Effect of Water Potential on Survival of Sclerotia of *Sclerotinia minor* in Two California Soils

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We wish to thank B. Aldwinckle, R. McMillen-Sticht, and J. Ogrodnick for preparation of the figures.
Accepted for publication 17 September 1984.

ABSTRACT

Abawi, G. S., Grogan, R. G., and Duniway, J. M. 1985. Effect of water potential on survival of sclerotia of *Sclerotinia minor* in two California soils. Phytopathology 75:217-221.

Survival of sclerotia of *Sclerotinia minor* was studied in two Salinas Valley soils, one with (soil A) and one without (soil B) a previous history of high incidence of lettuce drop. The soil matric water potentials (ψ_m) tested were 0, −0.1, −0.3, −1, −2, −5, −10, and −15 bars. Both soils were also maintained in an air-dried state (−400 bars) or were watered once a week and allowed to dry to give a fluctuating water regime (0 to −430 bars). All sclerotia either had disintegrated or failed to germinate after 8 wk of incubation at $\psi_m = 0$ in both soils. In contrast, no significant ($P = 0.05$) reduction in sclerotial germination occurred in either soil subjected to the fluctuating water regime after 52 wk of incubation, the longest period tested. At all other ψ_m values tested, viability of sclerotia in soil A progressively decreased with time and with increasing ψ_m, and only the fluctuating water treatment contained viable sclerotia after 52 wk of incubation. In soil B, most sclerotia were still viable after 52 wk of incubation at ψ_m values of −0.1 to −15 bars, but only 10% of those in the air-dried treatment were viable. When the ψ_m of soil B was allowed to fluctuate, nearly 100% of the sclerotia remained viable for 52 wk. The two soils differed in gas composition, water content, texture, organic matter, electrical conductivity, cropping history, and microorganisms isolated from sclerotia. However, the data obtained suggest that the differences in the biological activities of the two soils probably account for the differential survival of sclerotia in these soils. *Trichoderma* spp. represented 41 and 1% of total organisms recovered from sclerotia buried in soil A and B, respectively, using a sclerotium agar medium.

Sclerotinia minor Jagger is worldwide in distribution and has an extensive host range (15). In California, *S. minor* is most destructive on lettuce (*Lactuca sativa* L.), causing disease in most lettuce-growing areas of the state, but it occurs most often in the Salinas Valley where about 22,000 ha of lettuce are planted annually. Considerable variation in the incidence of lettuce drop (0 to 30%) occurs among lettuce fields (11); however, the overall incidence average is about 5% (13).

Sclerotia are the main survival structures of *S. minor* and other *Sclerotinia* spp. (4,7). Sclerotia of *S. minor* are capable of carpogenic, hyphal, or eruptive mycelial germination (6). Direct infection of lettuce and the epidemic development of lettuce drop generally results from preconditioned sclerotia that undergo eruptive mycelial germination (2,11). A close correlation between the incidence of lettuce drop and percentage of eruptive mycelial germination was demonstrated under greenhouse (6) and field conditions (11). Thus, the infective propagule for lettuce drop caused by *S. minor* is the sclerotium per se. Detailed information on the longevity of sclerotia in soil is essential to forecast the potential for disease occurrence and to recommend appropriate control measures.

Many factors are known to affect survival and germination of fungal sclerotia in soil (7). Considerable information is also available on the factors that affect germination of sclerotia and the incidence of lettuce drop (2,11). However, information on survival of sclerotia of *S. minor* in soil and factors that affect their longevity are limited. The survival time of sclerotia of *Sclerotinia* spp. is variable but a portion of the soilborne population may survive in soil for 3–8 yr (4). Soil pH, temperature, and moisture were reported to have little direct effect on survival of sclerotia, and the biological component of soil was thought to be more important (4). Imolehin and Grogan (11) concluded that sclerotia survived better in drier soils, but germination decreased progressively with time and depth of burial.

