Effect of Temperature and Moisture Tension on Growth, Sclerotial Production, Germination, and Infection by *Sclerotinia minor*

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We are grateful to K. A. Kimble, Curt Waters, and Diane Zumwalt for helpful discussion and technical assistance and to Jeff Hall for preparation of illustrations.
Accepted for publication 19 May 1980.

ABSTRACT

IMOLEHIN, E. D., R. G. GROGAN, and J. M. DUNIWAY. 1980. Effect of temperature and moisture tension on growth, sclerotial production, germination, and infection by *Sclerotinia minor*. Phytopathology 70:1153-1157.

Sclerotial germination and mycelial growth of *Sclerotinia minor* occurred from 6 to 30 C; the optimum for each was at 18 C. Infection of lettuce tissue by hyphae from germinating sclerotia occurred from 6 to 24 C with an optimum at 18 C. The temperature range for sclerotial formation was from 12 to 24 C with more sclerotia produced at 12 C than at other temperatures, but sclerotia produced at the higher temperatures were larger. Mycelial growth on cornmeal agar occurred at solute potentials of −1 to −73 bars, but sclerotia were formed only within the range of −1 to −43 bars. Growth in liquid media was maximal at about −7 bars and declined at lower solute potentials. Sclerotia produced at various solute potentials and temperatures germinated equally well. Sclerotia were capable of eruptive germination at soil moisture tensions ranging from −1/3 to −15 bars, but the highest percent germination occurred at −1/3 bar. Infection of lettuce tissue by sclerotia at temperatures between 6 and 24 C was dependent on the ability of sclerotia to germinate eruptively. All tissues inoculated with mycelial plugs instead of sclerotia at 6 to 24 C became infected.

Additional key words: Lactuca sativa.

Following infection of lettuce by *Sclerotinia minor* Jagger, sclerotia are formed on the infected tissue which when disked under replenish the soil with inoculum (1–3,7,14). However, there are no quantitative reports on the influence of environmental factors on sclerotial formation. Chivers (8) reported that size of sclerotia produced by *S. minor* and *S. intermedia* was affected by temperature, but the numbers and infectivity of sclerotia produced at these temperatures were not discussed. Most references on the germination of sclerotia of *Sclerotinia* spp. are on carpogenic germination by *S. sclerotiorum, S. trifoliorum* (6,7,11,18,25), and *S. minor* (5,10,16,23). Prolonged wet periods are necessary (1,2,6,24,28) and temperatures between 10 and 15 C are optimum (1,7,9,18,19,25) for production of apothecia. Although apothecial production by *S. minor* in the laboratory has been reported (10, 21–23), there is only one report of *S. minor* forming apothecia in nature (16). Sclerotia of *S. minor* usually germinate by an eruptive growth of mycelium, providing a means of infection that does not require a food base. Reports of field observations and laboratory experiments on eruptive germination by sclerotia of *S. minor* (3–5, 17,27) have not included effects of temperature and moisture. Abawi and Grogan (1) reported that lesion initiation and development by *S. sclerotiorum* on bean were optimum at 20–25 C, but similar reports on *S. minor* are lacking. The present investigation is concerned with the effects of temperature and moisture tension on growth, sclerotial production, eruptive germination, and infection by *S. minor*.

0031-949X/80/12115305/$03.00/0

MATERIALS AND METHODS

Effect of temperature and solute potential on radial growth, sclerotial production, and mycelial dry weight. Three isolates of *S. minor* (Ess-1 and SM-1 from New York, and SV-1 from Salinas Valley, California, all isolated from infected lettuce (*Lactuca sativa* L.), were used. The centers of 90-mm diameter glass petri plates containing 20 ml of PDA (200 g potato, 10 g dextrose, and 15 g of agar per liter of tap water) were inoculated with 5-mm-diameter mycelial disks from 3- to 5-day-old cultures of *S. minor* on PDA. Each of five plates were incubated at 6, 12, 18, 24, and 30 C. Radial growth was measured after 48 and 72 hr and the number of sclerotia was assessed after 30 days. After 6 wk of aging in the petri plates at room temperature (about 20 C), the sclerotia were air dried for 24 hr and tested for eruptive germination by placing them on moist quartz sand for 5 days.

