

# Eradication of Primary Inoculum of *Botrytis cinerea* by Soil Solarization

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## ABSTRACT

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The primary inoculum source of *Botrytis cinerea* can be eradicated by soil solarization, thus retarding the development of gray mold in many horticultural crops. The effect of soil solarization on the loss of viability of inoculum of *B. cinerea* buried in sand-mulched soil at different depths was studied in two different years. In 1989, solarization during the last week of July eradicated the pathogen. The maximum average temperatures for the period were 36.4, 40.3, and 46.8 C at 25-, 15-, and 5-cm depths, respectively. Soil solarization during the first week of July 1990 resulted in maximum temperature increments of +7.4, +4.3, and +9.4 C, depending on depth. Complete loss of viability of mycelium at depths of 5–25 cm, as well as of sclerotia buried at 5 cm, was observed after 2 days in solarized plots. Viability of sclerotia at 15- and 25-cm depth decreased linearly with temperature–time (degree–hour). Estimations of solarization periods required for eradication of sclerotia of *B. cinerea*, under the thermal regime of the first week of July 1990, were 6 and 10 days at 15- and 25-cm depths, respectively, for the conditions stated above.

Additional keywords: vegetable crops

*Botrytis cinerea* Pers.:Fr., a pathogen of many horticultural crops, can survive in soil either as mycelium or as sclerotia (2). The latter are the fungal structures less affected by unfavorable environment (8), their survival being longer under dry conditions and low temperatures. Mycelium is reported to survive in vitro for more than 1 yr at 0 C. Survival is affected by temperature and humidity, their effects varying with different isolates (15).

In vegetable crop production of the Southern Mediterranean Coastal Plain of Spain, sand-mulching is the usual practice to increase soil temperature and plant growth. Natural soil is amended with manure and overlaid with a 15-cm layer of sand where planting is done. Greenhouses are permanent structures with plastic covers that are replaced every 3–5 yr and are ventilated by means of lateral openings. These greenhouses are usually close to each other (3–8 m) within a given area.

Cultural practices and harvesting cause disturbances to the soil layer that facilitate dispersal of the pathogen. Crop debris (either inside or in the proximity of the plastic greenhouses where plant residue piles are usually kept for long periods) is the main inoculum source for the development of gray mold in most crops grown in that important horticultural area (*personal observation*). Sanitation practices outside the plastic greenhouses are one of the targets in con-

trolling gray mold. Another target is the eradication of inoculum sources of *B. cinerea* overwintering inside the plastic greenhouse.

The practice of soil solarization in these plastic greenhouses is being increasingly adopted to achieve control of soil-borne plant pathogens while reducing the use of fumigants (5). Epidemic development of gray mold would probably be retarded after a drastic reduction of infested crop residues in the greenhouses, similar to reports on leaf blight of onion (3). Conventional methods for controlling gray mold have been disappointing because of the tolerance to fungicides in *B. cinerea* (1) and the persistence of inoculum in the soil. Previous in vitro studies indicated the loss of viability of sclerotia of *B. cinerea* after incubation in water at 45 C for 1 hr (10). It was thought that soil solarization would be an effective control tactic, because it is an efficient method for the control of many soilborne plant pathogens (9). The present study evaluated soil solarization for controlling the primary inoculum of *B. cinerea* in plastic greenhouses in southern Spain.

## MATERIALS AND METHODS

Two isolates of *B. cinerea* (BC-132 and BC-134) that produced abundant sclerotia were selected from 28 isolates obtained from different vegetable crops affected by gray mold in the Mediterranean Coastal Plain. Mycelia and sclerotia of these two isolates were produced on acidified (pH 4.8) potato-dextrose agar (PDA) plates incubated at 24 C in the dark. Sets of 10 mycelial disks (8 mm diameter) were cut from the edge of the

colonies after a 7-day incubation period and mixed with 20-g aliquots of golden beach sand (siliceous particles 0.1–0.4 mm) in nylon bags. Similarly, sets of 10 small sclerotia of *B. cinerea* from 30-day-old cultures with a total weight 0.24–0.32 g were mixed in another bag containing 20 g of sand.