This paper describes effects of soil water potentials on survival of sclerotia of *S. minor* in two Salinas Valley field soils with and without a long history of high incidence of lettuce drop. Summaries of this investigation were reported previously (1,3).

MATERIALS AND METHODS

An isolate (CSS-7) of *S. minor* obtained from a naturally infected lettuce plant near Salinas, CA, was used throughout this study. The fungus was maintained by periodic transfers on potato-dextrose agar (PDA). Sclerotia were produced on autoclaved celery segments in flasks with 5–10 ml of water. After 2 wk of incubation at room temperature, the flask contents were blended for 15 sec and placed in a large glass bottle with a long neck. The bottle was filled with sterile distilled water, mixed thoroughly, and the suspension was decanted immediately leaving the sclerotia on the bottom of the bottle. The process was repeated several times until the sclerotia appeared to be free of plant debris. Sclerotia then were aseptically air-dried, placed in glass jars, and stored at 5 C until used.

Soil from two commercial lettuce fields located near Salinas, CA, were used in this study. Serious outbreaks of lettuce drop have occurred on plants grown in Field A, whereas there is no known history of such outbreaks in Field B, although both fields have been planted to lettuce for many years. A large composite soil sample (~100 L) from each lettuce field was randomly collected, passed through a 6.4-mm screen, and mixed thoroughly. Physical and chemical properties of the two soils were analyzed by the Agricultural Extension Laboratory, University of California, Davis 95616.

Soil matric potentials (ψ_m) were adjusted to −0.1, −0.3, −1, −2, −5, −10, and −15 bars by placing mixtures of soil and sclerotia on pressure-plate extractors (Soil Moisture Equipment Corp., Santa Barbara, CA). About 50 sclerotia (12-wk-old) were added to soil (about 3–4 cm^3) and placed in small sieves. These sieves were made from 1-cm sections of PVC tubing (2 cm in diameter) with a fine nylon mesh sealed to one end by heating. The sclerotia-containing sieves were placed on pressure plates and enough soil was added to

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cover the surface of the plate with about a 1-cm layer. The soil was thoroughly saturated with water and the plates were then exposed to the pressure required to obtain each ψ_m. After about 16 hr of equilibration time, each sieve was removed from the pressure plate and placed in the center of a 5-cm column of soil, with the same ψ_m, in a glass vial (Fig. 1). The vial was closed with a rubber stopper through which two surgical needles were inserted. One needle provided gas exchange on top of the soil column. The other needle was connected to a fine plastic tube that extended to the level of sclerotia and was used for withdrawing gas samples. The opening of the latter needle was closed with silicone rubber (Fig. 1). Other vials were filled similarly with sclerotia and saturated ($\psi_m = 0$ bar) or air-dried ($\psi_m \simeq -400$ bars) soil. In vials with soil at $\psi_m = 0$, a fine glass tube with one end closed with silicone rubber was substituted for the needle and plastic tube to allow sampling of air equilibrated with saturated soils. All vials were placed in a large insulated chest inside a wooden cabinet. A constant flow of humidified air at 23 C was maintained inside the insulated chest. In addition, sieves containing sclerotia and soil were buried in the top 2.5-cm soil layer of 12.5-cm clay pots that were placed on a shaded window sill and watered to saturation once a week. Soil at the level of the sclerotia dried to about −430 bars before watering and the temperature in pots ranged between 17 and 32 C.

The total water potential (ψ) of soil in the vials was determined with a psychrometer (9). The relationship between ψ_m and water content of the soils (Fig. 2) was determined by drying samples from each ψ_m value to a constant weight at 105 C. Viability of sclerotia at all ψ_m tested was determined after 2, 5, 8, 12, 16, and 52 wk of incubation. Gas samples were collected before the vials were opened. The gas samples were taken with 1-ml plastic syringes and O_2, CO_2, and N_2 concentrations were determined with a Carle model 8800 gas chromatograph (Carle, Inc., Fullerton, CA). The ψ of two samples per treatment was then determined by placing soil from near the sclerotia in the psychrometer. Sclerotia were recovered by wet sieving, washed in running tap water for at least 2 hr, surface-sterilized in 0.5% NaOCl for 10 min, and blotted dry. Twenty sclerotia from each replicate were plated (five per plate) on acidified PDA (APDA) (pH = 3.5–4.0). The number of germinating sclerotia was recorded 10 days after plating.