A basal Bacto cornmeal agar medium (CMA — pH 6.0) was used for studies on the effect of solute potential (ψ_s) on radial growth and sclerotial production, whereas potato dextrose broth (PDB — pH 6.5) 200 g potato and 10 g of dextrose per liter of tap water was used for all experiments on the effect of ψ_s on dry weight production. Both types of media were adjusted to the desired ψ_s values by adding different amounts of NaCl, KCl, or sucrose, the amounts being determined by their water activites (26). Media were autoclaved for 15 min at 120 C and 1.06 Kg/cm^2 steam pressure, after which their actual ψ_s values were determined with a thermocouple psychrometer as described by Duniway (12). Petri plates containing 20 ml of the osmotically adjusted media were inoculated with *S. minor* as described above. Three plates at each ψ_s value were incubated and radial growth was determined after 50 hr at 20 C. Sclerotial production and percent germination were determined as described above.

To determine growth as dry weight, 75 ml of PDB in 250-ml Erlenmeyer flasks were inoculated with 5-mm diameter mycelial disks from 3- to 5-day-old cultures of *S. minor* and three flasks were incubated for 8 days at each of the selected temperatures. For ψ_s studies, the medium was adjusted to the different ψ_s values prior to inoculation with *S. minor*, and the flasks were incubated on a reciprocating shaker at 400 rpm at room temperature (20 C) for 8 days. The contents of each flask (no sclerotia had formed) were filtered onto tared No. 1 Whatman filter paper and washed five times with distilled water. The mycelial mats were dried in the oven at 110 C for 24 hr and dry weights were determined.

Effect of temperature and soil water potential on germination of sclerotia. Sclerotia were produced on autoclaved oat seeds with or without amendment with nutrient broth (NB) (3 g beef extract, 10 g dextrose, and 10 g Bacto peptone per liter of distilled water). Sixty milliliters of tap water or NB were added to 50 g of oat seed in a 250-ml flask and autoclaved on two successive days for 20 min at 120 C and 1.06 kg/cm^2 steam pressure.

The autoclaved oat seeds were inoculated with mycelial disks from 3-day-old cultures of *S. minor*. Cultures were aged in the flasks at room temperature (about 20 C) for 12 – 24 wk before use. Sclerotia were recovered by using 1.98 mm (to retain coarse oat debris) and 0.25 mm (to retain sclerotia) sieve sizes. Sclerotia, transferred from the 0.25-mm sieve into a 1,000-ml beaker, were rinsed eight times with tap water to remove debris and were submerged in 0.5% NaOCl for 3 min. Finally the sclerotia were dried at room temperature for 24 hr and stored at 4 C until needed.

To determine percent germination, 25 air-dried sclerotia were placed on moist quartz sand in a 9.0-cm diameter petri plate. Four plates were incubated at five different temperatures (6, 12, 18, 24, and 30 C). Plates were kept in sealed plastic bags to prevent water loss. Percent eruptive germination as described by Adams (4) was recorded daily.

To determine the combined effect of soil water potential and temperature on germination of sclerotia, an unsterilized soil (clay loam) from a lettuce field in the Salinas Valley, California was placed on pressure plates and saturated with distilled water. The plates were allowed to equilibrate for 24 hr before imposition of pressures for 24 hr to obtain the desired soil matric potentials (ψ_m) −0.33, −1, −5, and −15 bars. A thermocouple psychrometer was used to determine the total water potential (Ψ) of soils from the pressure plates. The respective Ψ values and water contents were −0.9, −2.8, −7, and −15.8 bars and 0.21, 0.19, 0.16, and 0.12 g water per gram of dry soil.