Bags with mycelial disks and those with sclerotia were buried at 5-, 15-, and 25-cm depths in a sand-mulched soil (15-cm layer of sand on top of a sandy-loam colluvial soil) typical of the plastic houses in the area in two 2 × 2 m plots located 1 m apart. One of these was irrigated and maintained at field capacity and thereafter mulched with a transparent polyethylene film 75 μm thick to achieve solarization; the other unmulched, non-irrigated plot served as a control. There were four replicated bags for each combination of isolate, inoculum type, depth, and time of sampling. A thermistor was placed at each of the given depths. These were connected to a datalogger (Campbell 21 X, Campbell Scientific, Ltd., Loughborough, UK) set to record temperatures every 5 min. The temperatures were averaged hourly during the entire period of solarization to get hourly temperatures ( $T_h$ ). In 1989, five samplings were made at weekly intervals starting 24 July; in 1990, daily samplings were performed during the first week after soil tarping starting on 2 July. Temperature analyses consisted of calculating temperature–time accumulated from the beginning of solarization until sampling using 11 minimum temperature thresholds from 30 to 40 C. These were calculated by the equation  $DH = \sum_i (T_h - T_i)_i$  where  $DH$  = temperature–time;  $T_h$  = hourly temperature;  $T_i$  = minimum threshold temperature; and  $i$  = each hour of the period considered in which  $T_h > T_i$ .

Mycelial disks and sclerotia were separated from soil on a 0.5-mm-mesh screen, rinsed with tap water to remove soil particles, and washed in sterile water. Afterwards, sclerotia were surface sterilized by dipping for 3 min in a solution of 1% NaClO, followed by rinsing in sterile water. After drying on a laminar flow bench for 24 hr at room temperature, sclerotia and mycelial disks were plated on acidified (pH 4.8) PDA and incubated at 24 C in the dark. Viability, as measured by hyphal growth into the agar, was determined over a period of 6 days.

A total of 53 isolates of *B. cinerea* from viable sclerotia recovered from sequential soil samplings at different depths

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was randomly selected for pathogenicity tests. Inoculations were done by the deposition of one mycelial disk from each of the isolates on the hypocotyl of 10-day-old seedlings of *Phaseolus vulgaris* L. cv. Garrafal oro (10). Three plants were inoculated with each isolate and incubated in a growth chamber at 24 C with a 12 hr/day photoperiod of fluorescent light at  $45 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for 7 days. Thereafter, hypocotyl lesions were rated according to a severity scale of 0–3 (0 = no symptoms, 1 = restricted lesion <5 mm, 2 = lesion 5–10 mm, 3 = extensive lesion >10 mm).

## RESULTS

In 1989, mean hourly temperature in the control plot did not exceed 33.2 C, except for the 5-cm soil depth, where it varied between 25.1 and 44.6 C; whereas in the solarized plot the ranges were 26.3–46.8 C, 29.5–40.3 C, and 30.8–36.4 C at 5, 15, and 25 cm, respectively (Fig. 1A). The differences of average maximum temperature between solarized and control plots were +1.8, +7.1, and +6.5 C at the three depths, respectively. In 1990, the mean hourly temperature in the control plot did not exceed 30.2 C at 15 and 25 cm and varied from 25.4 to 41.3 C at 5 cm; whereas in the solarized plot they were 26.8–45.6 C, 29.2–39.6 C, and 30.6–35.5 C at 5, 15, and 25 cm, respectively (Fig. 1B). In the solarized plot, average maximum temperatures were 4.3, 9.4, and 7.4 C higher than their respective in the control plot at 5, 15, and 25 cm.