Microorganisms associated with sclerotia that were retrieved after burial were determined by plating washed (a minimum of 2 hr in running tap water) sclerotia on a sclerotium-agar medium. This medium was prepared by steaming 10 g of sclerotia of either *S. minor* or *S. sclerotiorum* in 500 ml of glass-distilled water for 1 hr. The volume was adjusted to 1 L with glass-distilled water, 15 g of Oxoid agar was added, and solutions were autoclaved for 20 min at 121 C. Ground sclerotial tissue was unsatisfactory because it coagulated and stuck to the sides of the flasks upon autoclaving. The use of sclerotia of *S. minor* or *S. sclerotiorum* as the nutrient source in the sclerotium agar medium had no apparent effect on the recovery of organisms associated with sclerotia in soil. Isolations from buried sclerotia were made after 2, 5, and 8 wk of incubation. Twenty sclerotia per soil per ψ_m treatment per replicate ($20 \times 2 \times 10 \times 3$ for a total of 1,200) were plated per sampling date. Isolated fungi and bacteria were maintained by periodic transfers to sclerotial agar, APDA, or nutrient agar.

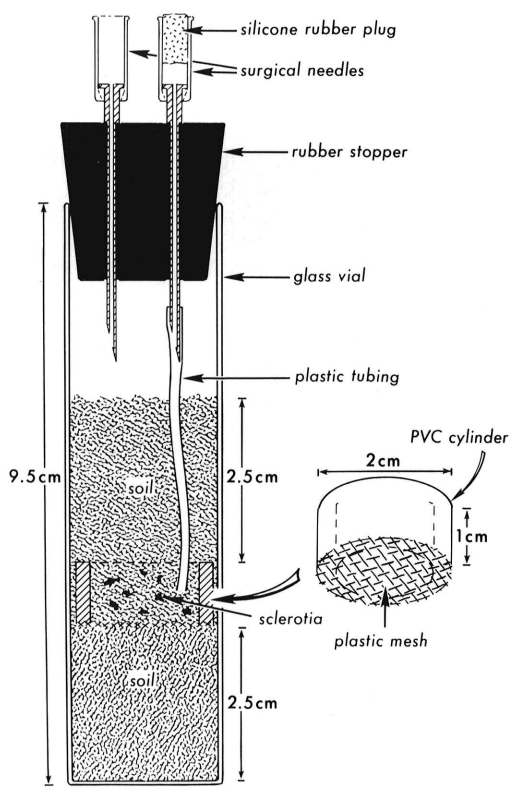

Fig. 1. Diagram of vials used to study survival of sclerotia in soil.

RESULTS

Survival of sclerotia. All sclerotia had disintegraged or failed to undergo hyphal germination after 8 wk of incubation in either soil A or B at $\psi_m = 0$ (Fig. 3). After 5 wk, sclerotial germination at $\psi_m = 0$ was only 18% and 13% in soil A (with) and soil B (without) a history of lettuce drop, respectively. At $\psi_m < 0$, survival of sclerotia (viability measured as hyphal germination) progressively decreased with time in the soil with a history of lettuce drop (soil A), and survival also decreased as soil ψ_m was increased (Fig. 3). Only sclerotia incubated in soil with the fluctuating moisture regime (saturation to air-dry, $\psi_m = 0$ to −430 bars) survived (88% hyphal germination) for 1 yr. In contrast, most of the sclerotia incubated in the soil with no history of lettuce drop (soil B) were still viable after 1 yr at all ψ_m values <0. However, hyphal germination of sclerotia from the $\psi_m = -400$ bars (air-dry) treatment was only 10%. Analysis of variance of the data (Table 1) showed that all interactions ($\psi_m \times$ soil, $\psi_m \times$ time, soil $\times$ time, and $\psi_m \times$ soil $\times$ time) were significant at $P = 0.01$.