Disks of soil (5.0 cm in diameter and 1 cm in depth) of the desired ψ_m were placed in 9.0-cm plastic plates. Four plates, each with 25 dry sclerotia slightly pressed into the soil, were wrapped in cellophane and incubated at each of three temperatures (12, 18, and 24 C). Eruptive germination was determined after 5 days.

Infection of lettuce by *S. minor* and sclerotia production at various temperatures. Outer leaves of mature (3-mo-old) head lettuce were cut into 50-mm diameter disks weighing about 1.25 g. Each leaf disk in a 5.5-cm diameter petri plate was moistened with water and inoculated with either a sclerotium or a 5.0-mm mycelial disk from a 3- to 5-day-old PDA culture of *S. minor*. Five replicate plates per inoculum type were incubated at the different temperatures. The inoculated tissues were assessed daily for infection and for sclerotial formation after 30 days. After 6 wk of incubation, the sclerotia were harvested, washed, dried for 24 hr, and eruptive germination was determined on moist quartz sand as described above. The experiment was repeated twice.

RESULTS

Effect of temperature and ψ_s on radial growth, sclerotial production and mycelial dry weight. The optimum temperature for radial growth was between 12 and 24 C (Table 1). After incubation for 48 hr, there were no marked differences between 18 and 24 C, but after incubation for an additional 24 hr maximum radial growth of all isolates occurred at 18 C. Although there were significant differences between isolates, growth at the various temperatures was similarly affected. Optimum sclerotial formation was at 12 C, and data for isolates not shown in Table 1 were similar.

Optimum radial growth (58–63 mm after 50 hr) occurred on the basal medium with ψ_s of −12 bars, radial growth on the basal medium with ψ_s of −1 bar averaged 36 mm, and at ψ_s of −100 bars growth was nil (Fig. 1).

TABLE 1. Effect of temperature on radial growth and sclerotia formation on PDA by *Sclerotinia minor*[a]

Temperature (C)	Radial growth (mm) 48 hr SM-1	48 hr CSS-1	48 hr SV-1	72 hr SM-1	72 hr CSS-1	72 hr SV-1	No. of sclerotia SV-1[b]	Percent sclerotial germination[c]
6	16.0	15.0	11.6	19.8	18.0	12.4	0	0
12	21.4	22.8	18.0	34.6	36.4	33.6	1,952	26
18	32.0	37.0	25.4	54.4	60.0	59.4	1,684	29
24	30.2	28.0	25.8	37.6	35.0	37.2	892	27
30	14.6	12.6	12.2	17.6	18.0	18.2	0	0

[a] Radial growth of isolates SM-1, CSS-1, and SV-1 of *Sclerotinia minor* on PDA plates was measured after 48 and 72 hr and number of sclerotia produced was determined after 30 days.
[b] Results for isolates SM-1 and CSS-1 are not presented but were similar.
[c] After 6 wk of aging, sclerotia were harvested, washed, and dried for 24 hr and assessed for eruptive germination on moist quartz sand.

An average of 140 sclerotia were produced on five 2.3-cm-diameter disks cut at random from each of the 14-day-old cultures at −1 bar. Decreasing the ψ_s from −1 to −24 bars increased sclerotial production from 140 to 236. Further decreases in ψ_s resulted in decreased sclerotial production and none were produced at ψ_s ranging from −64 to −100 bars (Fig. 2). Results obtained with all three osmotica were similar. Sclerotia produced over the range of −1 to −43.5 bars did not differ significantly in ability to germinate eruptively when moistened (29–32%).

Optimum growth of all isolates on PDB occurred at 18 C (Fig. 3). The least growth was obtained at 30 C. Although growth rates of the three isolates at the different temperatures were similarly affected, there were significant differences in total growth among the isolates.

Dry weight of mycelium produced on unamended PDB (−2 bars) was 199 mg. Decreasing the ψ_s from −2 to −7 bars resulted in growth increase to a maximum of 341 mg. Further reduction in ψ_s resulted in reduced growth; mycelial growth was 22% of the maximum at −40 bars and was nil at ψ_s values below −73 bars (Fig. 4).