At the first sampling in 1989, 7 days after the treatment began, inocula of the two isolates of *B. cinerea* had completely lost viability in the solarized plot. In contrast, fungal viability in the control plot decreased to 46, 94, and 59% for mycelial disks and to 95, 96, and 73% for sclerotia at 5, 15, and 25 cm, respectively. In 1990, the viability of mycelial disks of *B. cinerea* at all depths and that of sclerotia at 5 cm were lost by the second day after solarization began. Table 1 summarizes the effects of soil solarization and depth of burial on the viability of sclerotia. Since there were no significant differences among the two isolates of *B. cinerea* used, pooled values are given. Sclerotia from control plots had significantly higher viability than those recovered from solarized soil for any of the sampling dates. Viability of sclerotia buried in solarized soil at 25 cm was significantly higher than that of sclerotia kept at 15 cm for every sampling time considered. Time of sampling also significantly influenced the viability of sclerotia buried in the solarized soil; without regard to isolate and depth, sampling at 3 days gave sclerotia with viability significantly lower than that of earlier samplings but similar to that of samples at 6 days. In contrast, there were no significant differences in viability of sclerotia buried in control soil (Table 1).

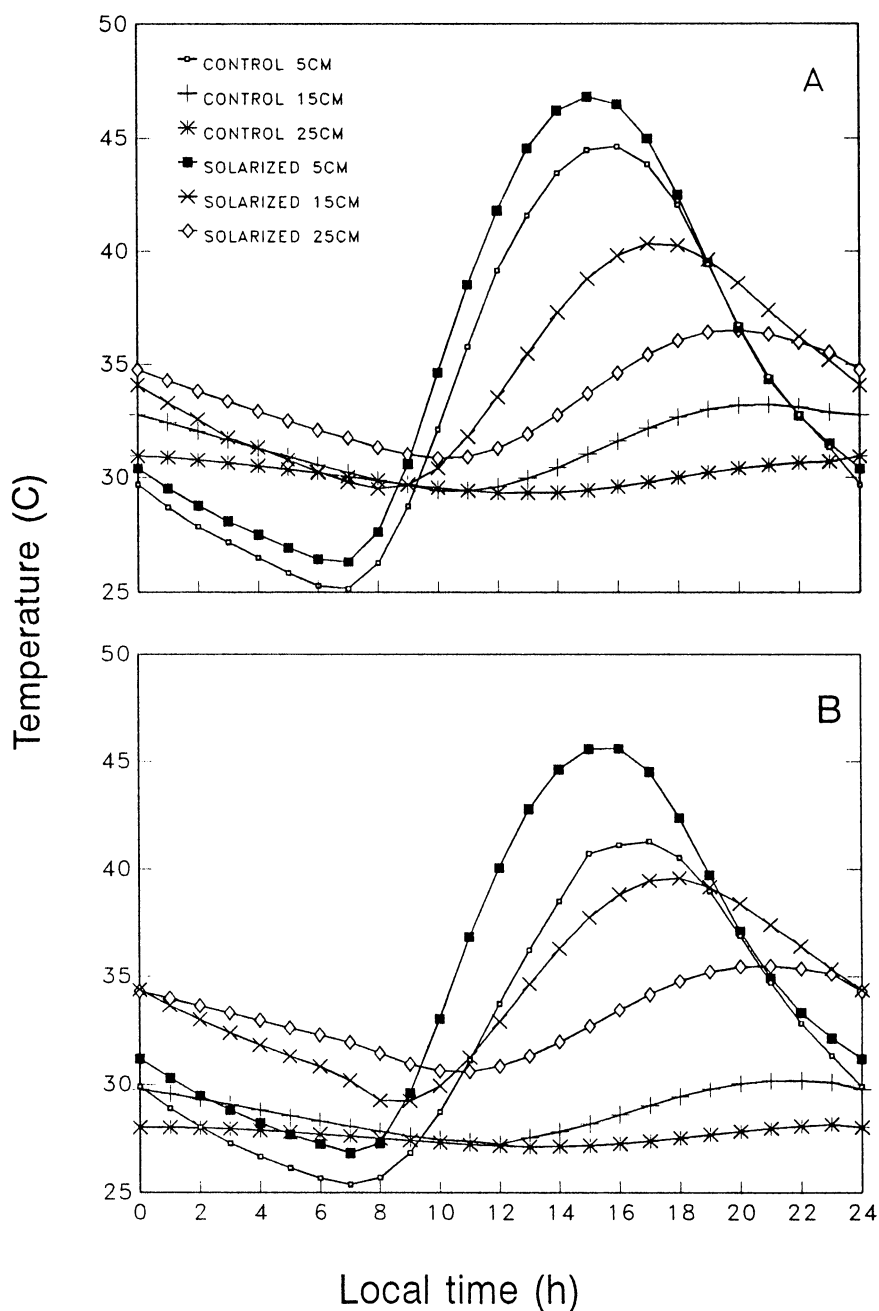
Viability of sclerotia of *B. cinerea* decreased linearly with temperature–time (accumulated degree–hours) calculated with minimum temperature thresholds of 38 and 34 C for 15 and 25 cm, respectively. The most significant regression equations are given in Table 2. Accordingly, complete loss of viability of the sclerotia of *B. cinerea* buried at 15 cm was obtained after the accumulation of 34.5 degree–hours (threshold temperature = 38 C); whereas for sclerotia at 25 cm depth, an accumulation of 53.6 degree–hours (threshold temperature = 34 C) was required. Under the thermal regime of the first week of July 1990, 6 and 10 days of solarization were needed to reach lethal temperature–time accumulations at 15 and 25 cm, respectively.

