergo mycelial germination. Sclerotia had a dormancy period after their production and required incubation in soil of 8–15 wk to break dormancy (5). Other reports (4,11,12) indicate that sclerotia of *S. minor* may require a period of drying before undergoing high levels of mycelial germination.

Relatively little else is known about the factors that induce mycelial germination by sclerotia of *S. minor*. Imolehin et al (12) found that the optimum temperature for mycelial germination for three isolates of *S. minor* was 18 C and the optimum soil matric potential was −0.33 bar. Imolehin and Grogan (11) concluded that in most soils lack of aeration (elevated CO_2 concentrations and/or depressed O_2 concentrations) or the presence of ethylene had no effect. Hau et al (9) observed that mycelial germination was increased when sclerotia were exposed to volatiles released from remoistened peanut leaves.

The present study was conducted to determine the effects of cold treatment and drying on mycelial germination by sclerotia of New York isolates of *S. minor*. A preliminary report of this work has been published (21).

MATERIALS AND METHODS

Sclerotia of *S. minor* collected from infected lettuce plants in New York lettuce fields were surface-disinfested for 5 min in 0.5% NaOCl. Some sclerotia were transferred to potato-dextrose agar (PDA) plates to establish laboratory isolates, and others were used directly in germination experiments. Additional sclerotia for some experiments were separated from organic soil using the wet-sieve glycerine-flotation technique (3).

Sclerotia of laboratory isolates were produced by growing *S. minor* on wheat kernels (95 g of wheat plus 90 ml of deionized water) in cotton-stoppered 500-ml Erlenmeyer flasks. The flasks were autoclaved twice with a 24-hr interval, seeded with mycelial disks, and incubated in the dark at 21 C.

After 4 wk, sclerotia were separated by wet-sieving the contents of the flasks through nested soil sieves. Sclerotia collected on a 50-mesh sieve (0.297-mm opening) were washed under running tap water in a cheesecloth-covered beaker for 10 min. Sclerotia with intact rinds sank to the bottom of the beaker and were collected for use in experiments without drying or after air-drying at room temperature and relative humidity (18–24 C, 40–50% RH).

Germination was tested by rehydrating air-dried (aged at room temperature) sclerotia in sterile deionized water for 5 min and placing surface-disinfested (0.5% NaOCl for 5 min followed by rinsing in sterile deionized water) sclerotia on moist, autoclaved, white quartz sand in glass petri dishes. Preliminary experiments indicated that exposure of sclerotia to 0.5% NaOCl for up to 20 min had no effect on mycelial germination. Fresh sclerotia (not air-dried before use) were surface-disinfested, rinsed, and placed on sand as above. Twenty-five sclerotia were placed in each 9-cm-diameter dish (40 cm^3 of washed sand, 14.5 ml of deionized water per dish) and 10 sclerotia were placed in each 4.8-cm-diameter dish (13 cm^3 of washed sand, 4.5 ml of deionized water per dish). For most experiments, 100 sclerotia (four plates of 25 or 10 plates of 10) were used for each treatment. Plates were incubated in sealed plastic bags at 21 C or cold-treated at different temperatures (3 or −12 C) before incubation at 21 C. Each sclerotium was periodically observed with a dissecting microscope (25×) for the occurrence of mycelial germination.

For infection experiments, 1.5-cm-diameter disks were cut from lettuce leaves (cultivar Montello, Harris Moran Seed Co., Rochester, NY) with a cork borer, rinsed in deionized water, and

placed over sclerotia of *S. minor* in sand plates. Plates with leaf disks were incubated at 21 C in an illuminated (12-hr photoperiod, 2,800 lx) incubator and were observed periodically for infection of lettuce tissues.

Results are presented as mean percent mycelial germination, eruptive mycelial germination, and noneruptive mycelial germination after 18 days of incubation at 21 C or at the conclusion of the experiment (42–54 days of incubation). Data were analyzed with a factorial analysis of variance and Duncan's *K*-ratio *t* test or multiple regression where appropriate.