Effect of temperature and soil water potential on germination of sclerotia. Sclerotia of *S. minor* germinated eruptively over a wide range (6–30 C) of temperature with the optimum at 18 C (Fig. 5). Germination of sclerotia from all isolates was similar. The highest temperature (30 C) tested was less favorable for sclerotial germination than was the lowest temperature (6 C). Incubation of sclerotia at 6 C delayed germination in comparison with 12–24 C.

In an unsterilized field soil, for each of the four ψ_m tested, the highest percent germination occurred at 18 C. Likewise, germination at all temperatures was optimum at the highest ψ_m value of −1/3 bar (Table 2).

Infection of lettuce and sclerotia production at different temperatures. The optimum temperature for infection was between 12 and 24 C. At 18 C all inoculated leaf disks were infected, but the extreme temperatures (6 and 30 C) were not favorable for infection, probably due to effects of these temperatures on sclerotial germination (Table 3). Inoculation of similar leaf disks with 5-mm diameter mycelial disks obtained from 3- to 5-day-old cultures of *S. minor* resulted in infection of all disks incubated at 6–24 C, but there was no infection at 30 C. Infection was delayed and the rate of lesion development was slower in leaf disks infected at the lower temperatures. All five inoculated disks were infected after 96, 72, 48, and 48 hr at 6, 12, 18, and 24 C, respectively.

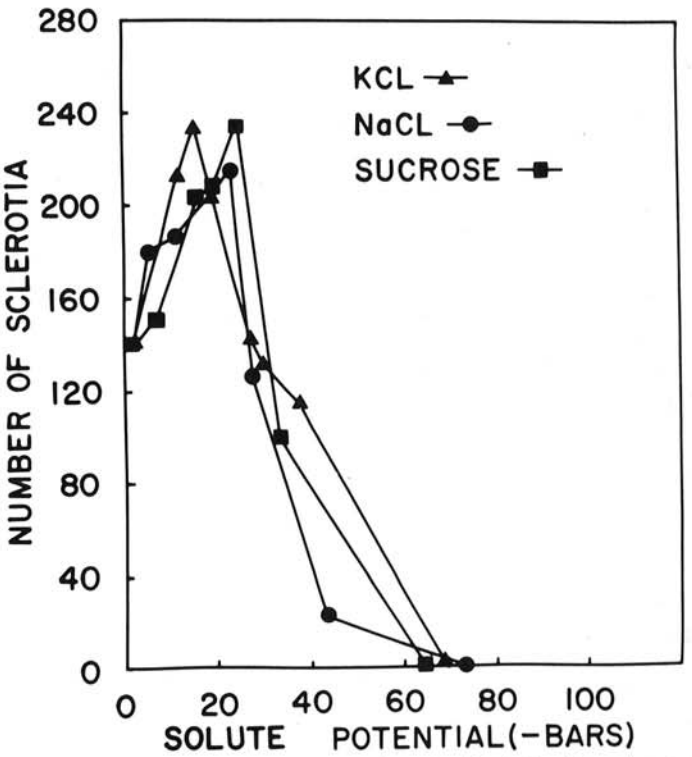

Fig. 2. Effect of solute potential on sclerotia production. Sclerotia produced on CMA adjusted to various levels of ψ_s was determined after 15 days of incubation.