**Table 1.** Effects of soil treatment and depth of burial on the viability of sclerotia of *Botrytis cinerea*<sup>y</sup>

Soil treatment	Depth (cm)	Viability <sup>z</sup> of sclerotia (%)	
		3 Days	6 Days
Control	5	97.5 a	88.8 a
	15	96.3 a	92.5 a
	25	100.0 a	96.3 a
Solarization	5	0.0 d	0.0 c
	15	27.5 c	16.7 c
	25	55.5 b	36.6 b

<sup>y</sup> Two isolates of *B. cinerea* (BC-132 and BC-134) were used; since there were no significant differences between them, pooled values of viability are given.

<sup>z</sup> Values in each column followed by the same letter are not significantly different according to Student's *t* test at *P* = 0.05.



**Fig. 1.** Mean hourly temperatures of soils at different depths either solarized or nonsolarized (A) 24–31 July 1989 and (B) 2–9 July 1990.

**Table 2.** Equations of regression on the viability of sclerotia of two isolates of *Botrytis cinerea* on accumulated temperature-time (degree-hours) calculated with a given threshold temperature

Isolate	Depth (cm)	Threshold temperature (C)	Regression equation	P
132	15	38	$V^y = 92.20 - 2.67 (DH^x)$	0.0008
134	15	38	$V = 94.96 - 2.84 (DH)$	0.0004
132	25	34	$V = 84.53 - 1.10 (DH)$	0.0313
134	25	34	$V = 98.03 - 1.83 (DH)$	0.0021

<sup>y</sup> V = viability (%).

<sup>x</sup> DH = temperature-time (degree-hour).

**Table 3.** Effects of solarization and burial depth of *Botrytis cinerea* sclerotia on their virulence to *Phaseolus vulgaris* cv. Garrafal oro

Soil treatment	Depth (cm)	Mean disease severity <sup>z</sup>
Control	15	1.38 a
	25	1.63 a
Solarization	15	0.63 b
	25	1.19 b

<sup>z</sup> Each value is the average of eight isolates of *B. cinerea* obtained from sclerotia of BC-132 and BC-134 recovered at four different sampling times. Three seedling replicates per isolate were evaluated according to a severity scale of 0-3, where 0 = no symptoms, 1 = restricted lesion <5 mm, 2 = lesion 5-10 mm, and 3 = extensive lesion >10 mm. Values followed by the same letter are not significantly different (Student's *t* test at *P* = 0.05).