Properties of the two soils. Soils A and B differed in physical and chemical characteristics (Table 1; Fig. 2). Soil A had a higher clay and lower sand content than soil B and they are classified as Salinas clay silt loam and silt loam, respectively. In addition, soil A had a higher water content at all ψ_m values used. In contrast, soil B had a much higher electrical conductivity, a greater percentage of exchangeable sodium, and a higher content of calcium, magnesium, sodium, and chloride ions. However, organic matter content of soil A was higher than in soil B. At the time soils were

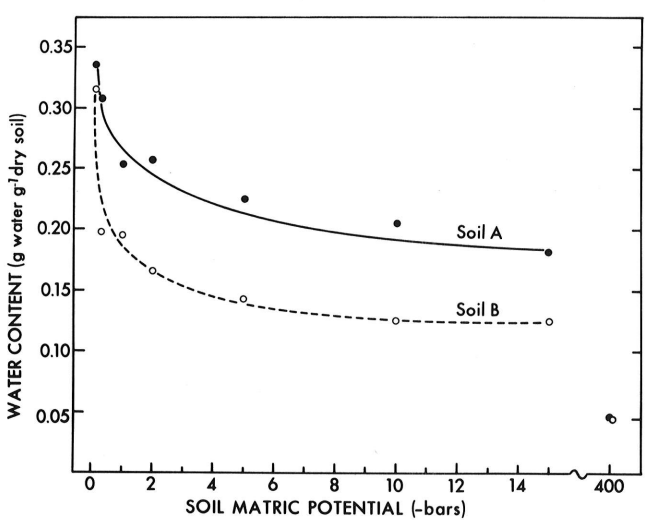

Fig. 2. Water contents of the two soils used to study survival of sclerotia plotted as functions of decreasing matric potential.

removed from pressure plates, ψ values were 0.7 to 2.5 bars lower than the ψ_m values in soil A; but in soil B, ψ values were 4.4 to 6.8 bars lower than ψ_m values in the −0.1 to −5.0 bar range and 10.6 to 15.6 bars lower than the ψ_m values of −10 and −15 bars. The greater differences between ψ_m and ψ in soil B are probably a reflection of its greater salt content (Table 2). During 52 wk of incubation, the ψ of soils initially at ψ_m values of −0.1 to −5.0 bars gradually decreased by 0.1–2.5 bars while the ψ of soils initially at ψ_m values of −10 and −15 bars gradually increased by 2.4–5.7 bars. Air-dried soil remained at $\psi \leqslant -400$ bars, and soil in pots dried to ψ values of about −4, −100, and −430 bars in 3, 5, and 7 days after watering, respectively.

Content of CO_2 varied considerably between sampling dates and treatments; however, the percentage CO_2 was always higher in soil A than soil B at ψ_m from −0.1 to −15.0 bars. For example, the content of CO_2 for soils A and B ranged from 1.5 to 3.2% and 0.5 to 1.9%, respectively, at 8 wk after incubation in the treatments at ψ_m values of −0.1 to −10 bars. Percentages CO_2 after 12 wk of incubation in soil A at ψ_m of −0.1, −0.3, −1, and −10 bars were 1.9, 1.7, 1.6, and 1.1, respectively. The corresponding CO_2 contents for soil B were 1.6, 1.0, 1.1, and 0.9. The percentage O_2 was essentially the same for both soils and varied between 19.5 and 20.5%.