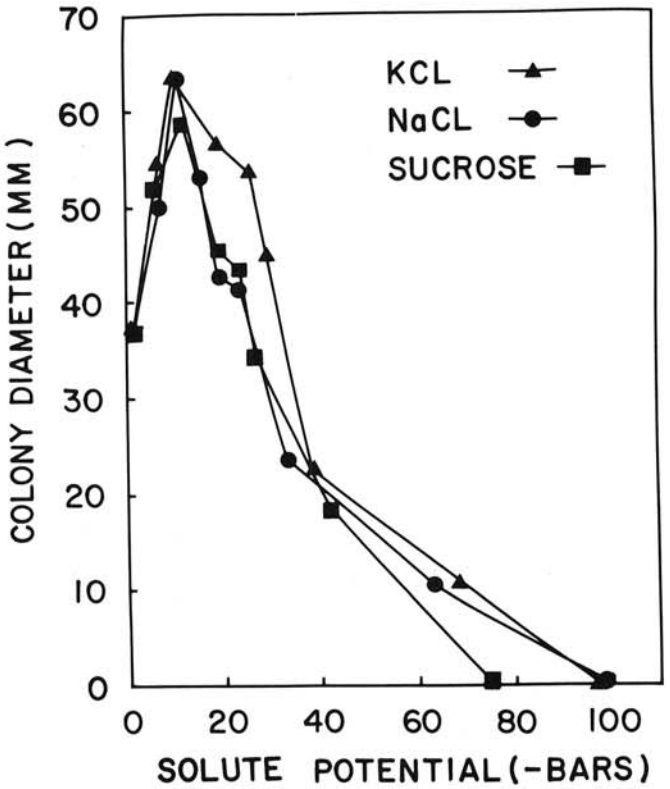

Fig. 1. Effect of solute potential on radial growth of *Sclerotiorum minor*. Radial growth on CMA adjusted to various ψ_s with three osmotica was determined after 50 hr of incubation.

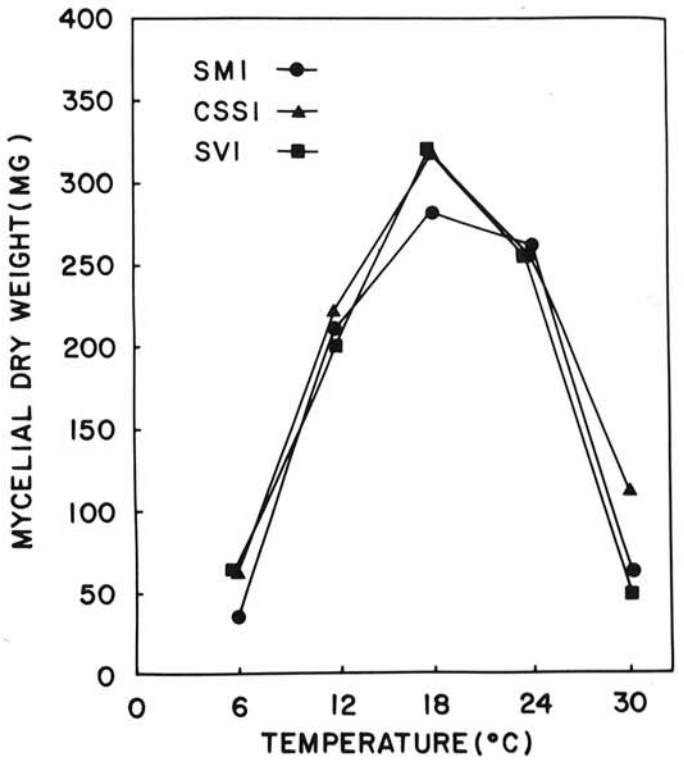

Fig. 3. Effect of temperature on mycelial dry weight production by *Sclerotiorum minor* after 8 days of growth. Mycelial production on PDB was greatest between 12 and 24 C with the optimum at 18 C.

No sclerotia were produced on infected leaf disks at 6 and 30 C (Table 3). Numbers of sclerotia produced on infected disks were fewer than those produced on PDA at the respective temperatures (Table 1). As temperature was increased from 12 to 24 C, fewer (but larger) sclerotia were produced.

DISCUSSION

Lettuce is susceptible to both *S. sclerotiorum* and *S. minor,* but lettuce drop in the Salinas Valley, California is caused almost exclusively by *S. minor* even though *S. sclerotiorum* also occurs there on other crops such as lima beans. The temperature range for near optimum growth of *S. minor* and *S. sclerotiorum* is similar (12–25 C), but carpogenic germination of *S. sclerotiorum* is favored by somewhat lower temperatures (10–15 C). Even so, it seems unlikely that difference in response to temperatures is the reason why *S. minor* usually predominates on lettuce in the Salinas Valley and elsewhere (2,3,5,7,14,27).

