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# Survival of *Botrytis cinerea* in southeastern Spanish greenhouses

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**Abstract**

The relative importance of sclerotia and mycelia of *Botrytis cinerea* Pers ex Fr. as structures of survival in southeastern Spanish greenhouses was investigated. Sclerotia were not found in the SE region, neither on plant debris nor on living plant material, suggesting it may serve only a minor role in epidemic development. *B. cinerea* survived mostly as mycelium. The percentage of artificially inoculated tomato stem pieces from which mycelium was recovered, was used to quantify its survival rate. Outside the greenhouses, mycelium survived in 33% and 5% of the tomato stem pieces 110 days after inoculation in 1995 and 1997, respectively. After the same number of days inside the greenhouses, no mycelium was recovered from stem pieces in 1995, and in 1997 only 7% of the stem pieces contained mycelium. Survival of mycelium outside and inside the greenhouses was significantly ($P < 0.05$) different after 47, 83, and 110 days of exposure to field conditions in 1995, but they were not different in 1997. Under controlled conditions, mycelium of *B. cinerea* lost viability at 100% relative humidity at temperatures ranging from 5 to 40 °C, suggesting that air temperature and relative humidity accounted for loss of viability of mycelium.

*Abbreviations:* BSM – *Botrytis* selective medium, cfu – colony forming units, $LD_{50}$ – lethal dose for 50% of the population, RH – relative humidity.

## Introduction

Epidemics of grey mould caused by *Botrytis cinerea* Pers ex Fr. occur every year in greenhouses in southeast Spain, usually from November to December through February. Crops grown at this time in the greenhouses are tomato, bean, pepper, cucumber, eggplant and squash, which are all susceptible to attack by *B. cinerea*. Conidia of *B. cinerea* infect the fruits of cucumber and squash plants, the leaves and stems of tomato plants, and leaves and pods of beans. *B. cinerea* must survive during summer under extreme conditions of high temperatures and very little precipitation.

The usual cycle of *B. cinerea* in northern Europe includes overwintering as sclerotia and as mycelium growing on plant debris in the soil (Smith et al., 1988). In tomato plants, sclerotia develop on woody tissues and on organic matter in the soil (Stall, 1991). In bean plants, sclerotia are produced on stem and pod tissue and remain on debris in the field (Jarvis, 1991). Conidia are produced throughout the growing season (in spring and summer), on germinating sclerotia or mycelium formed in dead tissues of numerous host species (Coley-Smith, 1980). Presence of ascospore inoculum that arises from spermatized sclerotia has been reported only in bean in New York (Pollach and Abawi, 1975).

The survival rate of mycelium and sclerotia in the soil is influenced by environmental conditions. Mycelium does not survive *in vitro* for more than one year at 0 °C and 95–100% relative humidity, and survives for less than one month at 20 °C when the relative humidity is below 95% (Van der Beerg and Lentz, 1968). Sclerotia have been reported to survive fluctuating

or extreme temperatures in laboratory studies (Coley-Smith, 1980). In northern regions of Europe and the USA, mycelium and sclerotia have been suggested to be able to survive for relatively long periods in colonised tissue above the soil surface (Coley-Smith, 1980), and their relative importance related to environmental conditions have been quantified in some cases. In Canada, in field grown strawberries, more than 90% of the inoculum of *B. cinerea* came from mycelium in old, overwintering leaves (Braun and Sutton, 1987), whereas in Scotland the importance of sclerotia was emphasized but not quantified (Jarvis, 1962). In Israel, where crop cycles in greenhouses are similar to the management cycles utilized in SE Spain, little importance is given to survival of *B. cinerea* as sclerotia (Elad et al., 1992). Under these conditions, *B. cinerea* has been reported to survive mostly as mycelium. It was found that mycelium had survived in the internal tissues of cucumber stems in up to 18% of the infected stems in open air conditions by the end of the summer (Yunis and Elad, 1989).

The survival rate for sclerotia in the soil is reported to be affected by depth of burial. Viability of sclerotia buried in January in Washington State (USA) was higher at a depth of 10 and 20 cm than at the surface level (Hsiang and Chastagner, 1992). In southern Spanish conditions viability of sclerotia buried in a sand-mulched soil in July was lower at 5 cm depth than at 15 and 25 cm depth, and PDA disks of mycelia survived better at 15 cm than at 5 or at 25 cm in the same soil (Lopez-Herrera et al., 1994).