RESULTS

Types of myceliogenic germination observed. Hyphal germination by sclerotia placed in sand plates was observed only infrequently and involved the production of a few very short hyphae (1 mm or less) that often disappeared within a few days. Mycelial germination was the most common type of germination by sclerotia in sand plates and consisted of a fluffy mass of persistent white hyphae originating from a more or less localized area on the sclerotium. Observations of germinating sclerotia with a dissecting microscope indicated mycelial germination was of two morphological types. In some cases, germination consisted of a plug of hyphae originating from within the sclerotium and erupting through the rind (Figs. 1A–D and 2A). Remnants of rind tissue were pushed aside by the emerging hyphae, and this type of mycelial germination is referred to as eruptive mycelial germination. In other cases, germination consisted of a mass of hyphae produced either from the surface rind cells or as individual hyphae emerging from between rind cells (Fig. 2B). Hyphae produced in this way could easily be teased away from the sclerotium leaving no obvious scars or breaks in the rind (Fig. 2C). This type of mycelial germination is referred to as noneruptive mycelial germination.

The two types of mycelial germination were difficult to distinguish without the aid of a dissecting microscope. Both resulted in consumption of the sclerotium contents. Sclerotia usually underwent only mycelial germination, but sometimes sclerotia underwent mycelial germination after initially undergoing hyphal germination. Only one type of mycelial germination was ever observed on a single sclerotium. Occasionally secondary sclerotia were formed after noneruptive mycelial germination but were never observed after eruptive mycelial germination (Fig. 2D).

For all experiments, in addition to the sclerotia placed on quartz sand, some sclerotia were placed on PDA. Hyphal germination was always near 100% on PDA, indicating high viability, but mycelial germination was never observed. Sclerotia produced on PDA in some preliminary experiments were observed to undergo noneruptive mycelial germination but rarely underwent eruptive mycelial germination.

Sclerotia were placed on organic soil in plates in other preliminary experiments and results were similar to those on sand. Because of difficulties in observing sclerotia on the black organic soil, subsequent experiments used white quartz sand.

Stimulation of mycelial germination by air-drying (aging) and/or cold treatment. In a representative experiment, six different durations of air-drying (aging) (0, 1, 7, 14, 28, and 56 days) followed by six different durations of cold treatment at 3 C (0, 1, 7, 14, 28, and 56 days) were tested in all combinations in a 6× 6 factorial design to determine their effects on mycelial germination by sclerotia of *S. minor* isolate S-1 (Table 1). Sclerotia were cold-treated by placing hydrated sclerotia in sand plates, enclosing the plates in plastic bags, and maintaining them at 3 C for the designated periods of time. After the treatments, all plates were incubated at 21 C.

The highest level of mycelial germination (93%) occurred after 7 days of air-drying and 28 days of cold treatment at 3 C. Statistical analysis indicated a significant increase in mycelial germination with increasing length of cold treatment up to 56 days and a significant increase in mycelial germination with increasing length of air-drying up to 28 days. After 56 days of cold treatment there was a decrease in mycelial germination with increasing air-drying

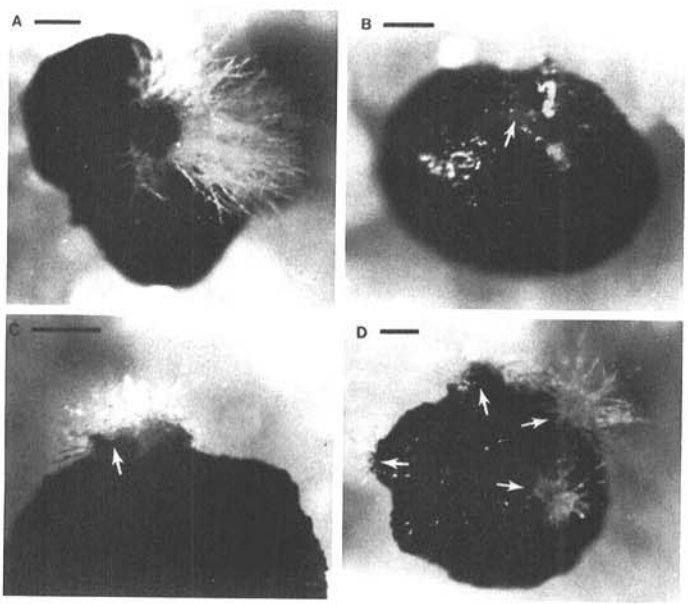

Fig. 1. Mycelial germination by sclerotia of *Sclerotinia minor*. Scale bars represent 125 μm. **A,** Eruptive mycelial germination. **B,** Early stage of eruptive mycelial germination—light-colored contents of sclerotium (arrow) are beginning to push out through the rind. **C,** Eruptive mycelial germination—hyphae have emerged from the sclerotium by pushing back remnants of the rind (arrow). **D,** Eruptive mycelial germination from multiple sites (arrows) on a single sclerotium.