Organisms associated with incubated sclerotia of *S. minor*. The frequency and kind of organisms isolated from washed sclerotia that had been retrieved and plated on the sclerotial agar did not differ within soils among the sampling dates or the different ψ_m treatments except for the $\psi_m = 0$ treatment. At the 5- and 8-wk sampling, mostly bacteria were isolated from sclerotia buried in both soils at $\psi_m = 0$. At $\psi_m < 0$, however, the frequency of isolation of antagonistic organisms differed greatly between the soils. Thus, the data for various ψ_m values and times of burial were pooled and presented as a comparison between soils A and B (Table 3). Fungal or bacterial isolates were obtained on the sclerotial agar from 54 and 48% of washed nonsterilized sclerotia that had been incubated previously in soils A and B, respectively. The kind and frequency of organisms isolated are summarized in Table 3. Isolates of *Trichoderma* spp. accounted for 41 and 1% of the total organisms associated with sclerotia that were incubated in soils A and B, respectively. Sclerotia that appeared to be colonized by *Trichoderma* spp. were retrieved from the sclerotial agar, surface-sterilized for 10 min in 0.5% NaOCl, and replated on APDA. *Trichoderma* spp. were isolated from 50% of these sclerotia, which suggested that the inner tissues of the sclerotia had been colonized by *Trichoderma* spp. Isolates of *Fusarium* spp. and *Cylindrocarpon* spp. were recovered more frequently from

TABLE 1. Analysis of variance of the data on the survival of sclerotia of *Sclerotinia minor*[a]

Source of variation	df	MS	Observed frequency[b]	Percent variation
Soil water potential (ψ_m)	9	679.3	105.9**	19.3
Soil (soils A and B)	1	9,413.5	1,467.1**	29.8
Time (incubation)	5	1,352.7	210.8**	21.4
$\psi_m \times$ soil	9	350.1	54.6**	10.0
$\psi_m \times$ time	45	62.8	9.8**	8.9
Soil × time	5	113.0	17.6**	1.8
$\psi_m \times$ soil × time	45	12.3	1.9**	1.7
Residual	359	6.4		7.3

[a] These data are presented in Fig. 3.
[b] ** = statistically significant, $P = 0.01$.

TABLE 2. Selected physical and chemical properties of the two Salinas Valley lettuce soils used to study the survival of sclerotia of *Sclerotinia minor*

Property	Soil A[a]	Soil B[a]
pH	7.1	7.0
Organic matter (%)	5.2	3.7
Electrical conductivity (millimhos/cm)	2.3	7.1
Exchangeable sodium (%)	2.0	6.0
Zn, mgm/kg soil	6.8	6.0
Cu, mgm/kg soil	0.8	0.4
Fe, mgm/kg soil	17.0	12.0
Ca, me/L	12.6	42.6
Mg, me/L	5.2	30.2
Na, me/L	7.2	29.0
Cl, me/L	3.4	26.5
Particle size analysis (%):		
Clay	37	30
Silt	49	45
Sand	14	25
Classification	clay silt loam	silt loam

[a] Severe outbreaks of lettuce drop are known to have occurred on plants grown in soil A but not soil B.