To design effective control strategies against grey mould, it is important to know the relative importance of sclerotia and mycelium as sources of inoculum of *B. cinerea*, but this remains unknown under the conditions in SE Spain. Furthermore, survival of the fungus on the soil surface has never been studied, even though ploughing is not a common cultural practice in the greenhouses. Microclimatic conditions within greenhouses may vary greatly during the summer, and periods of air temperature above 40 °C are frequently recorded in July and August, even when cultural practices include blanching of the polyethylene cover.

Differences in survival outside or inside greenhouses may have important consequences for population dynamics of *B. cinerea* in the region, especially if *B. cinerea* did not survive inside greenhouses. The specific objectives of this work were to identify the main survival structures of *B. cinerea* in SE Spain in summer time, and to quantify differences in the survival rates of mycelium and sclerotia of *B. cinerea* when exposed to environmental conditions encountered outside and inside greenhouses.

## Materials and methods

### *Survey of structures of survival*

Plant residues (tomato, squash, bean and cucumber grown the previous season) from 10 commercial greenhouses were examined for sclerotia production prior to the onset of grey mould epidemics. In October 1996, samples were taken from 10 small squares (each of 20 $cm^2$ size) over two diagonals of each greenhouse. All dead leaves, stems, fruits and any plant debris found on the soil were collected. Four trash piles of pruned plant material, dead plants, and fruits (tomato and pepper residue) that were in the surroundings of the surveyed greenhouses were also sampled. In October 1998, plant residues were collected outside greenhouses and they were examined for surviving mycelia of *B. cinerea*. All residues were examined for sclerotia of *B. cinerea* with a stereomicroscope (65–100×) magnification). The sampled plant residues were placed in Petri dishes (13 cm diameter) on filtre paper wetted with distilled water and incubated at 22–24 °C. They were examined daily with a stereomicroscope and checked for conidiophores and conidia of *B. cinerea*.

The production of sclerotia on plant tissue and on detached leaves and stems of tomato were also examined. In 1997/98 and 1998/99, the development and evolution of grey mould disease were examined weekly in two tomato (*Lycopersicon esculentum* Mill. cv. Daniela; Hazera) greenhouses. To study production of sclerotia on detached tissue, infected leaves from the greenhouse were collected as they displayed symptoms of grey mould. In the 1997/98 season whole leaves were collected on 3 October, 27 November 1997, and 15 January 1998. Each leaf was placed in a nylon mesh bag of 0.3 mm mesh size, and bags were left on the soil of the greenhouse until the crop was uprooted on 25 January 1998. In 1998/99, *B. cinerea* attacked later, and infected leaves were collected on 25 January 1999. Bags were placed on the soil of the greenhouse from this date until 15 April 1999. All plant material was examined carefully with a stereomicroscope for the presence of sclerotia of *B. cinerea*. Survival of the mycelium was also investigated after 15 April 1999 by the dilution plate method. Climatic conditions were

recorded during the two experimental periods using a data logger (Escort Junior Jcal v1.3, Messtechnik ag, Switzerland).

*Survival of mycelium under field conditions*

Survival of mycelium of *B. cinerea* was studied on tomato stem fragments artificially inoculated and placed under field conditions in 1994, 1995 and 1997 in polyethylene greenhouses located at the Experimental Station of Vegetable Crops in Almeria, Spain. The same greenhouse was used in 1994 and 1995, and another one in 1997.

To produce the stem fragments, tomato plants (cv. Lorena) were grown in pots until they had 5–7 leaves. Tomato stems of 2–6 mm in diameter were cut into 2 cm-long pieces and inoculated by immersion in a spore suspension of *B. cinerea*. Three isolates of the pathogen were used in 1994, and seven in 1995 and 1997. Each year the isolates were randomly taken from a collection derived from a 1992 survey of commercial greenhouses within the region. The isolates were maintained in sand with oat meal (3% v/v) at 4 °C. The stem fragments were incubated in Petri dishes with sterile filtre paper wetted with sterilized distilled water at 22 °C in the dark until the pieces showed symptoms of *B. cinerea* infection. Then, they were dried at 30 °C for 7 days, and placed in nylon bags of 0.3 mm mesh size. On 1 July 1994, 1 June 1995 and 17 June 1997, four bags per isolate were exposed to the environment at the soil surface. Two of the four bags were placed near the lateral border within a greenhouse, and two bags were placed outside on the soil surface between two greenhouses. A hygrothermograph was placed 1 m above the soil surface to record air temperature and relative humidity both inside and outside the greenhouse structures.