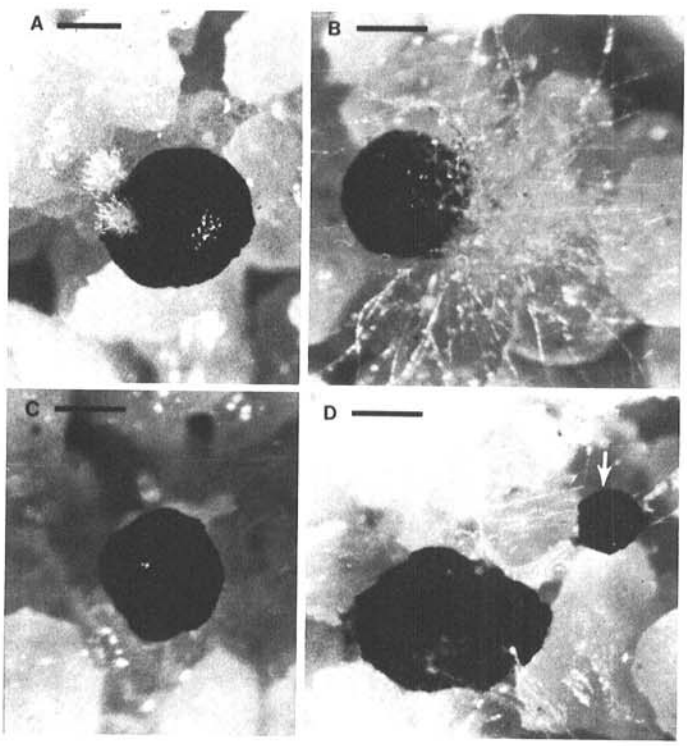

Fig. 2. Mycelial germination by sclerotia of *Sclerotinia minor*. Scale bars represent 250 μm. **A,** Eruptive mycelial germination. **B,** Noneruptive mycelial germination. **C,** Same sclerotium as in B after hyphae were teased away from the surface. **D,** Secondary sclerotium (arrow) produced after noneruptive mycelial germination of a sclerotium of *S. minor*.

time. For those sclerotia that germinated during this experiment, most (86–99%) underwent eruptive mycelial germination, and the remainder underwent noneruptive mycelial germination.

Cold treatment consistently stimulated mycelial germination by sclerotia of *S. minor* isolate S-1 in all experiments. Cold treatment was only effective when sclerotia were hydrated during the cold treatment; cold treatment of dried sclerotia did not stimulate mycelial germination. Air-drying sclerotia after production also stimulated mycelial germination but was generally not as effective as cold treatment. Neither treatment was required because some untreated sclerotia underwent mycelial germination (Table 1). Cold-treated sclerotia, however, underwent mycelial germination much more rapidly than untreated sclerotia.

Cold treatment at −12 C also stimulated rapid mycelial germination but resulted in a shift in the proportions of sclerotia that underwent eruptive and noneruptive mycelial germination (Table 2). Cold treatment at −12 C stimulated a higher proportion of noneruptive mycelial germination, whereas cold treatment at 3 C stimulated eruptive mycelial germination (Table 2). Noneruptive mycelial germination occurred very rapidly after sclerotia were placed under favorable conditions for germination (21 C). After 15 days of incubation all sclerotia preconditioned to germinate noneruptively had done so and any remaining sclerotia that germinated during the course of the experiment underwent eruptive mycelial germination. Regression analysis of log-probit transformed data indicated that 32 days of incubation were required for 50% of untreated sclerotia to germinate, and only 9 days of incubation were required for germination to reach 50% for sclerotia cold-treated at −12 C (Table 2). Untreated sclerotia eventually underwent high levels of mycelial germination (>90%) but required longer incubation periods (up to 50–60 days) to reach these levels.