Pathogenicity of the isolates of *B. cinerea* from sclerotia of BC-132 and BC-134 sampled at different times from the solarized soil at different depths was lowest (53% of inoculations were successful) for sclerotia buried at 15 cm. Severity of disease reactions of seedlings inoculated with these isolates was statistically analyzed. Since there was a reduced number of viable sclerotia from 5-cm depth in the solarized plot and from some of the times of sampling, only 15- and 25-cm depths and 2, 3, 4, and 6 days of solarization were considered in the analysis. There were no significant differences among times of sampling. Therefore, pooled values of severity were summarized in Table 3. Severity of disease reaction was significantly higher for isolates from sclerotia buried in control plots. Although there were no significant differences between depths of burial for any of the soil treatments used, isolates from sclerotia buried at 15 cm in solarized soil gave mean disease severities lower than those from sclerotia solarized at 25 cm.

## DISCUSSION

Primary inoculum of *B. cinerea* occurs inside the plastic greenhouses as sclerotia and mycelium in plant debris that remains in the soil. Its short-distance dispersal has been reported (6,11); therefore inoculum eradication is a valid approach to control, particularly when the rates of disease increase are not high (16).

However, experimental data indicate that an inverse relationship between initial inoculum and rate of disease progress occurs in some polycyclic epidemics, thereby reducing the usefulness of sanitation practices (12). Although gray mold of begonia was studied in this regard, no significant differences between rates of disease progress occurred for different initial disease proportions in most cases. Under this controversial situation, assuming epidemics in vegetables and begonia are essentially the same, the eradication seems to play a role in the satisfactory control of gray mold.

Because of the high thermal sensitivity of *B. cinerea*, particularly under wet conditions (7,15), soil solarization used in plastic greenhouses to control soilborne pathogens is thought to virtually eradicate *B. cinerea*. The higher thermal sensitivity of its mycelium (7) makes it easily eradicated because of the high temperature in a closed plastic greenhouse during the summer. However, sclerotia seem to require the hydrothermal effect achieved by soil solarization. The diurnal variation of temperature and humidity during this process makes it difficult to compare with constant conditions during laboratory studies on thermal inactivation (15). Our experiments confirmed the high thermal sensitivity of mycelium and sclerotia of *B. cinerea*, since only two typical summer days were required to achieve complete loss of mycelial viability under the conditions prevalent in the Southern Mediterranean Coastal Plain. The same treatment eradicated sclerotia of *B. cinerea* that were buried at 5 cm, but those at greater depths required longer periods of solarization for complete loss of viability. It is important that sclerotia in deep soil layers should also be destroyed, because they may come to the surface after cultural practices and then sporulate.

The fact that similar heat sensitivity was shown by sclerotia of the two isolates of *B. cinerea* speaks to the general efficacy of soil solarization against this fungus. This efficacy decreases with soil depth, where accumulated degree-hours decrease. A strong logarithmic relationship was found between time and temperature for the death of four soilborne pathogens, including *Verticillium dahliae*, in natural soil (13). A different approach has been taken in our study, applying the temperature-time concept

widely used in crop physiology. Linear regressions of viability of sclerotia on the accumulated degree-hours using different temperature thresholds were calculated. The best goodness-of-fit for the regression indicated which temperature thresholds should be considered in each case in order to calculate the degree-hours required for a given level of control of the inoculum. Then, decisions can be made as to the minimum duration of soil solarization, as long as the degree-hours over that threshold can be estimated in a field situation.