To study survival of mycelium on tomato stems over time, samples of 10 stem pieces from each bag were taken 30, 60 and 90 days after initiating the treatments in 1994; and 47, 71 and 106 days after the start of treatments in 1995 and 1997. Survival was evaluated on pieces at time 0, that is, before the bags were placed on the soil surface. Stem fragments were surface sterilized by dipping them in a 1% NaClO solution for 1 min and rinsing twice in sterile distilled water. After they were dried on a sterile filtre paper, each piece was split lengthwise, placed on PDA in Petri dishes with streptomycin (500 mg/l) and incubated in the dark at 22 °C. Survival was evaluated as the ability of hypha to grow on PDA within a 6 day period and as its ability to infect plants, and the results are presented as the percentage of stem pieces with viable (i.e. surviving and pathogenic) mycelium over a sample of 10. For each sampling date, there were two replications per isolate inside and outside the greenhouse. Mycelium viability at time 0 was used as reference (viability = 100). Pathogenicity tests were done on cucumber cotyledons (*Cucumis sativus* L., cv. Hyclos Mix F1 RS; Royal Sluis), detached and placed in Petri dishes on two filter papers soaked with sterile distilled water. Cotyledons were inoculated with 4 mm-diameter plugs of actively growing mycelium and incubated at 22 °C in the dark for 6 days. Isolates were considered pathogenic if cotyledons were soft rotted.

Percentage viability was compared inside and outside the greenhouse for each sampling date. An analysis of variance was performed with the General Linear Models Procedure (GLM) of SAS (1987) with the following factors considered in the model: the year of the experiment (1995 and 1997), the location of the stem samples (inside and outside the greenhouse), and isolates as a random factor nested in 'year' factor. The 'Isolates' factor was nested in the 'year' factor because different isolates were used each year. Respective interaction terms were also included in the model. Results from 1994 were not included in the analysis of variance, because only 3 isolates were used that year. Percentage of survival was transformed to arc sine of square root of the proportion prior to the analysis of variance. Appropriate F tests for each term of the model were derived from the Expected Mean of Squares, which were obtained by application of the rule for finding expected mean squares (Sokal and Rohlf, 1981). Since the factor interactions were always present, survival outside and inside were compared for each year by the Bonferroni method of multiple comparisons (Neter et al., 1985). The estimated variance for comparing the two locations for any given year was two times the Mean Square of the interaction term 'location by isolate' divided by the number of isolates (7) and the number of replications (2) (Sokal and Rohlf, 1980; Neter et al., 1985).

*Survival of mycelium under controlled temperature*

Infected tomato leaves of plants grown in two greenhouses were collected in January 1998 (one

greenhouse) and May 1999 (another greenhouse). Most lesions of *B. cinerea* were sporulating at the time of sampling, so samples included mycelium, conidiophores and conidia. Three or four leaves, which had healthy tissue as well, were placed in paper bags (6 bags in 1998 and 8 in 1999). The bags were introduced in incubators at five temperatures (5, 20, 30, 35 and 40 °C ± 2 °C), half of them previously enclosed in plastic containers (24 × 30 × 14 cm) containing two filter papers soaked in sterile water to obtain 100% RH. Thus, the bags were exposed to two humidity conditions, hereafter called 'ambient' or 'saturated' conditions.

After incubation for 15 weeks in darkness, the contents of each group of bags were pooled, grinded and mixed in an Osterizer appliance, and populations of *B. cinerea*, total bacterial flora and total mycoflora were estimated by the following dilution plate method. Two plant samples (5–8 g each) were taken from each group and added to 250 ml flasks containing 150 ml sterile 0.01 M phosphate buffer (pH 7.0). After shaking for 30 min at 150 rpm, the liquid was serial diluted to $10^5$-fold. Three aliquots (0.1 ml) per dilution were spread on PDA, on PDA+0.5 g/l streptomycin sulphate, and on *Botrytis* selective medium (BSM) (Kerssies, 1990). Plates were incubated at 20–25 °C and the number of colonies counted. Counts of colony-forming units (cfu) per gram of dry plant for each combination of temperature and humidity were obtained for *B. cinerea* (counted on BSM), for mycoflora in general (counted on PDA + streptomycin) and for bacteria (counted on PDA).