TABLE 1. Mycelial germination by sclerotia of isolate S-1 of *Sclerotinia minor* at 21 C after air-drying at room temperature and/or cold treatments at 3 C

Cold treatment time (days)	Air-drying time (days)					
	0	1	7	14	28	56
0	21[a]	10	9	23	9	21
1	32	32	29	32	25	55
7	62	73	82	84	60	61
14	75	83	81	84	60	74
28	72	88	93	84	85	82
56	72	89	78	82	81	72

[a] Values are mean percent mycelial germination of four replicates of 25 sclerotia each and were recorded after 18 days of incubation at 21 C. Regression equation: Mycelial germination = $20.0204 + 7.7013C - 0.2773C^2 + 0.0028C^3 + 1.4339D - 0.0888D^2 + 0.0011D^3$ where C = cold treatment time in days and D = drying time in days. $r^2 = 0.81$.

TABLE 2. Total mycelial germination (MG), eruptive mycelial germination (EMG), and noneruptive mycelial germination (NMG) by sclerotia of isolate S-1 of *Sclerotinia minor* after different cold treatments

Cold treatment temperature[a]	Germination (%)[b]			Incubation time required for 50% MG (days)[c]
	Total MG	EMG	NMG	
None	81 a	79 a	2 a	32
3 C	96 b	88 a	8 a	13
−12 C	100 b	61 b	39 b	9

[a] Sclero ia were cold-treated at the indicated temperature for 7 days.

[b] Values are mean percent germination of 10 replicates of 10 sclerotia each and were recorded after 54 days of incubation at 21 C. Values in a column followed by the same letter are not significantly different ($P = 0.05$) according to a factorial analysis of variance and Duncan's K-ratio t test.

[c] Values are the length of incubation required for 50% of the germinating sclerotia to undergo mycelial germination and were calculated from regression equations generated following log-probit transformation of the data.

Alternately air-drying and rehydrating sclerotia of *S. minor* isolate S-1 for up to three cycles did not affect total mycelial germination but increased the proportion of germinating sclerotia that underwent noneruptive mycelial germination (Table 3).

Mycelial germination by different laboratory isolates of *S. minor*. Twenty-two isolates of *S. minor* originating from different New York lettuce fields underwent dissimilar levels of mycelial germination after air-drying and cold treatments (Table 4). Sclerotia of six of the 22 New York isolates were viable but underwent very low levels of mycelial germination (0 to 1%) after all cold treatments and air-drying treatments. These six isolates are not included in Table 4. For 12 of the remaining 16 isolates, there was a significant increase in total mycelial germination after cold treatment at 3 C, whereas air-drying significantly increased mycelial germination for 15 isolates (Table 4). There were also differences among isolates in the proportions of germinating sclerotia that underwent eruptive and noneruptive mycelial germination (Table 4). Some isolates underwent primarily eruptive mycelial germination (e.g., isolate 82-18 with 78% mycelial germination and 73% eruptive mycelial germination after 56 days of air-drying and 28 days of cold treatment), whereas for others, a higher proportion of sclerotia underwent noneruptive mycelial germination (e.g., isolate 82-3 with 100% mycelial germination and only 31% eruptive mycelial germination). All 22 isolates were pathogenic to lettuce when agar disks bearing mycelium were applied to the bases of lettuce plants in pots in the greenhouse.

Mycelial germination by sclerotia collected in the field. A cold treatment at 3 C for 7 days resulted in a significant increase in total mycelial germination and eruptive mycelial germination by sclerotia of *S. minor* separated from organic soil collected from a field in which lettuce drop was severe (Table 5). Increasing air-drying time, however, decreased mycelial germination and noneruptive mycelial germination. Although most sclerotia were viable as indicated by germination on PDA, some sclerotia failed to undergo mycelial germination or hyphal germination during the course of this experiment.

Direct infection of lettuce tissues by hyphae from germinated sclerotia of *S. minor*. Both eruptive and noneruptive mycelial germination by sclerotia of *S. minor* resulted in infection of lettuce tissue. Infection of lettuce leaf disks by sclerotia that had been cold-treated at 3 C or −12 C occurred at a rate almost identical with that of mycelial germination by similarly treated sclerotia (Fig. 3). Infection occurred very rapidly when sclerotia were cold-treated at −12 C because these sclerotia usually underwent noneruptive mycelial germination. Infection by hyphae from sclerotia cold-treated at 3 C occurred more slowly because these sclerotia usually underwent eruptive mycelial germination.