TABLE 2. Influence of soil-moisture potential (ψ_m) and temperature on germination of sclerotia of *Sclerotinia minor*

Water potential (−bars)	Germination (%)		
	12 C	18 C	24 C
1/3	69.5	95.5	72.0
1	53.0	77.5	63.0
5	44.9	66.0	54.0
15	38.0	54.0	47.0

Nonsterilized lettuce field soil from Salinas Valley, California, was adjusted to the different soil moisture tensions using pressure plates. Soil disks with the required ψ_m, placed in 9.0-cm diameter plastic plates, were seeded with 25 sclerotia. Four plates wrapped in cellophane were incubated at each of the three temperatures for 5 days and eruptive germination at the various ψ_m-temperature combinations was determined.

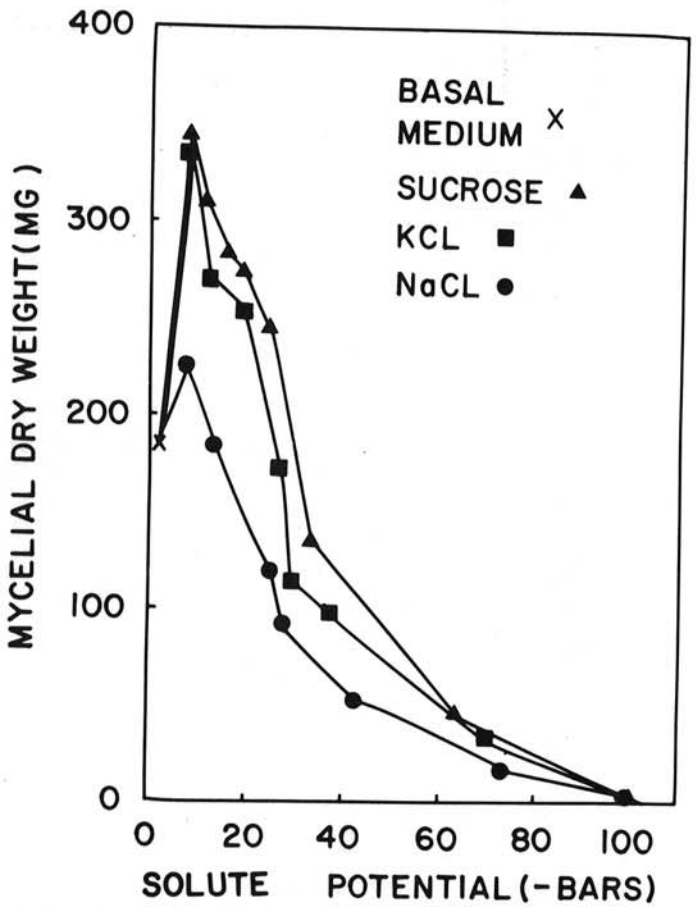

Fig. 4. Effect of ψ_s on mycelial dry weight on PDB adjusted to different ψ_s. Inoculated flasks were incubated on reciprocating shaker at 400 cpm for 8 days.

Many years ago Beach (5) reported that *S. minor* can initiate disease under drier conditions than *S. sclerotiorum* and attributed this difference to the ability of *S. minor* to renew growth in a vegetative manner. He noted that when rainfall occurred after a prolonged drought, *S. minor* developed, but *S. sclerotiorum* did not and he surmised that the wet weather was "either too brief or too late" for development of *S. sclerotiorum*. During the next 2 yr when the rainfall in Pennsylvania was more plentiful and soils were kept uniformly moist, prevalences of the two fungi on lettuce were more nearly equal (5). More recent reports (9,16,18,25) support these observations and show that carpogenic germination of *S. sclerotiorum* occurs only after several weeks of incubation at nearly optimum temperature and low moisture tension (1,10,15). Duniway et al (13) reported, for example, that maximum numbers of apothecia were produced after 17–21 days at −0.24 bar ψ_m for one isolate and between −0.08 and −0.16 bar for another. In contrast, results herein show that sclerotia of *S. minor* can germinate eruptively and probably produce infection at a moisture tension as low as −15 bars. Therefore they may infect at lower levels of soil