To test the pathogenicity of *B. cinerea*, four randomly identified colonies per plate of BSM were transferred to PDA plates and incubated at 20–25 °C in darkness. After 7–10 days, spores of colonies from all the plant samples maintained under the same conditions were pooled, and a suspension of $10^6$ spores/ml was prepared. Tomato leaflets were then submerged in the spore suspension for 15–30 min and placed in a Petri dish with two wetted filtre papers. After one week of incubation at 20–22 °C in the dark, pathogenicity was evaluated by the isolates´ability to produce soft rots.

Populations of total mycoflora and bacteria were compared by their respective analysis of variance performed with the General Linear Models Procedure (GLM) of SAS (1987). Factors considered in the model were year (1998, 1999), temperature (5, 20, 30, 35 and 40 °C), humidity (ambient, saturated), and interaction terms of second and third order. Differences in total mycoflora between ambient and saturated conditions were compared for each temperature by the Bonferroni method of multiple comparisons (Neter et al., 1985).

## Results

### *Survey of structures of survival*

No sclerotia were observed with a stereomicroscope in or on plant residue collected inside and outside greenhouses before the epidemic of *B. cinerea* began in 1996. When naturally infected tomato leaves were detached and placed in bags on the soil surface of the greenhouse, no sclerotia were produced within 1, 7 or 8 weeks in 1998, nor within 11 weeks in 1999. Sclerotia were not produced in tomato plants during the epidemic cycles in 1997/98 and 1998/99.

Mycelia of *B. cinerea* were found in samples of plant residues occasionally collected in October 1996 and 1998. Conidiophores and conidia were abundantly produced when placed in a moist chamber.

### *Survival of mycelium under field conditions*

Survival of mycelium in infected tomato stems varied ($P < 0.05$) with the year of the experiment and the environment in which samples were located on the soil surface (outside or inside the greenhouse). In 1995, survival outside the greenhouse was higher ($P < 0.05$) than inside after 47, 83 or 110 days of exposure at these field conditions (Table 1). However, no differences between survival outside and inside the greenhouses were detected in 1997. Furthermore, mycelium had not survived inside the greenhouse after 110 days in 1995, whereas outside it did in over 32% of the inoculated stem pieces (Table 1). In 1994, mean survival of mycelium decreased faster outside than inside the greenhouse (results not compared statistically). Although mycelium remained viable in 22% of the stem pieces that were subjected to conditions inside the greenhouse for 30 days, and in 11% of the pieces at 60 days, no mycelium survived the 90-day exposure. In contrast, only 9% of the stems contained viable mycelium at the end of the 30-day exposure to conditions outside the greenhouse, and no mycelium remained viable in any samples that were exposed for 60 and 90 days. All recovered mycelia were pathogenic on cucumber cotyledons.

*Table 1*. Percentage of survival of mycelium of *Botrytis cinerea* on the soil surface outside and inside the greenhouse after 47, 83 and 110 days of exposure in 1995 and 1997

| | Time and year of exposure | | | | | |
|---|---|---|---|---|---|---|
| | 47 days | | 83 days | | 110 days | |
| Location | 1995 | 1997 | 1995 | 1997 | 1995 | 1997 |
| Outside | 57.4[1] a[2] (15–97) | 26.4 a (6–57) | 44.8 a (6–88) | 12.2 a (0–15) | 32.5 a (11–90) | 5.1 a (0–24) |
| Inside | 7.1 b (0–29) | 14.1 a (0–29) | 2.3 b (0–7) | 6.3 a (0–22) | 0 b (0) | 7.4 a (0–20) |

[1]Mean percentage of 10 tomato stem pieces that contained viable mycelium. Stems were artificially inoculated with seven isolates each year, each isolate repeated twice. Values in parentheses are the minimum and maximum percentage of survival for that date and location.

[2]Values followed by the same letter within a column do not differ ($P < 0.05$) according to a Bonferroni procedure of multiple comparisons for each sampling date.