TABLE 3. Total mycelial germination (MG), eruptive mycelial germination (EMG), and noneruptive mycelial germination (NMG) by sclerotia of isolate S-1 of *Sclerotinia minor* at 21 C after different cycles of air-drying at room temperature and rehydrating

Wet/dry cycles[a] (no.)	Cold treatment[b]	Germination (%)[c]		
		Total MG	EMG	NMG
0	−	90 a	64 a	26 a
0	+	100 b	56 a	44 b
1	−	98 b	28 b	70 cd
1	+	100 b	30 b	70 cd
2	−	100 b	33 b	67 cd
2	+	100 b	22 b	78 cd
3	−	99 b	15 c	84 d
3	+	99 b	31 b	68 cd

[a] Air-dried sclerotia were alternately rehydrated for 24 hr and air-dried for 24 hr at room temperature for the indicated number of cycles.

[b] After the wet/dry cycles, sclerotia were cold-treated at 3 C for 7 days (+) or not cold-treated (−).

[c] Values are mean percent germination of four replicates of 25 sclerotia each and were recorded after 45 days of incubation at 21 C. Values in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's K-ratio t test.

DISCUSSION

The term "mycelial germination" was introduced by Adams and Tate (5) to describe the production of a mass of hyphae that emerges from within a sclerotium, bursts through the rind, and directly infects plant tissues. The term "eruptive germination" frequently has been equated with mycelial germination in the literature for *S. minor* as well as other fungi (9,11,12,17). Observations in the present study indicate that a mass of hyphae can be produced from sclerotia of *S. minor* with or without the eruption of the hyphae through the rind en masse. Mycelial germination as observed here is of two morphological types, and they differ in the length of time required to complete germination after the onset of favorable conditions. Noneruptive mycelial germination occurs more rapidly than eruptive mycelial germination and involves the production of a mass of hyphae that originates from the surface of the sclerotium or from hyphae that emerge individually between rind cells and aggregate on the surface of the sclerotium. Noneruptive mycelial germination has previously been referred to as "extensive hyphal germination" (21). Eruptive mycelial germination involves the production from within the sclerotium of a mass of hyphae that bursts through the rind en masse. Both forms of mycelial germination are capable of infecting lettuce tissues without prior colonization of a food base.

The optimum temperature (21 C) for mycelial germination for isolate S-1 was similar to that found by Imolehin et al (12). They observed high levels of mycelial germination, however, after only 5 days of incubation on moist sand. Hau et al (9) recorded mycelial germination after only 4 days of incubation and observed low (<50%) levels of germination in all but a few treatments. In the present study, high levels of mycelial germination did not occur until after 14–21 days of incubation, depending on the type of germination. The reason for these differences is not known.

Cold or chilling treatments have been used to stimulate carpogenic germination by *S. sclerotiorum* and *S. trifoliorum* (1,6,18,19) and other fungi (16). In the present study, cold treatment also was effective in stimulating mycelial germination by sclerotia of many isolates of *S. minor*. Cold treatment stimulated a more rapid rate of mycelial germination but was effective only if sclerotia were hydrated during the cold treatment. Cold treatment stimulation of carpogenic germination by sclerotia of *S. sclerotiorum* also has been reported to be effective only when sclerotia are hydrated during the cold treatment (18,19).

The cold treatment temperature appears to be a critical factor for stimulating germination by sclerotia of both *S. sclerotiorum* and *S. minor*. Bedi (6) reported that a cold treatment at 0 C stimulated carpogenic germination by sclerotia of *S. sclerotiorum*, whereas cold treatment at −15 C resulted in loss of the ability to undergo carpogenic germination although the sclerotia were still viable. A qualitative difference in the effect of cold treatment temperature on sclerotia of *S. minor* also was observed in the present study. Cold treatment at −12 C stimulated sclerotia to undergo a higher proportion of noneruptive mycelial germination than similar sclerotia that were not cold-treated or were cold-treated at 3 C. Chilling treatments have been suggested to stimulate germination of sclerotia of *Claviceps* by removing a restraint on the glyoxylate pathway (8). Sclerotia of *Sclerotinia* species,

TABLE 5. Mycelial germination (MG), eruptive mycelial germination (EMG), and noneruptive mycelial germination (NMG) by sclerotia of *Sclerotinia minor* retrieved from organic soil

Air-drying time (days)	Cold treatment[a]	Germination (%)[b]		
		Total MG[c]	EMG[d]	NMG[e]
0	−	54	19	35
0	+	61	23	38
1	−	57	22	35
1	+	69	30	39
14	−	54	25	29
14	+	71	41	30
56	−	39	17	22
56	+	57	39	18