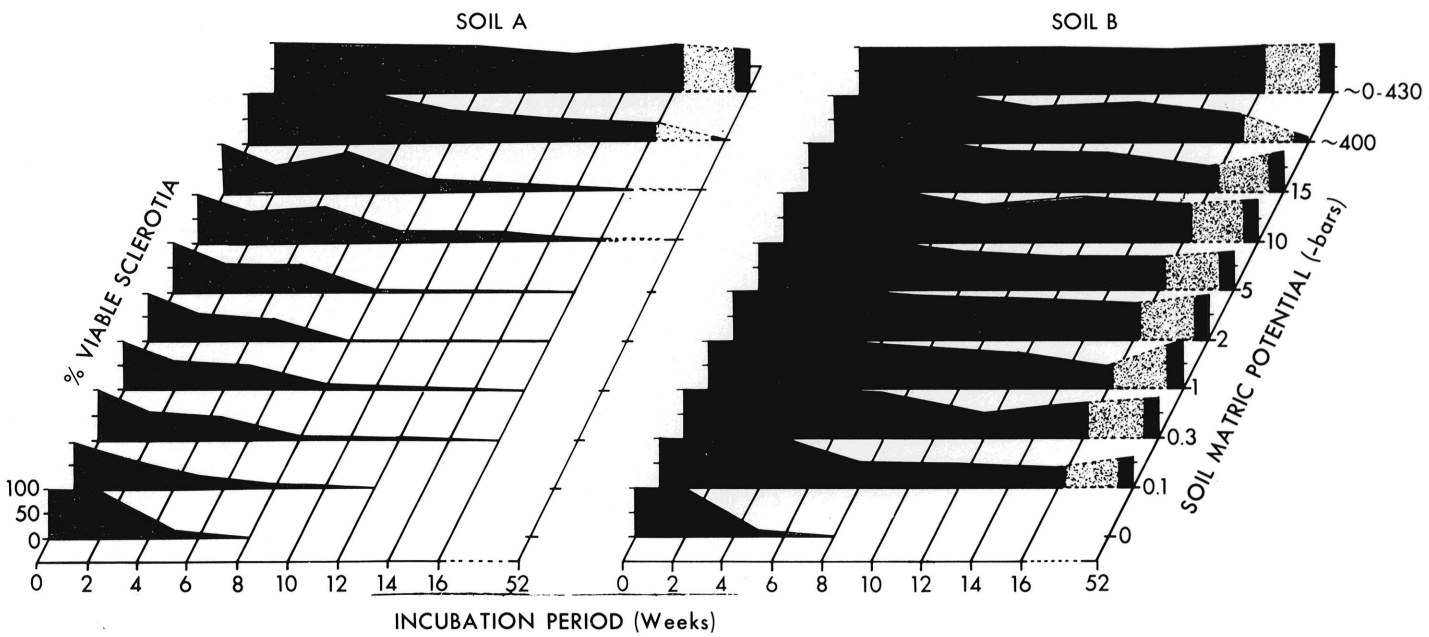

Fig. 3. Survival of sclerotia of *Sclerotinia minor* at different matric potentials (ψ_m) under controlled conditions in a soil where severe outbreaks of lettuce drop were known to occur (soil A) and a soil without known history of such outbreaks (soil B).

sclerotia buried in soil B than those buried in soil A. About 31% of the bacterial isolates obtained from sclerotia buried in either soil were fluorescent *Pseudomonas* spp., 41% were oxidase positive, and 12% were capable of rotting thin potato slices.

In contrast, additional isolations made from surface-sterilized sclerotia previously incubated in soil B failed to yield any fungal growth on APDA. However, from comparable sclerotia incubated in soil A, 234 isolates of *Trichoderma* spp. (including *T. harzianum*), one of *Penicillium* sp., and one of *Fusarium* sp. were recovered for a total isolation rate of about 20%.

DISCUSSION

Our results show clearly that survival of sclerotia of *S. minor* is greatly influenced by the type and water status of the soil in which the sclerotia are incubated. Other factors such as depth of burial, soil temperature, soil gases, and antagonistic and mycoparasitic organisms also are known to affect survival of sclerotia of *Sclerotinia* spp. (4,7,11,16). Longevity of sclerotia of *Sclerotinia* spp. in soil has been reported to be as short as a few weeks to as long as 8 yr or more (4,7,14). This variability is not surprising because the studies were conducted in different soils and under a wide range of environmental conditions. It may be that accurate and biologically meaningful data on survival of sclerotia of *Sclerotinia* spp. can best be obtained in situ and under the prevailing soil conditions and production practices.

Our data showed that sclerotia of *S. minor* survived less than 8 wk at soil ψ_m of 0. This finding agrees with an earlier report of Moore (14) from Florida showing that the longevity of sclerotia of *S. sclerotiorum* under flooded conditions ranged between 23 and 45 days. As a result, flooding has been used in Florida for control of diseases caused by *Sclerotinia* on a number of crops (14,18). This practice is possible and practical when water for flooding can be applied cheaply and is more effective when flooding is done during the warm summer season. Observations of heavily infested fields suggest that flooding during cool periods, as during the winter months in New York State, is not effective (G. S. Abawi, *unpublished*).