There was a high degree of variability in the survival of mycelium of *B. cinerea* among individual isolates each time and year of exposure inside or outside the greenhouses (indicated by the maximum and minimum values of percentage of survival, Table 1). Moreover, in 1997 there were some isolates that survived for a longer time in stem pieces located outside, whereas other isolates survived longer inside. This was not observed in 1995, when each isolate always survived a longer period outside than inside the greenhouse. These observations were also concluded from the analysis of variance performed for each sampling date, as variation in survival among isolates was always significant ($P < 0.05$), and so the interaction term of the factors year and location.

Maximum daily temperature inside the greenhouse was above 30 °C every day between 12:00 and 16:00 hours in July and August in 1995 and 1997 (Figure 1). The air temperatures inside the greenhouse exceeded 40 °C for a total of 1486 h between 1 June and 17 September 1995, but only for 154 h between 16 June and 6 October 1997. Absolute maximum temperatures outside the greenhouse were 37.8 and 30.9 °C in July 1995 and 1997, respectively, and 36.1 and 35.8 °C in August in 1995 and 1997, respectively. The relative humidity recorded inside the greenhouse exceeded 80% every night, and minimum daily relative humidities were between 20 and 30% when air temperatures reached their maximum.

*Survival of mycelium under controlled temperature*

Mycelium of *B. cinerea* on naturally infected leaves remained viable following a 15 week (2520 h) exposure under 'ambient' conditions to temperatures of 5, 20, 30, 35 and 40 °C (Table 2). However, under saturated conditions, mycelium of *B. cinerea* did not survive at any temperature in the 1998 experiment. In the 1999 experiment, *B. cinerea* appeared occasionally (cfu/g of dry plant $< 400$) and was pathogenic only at 20 and 35 °C. At the same time, there was a significant ($P < 0.05$) increase in cfu of total bacteria when naturally infected leaves were incubated under saturated humidity conditions at 35 and 40 °C. No significant increase of total bacteria was estimated under 'ambient' conditions of humidity. Saturated conditions gave a significant increase in total mycoflora at 30 °C. Only at 5 °C there was a decrease in the total population of mycoflora under saturated conditions, whereas at 20 °C no significant effect on mycoflora or bacteria was detected (Table 2).

## Discussion

The relative importance of sclerotia and mycelium of *Botrytis cinerea* as structures of survival in southeastern Spanish greenhouses was investigated. Our work shows that *B. cinerea* survives as mycelium during summer. It was able to survive (33% in 1995 and 5% in 1997) following a 110 day period of exposure (until 20 September 1995 and 6 October 1997) at the environmental conditions outside the greenhouse. By that time vegetable crops were already being cultivated and infection by *B. cinerea* may have occurred if weather conditions had been favourable. Furthermore, survival outside and inside greenhouses varied between years. In 1997, the survival rates between locations were similar, but in 1995 the survival rates for mycelium maintained outside the greenhouse was higher than the rates observed for stems exposed to the environment inside a greenhouse. In addition, mycelium of *B. cinerea* inside the greenhouse did not survive 110 days in 1995, but it survived in 1997.

Large differences in temperature were recorded within the greenhouses used in the 1995 experiment (1486 h above 40 °C) and the 1997 experiment (154 h above 40 °C). The greenhouse used in the 1997 study was larger and shaded more hours during the day than