[a] After air-drying at room temperature, sclerotia were cold-treated at 3 C for 7 days (+) or not cold-treated (−).
[b] Values are mean percent germination of 10 replicates of 10 sclerotia each and were recorded after 18 days of incubation at 21 C.
[c] Factorial analysis of variance and regression analysis indicated a significant ($P = 0.01$) increase due to cold treatment and a significant ($P = 0.01$) decrease due to increasing air-drying time.
[d] Factorial analysis of variance indicated a significant ($P = 0.01$) increase due to cold treatment and no effect due to increasing air-drying time.
[e] Factorial analysis of variance and regression analysis indicated a significant ($P = 0.01$) decrease due to increasing air-drying time and no effect due to cold treatment.

TABLE 4. Mycelial germination by sclerotia of 16 New York isolates of *Sclerotinia minor* following different durations of cold treatment at 3 C and air-drying at room temperature

	Air-drying time (days)					
	0			56		
	Cold treatment time (days)					
Isolate	0	7	28	0	7	28
82-3[a,b]	75[c] (63)[d]	97 (90)	94 (91)	97 (41)	98 (38)	100 (31)
82-21[a,b]	39 (26)	52 (46)	89 (86)	78 (35)	98 (17)	100 (34)
82-26[a,b]	7 (7)	16 (16)	18 (15)	91 (53)	95 (42)	99 (20)
82-29[a,b]	2 (1)	14 (5)	8 (8)	88 (24)	88 (17)	99 (18)
82-14[a,b]	2 (1)	15 (13)	0 (0)	50 (43)	83 (83)	92 (82)
82-31[b]	2 (1)	7 (6)	4 (4)	76 (29)	88 (34)	85 (38)
82-27[a,b]	8 (7)	11 (11)	31 (21)	47 (38)	75 (59)	85 (76)
82-18[a,b]	10 (8)	12 (11)	23 (21)	34 (29)	58 (56)	78 (73)
82-15[a,b]	2 (1)	16 (14)	20 (19)	39 (38)	54 (53)	61 (54)
82-2[b]	17 (16)	16 (13)	8 (7)	35 (35)	44 (42)	51 (50)
82-16[a,b]	1 (0)	1 (1)	1 (0)	6 (6)	14 (14)	49 (48)
82-30[b]	1 (1)	5 (4)	1 (0)	65 (42)	73 (35)	46 (16)
82-22[a,b]	2 (0)	6 (4)	7 (5)	9 (6)	26 (24)	42 (40)
82-6[b]	4 (4)	5 (4)	9 (6)	36 (32)	47 (41)	34 (32)
82-17[a,b]	0 (0)	5 (2)	4 (3)	9 (1)	13 (8)	33 (20)
82-1[a]	0 (0)	11 (2)	11 (6)	9 (7)	7 (6)	10 (9)

[a] For this isolate, factorial analysis of variance indicated a significant ($P = 0.01$) increase in mycelial germination due to cold treatment.
[b] For this isolate, factorial analysis of variance indicated a significant ($P = 0.01$) increase in mycelial germination due to air-drying.
[c] Values are mean percent mycelial germination of four replicates of 25 sclerotia each and were recorded after 42 days of incubation at 21 C.
[d] Values in parentheses are mean percent eruptive mycelial germination of four replicates of 25 sclerotia each and were recorded after 42 days of incubation at 21 C.

however, do not contain large lipid reserves (7,13) and cold treatment may act on some other physiological process(es). Saito (19) reported stimulation of myceliogenic germination by sclerotia of *S. sclerotiorum* after wounding of the rind. Also, rind cells of sclerotia of *S. sclerotiorum* have been reported to resume growth after penetration of the sclerotium by other fungi (14). Cold treatment of *S. minor* sclerotia by freezing at −12 C may act by disrupting the rind sufficiently to allow individual hyphae to emerge when temperature and moisture conditions become favorable for growth.