Survival time of sclerotia buried in the disease-conducive soil (soil A) was negatively correlated with soil ψ_m at values <0 bar. Several other studies (4,8,11) have shown that germination and production of sclerotia, as well as mycelial growth of both *S. minor* and *S. sclerotiorum*, occurred at low water potentials (10,12,19). The most interesting finding of our data concerning ψ_m, however, is that sclerotia in a fluctuating water regime survived best. The soil ψ in this treatment varied weekly between 0 and -430 bars, yet sclerotia were viable after 1 yr of incubation, the longest period tested. This result is compatible with other epidemiological information showing that sclerotia in the top layers of soil, where water content may fluctuate greatly, are the principal inoculum for lettuce drop and white mold of beans incited by *S. sclerotiorum*. It has been shown, for example, that infection of lettuce occurs most abundantly in the top 5-cm of soil from eruptively germinating sclerotia (11,13). Likewise, ascosporic inoculum for infection of bean blossoms is produced by conditioned sclerotia located within the top 5 cm of soil (2). However, our results showing longer survival under fluctuating moisture conditions contradict those by Smith (17). He reported that drying and wetting of sclerotia resulted in enhanced decomposition due to increased leaching of nutrients which increased microbial activities of antagonists and/or mycoparasites.

Surprisingly, viability of sclerotia of *S. minor* buried in the soil without a known history of lettuce drop (soil B) remained high and was significantly greater than the viability of sclerotia buried in the soil with a long history of lettuce drop (soil A). This difference occurred in all ψ_m treatments except those at $\psi_m = 0$ and 0 to -430 bars (Fig. 3). Sclerotia recovered from soil B were firm and had essentially no fungal parasite growth after surface sterilization. In contrast, sclerotia recovered from soil A became progressively softer and progressed through stages of decomposition as the incubation period increased. *Trichoderma* spp. were predominantly and frequently isolated from washed as well as surface-sterilized sclerotia buried in soil A but not from those buried in soil B. Although other factors may be important, our data suggest that the differential survival of sclerotia in the two soils is probably due to differences in the biological activities of the two soils. Evolution of CO_2 was higher from soil A than from soil B at all sampling dates and ψ_m values. *Trichoderma* spp. were the most commonly associated with sclerotia buried in soil A and the higher salt content of soil B may have reduced their activities and deleterious effects on sclerotia of *S. minor*. Also, the activities of organisms inhibitory to the *Trichoderma* spp. may have been enhanced in soil B. Germination of sclerotia of *S. minor* may have been inhibited by one or more of the ions present at high levels in soil B. If so, infection of lettuce would be prevented and sclerotial inoculum would not be replenished by each lettuce crop. As a result, sclerotial inoculum would eventually decrease in soil B even though the time of sclerotial survival was greater.

Imolehin and Grogan (11) studied survival of sclerotia of *S. minor* at different depths in the same two commercial lettuce fields from which the soils used in this study were obtained. They also found similar differences in time of survival, and more frequent association with *Trichoderma* spp., of sclerotia buried in soil A than those buried in soil B. Many antagonists and mycoparasites have been shown to be deleterious to sclerotia of *Sclerotinia* spp. but largely under controlled conditions (4,18). Considerable field testing has been conducted using *Coniothyrium minitans* Campbell and more recently with *Sporidesmium sclerotivorum* Uecker, Ayers, and Adams (5) as parasites of sclerotia, but neither of these mycoparasites has been used commercially.

All sclerotia used in this study were produced under laboratory conditions on autoclaved celery stem segments. Imolehin and Grogan (11) found no difference in the survival of laboratory-produced sclerotia and those produced on naturally infected plants. In our studies, survival of sclerotia was demonstrated by their recovery from soil and their hyphal germination on APDA. Although the ability of sclerotia to undergo hyphal germination may not predict their infective potential as inoculum, it does indicate viability. Sclerotia that are capable of hyphal germination require exogenous energy sources for infection of lettuce, whereas those capable of eruptive mycelial germination can infect directly (2,4,6,12).