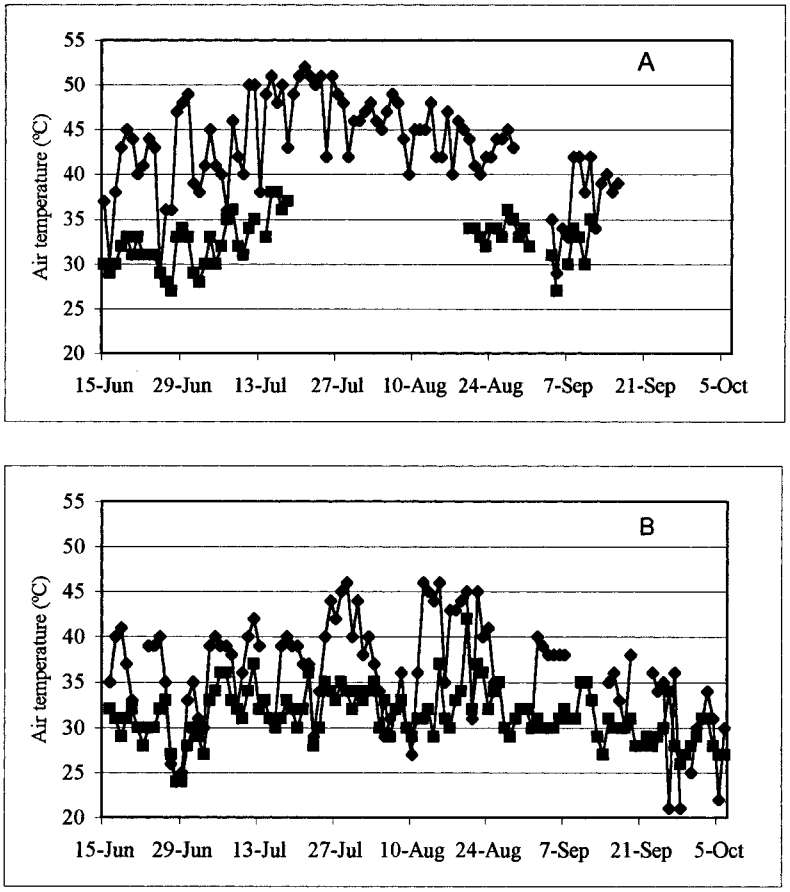


*Figure 1.* Daily maximum temperature recorded outside (■) and inside (♦) the greenhouse during the experimental period to measure the survival rate of mycelium of *Botrytis cinerea* in tomato stem fragments in 1995 (**A**) and 1997 (**B**).

*Table 2.* Populations of *Botrytis cinerea*, total mycoflora and bacterial flora (cfu $g^{-1}$ of dry plant) in infected tomato leaves after a 15-week exposure to controlled conditions of temperature and humidity[a]

| | *B. cinerea* | | Mycoflora | | Bacterial flora | |
|---|---|---|---|---|---|---|
| | Dry | Wet[b] | Dry | Wet[b] | Dry | Wet[b] |
| 5 °C | 48855 a[c] | 0 b[c] | $25.2 \times 10^6$ a | $1.7 \times 10^6$ b | $3.8 \times 10^8$ a | $13.7 \times 10^8$ a |
| 20 °C | 42478 a | 72 b | $3.0 \times 10^6$ a | $4.1 \times 10^6$ a | $12.6 \times 10^8$ a | $2.6 \times 10^8$ a |
| 30 °C | 31487 a | 0 b | $2.1 \times 10^6$ a | $5.8 \times 10^6$ b | $2.1 \times 10^8$ a | $1.1 \times 10^8$ a |
| 35 °C | 7798 a | 189 b | $0.2 \times 10^6$ a | $0.1 \times 10^6$ a | $2.0 \times 10^8$ a | $26.2 \times 10^8$ b |
| 40 °C | 3407 a | 0 b | $0.8 \times 10^6$ a | $0.8 \times 10^6$ a | $5.3 \times 10^8$ a | $32.3 \times 10^8$ b |

[a]The data values are combined populations from leaves collected from two tomato greenhouses in 1998 and 1999.

[b]Wet conditions were established in an enclosed plastic container with filter paper moistened with distilled water.

[c]Values within columns representing 'dry' and 'wet' conditions for each of the three groups that are followed by the same letter do not differ ($P < 0.05$) for a given temperature treatment according to a Bonferroni procedure of multiple comparisons.

was the greenhouse used in 1995. However, the temperature cannot be the only explanation of differences in survival of mycelium between both years of the study. In 1995, the mycelium exposed to temperatures, which exceeded 40 °C for 1486 h inside the greenhouse, lost its pathogenicity prior to observations made on day 110. Under controlled conditions, mycelium lost viability when exposed continuously to constant temperatures for 2520 h and saturated air (100% RH). In the case of exposure to 'ambient' conditions (variable humidity that never reached 100% RH) at the same constant temperatures, mycelium remained viable after 2520 h. Relative humidities inside the greenhouses were usually above 80% RH at night and dew occasionally formed. Saturated conditions over continuous periods of time seem to be necessary to reduce viability of mycelium. Periods of high RH inside the greenhouses may have speeded up the loss of viability.