Air-drying or aging of sclerotia have been suggested to be important in stimulating mycelial germination. Some reports indicate that newly produced sclerotia of many isolates of *S. minor* require a dormancy period before undergoing mycelial germination (2,4). In the present study, the effects of air-drying sclerotia at room temperature for different periods of time following their production may be attributed to aging. Although air-drying of sclerotia stimulated mycelial germination by many isolates of *S. minor*, neither air-drying nor cold treatment were essential for mycelial germination to occur. Some isolates of *S. minor* underwent relatively high levels of mycelial germination under all the cold and air-drying treatment combinations. Other isolates exhibited very little or no mycelial germination under the same conditions. Adams and Tate (5) also reported such differences between isolates with respect to dormancy periods, but Imolehin et al (12) obtained similar results with all the isolates they tested. Because not all isolates responded to cold treatment or air-drying, other factors must also be involved in stimulating mycelial germination by sclerotia of *S. minor*.

Germination experiments in the present study were carried out primarily with laboratory-produced sclerotia. Sclerotia produced in axenic culture differ from those produced on infected plants in the field (13,15,20). Cold treatment, however, stimulated mycelial germination by sclerotia of *S. minor* separated from field-collected organic soil or produced in laboratory culture. Also, both eruptive and noneruptive mycelial germination were observed on sclerotia from both infected plants and soil and on sclerotia produced on wheat kernels. The potential effect on germination of leaching due to washing laboratory-produced sclerotia under running water, however, might be important and should be investigated.

The cycling of soil moisture may produce conditions that allow for noneruptive mycelial germination by sclerotia of *S. minor* to result in direct infection of lettuce plants. Adams and Tate (4) reported that cycling soil moisture of infested soil from saturation to 30% of saturation resulted in increased levels of lettuce drop in greenhouse experiments. In the present study, alternately air-drying and rehydrating sclerotia did not increase total mycelial germination by these sclerotia but did cause an increase in noneruptive mycelial germination.

Noneruptive mycelial germination occurs more rapidly than eruptive mycelial germination and would allow sclerotia of some isolates of *S. minor* to take advantage of short periods of favorable temperature and moisture to germinate and infect lettuce plants. Thus, high levels of lettuce drop might be expected in years following cold winters and/or springs with large fluctuations in soil moisture.

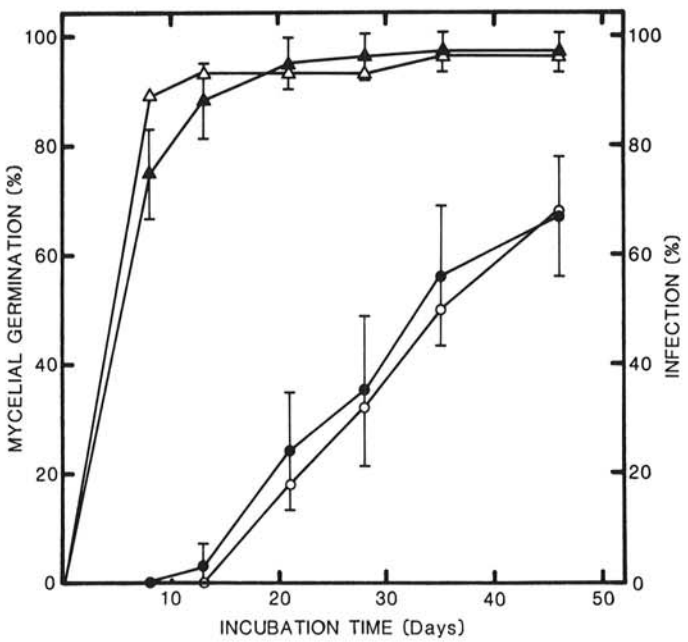

Fig. 3. Mycelial germination by sclerotia of *Sclerotinia minor* isolate S-1 and infection of lettuce leaf disks. (●—●) = Infection (%) of lettuce leaf disks placed over sclerotia cold-treated at 3 C for 7 days; (o—o) = mycelial germination (%) by sclerotia cold-treated at 3 C for 7 days and incubated (all germinating sclerotia underwent eruptive mycelial germination); (▲—▲) = infection (%) of lettuce leaf disks placed over sclerotia cold-treated at −12 C for 7 days; (Δ—Δ) = mycelial germination (%) by sclerotia cold-treated at −12 C for 7 days and incubated (89% of germinating sclerotia underwent noneruptive mycelial germination, 11% underwent eruptive mycelial germination). Closed symbols (●, ▲) represent means of 15 replicates of five leaf disks and sclerotia each, and vertical bars represent ± 2 standard errors of the means. Open symbols (o, Δ) represent percent germination in two plates of 28 sclerotia each (sclerotia in one plate cold-treated at 3 C, sclerotia in the other plate cold-treated at −12 C).

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