Soil solarization applied to sand-mulched soils in plastic greenhouses of the Southern Mediterranean Coast of Spain for the control of soilborne plant pathogens will eventually achieve eradication of mycelium and sclerotia of *B. cinerea* that remain in infected crop residues incorporated into the soil. Moreover, sandy soils are more suited to solarization than are coarse soils, in which the effectiveness of solarization may be impaired (14). In addition, a slight reduction of the pathogenic ability of sclerotia from solarized soil was observed independently of the isolates and soil depths studied. This effect, known as the weakening phenomenon, has previously been reported in the case of *Fusarium oxysporum* f. sp. *niveum* and was attributed to sublethal temperatures that affect virulence (4).

Soil solarization may retard epidemic development of gray mold in vegetable crops, but concerted eradication in an area might be required because of the possible immigration of conidia from outside the greenhouse. Therefore, sanitation practices outside the plastic greenhouses, i.e., removal or destruction of plant residue piles in the area between the greenhouses, would be complementary to solarization. The combination of these two eradication methods would lower the incidence of gray mold and reduce the number of fungicide treatments required.

## LITERATURE CITED

- Bollen, G. J., and Scholten, G. 1971. Acquired resistance to benomyl and some other fungicides in a strain of *Botrytis cinerea* in cyclamen. Neth. J. Plant Pathol. 77:83-90.
- Coley-Smith, J. R. 1980. Sclerotia and other structures in survival. Pages 85-114 in: The Biology of *Botrytis*. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, London.
- Ellerbrock, L. A., and Lorbeer, J. W. 1977. Sources of primary inoculum of *Botrytis squamosa*. Phytopathology 67:363-372.
- Freeman, S., and Katan, J. 1988. Weakening effect on propagules of *Fusarium* by sublethal heating. Phytopathology 78:1656-1661.
- González-Torres, R., Melero-Vara, J. M., Gómez-Vázquez, J., and Jiménez-Díaz, R. M. 1993. Use of soil solarization and fumigation to control *Fusarium* wilt of watermelon in plastic houses. Plant Pathol. 42:858-864.
- Jarvis, W. R. 1962. Splash dispersal of spores of *Botrytis cinerea*. Nature 193:599.
- Jarvis, W. R. 1977. *Botryotinia* and *Botrytis* species: Taxonomy, physiology and pathogenicity. Can. Dep. Agric. Monogr. No. 15.

8. Javed, Z. U. R. 1977. The effect of various conditions on survival of sclerotia and conidia of *Botrytis cinerea* f. sp. *coffee* the cause of "wart disease" on coffee in Kenya. *Kenya Coffee* 42:53-62.
9. Katan, J. 1981. Solar heating (solarization) of soil for control of soilborne pests. *Annu. Rev. Phytopathol.* 19:211-236.
10. López-Herrera, C. J., Sagasta-Azpeitia, E. M., and Grana Enciso, E. 1986. Estudio de la facies esclerocial de *Botrytis cinerea*. *Phytopathol. Mediterr.* 25:19-25.
11. Miller, P. M., and Waggoner, P. E. 1957. Dispersal of *Botrytis cinerea* among strawberries. (Abstr.) *Phytopathology* 47:24.
12. Plaut, J. L., and Berger, R. D. 1981. Infection rates in three pathosystem epidemics initiated with reduced disease severities. *Phytopathology* 71:917-921.
13. Pullman, G. S., DeVay, J. E., and Garber, R. H. 1981. Soil solarization and thermal death: A logarithmic relation between time and temperature for four soilborne plant pathogens. *Phytopathology* 71:959-964.
14. Tjamos, E. C., and Faridis, A. 1980. Control of soilborne pathogens by solar heating in plastic houses. *Proc. Congr. Mediterr. Phytopathol. Union* 5:82.
15. Van den Berg, L., and Lentz, C. P. 1968. The effect of relative humidity and temperature on survival and growth of *Botrytis cinerea* and *Sclerotinia sclerotiorum*. *Can. J. Bot.* 46:1477-1481.
16. Vanderplank, J. E. 1963. *Plant Diseases: Epidemic and Control*. Academic Press, New York.