The survival of mycelium of *B. cinerea* has been little investigated. However, a few laboratory studies of survival of sclerotia under extreme conditions have been made that corroborate our results. Coley-Smith (1980) reported the effect of temperature on survival of sclerotia of *B. cinerea* in the laboratory. With dry heat, high temperatures resulted in a slow death rate of sclerotia (value of lethal doses for 50% of the sclerotia, $LD_{50}$, was five months at 20 °C, 103 days at 40 °C, and 3 days at 80 °C). With wet heat (sclerotia immersed in distilled water in test tubes) sclerotia were killed much more rapidly ($LD_{50}$ = 1 min at 60 °C, and 30 min at 40 °C). Also, results obtained with conidia support conclusions obtained here with mycelium. Brodie and Blakeman (1975) reported a rapid loss of viability of moist conidia of *B. cinerea*, and suggested that this may be a result of carbohydrate exhaustion due to a combination of increased competition and loss of nutrients through leakage. Coley-Smith et al. (1980) also reported that the deleterious effect of high temperature on survival of conidia of *B. tulipae* is greater in wet than in dry soils.

Brodie and Blakeman (1975) also associated loss of viability of conidia of *Botrytis* species in moist conditions with microbiological activity in soil. Such activity is reduced in dry soil. Our results suggest that an important cause for loss of viability of mycelium would be antagonism and competition by other micro-organisms, but saturated conditions would be required. Humidity had a varied influence on the population levels of the mycoflora, in the sense that higher populations were observed at the 30 °C treatment, lower populations at 5 °C at saturated conditions, and there were no differences among populations between the saturated and ambient environment at temperatures of 20, 35 and 40 °C. Bacterial populations increased due to high moisture at 35 and 40 °C, but were not altered at 5, 20, and 30 °C. Cardinal temperatures of growth of specific fungi or bacteria present in debris may account for this.

Implications of the results presented in this work are important for disease management. Primary inoculum for grey mould will come mostly, if not only, from outside the greenhouses depending on the environmental conditions. This implies that sanitary practices outside the greenhouses and not only inside, which is sometimes the usual practice, can contribute in delaying the beginning of grey mould epidemics. Since the density of the greenhouses is very high in this region, there is a considerable exchange of inoculum among greenhouses in the initial stages of the grey mould disease.

Oversummering of *B. cinerea* has been previously studied (Yunis and Elad, 1989). Although this work did not evaluate differences in survival between outside and inside conditions, results concerning survival outside the greenhouses are in agreement with those obtained here. *B. cinerea* is said to survive in Israel mostly as mycelium in plant stems where it is protected from desiccation. Our work did not distinguish between internal and external mycelium, because stems were split lengthwise or crushed to quantify viability; thus, this point cannot be evaluated.

Sclerotia are considered to be a survival structure of *Botrytis* species, with a relative importance not quantified in most of the cases (Coley-Smith, 1980). Overwintering of sclerotia of *B. cinerea* has been widely reported (Smith et al., 1988) as well as that of *Botrytis squamosa* (Ellerbrock and Lorbeer, 1977) and *Botrytis tulipae* (Hsiang and Chastagner, 1992). Sclerotia were not found in the SE region of Spain, either on plant debris or on living plant material. Although there are methods to look for sclerotia in soil (Braun and Sutton, 1987; Thomas et al., 1983) we have not considered using them since no signs of sclerotia formation were detected. Even so, we cannot rule out their occasional formation. If this was the case, sclerotia would play a minor role as a structure of oversummering and raises a question about the occurrence of the teleomorphic stage (*Botryotinia fuckeliana*). The two mating types required for sexual compatibility (MAT1-1 and MAT1-2) have been found in SE Spain (Delcán, 1997). However, apothecia of *B. fuckeliana* have never been reported in this region. Probably, the rare occurrence

of sclerotia in SE Spain could be a cause for the lack of apothecia, since previous production of sclerotia is usually a prerequisite to formation of apothecia. In other parts of the world the teleomorph of the fungus also occurs rarely in the field (Polach and Abawi, 1975), thus the anamorph is the predominant stage .

Survival of *B. cinerea* in SE Spanish conditions is different from that described in northern Europe. Differences in the crop cycles and weather conditions (*B. cinerea* should overwinter in northern Europe while oversummer in SE Spain) may account for this, playing an important role in the epidemiology of the pathogen.

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