TABLE 3. Identity and frequency of organisms isolated from sclerotia buried in soils A and B[a]

Organism	Organisms isolated (% of total)[b]	
	Soil A	Soil B
Trichoderma spp.	41	1
Penicillium spp.	2	8
Fusarium spp.	6	19
Cylindrocarpon spp.	7	21
Gliocladium spp.	2	0
Bacterial isolates[c]	41	49
Others[d]	1	2

[a] Field soils with (soil A) and without (soil B), respectively, a previous history of high incidence of lettuce drop. Sclerotia were retrieved from soil by sieving, washed in running tap water for a minimum of 2 hr, and plated on the sclerotial agar. The data shown represent averages of all isolations made from the various soil treatment at $\psi_m < 0$ after 2, 5, and 8 wk of burial.

[b] The total number of organisms isolated from 1,800 sclerotia buried in each of soil A and B was 972 and 860, respectively.

[c] 31% of these isolates were fluorescent *Pseudomonas* spp.

[d] Includes isolates of *Cephalosporium* spp., *Aspergillus* spp., *Pythium* spp., and *Rhizopus* spp.

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Etiology

Pathogenicity and Relative Virulence of Seven *Phytophthora* spp. on Mahaleb and Mazzard Cherry

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Based on a portion of a dissertation submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, University of California, Davis.

Accepted for publication 21 June 1984.

ABSTRACT

Wilcox, W. F., and Mircetich, S. M. 1985. Pathogenicity and relative virulence of seven *Phytophthora* spp. on Mahaleb and Mazzard cherry. Phytopathology 75:221-226.

Isolates of *Phytophthora cryptogea*, *P. cambivora*, and *P. megasperma* from cherry, isolates of *P. cinnamomi* and *P. citricola* from walnut, and an isolate of *P. cryptogea* from safflower individually caused 88–100% root rot and 27–100% crown rot on Mahaleb cherry seedlings that were grown for 15 wk in artificially infested UC mix and periodically flooded. In contrast, cherry isolates of *P. drechsleri* and an unidentified *Phytophthora* sp. caused 62 and 41% root rot, respectively, but caused no crown rot under the same conditions. Mazzard cherry seedlings frequently appeared less susceptible than Mahaleb seedlings to root and crown rot, although this varied with the *Phytophthora* species involved. Mazzard appeared to be significantly more resistant than Mahaleb to both root rot and crown rot caused by *P. cambivora*, *P. megasperma*, and the safflower isolate of *P. cryptogea*, and to crown rot caused by *P. cinnamomi* and *P. citricola*. However, Mazzard roots appeared nearly as susceptible as Mahaleb roots to the latter two *Phytophthora* spp., and roots of both cherry species appeared moderately susceptible to *P. drechsleri* and the unidentified *Phytophthora* sp. Roots and crowns of Mazzard also appeared as highly susceptible as Mahaleb to the cherry isolate of *P. cryptogea*. This is believed to be the first report experimentally implicating *P. cryptogea* as a pathogen of a commercial stone fruit tree species in the United States.

Additional key words: *Prunus avium*, *Prunus mahaleb*, soilborne diseases, wet feet.

Phytophthora root and crown rots reached epidemic proportions in California sweet cherry (*Prunus avium* L.) orchards during the early 1970s, and by 1976, *P. megasperma*, *P. cambivora*, and *P. drechsleri* had been identified as causal agents of these diseases (11). Subsequent isolations from numerous sweet cherry trees showing typical root or crown rot symptoms (11) have yielded additional unidentified *Phytophthora* spp. (8,9), although the pathogenicity of these species has not been previously demonstrated. A number of *Phytophthora* spp. have also been isolated from dead and declining peach, apricot, prune, apple, walnut, and almond trees throughout the deciduous fruit and nut tree-growing districts of California (8,9,11,13,14). These isolations have raised concern that such orchards might serve as a source of

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