TABLE 3. Effect of temperature on infection of lettuce tissue and sclerotia formation by *Sclerotinia minor*[a]

Temperature	Leaf disks infected[b] (no.)	Sclerotia formed[b] (no.)	Sclerotia germination[c] (%)
6	1	0	0
12	4	131	35
18	5	107	32
24	3	83	33
30	0	0	0

[a] Lettuce tissues were inoculated by placing dried sclerotia on tissue disks moistened with sterile water. The inoculated tissues were assessed daily for infection for 7 days, and number of sclerotia formed was assessed after 30 days. Percent germination of sclerotia after 6 wk of aging on the infected tissue was determined by incubation for 5 days at 18 C on moist quartz sand.

[b] Of five leaf disks inoculated and incubated.

[c] Average of three trials with five replicates per treatment.

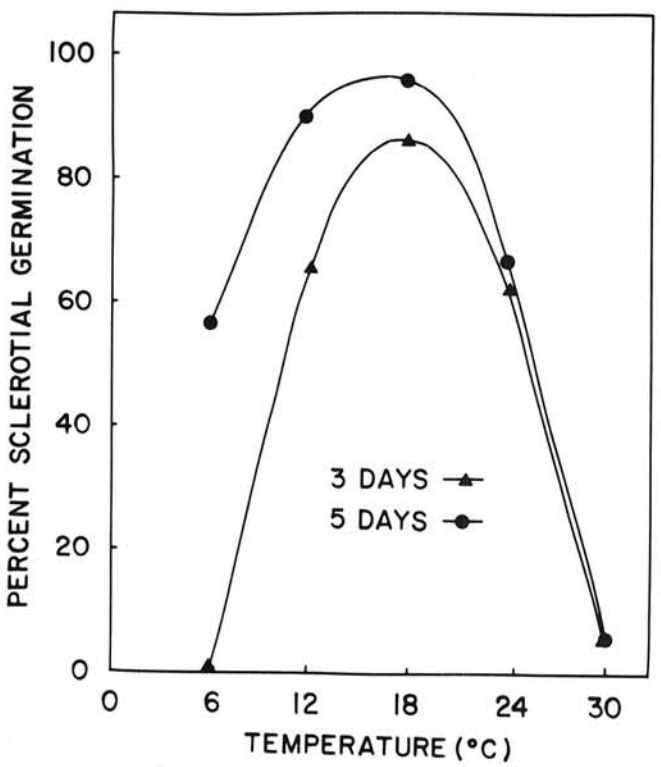

Fig. 5. Effect of temperature on sclerotial germination. Maximum germination was at 18 C regardless of the length of incubation period and the germination at 30 C was nearly nil.

moisture.

Despite repeated and thorough searches, we have never observed apothecial production in lettuce fields in the Salinas Valley. However, apothecia of *S. sclerotiorum* are produced abundantly in lima bean fields of the Salinas Valley after the soil surface is covered by a heavy canopy of foliage. Inasmuch as both lettuce and lima beans are irrigated (rainfall usually is nil during the growing season), the production of apothecia in lima bean fields but not in lettuce fields is attributable to the slower loss of moisture at the soil surface under the heavy foliage of lima beans as compared with a more rapid drying of the soil surface in the relatively open and exposed lettuce field soils.

Although most reports indicate that the primary inoculum of *S. sclerotiorum* is ascospores (1,2,11) and the inoculum of *S. minor* is individual sclerotia that germinate eruptively and produce mycelium instead of apothecia, apparently there are exceptions for both fungi. Hawthorne (16) reported the natural occurrence of apothecia of *S. minor* and Huang and Hoes (20) presented evidence for direct infection of sunflower by mycelium from sclerotia of *S. sclerotiorum*. The exceptions noted above indicate the potential for variability of these fungi and the need for additional comparative studies on their biology and epidemiology.

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